

CLINICAL CHEMISTRY
IN
PRACTICAL MEDICINE

BY

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SECOND EDITION

EDINBURGH

E. & S. LIVINGSTONE

16 & 17 TEVIOT PLACE

1937

Pharmacology

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Computerised

D. O

NLVS/IVRI



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Printed in Great Britain

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PREFACE

DURING the past twenty years biochemistry has become a subject of ever-increasing importance in medicine ; especially is this the case in the diagnosis, prognosis, and treatment of the so-called metabolic diseases. But there is hardly a branch of medicine or surgery which does not, at some time, have need of the information which can be supplied by the biochemical laboratory ; and no student or practitioner can be regarded as well equipped to-day who does not possess a knowledge of the circumstances in which a chemical examination may be of service, of the interpretation and significance to be placed upon the results of such an examination, and of the technique of obtaining the specimens to be sent to the laboratory for analysis. In order to appreciate the rationale of chemical pathology in its application to practical medicine, it would also be ideal for him to have a broad knowledge of how the various chemical analyses are carried out, and a detailed knowledge of the method of performing many of the simpler tests which do not require much time or equipment, and which may be performed, if he so wishes, in his own surgery or dispensary. This book has been written with a view to giving information on these points to the practitioner, house-physician, and senior student. It is not an elaborate text-book of biochemistry for the laboratory worker, but it aims at something more than a short description of side-room methods, or the

interpretation alone of laboratory results. It does not attempt a comprehensive account of the well-nigh innumerable tests which have been proposed at one time or another for investigating the various bodily functions ; it selects those which are more important, and which, in the authors' experience, have proved useful and reliable. There are, indeed, a number of branches of the subject on which the book does not touch, but the authors' aim has been rather to treat adequately, within a small compass, the more everyday problems requiring chemical investigation, than to produce an exhaustive treatise on the capabilities of the biochemical laboratory in medicine. They have attempted further to explain the rational foundation of the tests in the hope that such an explanation will help in understanding the mechanism of many of the pathological processes concerned.

Laboratory workers, like many other specialists, are apt to be over-enthusiastic about their own branch of work, and to attach undue weight to the information they are able to give ; and there is little doubt that there is a tendency nowadays, with the increased facilities for chemical and other investigations, for some deterioration to take place in bedside observation as compared with former days. The chemical laboratory, by itself, cannot supply a ready-made diagnosis, but only a link — on some occasions a valuable one — in the chain of evidence which leads the physician to his diagnosis, prognosis, and treatment. The authors, therefore, have been at pains not only to point out the value of certain chemical investigations, but also to stress their limitations. If only the nature of the information which these investigations afford is realised, then, even if the methods are

multiplied, as they are certain to be, far beyond those in use to-day, there is yet no reason why they should divert attention from the bedside study of disease.

The second edition has been thoroughly revised in the light of the authors' own and others' experience; some tests which have been superseded or have not stood the test of time have been deleted; others which have proved more valuable have been introduced. Certain sins of omission, to which reviewers of the first edition called our attention, have, it is hoped, been remedied.

The arrangement of the book has been so altered as to allow the technical details to be collected into an Appendix. This has enabled the argument in the text to continue without interruption, and the new Appendix, with the additional methods we have added, includes all the side-room tests (apart from bacteriological) which the student is usually required to know.

The authors wish to take the opportunity of thanking Dr. Harold Scarborough for his most helpful criticism of the manuscript, for his aid in proof-reading, and for contributing the haematological section in Appendix I.

They also wish to acknowledge the courtesy of Messrs. Heffer of Cambridge, who granted permission for the adaptation of the diagram appearing on p. 185 of Cole's *Practical Physiological Chemistry*, 8th edition.

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October 1937.

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CHAPTER I

INTRODUCTION

SIMPLE chemical tests such as the detection of sugar and protein in urine have long been used as aids in the diagnosis of disease. The last twenty or twenty-five years, however, have witnessed an enormous increase in our knowledge of the chemical abnormalities to be found in disease. This increase has occurred along with (though beginning rather later than) the growth of biochemistry, and has been greatly aided by the development of analytical methods suitable for estimating on a micro-scale many of the substances present in the body fluids and excreta.

As usually happens when a new department of knowledge is rapidly made available, there is difficulty at first in appreciating its real worth. The enthusiasm of its sponsors and the hostility of its critics both tend for a while to hinder formation of an unbiased judgment. In the case of biochemistry as applied to medicine that phase is now, happily, passing, and it is possible to assess the new science at its true value, and to rank it as one of many valuable aids in the diagnosis and prognosis of disease. It is not, has never been, and almost certainly never will be, the philosopher's stone of medicine; its methods, both as regards number and accuracy, are still far from perfect; it is still growing so rapidly that many of its conclusions may require modification; even with

accurate methods of analysis the normal biological range of variation and the reserve of power possessed by all healthy tissues limit, and must continue to limit, the usefulness of most chemical tests. Nevertheless, properly used and interpreted with due regard to these limitations, chemical methods have established their right to be considered among the major aids of diagnostic medicine. Like other laboratory methods, they must be used simply to supply evidence which the diagnostician has then to consider along with his clinical findings and all other available information ; in other words, like other laboratory methods, they supplement, but do not replace clinical examination.

As in radiological, bacteriological, or serological examinations, many techniques available for biochemical examination are necessarily the province of the technical expert. Radiology, of course, occupies a somewhat special position on account of the complicated and expensive equipment which is required, but a number of biochemical, as of bacteriological, tests, can well be made by the practitioner without specialised biochemical training. It is perfectly obvious that the accumulation of data regarding any particular case is quite useless unless the data can be interpreted ; diagnosis consists in collecting and interpreting all relevant data. It follows that the practitioner of these days must understand the meaning not only of the signs he elicits at the bedside, but of the further information he obtains from laboratory procedures to supplement his clinical examination.

The physician, confronted by a certain group of clinical signs and symptoms, and desirous of obtaining the additional data which biochemical methods can give him, must know what particular biochemical

investigation, if any, may throw further light on his problem. This holds whether he is to carry out the actual analysis himself or to delegate that part of the work to a biochemist. He must not order, as is sometimes done, a "complete" or "routine" examination of some biological fluid; such an order is usually merely a cloak for ignorance. Even though he does not carry out the actual chemical analysis himself, the physician should have some general idea of the procedures involved. Otherwise he can hardly appreciate the meaning of, and the necessity for, the various precautions and conditions which the chemist demands in the preparation of the patient and the obtaining of samples. Yet this part of the test is just as important as the chemical analysis, and is, of course, essentially within the province of the clinician. The clinician must, therefore, know how to obtain the specimens for chemical examination, how to preserve them from contamination or alteration, how to send them to the laboratory, and under what conditions they must be obtained so as to exclude fallacies as far as possible. He must know the meaning of the results obtained, and the significance to be attached to a variation from the normal or to the absence of such a variation. This requirement implies a knowledge of the normal with the physiological variations from it, and of the conditions, other than that suspected, which may cause a result similar to that obtained.

Valuable as a biochemical investigation often is, it cannot be over-emphasised that its results must always be considered in conjunction with those of clinical examination, and that taken alone it can rarely, if ever, justify a diagnosis. Just as a systolic murmur

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heard over the apex-beat of the heart is not, by itself, evidence of mitral incompetence, so a high blood urea is not itself sufficient justification for a diagnosis of nephritis. A high blood urea may be due to cardiac failure, intestinal obstruction, dehydration from physiological or pathological causes, or any acute fever; and only a consideration of other clinical factors can differentiate amongst these possible causes. Similarly, a rather high fasting blood sugar may be due merely to nervousness on the part of the patient, but if it is accompanied by polyuria, thirst, or emaciation, it becomes valuable evidence in support of a diagnosis of diabetes mellitus. Hence, *per se*, the chemical findings may mean little, but considered as a link in the chain of evidence they may be of great importance. It is important also to realise that though a chemical analysis giving abnormal results may support a certain diagnosis, one giving normal results does not necessarily exclude it. The organs of the human body are mostly constructed on a very generous scale. Thus it seems that half, or more, of the kidney may be destroyed by disease before its function becomes markedly impaired. Even then impairment of one function does not necessarily mean impairment of all; so that clinical signs may lead to a suspicion of nephritis, and quite rightly, before the blood urea is raised. Hence a normal value for the blood urea or non-protein nitrogen is not incompatible with the existence of chronic Bright's disease. It has further to be remembered that most substances of biological importance have, even in health, a considerable range of variation on either side of the normal mean. Hence an analytical result within the normal range may actually be pathological in a particular case, although of course

there is usually no means of knowing whether it is so or not. Thus the normal carbon-dioxide combining power of the blood lies between 53 and 73 volumes per cent., and a result of 56 volumes per cent. must be taken as normal. Actually, however, it may represent a pathological fall from an original value of 70 volumes per cent., a fall which might be of considerable significance if the original value of 70 were known. It is rarely that such values within the normal range are known to be actually pathological, and this wide range of physiological variation therefore constitutes an important limitation of the biochemical method of investigation.

The value of biochemical methods of investigation is not confined to diagnosis, but extends to prognosis and the control of treatment, where, indeed, some of their chief uses are found. It sometimes happens that after a diagnosis has been made, and treatment has been instituted, considerable improvement takes place, and can be demonstrated by chemical means before the clinical picture has shown much alteration. Thus in a patient with meningococcal meningitis the first sign of improvement after serum treatment may be the return of sugar in the cerebro-spinal fluid, on which a more favourable prognosis may be given. On the other hand, an apparent clinical improvement may occur under treatment without any real amelioration in the underlying disease, and the true state of affairs may be revealed by chemical examination. An instance of this is found in the advanced nephritic, who apparently improves under rest and dietetic treatment, but for whom the prognosis remains desperately bad, as is evidenced by the blood creatinine remaining at a high level.

While in some diseases chemical examinations are

an aid in the control of treatment, in others successful treatment can hardly be carried out without them. No physician, for example, would care to treat a case of diabetes mellitus without frequent recourse to chemical examination of the urine, if not of the blood, and no surgeon should nowadays undertake the risk of radical prostatectomy without previous investigation of the blood urea.

Most of the investigations mentioned above, and many others like them, may be successfully carried out in general practice—with, perhaps, the technical aid of the biochemist—and do not necessitate the sending of the patient to hospital. In some cases even the actual analysis can be carried out by the practitioner himself, if he has the time and inclination to do so. When the samples are sent to a biochemist for analysis they should invariably be accompanied by a short note of the clinical condition of the patient, since the biochemist's experience may then suggest some further useful test. Close co-operation between the chemist and the clinician is always necessary, for both are partners to the transaction, and a successful accomplishment of any investigation can be achieved only if each does his own part efficiently, and knows the full facts of the case. Besides being of service in the better-known and more usual methods of chemical investigation, such as are dealt with in this book, it sometimes happens that the biochemist is able to render assistance in the elucidation of obscure and difficult cases. No single book could deal with all such contingencies, and this one pretends to no more than a treatment of the commoner and more generally useful tests, but in cases of difficulty a consultation between the clinician and the biochemist may be fruitful.

CHAPTER II

THE COLLECTION AND PRESERVATION OF SAMPLES

ALTHOUGH in many cases, perhaps the great majority, the physician will not himself carry out the actual chemical analysis involved in the various tests of bodily functions, it is always his province to obtain the material for analysis and to see that it arrives at the laboratory in such a condition that it can be analysed with trustworthy results. We have therefore thought it advisable to collect into one chapter the methods used for obtaining samples of blood, urine, cerebro-spinal fluid, etc., for preserving them from alteration, and for dispatching them to the laboratory. Exceptions to this are the collection of gastric contents and of expired air, which belong more appropriately to the chapters on gastric analysis, and the determination of the basal metabolic rate respectively.

URINE

Collection.—For chemical tests it is usually unnecessary to take aseptic precautions in the collection of urine, provided that the analyses are carried out with the minimum of delay, or, where a day or more must necessarily elapse between the collection and the analysis, provided some appropriate preservative is added. For many purposes a twenty-four hour sample

is required, and where quantitative results are sought, as in determining the daily output of sugar or of urea, the period must be accurately measured and precautions must be taken to prevent loss of urine. Thus at the beginning and at the end of the period the patient must be instructed to empty his bladder completely, and should be told always to micturate into a separate container before defaecation. After the sample has been measured, and any preservative has been added, a certain amount of it is sent to the laboratory for analysis, with a note of the total volume passed. For most purposes the sample need not exceed 200 c.c., though some such generous amount should be sent rather than a few cubic centimetres in a test-tube. When there is any doubt as to the volume needed for an analysis, it is obviously best to send the maximum available. For some analyses, of course, very considerable amounts are required, and one may instance the estimation of lead in cases of lead-poisoning, for which the whole twenty-four hour specimen is needed in order to obtain reliable results.

In some conditions more information can be obtained by the analysis of separate samples of urine than by the analysis of the twenty-four hour collection. Thus in the investigation of cases of glycosuria it is often desirable to know whether sugar is being excreted continuously or only after meals, and in suspected chronic interstitial nephritis the testing of separate urine samples for variation in specific gravity and urea content may give information of great value. Tests of this kind need not, of course, prevent the simultaneous carrying out of investigations into the total daily output of the various urinary constituents; a measured volume of each sample of urine passed

may be used for separate testing, and if this is kept as low as possible—only a few cubic centimetres need be used—the error in the analysis of the remainder of the twenty-four hour sample will be slight.

Preservation of Urine for Analysis.—For hospital work, probably the most efficient method of preserving the urine for analysis consists in the addition of a little toluene—enough to form a continuous film over the surface of the fluid—and the keeping of the samples in a refrigerator. Toluene alone is not very satisfactory, for it does not always prevent the growth of bacteria completely. It is to be remembered that the great object of preservatives is to guard against the growth of bacteria, since the organisms live at the expense of the urinary constituents, and so, if allowed to grow, may profoundly alter the composition of the urine. Thus, in particular, sugar tends to disappear and ammonia to be produced at the expense of urea. Hence, unless the urine is examined while it is fresh, or with adequate precautions against bacterial growth, estimations of the output of glucose, or of the ratio between ammonia and urea, may be entirely fallacious and misleading.

The choice of a suitable preservative in private practice is not so easy. Chloroform (5 c.c. to each 100 c.c. of urine) is more efficient than toluene in retarding bacterial growth, but has the objection that it reduces copper solutions and so simulates sugar. If it be used, therefore, the urine must be boiled to remove the chloroform before tests are applied for sugar. Formalin, which also has been suggested as a preservative of urine, similarly reduces copper solutions, and as it is not removed completely by boiling it is therefore definitely undesirable in cases suspected

of glycosuria. Thymol, too, though efficient as regards prevention of bacterial growth, interferes with certain tests. Possibly the best means of preserving urine for chemical examination is simple acidification to a reaction at which the ordinary bacteria cannot grow. For this purpose the addition of 1 c.c. of concentrated hydrochloric acid to 100 c.c. of urine suffices. The acid usually precipitates uric acid and destroys casts (so that for microscopical examination it cannot be used). Before the urine is examined chemically it is advisable to neutralise it by the addition of sodium hydroxide (1 c.c. of 40 per cent. NaOH per 100 c.c. of sample is a suitable amount), and the volume of alkali so added must be taken into account in the subsequent calculations. Use of acid for preserving the urine prevents any estimation of the titratable acidity or pH; but this disadvantage is far outweighed by the usefulness of the method with respect to the commoner tests.

BLOOD

Amount.—It is impossible to lay down any simple hard-and-fast rule as to the amount of blood required for chemical analysis, though in the majority of cases 2 c.c. for each separate estimation is an adequate amount. The actual amount needed for a number of the commoner estimations is given in Table I, but it must be remembered that these are minimal and do not allow for any duplication of analyses. Wherever possible it is wise to send more than the bare amount required for a single estimation, for it is most annoying to be deprived of useful information through an accident during an analysis which cannot be repeated. Again, it is annoying to the chemist to receive, as he so

TABLE I

<i>Test</i>	<i>Method</i>	<i>Quantity</i>	<i>Coagulation</i>
CO ₂ combining power	Van Slyke	4 c.c.	Non-coagulated
Total Sugar	Benedict or Folin and Wu Hagedorn and Jensen	2 c.c.	Non-coagulated
		0.2 c.c.	Non-coagulated
Lacvulose	Micro-Kjeldahl	2 c.c.	Non-coagulated
Non-protein nitrogen		2 c.c.	Non-coagulated
Urea nitrogen	Urease	1 c.c.	Non-coagulated
Creatinine	Folin	2 c.c.	Non-coagulated
Uric acid	Benedict	5 c.c.	Non-coagulated
Inorganic phosphate	Fiske and Subarrow	1 c.c.	Non-coagulated
Chlorides	Silver-nitrate methods	1 c.c.	Non-coagulated
Methaemoglobin, etc.	Spectroscope	2 c.c.	Non-coagulated
Plasma Proteins	Micro-Kjeldahl	5 c.c.	Non-coagulated
Cholesterol	Bloor	1 c.c.	Non-coagulated
Total fatty acids	Stewart and Hendry	2 c.c.	Non-coagulated
Lipoid Phosphorus	Stewart and Hendry	1 c.c.	Non-coagulated
Bilirubin	Van den Bergh	7 c.c.	{ Non-coagulated or coagulated
Icteric index	Moulengracht	3 c.c.	
Phenol-tetrachlorophthalein	Rosenthal	{ 10 c.c. first sample 2 c.c. subsequently }	Non-coagulated
Calcium and Magnesium	Kramer and Tisdall		5 c.c.
Carbon monoxide	{ Tannin Spectroscope Van Slyke }	3 c.c.	Non-coagulated

often does, a minute amount of blood with a demand that several complex analyses be carried out on it. It is usually a simple matter to obtain sufficient

blood by venepuncture, but when only very small amounts of blood are available it is usually possible, as in the case of sugar, to employ special micro-methods.

Anti-coagulants.—Most analyses are carried out upon whole blood, and therefore the sample must be prevented from clotting. The agents most commonly used for this purpose are sodium citrate and sodium or potassium oxalate. These salts act on the calcium which is essential for blood coagulation, the citrate by converting all the calcium to the very slightly ionised but soluble calcium citrate, and the oxalate by forming the insoluble calcium oxalate which is precipitated. The amounts of anti-coagulant required are very different in the two cases. Citrates prevent coagulation only when present in considerable amount, smaller amounts actually hastening clotting, and at least 60 mg. of sodium citrate (enough to cover the surface of a threepenny-piece) must be used for each 10 c.c. of blood.

The most usual anti-coagulant is sodium oxalate, of which relatively little is needed; 10 mg. (a small penknife-point) is ample to prevent 10 c.c. of blood from clotting. Whichever anti-coagulant is used the salt should be in as finely powdered a condition as possible, since it can only fulfil its function if it is rapidly dissolved in the blood before clotting can take place. For the same reason the blood and anti-coagulant should be thoroughly shaken together at once; it is no uncommon experience in the laboratory to receive a sample with solid oxalate at the bottom of the test-tube and a firm clot of blood above. An equally common fault is the use of an excessive quantity of oxalate, and this it is also important to avoid, since a high concentration of oxalate may

seriously interfere with certain chemical estimations, such as those of sugar, non-protein nitrogen, and creatinine. A useful method, and one which is almost essential in the successful collection of capillary blood, is to prepare a test-tube thinly coated with a film of sodium oxalate. The required amount of oxalate is placed in the tube, dissolved in a drop or two of water. This solution is then evaporated over a small gas flame, the tube, meanwhile, being held at an angle and rotated continuously, so that the oxalate is deposited very thinly over the walls of the tube. Though oxalate so deposited dissolves more rapidly in the blood than does finely powdered oxalate, the use of test-tubes so prepared does not absolve the operator from the duty of shaking the blood with the anti-coagulant.

For the determination of calcium, of course, oxalates cannot be used as anti-coagulants. It is often recommended that citrate be used for this purpose, on the ground that calcium citrate is soluble. It has been found, however, that amounts of citrate sufficient to prevent coagulation of the blood may precipitate a little of the calcium, and that with considerable excess of citrate, such as is often used in practice, the loss of calcium in this way may be serious. Even when citrate is used to prevent clotting, the actual calcium estimation is carried out on the plasma, since the results are more uniform and informative than those obtained from analysis of whole blood. It seems preferable, therefore, to avoid the possible loss of calcium resulting from the use of sodium citrate, and to use the serum obtained from blood which has been allowed to clot. This is indeed essential if information is required as to the proportion of calcium which is

present in the diffusible state, since citrate completely upsets the distribution of the blood calcium even if it does not alter the total amount. The determination of sodium, of course, cannot usefully be made after addition of any sodium salt, and that of potassium is best done without addition of any anti-coagulant, since added salts may alter the distribution of potassium between cells and plasma. For these estimations also the blood should be allowed to clot.

Some tests, such as that of Van den Bergh, and the determination of the icteric index, demand the use of blood in which no haemolysis has occurred. Hence in obtaining samples, for such tests particularly, neither ether nor alcohol must be allowed to come in contact with the needle and syringe, or with the collecting vessel. Water also must be avoided, and so all the apparatus should be clean and dry. If the syringe must be washed out, sterile physiological salt solution should be used for that purpose. The older practice in carrying out the Van den Bergh test was to use blood serum, but it is now recommended that plasma be employed, since with the use of a suitable anti-coagulant such as sodium oxalate haemolysis is less likely to occur than when the blood is allowed to clot spontaneously. Sodium oxalate is to be preferred to the potassium salt. A second important reason for the use of plasma in the Van den Bergh reaction is the desirability of carrying out the test as soon as possible after the blood has been drawn. It is stated that if more than two hours elapse before the test is made the results may often be misleading.

Preservation of Blood Samples.—All blood analyses should be carried out as soon as possible. Besides the Van den Bergh reaction, this is particularly true of

sugar estimations. Loss of sugar from shed blood may begin within a few minutes of withdrawal, though usually the loss is inappreciable for the first hour or so. Sugar estimation is valueless in blood which has been kept overnight without special precautions, since by morning half or more of the sugar may have disappeared. For sugar estimation it is a usual, and good, procedure to add the blood at once, without the use even of anti-coagulants, to the protein precipitant. After this treatment the mixture may safely be kept for a few hours, and may even be sent through the post. Many preservatives have been suggested for blood, and, for example, a trace of formalin has been found to prevent loss of sugar for a considerable time, even when added in amount far too small to interfere with the estimation. Possibly the most useful preservative—which, however, cannot be used if urea estimation is required (since fluoride is an enzyme poison)—is a mixture of one part of sodium fluoride to three parts of sodium or potassium oxalate, 40 mg. of the mixture being added, without other anti-coagulant, to each 10 c.c. of blood. With blood preserved in this way, estimations of sugar, non-protein nitrogen, and creatinine can safely be carried out after two or three days, so that samples so protected may be sent by post to the laboratory. If urea estimation is required, and the analysis cannot be done within a few hours of drawing the blood, a drop or two of formalin may be used as a preservative.

The inorganic phosphate rapidly increases in shed blood owing to hydrolysis of phosphate esters, and it is doubtful if any really satisfactory method exists for preventing this action over a long period. The first step in the estimation of inorganic phosphate

is the precipitation of the blood proteins by addition of an equal volume of 25 per cent. trichloroacetic acid. After the addition of this reagent the hydrolysis of phosphate is very much slower, although it still proceeds, owing, probably, to the acid reaction of the mixture. The immediate addition of trichloroacetic acid to the blood sample (using accurately measured amounts of each) allows the remainder of the analysis to be postponed for some hours.

No satisfactory method is known for preserving blood required for determination of the carbon-dioxide combining power. If really accurate results are to be obtained this analysis must be carried out within an hour or two of the blood being withdrawn.

As in the cases of other samples for analysis, the chemist should always be informed what preservatives and anti-coagulants have been added to the samples of blood he receives.

Collection of Blood Samples.—When only a small amount of blood is required, or when other methods are unsuitable, it may be obtained by pricking the finger or the lobe of the ear. In order to ensure a free flow of blood the part selected should be warm, and should be congested by slight constriction, or, in the case of the finger, by swinging the arm. The stab should be made with a blood gun or a small scalpel, which are much more efficient than a needle and are no more painful. The stab should be resolute and firm, since half-hearted efforts are really more uncomfortable to the patient and generally have to be repeated. After the stab has been made the blood is massaged out into a suitable vessel. When the finger is selected as the source of the blood it is advisable to make the stab at the tip rather than at the base of the nail, since it is

usually possible to obtain a greater flow of blood, and to obtain it more easily, from this position. In using capillary blood for the estimation of sugar it must be borne in mind that though during fasting the sugar concentration is usually the same as that of venous blood, after ingestion of glucose the sugar concentration is rather higher in the capillary blood. This is due to synthesis of glycogen in the muscles at the expense of the sugar brought to them by the capillaries.

When a larger quantity of blood is required for analysis it is usually obtained, by means of a hollow needle and syringe, from one of the superficial veins at the bend of the elbow. The syringe is not absolutely essential, but we much prefer its use to the method of simply inserting a needle in the vein and allowing the blood to drip through it into the test-tube. With this latter method blood is apt to be spilt, and unless there is to be danger of the blood clotting a large-bore needle must be used, with consequent increased discomfort to the patient.

The withdrawal of blood from a vein should be perfectly simple in the vast majority of cases, but it is surprising how frequently it is inefficiently performed, with resulting undue discomfort to the patient, the formation of a haematoma, and partial, or even total, failure to procure an adequate specimen. Venepuncture should, of course, be performed with scrupulous aseptic precautions, and it is advisable to boil the needle rather than to sterilise it with ether or alcohol, since the introduction of these substances under the patient's skin is apt to be painful, and may, moreover, lead to haemolysis of the blood. After sterilisation only the collar of the needle must be touched in fitting it on to the syringe. It is quite unnecessary to

use a needle with a very big bore, though it must of course have a somewhat wider lumen than the very fine needles commonly used for hypodermic injections. Much more control can be obtained if the needle is short rather than long, and if it has a short bevel. A No. 19 S.W.G. needle, with a length of one and a half inches, is recommended.

The selected vein is made prominent by suitable constriction of the upper arm with a soft piece of rubber-tubing. After the surface of the skin has been cleansed with ether, and with the thumb of the operator's left hand controlling the vein, the needle is inserted and the requisite quantity of blood withdrawn into the syringe. The best results are obtained by slow traction on the piston of the syringe.

If the vein does not become sufficiently prominent after the application of the tourniquet, the patient should be asked to open and clench his hand several times, which will drive more venous blood upwards into the veins of the forearm. When the skin is punctured immediately over the distended vein some bleeding may occur from the puncture-hole when the needle is withdrawn. This may be avoided by inserting the needle alongside, rather than directly over, the vein, and then turning the point inwards so as to pierce the vessel. After a little practice one grows accustomed to the "feel" of the needle as it slips into the vein. It is essential to remember to release the tourniquet before taking out the needle, since failure to do this is the commonest cause of an unsightly bruise or haematoma at the bend of the elbow. On withdrawal of the needle pressure is immediately applied with a small pad of cotton-wool over the puncture-hole, and no bleeding should ordinarily

occur. Once the sample of blood has been procured it is immediately transferred to a test-tube and thoroughly shaken up with a knife-point of sodium oxalate, or left to clot according to the nature of the analysis in view. The syringe and needle should, as soon as possible, be washed out with water and then with acetone, and the syringe should be lubricated with a little liquid paraffin.

Difficulty may sometimes be experienced with very emaciated patients, whose veins are not anchored by subcutaneous fat, and thus tend to be pushed away from the point of the needle. If the vein is sufficiently steadied by tightening the skin over it with the thumb of the left hand this difficulty can be overcome. In many fat subjects, on the other hand, especially women, the vein never becomes visible, even after considerable constriction has been applied to the upper arm. It is usually palpable, however, and often much less difficult to puncture than the freely mobile vein of a thin subject. If it is neither visible nor palpable, the arm should be immersed for a little while in hot water, which almost invariably makes the vein more prominent. If this fails, some other vein, which may be more obvious, on another part of the body, such as the dorsum of the foot, may be tried.

In young children it is usually impossible to obtain blood by puncture of the veins of the arm, and the external jugular vein is generally selected, with the child's head turned so as to steady the vessel over the sterno-mastoid muscle. The fact that the child usually cries during the proceeding causes the vein to be satisfactorily distended. If a large quantity of the child's blood is not required, a specimen of 2-3 c.c. may easily be collected from a small stab wound in the

heel. The ankle is grasped so as to congest the heel, and, the stab having been made with a sterile scalpel, the resulting blood is massaged into a test-tube.

In infants, blood may be obtained by puncture of the superior longitudinal sinus through the anterior fontanelle. This procedure, which sounds rather formidable, is in reality perfectly simple and safe, provided the child's head is held very still by an assistant and the needle is not inserted farther than the depth of its bevel, since the sinus lies immediately beneath the surface of the skin. The needle should be inserted in the middle line through the posterior angle of the fontanelle.

It is important to remember that many of the blood constituents are increased in amount for some time after a meal, and in estimating these substances, therefore, it is absolutely essential to obtain the blood while the patient is fasting. Though for other analyses fasting blood is not really necessary, its use is not harmful, and for routine analysis it is always desirable to use fasting blood, the blood being withdrawn in the morning before the patient has had breakfast.

FAECES

For chemical examination it is desirable that the complete stool be sent to the laboratory and that the analysis be commenced as quickly as possible, since, even more than in the case of urine, the chemical composition of faeces is liable to undergo alteration owing to bacterial action. Probably the best preservative which will prevent the alteration in amount and distribution of the fats of the faeces is alcohol, which, if added in considerable amount, will not only kill

bacteria, but prevent the continued action of lipase. When the faeces have to be sent through the post, however, this procedure is inadmissible, and formalin should be substituted. The chemist should be informed that preservative has been added. Samples of faeces obtained by the use of purgatives or of an enema are of no use for chemical examination, and no liquid paraffin or other oil should be given for at least three or four days prior to the collection of the sample.

CEREBRO-SPINAL FLUID

The cerebro-spinal fluid may be withdrawn from the lumbar theca, the cistern space, or from the lateral ventricles. Of these the safest and most usual method is withdrawal from the lumbar theca. In performing the operation of lumbar puncture, absolute asepsis is again essential, since the most disastrous results may be occasioned by an artificial infection of the meninges.

The ideal lumbar puncture needle is some 7-9 cm. (3-4 in.) long, moderately pliable and certainly not brittle. The bevel must be absolutely smooth and the shaft perfectly rustless. It is provided with a handle and a stylet, and its bore should be capable of being fitted on to a "record" syringe. The needle should be sterilised by boiling rather than by ether or alcohol.

Two types of needle are in general use. The Graham type, a straight needle, is the more suitable when a manometer is being used; the White-Jenselme type, provided with a T-handle through which the cerebro-spinal fluid drips, is perhaps easier to introduce.

The position of the patient during the operation is exceedingly important, since, if this is faulty, an easy

operation becomes one of considerable difficulty, if not impossible. Preferably the patient should be lying down with his back on the edge of the bed, his hands round his knees pulling them up towards his chin, and his head bent downwards, so as to ensure the greatest possible degree of flexion of the spine, thereby opening up the inter-vertebral spaces to their maximal extent.

Lumbar puncture is usually performed in the space between the third and fourth lumbar vertebrae. In order to identify this space a line is drawn with an iodine swab joining up the iliac crests. This line will cut across the spinal column at or near the spine of the fourth lumbar vertebra, and since the spinal cord ends about the level of the second, it will be quite safe to puncture the theca in the space above, or in the space below, the fourth lumbar vertebra. After the operator has washed his hands as for a surgical operation, and the patient's skin in the lumbar region has been thoroughly disinfected with iodine, the needle is inserted in the third or fourth interspace. A point on the mid-line should be chosen in children, and one 5-10 mm. to one side of it in adults. The only real pain the patient should feel if the operation is properly conducted is the discomfort of skin puncture, and this may be prevented in nervous patients by injecting a few drops of novocaine under the skin, so as to cause a small blister over the site of the puncture, or by freezing the region with ethyl chloride. A general anaesthetic is unnecessary save in maniacal cases.

On introducing the needle through the skin there is often an involuntary straightening of the spine, and the patient should always be made to flex the spine again thoroughly before the operation is proceeded with. The needle should then be inserted steadily forwards

and slightly upwards, and should slip easily into the spinal canal, encountering little resistance on the way, except just as it goes through the ligamentum flavum. The dura of the spinal canal should be punctured at a depth of 4-6 cm. in adults, unless they are very fat, when the needle may have to be inserted to a depth of 7 cm. In children the canal is reached at a depth of 2-4 cm., depending on the age and development of the child. Just as it is possible to tell, after a little experience, when a needle has entered a vein, so one quickly learns to appreciate the "feel" of the needle as it punctures the dura of the spinal canal. When this occurs the stylet should be withdrawn and the cerebro-spinal fluid allowed to run into a clean sterile test-tube, which is at once stoppered. Several of these tubes should be ready, since the first few cubic centimetres of the fluid may be contaminated with blood and so be rendered unsuitable for certain of the analyses. The first one or two cubic centimetres should therefore be received into a separate test-tube. In cases in which the pressure of the fluid is not very obviously increased no more than 10 c.c. of fluid should be withdrawn. Where it is obviously under greatly increased tension, as in meningitis, considerably more may be drawn off with benefit to the patient, but certainly never more than 40 c.c., and seldom so much. The quality of the patient's pulse serves as a good guide as to the amount of fluid to be taken.

When the needle encounters a hard resistance during the course of puncture no attempt should be made to force it onwards, as it is no doubt encountering bone, and traumatising of the periosteum will cause the patient considerable pain. Under such circumstances

the needle should be withdrawn until its point is just under the skin, and a new attempt made.

If no fluid is obtained although it is believed that the needle has reached the spinal canal, haphazard plunging of the needle in all directions should on no account be made, as this will undoubtedly cause bleeding and post-operative headaches. Rotation of the bevel of the needle should be tried, after which the fluid may escape easily, since the point may have become obstructed by a nerve root; or slight constriction may be applied to the veins of the neck, thereby raising the intracranial venous pressure and so increasing the tension of the cerebro-spinal fluid. If these measures fail, the needle should be inserted a little farther. If this also fails, and if by means of the stylet it is ascertained that the lumen of the needle is quite clear, the needle should be withdrawn and an attempt made in the next interspace.

Occasionally all efforts to withdraw cerebro-spinal fluid by lumbar puncture fail. Such a "dry puncture" may be due to absence of fluid in the lumbar theca, as may be caused by an obstruction in the spinal canal; or to thick pus blocking the lumen of the needle, as occasionally happens in suppurative meningitis; but one should be very chary of ascribing one's failure in obtaining fluid to these causes, since a "dry puncture" is much more usually due to failure of the operator to puncture the dura and enter the spinal canal.

In very fat persons, or in patients with some degree of spinal arthritis, it may be impossible with the patient lying in bed to get the back sufficiently arched to carry out the puncture successfully. Under such circumstances lumbar puncture is more easily performed with the patient sitting on a stool, his legs

separated, and his head and shoulders bent downwards towards his knees as much as possible.

Provided the needle is not inserted to a very excessive depth, so as to pierce the aorta, and provided excessive force is not used, so as to break the needle, the dangers of lumbar puncture are very slight. A few cases of death from cerebral hernia have been reported following its performance, but in these the puncture was done with the patient in a sitting posture. The rapid removal of 15–20 c.c. of cerebro-spinal fluid from any case suspected of suffering from a cerebellar or cerebral tumour, whether associated with papilloedema or not, is an exceedingly dangerous procedure. When a lumbar puncture is performed in such cases a simple manometer should always be attached to the needle, the intelligent use of which will obviate the dangers of lumbar puncture under such circumstances. Owing to the presence of the manometer the fluid will not be rapidly withdrawn, and an undue lowering of the cerebro-spinal fluid pressure can be prevented. (See p. 222.)

Lumbar puncture is definitely contra-indicated in erysipelas or septic skin conditions of the lumbar region, and in cases of the exanthemata it should be performed only when *absolutely* necessary.

It is ideal to keep the patient in bed for twenty-four hours after the operation, and to tell him to take things easily for a day after that. It is essential, if headache is to be avoided, that he should at any rate lie flat for two or three hours afterwards. Headache is the commonest sequel to the puncture, and may indicate that too much fluid has been removed, or may be due to non-closure of the puncture-hole in the arachnoid, so that leakage of fluid into the tissues

occurs for some time afterwards. This headache is usually satisfactorily treated by keeping the patient at rest with the foot of the bed raised, giving him plenty of water to drink and prescribing aspirin. Occasionally an intramuscular injection of pituitrin relieves it. Pain in the extremities is a less common sequel, and is probably due to injury of one of the filaments of the cauda equina. It is of a very temporary character, and yields to rest in bed and some mild sedative.

Cerebro-spinal fluid may also be withdrawn through the occipito-atlantoid ligament from the cistern space. This procedure may be called for in order to procure fluid in spinal-subarachnoid block, and may occasionally be useful in the diagnosis of tuberculous meningitis, since the tubercle bacilli are more easily demonstrated in the cistern than in the lumbar fluid. Owing to the proximity of the vital centres the procedure is not free from risk in the hands of an inexperienced operator. The technique should not, therefore, be learned from a book, but from repeated trials on the cadaver.

Puncture of the lateral ventricle, which is usually performed for therapeutic purposes in hydrocephalus, is a much more formidable operation, and calls for great care and precision. It should not, therefore, be undertaken without skilled surgical aid.

CHAPTER III

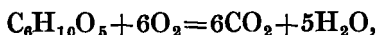
THE BASAL METABOLIC RATE

THE word "metabolism" is a general term implying the utilisation of foodstuffs by the body tissues. Since food is the fuel, the combustion of which gives to the body the energy by which it is able to perform its various activities, and since combustion involves the giving out of heat, we may measure the metabolism of an individual by the amount of heat he produces in a given time. Such a direct measurement of metabolism, however, involves the use of an exceedingly elaborate and expensive calorimeter chamber, with at least four people in constant attendance to supervise its working. It is, therefore, impracticable in the vast majority of hospitals to measure metabolism by this direct method, ideal though it may be to do so.

Since, however, the energy production of an organism is due to processes of combustion which involve the utilisation of oxygen, it is, subject to certain conditions, directly related to its oxygen consumption and carbon-dioxide elimination. The metabolism of an individual may thus be calculated by the indirect process of ascertaining his consumption of oxygen in a given time, and calculating from the result the amount of heat produced. In order to do this it is necessary to know the relationship between the oxygen consumption and the heat production. This depends on the type of food being oxidised, and to

understand this point it is necessary to consider briefly the *respiratory quotient* ("R.Q."), the ratio between the amount of carbon dioxide eliminated and the amount of oxygen used.

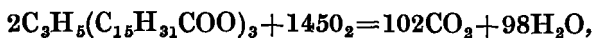
Fat, carbohydrate, and protein are all oxidised in the body with production of carbon dioxide and water. In the case of carbohydrate the relation between the volume of oxygen used and the volume of carbon dioxide formed can be calculated very readily from the equation :



whence it appears that each gramme molecule of oxygen absorbed gives rise to 1 g. molecule of carbon dioxide. Since 1 g. molecule of any gas occupies 22.4 litres at standard temperature and pressure, we have :

$$\frac{\text{vol. of } CO_2 \text{ produced}}{\text{vol. of } O_2 \text{ used}} \text{ (i.e. R.Q.)} = \frac{6 \times 22.4}{6 \times 22.4} = 1.$$

For a simple fat, such as tripalmitin, the R.Q. may be similarly calculated from the equation of combustion :



$$\text{whence, } R.Q. = \frac{102 \times 22.4}{145 \times 22.4} = \frac{102}{145} = 0.703.$$

The fat actually oxidised in the body is usually a mixture, for which careful calculation, checked by experiment, shows the average respiratory quotient to be 0.707.

In the case of protein the position is complicated by the fact that carbon dioxide and water are not the only products of combustion, urea, creatinine, etc., being also formed. With allowance for this factor,

however, the R.Q. for an average protein is found to be 0.80.

The actual R.Q. found in the course of metabolism is due to the oxidation of a mixture of the three types of foodstuff; but it is possible, knowing the urinary nitrogen excretion, from which the amount of protein metabolised may be obtained, to calculate the relative amounts of fat, carbohydrate, and protein oxidised, with any given R.Q. In practice it is found that the rate of protein metabolism under basal conditions varies so slightly that, under these conditions, it need not be separately determined. For clinical purposes it is sufficiently accurate to assume it to have the normal value.

The amount of heat evolved during the combustion of each of the foodstuffs has been determined experimentally, and from the data so obtained the amount of heat evolved per litre of oxygen—the “calorific value of oxygen”—has been calculated. Thus experiment shows that 1 g. of carbohydrate in the form of glycogen or starch gives 4.18 cal. when it is burnt to carbon dioxide and water. The equation for the combustion allows it to be calculated that 1 g. of either of these substances uses 0.828 litres of oxygen during the combustion, so that for each litre of oxygen used in the combustion of carbohydrate $\frac{4.18}{0.828}$, or 5.047 cal., must be evolved. In other words, the *calorific value of oxygen* used in the combustion of carbohydrate is 5.047 cal. per litre. Similarly it appears that the calorific value of a litre of oxygen used in the combustion of fat is 4.686 cal., and of protein, 4.463 cal. Using these figures, and the knowledge of the relative amounts of the different foodstuffs oxidised

at a given R.Q., we are able to calculate the calorific value of oxygen for all possible values of the R.Q. This has been done in Table II. It need hardly be said that experimental work has amply shown that the heat production in metabolism calculated in this way from the R.Q and oxygen consumption agrees with that found by direct measurement in the calorimeter.

TABLE II

THE RELATION BETWEEN THE RESPIRATORY QUOTIENT AND THE CALORIFIC VALUE OF OXYGEN

The figures given here are calculated on the assumption that only fat and carbohydrate are being oxidised. Actually, of course, protein also is being burned, but the error introduced by this simplification of the calculations is not considerable.

<i>Respiratory Quotient</i>	<i>Cals. per litre of Oxygen used</i>	<i>Respiratory Quotient</i>	<i>Cals. per litre of Oxygen used</i>
0.70	4.686	0.86	4.875
0.71	4.690	0.87	4.887
0.72	4.702	0.88	4.900
0.73	4.714	0.89	4.912
0.74	4.727	0.90	4.924
0.75	4.739	0.91	4.936
0.76	4.752	0.92	4.948
0.77	4.764	0.93	4.960
0.78	4.776	0.94	4.973
0.79	4.789	0.95	4.985
0.80	4.801	0.96	4.997
0.81	4.813	0.97	5.010
0.82	4.825	0.98	5.022
0.83	4.838	0.99	5.034
0.84	4.850	1.00	5.047
0.85	4.863		

The rate of oxygen consumption will, of course, be enormously modified in any individual by a number of circumstances: it will be profoundly affected by the degree of muscular activity; by the nature and

amount of food taken ; by changes in temperature ; and by the presence or absence of mental exertion or excitement. In order, therefore, to compare the metabolism of one person with that of another, it is necessary that the circumstances under which the comparison is made should, so far as possible, be identical—that is, we should try to keep those factors which modify the rate of metabolism constant in each case. Such standard conditions may be obtained by keeping the patient in bed at a constant temperature, completely relaxed mentally and physically, and with glandular and peristaltic activity reduced to a minimum by previous starvation. Practically the whole energy expenditure under such conditions will be represented by that necessary to maintain the vital functions, such as the respiratory and cardiac movements, the tonus of the muscles, and the body temperature. Metabolism measured under such conditions is called the *basal metabolism*, which may therefore be defined as the energy expenditure of an organism in a state of complete physical and mental rest.

In order to maintain the body temperature at 37°C . a certain amount of energy must be continually produced, since heat is always being lost from the surface of the body. The heat produced to replace that lost from the body surface forms, in fact, a large part of the total production under basal conditions. To a considerable extent, therefore, the amount of energy produced by a person under basal conditions depends on his surface area. The smaller the weight of the subject, for example, the greater will be his surface proportionately. Hence combustion will have to take place at a greater rate in a small man in order to maintain his body temperature constant

than in a large one. This apparently paradoxical relationship between weight and surface area will, perhaps, be made clearer by a concrete example. Imagine a cube of some substance, having sides of 1 cm. and a weight of 10 g. The surface area of the cube will then be 6 sq. cm., and the surface area per gramme will be 0.6 sq. cm. Now imagine the cube divided by three cuts into eight cubes, each having a side of 0.5 cm. length. The surface area of each of these small cubes will be 6×0.5^2 —i.e. 1.5 sq. cm.—and the weight will be 1.25 g. Hence the surface area per gramme of small cube is $\frac{1.5}{1.25}$, or 1.2 sq. cm.,

so that with the smaller weight the surface is relatively greater. Similarly, a man of 60 kilos. has a greater surface area in proportion to his weight than a man of 70 kilos.

Again, the age and sex of a person have an effect on the basal metabolism. A healthy man of forty has a different basal metabolism from a healthy man of twenty, and he has also a slightly different basal metabolism from a healthy woman of his own age.

If, however, the surface area and sex and age of the individual be taken into account, it is found that the amount of energy produced per unit of surface area per hour under basal conditions varies among healthy persons only within narrow limits. This value—the number of calories produced under basal conditions per square metre of body surface per hour—is known as the *basal metabolic rate* (“B.M.R.”), and is usually expressed as a plus or minus percentage of the normal. Tables showing the normal B.M.R. for persons of different ages and sex have been compiled from a large

number of observations on healthy subjects and are given below (Table III).

TABLE III

VARIATION OF BASAL METABOLIC RATE WITH AGE AND SEX IN NORMAL HEALTHY PERSONS. (After AUB and DU BOIS)

<i>Age in Years</i>	<i>Cals. per Sq. M. per Hour</i>	
	<i>Males</i>	<i>Females</i>
14-16	46.0	43.0
16-18	43.0	40.0
18-20	41.0	38.0
20-30	39.5	37.0
30-40	39.5	36.5
40-50	38.5	36.0
50-60	37.5	35.0
60-70	36.5	34.0
70-80	35.5	33.0

It may safely be said that there can be few laboratory undertakings of a routine practical nature which call for greater technical proficiency and attention to detail than a determination of the B.M.R. Otherwise an experimental error so easily creeps in that the result obtained is valueless and may even be grossly misleading if any consequence is laid upon it. Not only must the various analyses entailed be carried out in the laboratory with the greatest care by someone practised in the use of the somewhat complex Haldane gas analysis apparatus, but the doctor or nurse in charge of the patient must pay the most scrupulous attention to the details of preserving in him a basal state until the samples of expired air have been obtained. It is this latter necessity which is perhaps most frequently overlooked and which is most difficult to attain.

PREPARATION OF THE PATIENT

The determination of the B.M.R. is made ideally as soon as possible after the patient has wakened in the morning. He should have taken no exercise of any sort since the preceding evening, not even being allowed out of bed in the morning to wash himself or to brush his teeth. Occasionally it may be necessary to determine the B.M.R. of an out-patient who cannot be taken into the hospital for the night. Such a determination on an ambulant case tends to be unsatisfactory, but the patient may be rendered approximately basal by being brought straight from his house to the hospital in a conveyance as early as possible in the morning, and being put at complete rest, when he gets there, for at least an hour before the test is made.

The patient should fast for at least twelve hours before the test so that glandular and peristaltic activity may be reduced to a minimum. He should have his temperature taken before the test, as it has been shown that the metabolic rate rises approximately 10 per cent. for each rise of 1° C. of body temperature. It would thus be a waste of time to estimate the patient's basal metabolic rate if he were suffering from any degree of fever. On the other hand, the patient should be comfortably warm, to exclude the converse fallacy of a raised metabolic rate due to shivering or to the invisible muscular hypertonus caused by cold.

Lastly, the patient should be mentally relaxed, and of all conditions this is the most difficult to attain. Any unknown test is apt to excite a patient—if his confidence is not fully obtained—and when such a

test is associated with a somewhat awe-inspiring paraphernalia of masks and bags his nervousness may become extreme. It is thus essential to have explained to the patient the previous day the scope and full details of the test, and if possible to have allowed him to try on the mask and to become accustomed to breathing through valves. If the test is performed in the ward, screens should be put round the bed, and both the patient and the ward should be kept as quiet as possible until the specimen has been procured. Too often the subject of the test is discovered by the operator engaged in an animated conversation with a friend in the next bed or engrossed in an enthralling novel.

It is, indeed, questionable whether all these conditions can usually be procured in ordinary hospital wards. It would be ideal if the determination could always be made in some quiet room adjacent to the laboratory. The patient should be wheeled there in his bed, and left for an hour before the test is started, to accustom him to his new surroundings.

TECHNIQUE

During the last few years numerous methods have been devised for determining the B.M.R.. Most of these have aimed at simplification of technique, and the majority are quite incapable of giving accurate results. Only two methods of proved value need be considered here—an "open" and a "closed" method. In the former the patient expires into a specially constructed bag; this expired air is analysed, and from the results of the analysis the oxygen consumption and carbon-dioxide production of the patient

are calculated. In the latter the subject rebreathes oxygen from a reservoir, carbon dioxide in the expired air is absorbed by means of soda lime, and the oxygen consumption is measured directly by the diminution in the volume of the gas in the reservoir.

The closed method possesses certain advantages, and at least one grave defect: it is easy to perform, no knowledge of gas analysis being necessary; only a single piece of portable apparatus is required, so that the complete determination may be made in a ward or even in a private house; lastly, three or four determinations may be carried out at one sitting. Moreover, by using a recording drum attached to the apparatus a complete record of the patient's respiration during the tests can be obtained, and any disturbance can be eliminated. On the other hand, a very grave criticism of the method is that it allows only a determination of the subject's oxygen consumption, and his carbon-dioxide elimination is not measured. Hence it is necessary to assume an arbitrary value for the respiratory quotient in calculating the calorific value of the oxygen used. We know, however, that the respiratory quotient of even normal individuals under basal conditions may vary considerably. This variation is often greatly accentuated pathologically, and we have already seen how the calorific value of oxygen varies with fluctuations in the respiratory quotient. It is, therefore, not surprising to find that this method, which postulates a fixed respiratory quotient of 0.82, gives accurate results only in about three-quarters of the cases studied, and in the remaining quarter the results are apt to be fallacious.

The open method, on the other hand, though re-

quiring a number of somewhat bulky or complex pieces of apparatus, and demanding considerable technical skill in gas analysis on the part of the operator, allows of the estimation not only of the oxygen used but of the carbon dioxide eliminated. Hence it permits an accurate determination of the amount of heat actually produced, and even of the amounts of the different foodstuffs oxidised. It is thus more informative as well as more accurate than the closed method.

In order to collect the expired air some sort of mask or mouthpiece, which can be fitted on to the subject, is necessary, whichever method is used. Probably the most suitable face-mask is a modified Haldane oxygen administration mask. This consists of a metal body with a pneumatic cushion round its edges, and is fitted with inspiratory and expiratory valves, which may be connected to the apparatus used for collection by flexible, fabric-covered, corrugated rubber-tubing. This mask may be strapped to the patient's face quite comfortably, so that he may breathe at will through his mouth or nose. The pneumatic rubber cushion can be made to fit the face so closely that no leakage occurs round the edges of the mask. After the mask has been fitted the operator should test it for leaks. If the inspiratory valve is permitting inspiration only and no expiration, and if the mask is fitting the face accurately, it should be impossible for the patient to expire if the end of the expiratory tube be temporarily closed by the operator's hand. Again, if the expiratory valve is functioning efficiently, no inspiration should be possible if the inlet for inspiration is closed in the same way.

On some patients with an unusual physiognomy,

such as a very protuberant nose or very hollow cheeks, it may be impossible to fit a mask so that no leak occurs round about its rubber edge. In such cases it is convenient to use a simple mouthpiece containing inspiratory and expiratory valves, and to prevent nasal respiration by means of a nose-clip. This mouthpiece consists of a rubber flange which fits over the gums and teeth behind the lips, and has two rubber projections which are clenched by the teeth. No leaks can occur with this apparatus, which, however, becomes exceedingly irksome to the patient if worn for any length of time, the discomfort produced giving rise to a fallacious B.M.R. reading.

Closed Method.—In the closed method the inspiratory valve is connected to a spirometer with a water seal, which has previously been filled with oxygen. The expiratory valve is connected to a soda-lime chamber, through which the expired air passes, becoming denuded of its carbon dioxide in the process before re-entering the reservoir. The oxygen in the reservoir will thus be gradually used up, and the floating chamber which contains it will fall, setting in motion the pointer of a dial above the apparatus which registers the volume of oxygen in the reservoir (Fig. 1). The reservoir is first of all filled with oxygen, tested for leaks, and the volume of oxygen which it contains read off on the dial. After the pulse and respiration rates of the patient have been noted the mask is adjusted, and this also is tested for leaks or for inefficient valve action in the manner already described. Breathing through valves is apt to disturb the normal respiration a little, so a preliminary period must be allowed for any such disturbance to pass off. When the respiration and pulse rates have

returned to their original value the patient is connected to the spirometer, and at some convenient moment the exact time is noted by starting a stop-watch. Simultaneously the volume of oxygen in the reservoir is read. It is essential that the test

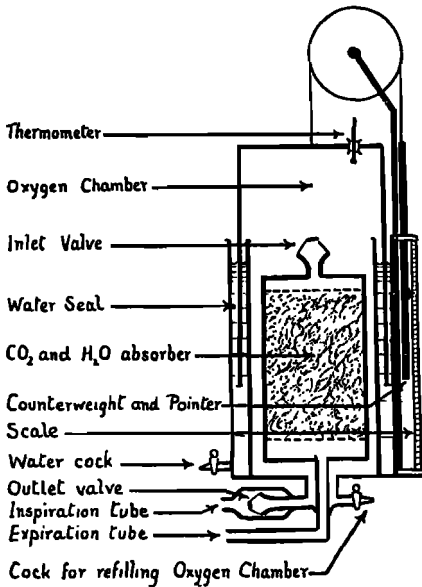


FIG. 1.—Sectional view of closed apparatus for determination of the Basal Metabolic Rate.

should be started and stopped at the same phase of respiration, and the best moment to select is probably just at the completion of expiration. After a suitable length of time, and again just when expiration ends, the volume of oxygen is read off and simultaneously the watch is stopped. Thus the volume of oxygen used in a measured time, measured at atmospheric

pressure and at the temperature of the spirometer, has been obtained. After repetition of the test the mean of two or three observations is reduced to standard temperature and pressure by means of the Tables supplied with the machine or, less conveniently, by means of the formula :

$$\text{vol. at S.T.P.} = \frac{\text{vol. observed} \times 273 \times \text{press. observed}}{(\text{temp. in } ^\circ\text{C.} + 273) \times 760}$$

The B.M.R. is then calculated thus :

Let the volume of oxygen used at S.T.P. be x litres, and let the time during which this oxygen is used be T minutes. With an arbitrary R.Q. of 0.82 it will be seen from Table II that 1 litre of oxygen is equivalent to 4.83 calories. Hence the number of calories produced per hour is $\frac{4.83x \times 60}{T}$, and the B.M.R. is equal

to this number divided by the body surface in square metres. The surface of the individual is obtained, the height and weight being known, from Du Bois' formula :

$S = (\text{wt. in kilos.})^{0.425} \times (\text{ht. in cm.})^{0.725} \times 71.84 \times 10^{-4}$,
or from the graph (Fig. 2) constructed from this formula. The result may then be expressed as a percentage increase or decrease on the figure for a normal person of that age and sex. The normal figures are given in Table III.

“Open” Method.—In determining the B.M.R. by the open method a similar mask is employed to that already described. The inlet valve is not connected to any reservoir, so that the patient breathes the ordinary air of the room. The expiratory outlet of the mask is connected by fabric-covered, corrugated

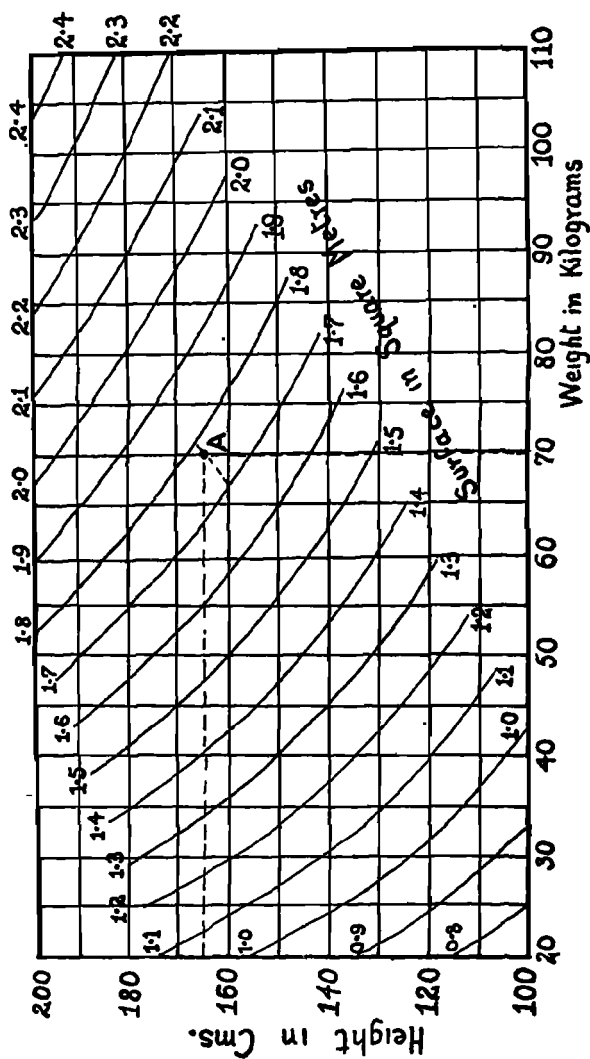


FIG. 2.—Chart for determining surface area from height and weight (after Du Bois). In the example shown, with height = 165 cm. and weight = 70 kilos., the surface area (point A) = 1.77 sq. m.

rubber-tubing to a large gas-tight bag made of rubber-coated canvas. The type of bag devised by Douglas, and usually known by his name, is most suitable. It is of large capacity—being capable of holding about 100 litres—is provided with a narrow side-tube and clip, so that specimens may readily be removed from the bag for analysis, and has a large three-way tap interposed between the bag and the mask, so that the expired air may be directed at will either into the bag or back again into the atmosphere.

The mask having been adjusted and tested for leaks exactly as in the closed method, the patient is connected with the Douglas bag, with the three-way tap turned so as to direct all the expired air into the atmosphere and to shut it off from the bag. When the patient's respiration and pulse have resumed their normal rate the tap is turned so as to admit the expired air into the bag. This is done just at the end of expiration, and the stop-watch is started at the instant the tap is turned. When the bag is reasonably full—containing not less than 40 litres, but not enough to cause undue distension—the tap is turned so as to shut off the bag, again at the end of expiration, and simultaneously the watch is stopped. The time required for the collection of the air sample varies from six to ten minutes, according to the patient's respiration. The patient's part in the test is now at an end, and the bag is removed to the laboratory, where the total volume of air is measured and samples are taken for estimation of oxygen and carbon-dioxide content. From the percentages of these gases in the expired air, and a knowledge of the composition of the ordinary room air, calculation gives the volumes of oxygen used and of carbon dioxide produced during the ex-

periment, and thence the R.Q. The calculation of the B.M.R. is then the same as for the closed method, except that the calorific value of oxygen at the R.Q. actually found is substituted for the arbitrary value of 4.83.

INTERPRETATION OF RESULTS

Although healthy normal persons of the same age and sex have basal metabolic rates which are fairly similar, it is essential to remember that such values are at best only approximate. The personal idiosyncrasies of the subject, and the slight experimental errors that in practice are inseparable from even the best-regulated determination, result in considerable variations above and below the normal mean value, which variations may be perfectly consistent with health. Hence it is the usual practice to disregard a variation of 15 per cent. on either side of the normal mean when the closed method is employed, and one of 10 per cent. with the open method. Even when these limits are exceeded a determination of the B.M.R. must not be taken as of great significance until a second and preferably a third determination has shown an approximately equal variation from the normal. The patient's apprehension of the first test is often so great and so difficult to control that, whatever the result of the first test, it should be regarded with suspicion; but once the patient has got used to the procedure, and provided the test is carefully carried out in every particular, then a variation of more than 15 per cent. from the normal becomes a matter of considerable significance.

The great fall in the basal metabolic rate which may occur simply by the habituation of the patient

to the procedure is insufficiently realised and, in consequence, far too much stress is often laid upon a single initial estimation, which is usually much higher than the true B.M.R. Once the patient has become thoroughly habituated, with skilful technique the experimental error should not be much more than 5 per cent.

The basal metabolic rate is significantly raised in cases of hyperthyroidism, fevers, and the leukaemias and in functional or organic neurological conditions producing spasticity, tremor, or excitement. It is usually mildly increased in acromegaly and in Paget's disease. It is lowered in hypothyroidism and in the terminal stages of wasting diseases. Much less constantly, or significantly, it is lowered in hypopituitarism. In hydraemic nephritis a low value of the B.M.R. has been fairly frequently recorded and has been made the basis of thyroid therapy in this condition. We believe, however, that such low readings are due to the simple fallacy that the inactive oedema fluid may artificially raise the body weight and, therefore, the estimated body surface on which the B.M.R. is calculated. Thus, low readings are obtained in spite of the fact that the patient, apart from his oedema, may have a normal rate of metabolism.

It is a curious fact in this connection that cases of exogenous obesity, unassociated with gross endocrine disorders, have almost invariably a normal B.M.R. Because of the great proportion of inactive fat in the obese, one would have expected a low result, and the normal rate would seem to indicate that the obese patient is actually using up a greater amount of energy than would a healthy person of "ideal" weight, of the same height and sex.

The B.M.R. is invariably raised in cases of hyperthyroidism, sometimes to as much as 100 per cent. above normal, and the extent of the increase furnishes the most accurate single sign at our disposal for gauging the severity of the disease, besides affording a valuable aid to diagnosis in those cases where the signs observed at the bedside are suggestive but inconclusive of the condition. A simple enlargement of the thyroid gland or benign goitre causes no increase, but, if anything, a decrease in the B.M.R., which result may be used to differentiate this condition from true hyperthyroidism, if such a differential diagnosis is not entirely apparent clinically.

The severity of exophthalmic goitre, as is well known, is not constant or regularly progressive, but tends to assume a cyclical periodicity, in which periods of exacerbation are followed by periods of comparative remission. These fluctuations in the severity of the disease are accurately demonstrated by readings of the B.M.R., which may thus aid in furnishing an indication of the effects of treatment and of the time at which operative interference may be undertaken with the best chance of success. It may be taken as a general rule that operation is undesirable if the B.M.R. is persistently increased by 40 per cent. or more. Operative procedures should in any case be undertaken while the B.M.R. is falling rather than rising. When iodine therapy is used as a pre-operative treatment of toxic goitre it is usual to follow the effect of treatment by frequent determinations of the B.M.R. In this way it is possible to choose the optimum time for operation, which is when the B.M.R. is at its lowest and before it has begun to rise again, as it will do with continued administration of iodine.

This procedure is also of value in differentiating thyrotoxicosis from other conditions in which the B.M.R. is also raised, since only thyrotoxicosis responds to iodine by a fall in the B.M.R. The condition known as toxic adenoma, on the other hand, gets slowly and progressively worse, and the B.M.R. accordingly tends to increase steadily, or at any rate does not show the same periodicity (Fig. 3). It is

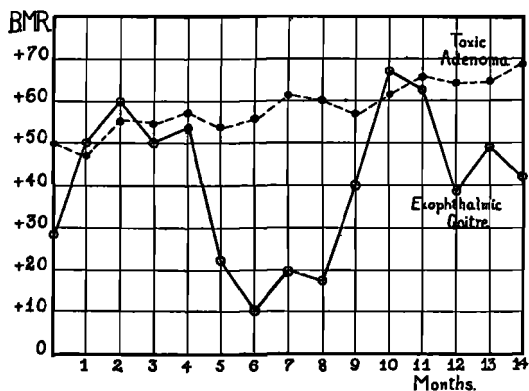


FIG. 3.—Variations in the Basal Metabolic Rate over a period of eighteen months, (a) in a case of exophthalmic goitre, (b) in a case of toxic adenoma.

claimed by some authorities that a differentiation may thus be made between the two conditions, though it would seem that such a differential diagnosis could be made as effectually and less tediously from the history of the case and the clinical examination.

Indeed, there is nowadays a strong tendency to consider toxic adenoma and exophthalmic goitre, or Graves' disease, under one heading of "Thyroid enlargement associated with thyrotoxicosis," since

small adenomata may be found in practically all cases of Graves' disease.

Just as hyperthyroidism is accompanied by a rise, so hypothyroidism is accompanied by a fall in the B.M.R., and in extreme cases of myxoedema this fall may be as much as 40 per cent. Such extreme cases are of course readily diagnosed without the use of the test, but the cases of more moderate hypothyroidism, which it is far more usual to encounter nowadays, often present an extremely difficult diagnostic problem to the physician, and in these cases the discovery of a persistently low B.M.R. is most significant. The treatment of myxoedema by the administration of thyroid may be accurately controlled by determination of the B.M.R. at intervals.

In thyroid disease, determination of the B.M.R. is thus of value :—

(a) As an aid to diagnosis, especially in border-line cases suffering from either hyper- or hypo-thyroidism.

(b) As a measure of the severity of well-established cases of thyrotoxicosis or of myxoedema.

(c) When repeated at intervals, as an indication of the efficacy of treatment and as a guide to dosage, or to the optimum moment for surgical interference.

Speaking very broadly, values of the B.M.R. in diseases of the thyroid may be interpreted as shown in Table IV.

TABLE IV

THE BASAL METABOLIC RATE IN DISEASES OF THE THYROID

Severe myxoedema	—40 to —25
Milder degrees of hypothyroidism	—25 to —15
Normal values	—15 to +15
Moderate hyperthyroidism	+15 to +30
Fairly severe hyperthyroidism	+30 to +50
Very severe hyperthyroidism	+50 and upwards

REED'S FORMULA

It is well known that the pulse rate and pulse pressure are greatly affected in cases of hyperthyroidism, the pulse pressure being the difference between the systolic and diastolic blood pressures. The greater the pulse rate and the higher the pulse pressure the more severe the disease. *Vice versa*, improvement is indicated by a slowing of the pulse and a decrease in the pressure. It is not surprising, therefore, that attempts should have been made to correlate these two factors with the basal metabolic rate, and that a fair degree of correlation has been found to exist. Reed has devised a formula from which the B.M.R. may be calculated if the pulse rate and the pulse pressure are known :

$$\text{B.M.R.} = 0.683 \times (\text{P.R.} + 0.9 \times \text{P.P.}) - 71.5.$$

For example, if the pulse rate is 100 and the pulse pressure 60,

$$\text{B.M.R.} = 0.683 (100 + 0.9 \times 60) - 71.5 = +33.7.$$

Apart, however, from the difficulty of determining the diastolic pressure accurately in many cases of exophthalmic goitre, Reed's formula is not sufficiently reliable to give a trustworthy idea of the B.M.R. in just those cases of small variation from the normal where such knowledge is of greatest service. Gross variations from the normal are readily detected by this somewhat crude method. When such gross abnormalities are present, however, the clinical signs are usually sufficiently obvious to allow a correct diagnosis, and assessment of the severity of the disease, to be made without recourse to other means.

CHAPTER IV

THE MECHANISM OF NEUTRALITY REGULATION

THEORETICAL CONSIDERATIONS

THE blood is very slightly alkaline—the arterial blood being somewhat more alkaline than the venous—and in order that the bodily functions may be properly carried out it must be kept in that condition. A small deviation from the normal seems to be permissible without the production of immediate ill-effects, but rather larger deviations in either direction are sufficient to cause serious derangement of many functions. Indeed, it is only within a moderately narrow range of alkalinity that life is possible, and certainly the blood must never be allowed to become acid or even neutral. It is obvious, therefore, that the mechanism for maintaining the blood—and through it the tissues—at the correct reaction must be at the same time delicately balanced and robust. A proper understanding of this mechanism and of the ways in which it may be strained in disease can best be obtained by a consideration of the chemical principles involved.

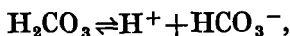
Acids and alkalis are among those substances whose aqueous solutions conduct electricity. Such substances, in solution, are dissociated to a greater or less degree into electrically charged particles called *ions*. Thus a solution of hydrochloric acid contains,

besides undissociated molecules, positively charged hydrogen ions (H^+) and negatively charged chlorine ions (Cl^-); a solution of sodium hydroxide contains molecules of that substance, and also positively charged sodium ions (Na^+) and negatively charged hydroxyl ions (OH^-). This dissociation into ions—*ionisation*—is reversible, and is never absolutely complete, so that undissociated molecules are always present, and by suitable means, such as evaporation of the solution, the ions can be made to recombine.

All acids, when dissolved in water, produce hydrogen ions, and indeed the definition of the term "acid" is based on this property. Since the characteristic property of an acid is the production of hydrogen ions, it follows that the strength of an individual acid depends on its capacity for ionisation. A solution of a strong acid containing 1 g. molecule per litre contains more hydrogen ions than one of a weak acid which also contains 1 g. molecule of acid per litre of solution. This can only mean that in the case of the strong acid a great many molecules have dissociated and so given rise to free hydrogen ions, while relatively few molecules of the weak acid have dissociated. In an extreme case a very strong acid would be almost completely ionised, an exceedingly weak one hardly at all. Hence the strength of an acid is determined by its degree of dissociation—that is, the extent to which it is ionised.

This conclusion is quite independent of the number of hydrogen atoms in the acid molecule, and, for instance, hydrochloric acid (HCl), with one atom of hydrogen per molecule, but largely ionised in solution, is a much stronger acid than phosphoric acid, which contains three atoms of hydrogen in each molecule (H_3PO_4)

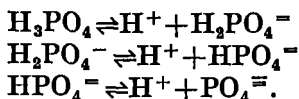
but is only slightly ionised. Such acids as carbonic (H_2CO_3) and phosphoric, which contain more than one atom of hydrogen in the molecule, dissociate in stages. Thus carbonic acid is dissociated, though, being a weak acid, only slightly, to H^+ and HCO_3^- ,



and the bicarbonate ion, HCO_3^- , is still further dissociated, though to an almost infinitesimal degree, according to the equation :



Similarly, phosphoric acid dissociates in three stages :

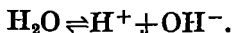


Phosphoric acid is a weak acid, and even in the first stage the amount of dissociation is small; in the second and third it is extremely slight.

In the same way we find that the strength of a base, such as sodium hydroxide, is also dependent on its degree of ionisation, a base being essentially a substance which, in solution, gives rise to hydroxyl ions (OH^-).

Before the implications of these considerations and their bearing on the mechanism of neutrality regulation are discussed, it is desirable to explain the nomenclature in general use for the quantitative expression of acidity and alkalinity.

Pure water, though it conducts electricity very badly, does so slightly in virtue of the fact that a very few molecules are dissociated according to the equation :



Evidently water is an acid, since it dissociates with production of hydrogen ions, and it must also be a base, since its ionisation gives rise to hydroxyl ions—that is, it is a so-called *amphoteric* substance. But water, an essentially neutral substance, must, as the equation shows, always produce equal numbers of the two ions. It is, indeed, this exact equality of hydrogen ion and hydroxyl ion concentration which we term *neutrality*. *Acidity* consists in the presence of an excess of hydrogen ions over hydroxyl ions, so that a convenient means of expressing the hydrogen ion concentration quantitatively will provide a measure of acidity. It is possible, however, to extend this, and to show that *alkalinity*, which consists essentially in an excess of hydroxyl ions over hydrogen ions, involves an actual deficiency of hydrogen ions, so that the concentration of hydrogen ions can be used as a measure applicable over the whole range of acidity and alkalinity. The reasoning which leads to this conclusion, and to the scale of measurement employed to express degrees of acidity and alkalinity, is as follows.

Consider what is happening in pure water. The water contains a certain number of free hydrogen ions, an equal number of free hydroxyl ions, and a relatively enormous number of undissociated water molecules. The amount of each is constant, but this constancy is not due to a mere passive static existence of molecules and free ions. On the contrary, water molecules are continually dissociating, but hydrogen ions and hydroxyl ions are recombining with equal rapidity, and so the balance is maintained. In order that a water molecule may be formed a hydrogen ion must collide with a hydroxyl ion, and therefore the rate of recombination of ions depends on the number

of collisions per unit of time. Now the more crowded the hydrogen ions—or, in other words, the greater the concentration of hydrogen ions—the greater is the chance of a hydroxyl ion colliding with one, and conversely, the greater the concentration of hydroxyl ions the greater the chance of a hydrogen ion colliding with one—just as, in looking for a needle in a haystack, one's chance of success is proportional to the number of needles in the stack. Hence the rate of recombination of ions is proportional to the number of collisions per unit of time, and this in turn is proportional to the concentration of each of the ions concerned.

Bearing this in mind, imagine the effect of adding acid to the water. This produces, of course, a great increase in the concentration of hydrogen ions, and thus a proportionate increase in the number of collisions between hydrogen ions and hydroxyl ions—*i.e.* in the rate at which water molecules are being formed. There is, however, no corresponding increase in the rate at which water molecules dissociate, and so some hydroxyl ions (together with an equal number of hydrogen ions) must be removed. As this process continues the number of hydroxyl ions is diminishing, and so the number of collisions per second gradually decreases; but the process of removal of hydroxyl ions cannot cease until the number of collisions per second has fallen to its original value, when, once more, the rate of water formation is equal to the rate of water dissociation. At this point, however, the solution, which contains more hydrogen ions than did the pure water, contains fewer hydroxyl ions; if the hydrogen ions have been doubled the hydroxyl ions have been halved, and so on. The two ions, in fact, bear the same relationship to one another as do the

volume and pressure of a gas, which, according to Boyle's Law, are so connected that the pressure is inversely proportional to the volume. And, just as in the gas the product of the pressure and the volume is constant, so in the solution the product of the concentrations of hydrogen ions and hydroxyl ions is constant. Experiment shows that in pure water 1 g. of hydrogen ion is present in 10,000,000 litres of water, so that the concentration of hydrogen ion is

$\frac{1}{10,000,000}$, or 10^{-7} . The concentration of hydroxyl

ions is the same, and the product is therefore 10^{-14} . We have seen that this product is constant, and is not altered by the presence of acid (or, on similar reasoning, of alkali), so that we arrive at the important conclusion that under all circumstances

$$(\text{conc. of H}^+) \times (\text{conc. of OH}^-) = 10^{-14}.$$

If, then, the concentration of hydrogen ions is greater than in pure water, as in an acid solution, the concentration of hydroxyl ions must be less, and conversely, in an alkaline solution, where the concentration of hydroxyl ions is greater than in water, the concentration of hydrogen ions must be less.

The concentration of hydrogen ions, then, affords a quantitative measure of the degree of acidity or alkalinity, being 10^{-7} in neutral solutions, greater than 10^{-7} in acid solutions, and less than 10^{-7} in alkaline solutions. Thus a decinormal solution of hydrochloric acid, a strong acid, and therefore almost completely dissociated, contains very nearly 0.1 g. of hydrogen ions per litre, a hydrogen ion concentration of $\frac{1}{10}$ or 10^{-1} . Similarly a decinormal solution of

the strong base sodium hydroxide contains 0.1 g. molecules of hydroxyl ions per litre, so that the hydrogen ion concentration is only 10^{-13} . On the other hand a decinormal solution of acetic acid, which being a weak acid is slightly dissociated (1.36 per cent.), has a hydrogen ion concentration of only $\frac{1.36}{100} \times \frac{1}{10}$, or 1.36×10^{-3} , or $10^{-2.867}$. In practice it is customary to use only the indices of these figures, under the term "pH," and to say that pure water has a pH of 7, an acid solution a pH *less* than 7 (thus decinormal HCl has a pH of 1, decinormal acetic acid one of 2.867), and an alkaline solution a pH *greater* than 7 (thus decinormal sodium hydroxide has a pH of 13).

Though this is perhaps the simplest way of regarding the derivation of the term "pH," it is actually defined as the logarithm to the base 10 of the concentration of hydrogen ions, the negative sign being omitted. Thus decinormal acetic acid, 1.36 per cent. dissociated, has

$$\text{Conc. of H}^+ = \frac{1.36}{100} \times \frac{1}{10} = 1.36 \times \frac{1}{1000}.$$

Now, $\text{Log}_{10} 1.36 = 0.133$, and $\text{Log}_{10} \frac{1}{1000} = -3$.

Hence,

$$\begin{aligned} \text{Log}_{10}(\text{conc. of H}^+) &= 0.133 + (-3) = -2.867, \\ \text{i.e.} \quad \text{pH} &= 2.867. \end{aligned}$$

The use of the pH system of measurement suffers from the drawback that it makes a change in hydrogen ion concentration look less than it really is. It must be remembered that a pH decrease from 7 to 6.7

—*i.e.* a decrease of 0.3—involves a doubling of the hydrogen ion concentration. At pH 7 the hydrogen ion concentration is 10^{-7} . Twice this concentration, 2×10^{-7} , has Log_{10} equal to $\text{Log}_{10}2 + \text{Log}_{10}10^{-7}$ —*i.e.* $0.301 + (-7)$ —*i.e.* -6.7 —the pH being therefore 6.7. Similarly a decrease of 1 in the pH means that the hydrogen ion concentration has been multiplied by 10.

This small change in the pH (the *number* representing the hydrogen ion concentration) produced by a relatively large change in the actual concentration of hydrogen ions has the disadvantage that it is liable to obscure the real magnitude of changes in alkalinity or acidity. Thus, the pH of arterial blood varies from about 7.3 to 7.5. The range in pH is only 0.2, but actually this means that the concentration of hydrogen ions is more than 50 per cent. greater at one extreme of the range than at the other. A change of 50 per cent. in the blood sugar concentration would be regarded as considerable, yet the peculiarity of the pH system has led to the quite unjustifiable statement that the hydrogen ion concentration of the blood varies only very slightly. Actually it may vary to quite a considerable extent; the pH, however, *does* vary only slightly.

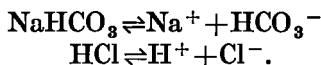
The addition of a small amount of acid to pure water causes a very considerable alteration in the pH. Thus 0.0365 g. of hydrochloric acid, which can liberate 0.001 g. of hydrogen ions, when dissolved in 1 litre of water gives a solution of pH 3 (*i.e.* a hydrogen ion

concentration of $\frac{1}{1000}$, or 10^{-3}). Yet this amount of

acid added to blood would produce a very much smaller change in pH, and therein lies the problem to

be solved—how is the blood able to prevent the acid from liberating its hydrogen ions ?

Consider a solution of sodium bicarbonate, which contains sodium ions and bicarbonate ions together with undissociated molecules, and suppose that hydrochloric acid, which is dissociated into hydrogen ions and chlorine ions, be added :



Now a collision between a hydrogen ion and a bicarbonate ion would form carbonic acid (H_2CO_3), which is a very weak acid and therefore has very little tendency to dissociate into free ions. Hence there will be formation of this acid in the form of undissociated molecules, and so free hydrogen ions will be removed from the solution, which will then contain undissociated molecules of carbonic acid, free sodium ions, free chlorine ions, and only a few hydrogen ions. Hence the solution is much less acid in reaction than one containing the same amount of hydrochloric acid alone. The sodium bicarbonate acts as a *buffer*, opposing, so to speak, the liberation of hydrogen ions by acid, as buffer springs oppose the momentum of the railway wagons which are shunted against them. Probably a better analogy is that of a sponge, which absorbs water, since the bicarbonate does not so much oppose the liberation of hydrogen ions as remove, or absorb, those already liberated. The buffer analogy, however, was first used, and the term buffer is now generally applied to substances which exert an action like that of sodium bicarbonate. The buffering action of sodium bicarbonate depends on the fact that it is a salt of a weak acid and a strong base, and it is

obvious that the salt of any weak acid can act in the same way, cutting down the fall in pH produced by the addition of acid. A salt formed from a strong acid and a weak base is able, by an exactly similar mechanism, to act as a buffer in the opposite direction, preventing an alkali from exerting its full effect in raising the pH.

NEUTRALITY REGULATION IN THE BLOOD

The blood is provided with a very efficient mechanism for preventing undue increase in the hydrogen ion concentration in spite of the very considerable amount of acids—end-products of metabolism—which it is called upon to carry.

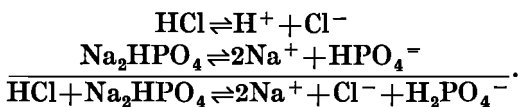
Of this mechanism haemoglobin forms an exceedingly important part. Like all proteins, haemoglobin can function either as an acid or as a base, according to circumstances; in solutions with a pH below a certain value (the iso-electric point) it behaves as a base, and in solutions more alkaline than that it behaves as an acid. In the pH range available to the more complex organisms such as ourselves, haemoglobin is a weak acid, and actually exists in the red cells as a potassium salt. Oxyhaemoglobin also exists as a potassium salt, but combines with more potassium than does reduced haemoglobin itself. Hence when oxyhaemoglobin gives up its oxygen to the tissues, potassium is liberated. It is then available to combine at once with the acids—mostly carbonic acid—which the tissues are producing and pouring into the blood-stream. In the lungs the process is reversed; the carbonic acid is excreted and the potassium left behind is available for the oxyhaemoglobin which is being re-formed. At the pH of arterial

blood the liberation of one gram molecule of oxygen involves the setting free of nearly 0.7 of a gram atom of potassium (which can combine with 0.7 of a gram molecule of carbon dioxide). Under normal metabolic conditions the R.Q. (the ratio of CO_2 produced to oxygen used) is about 0.8, so that without any change of pH about $\frac{7}{8}$ of the CO_2 produced is neutralised by the potassium liberated during the reduction of oxyhaemoglobin.

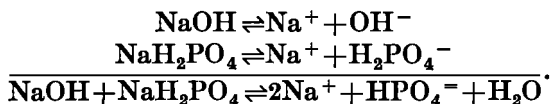
The remainder, together with the "fixed" acids (*i.e.* those other than carbonic acid, which decomposes to water and CO_2) of metabolism and those present in the absorbed foodstuffs (uric acid, phosphoric, sulphuric, etc.) is left to the remaining mechanism—the *buffering power* of the blood.

In blood there are three buffer systems present in sufficiently high concentration to be quantitatively important, and of these sodium bicarbonate is one. In addition, phosphates are present, and act as buffers, since phosphoric acid is weak and slightly dissociated. Not only is this so, however, but phosphoric acid contains three hydrogen atoms in its molecule, and can therefore give rise to three different sodium salts which dissociate in different ways. Two of these salts are of practical importance, sodium dihydrogen phosphate— NaH_2PO_4 —which ionises to Na^+ and H_2PO_4^- , and disodium hydrogen phosphate— Na_2HPO_4 —which ionises to 2Na^+ and HPO_4^- . Solutions of the former are slightly acid, since H_2PO_4^- tends to ionise further to HPO_4^- or PO_4^{\equiv} and H^+ ; while solutions of the latter are slightly alkaline, since the tendency of the HPO_4^- to dissociate further is overcome by its tendency, in the presence of many sodium ions, to combine with hydrogen ions to form H_2PO_4^- . The

hydrogen ions for this action are obtained from the dissociation of water, and necessarily, of course, leave an excess of hydroxyl ions. Evidently addition of acid to a solution of disodium hydrogen phosphate does not cause a very great fall in pH, since the dihydrogen phosphate is formed :



Conversely, addition of alkali to a solution of sodium dihydrogen phosphate produces a relatively small increase in pH on account of the formation of the only slightly alkaline disodium salt :



Although the actual amount of phosphate in blood is small, and its direct buffering action is correspondingly small, phosphates are of considerable importance in the excretion of acid—the second line of defence which we have to consider shortly.

The third buffer substance is protein. The blood proteins include albumin and globulin (mainly in the plasma) and, of course, haemoglobin, which can share in neutrality regulation as a general buffer as well as by virtue of the special property previously discussed.

Protein is built up from amino-acids, which are acids since they contain the carboxyl group, $-\text{COOH}$, and can give rise to hydrogen ions. They also contain the basic amino group, $-\text{NH}_2$, which can combine with water (just as ammonia does to form

ammonium hydroxide) and then give rise to hydroxyl ions. They are, in fact, like water—amphoteric substances. In the protein some of these carboxyl and amino groups remain free, although many are concerned in the linkages between the amino-acids, and so proteins themselves are amphoteric. Whether they behave as bases or as acids depends on circumstances, but at the pH of blood they are functioning as weak acids, and exist partly as salts (of sodium in the plasma, of potassium in the red cells).

It has been estimated that, per litre of blood, the various buffers are able to neutralise the following amount of strong acid under physiological conditions :

	<i>c.c. of N. acid neutralised per litre of blood</i>
Bicarbonate	18.0
Haemoglobin	8.0
Serum protein	1.7
Other buffers (mainly phosphate)3
TOTAL	28.0

These various buffer systems are, of course, interdependent and, except for haemoglobin, are not confined to the blood, but are present in tissues also (where, however, the protein salts are probably the most important). Since, so far as the blood is concerned, the buffers are partly in the plasma and partly in the cells, the diffusion of acid from tissues into the plasma will, in practice, set in motion a somewhat complex series of transferences of ions from the plasma to the cells and *vice versa*. Nevertheless, the brief outline of the neutrality regulating mechanisms given above, lacking though it is in detail, is sufficient for a general understanding of the processes and of the ways in which they may be overtaxed under abnormal conditions.

The blood, then, in virtue of the various buffer salts it contains, is able to take up considerable amounts of acid without any marked alteration in pH. This ability is of great importance—especially so since the blood reflects the conditions throughout the tissues—for the blood is constantly being called upon to deal with acid, in health no less than in disease. The ordinary metabolic changes in the tissues—the oxidation of fat, carbohydrate, and protein—produce carbonic acid, which the blood must carry to the lungs; purines are oxidised to uric acid; the sulphates of such amino-acids as cystine and methionine ultimately appear as sulphuric acid. Muscular effort causes the production of lactic acid in the working muscles and some of this diffuses into the blood; the deranged oxidation of fat in diabetes mellitus causes the accumulation of β -hydroxybutyric and acetoacetic acids, and the acid produced in these and other ways must be prevented from affecting the pH. Now although the buffer mechanism of the blood—and tissues—is normally so efficient that considerable amounts of acid can accumulate without much change in pH, its action is limited. It may be likened to a sponge, which can absorb considerable quantities of water, but eventually becomes saturated. It is essentially, however, a stop-gap, and the body's final method of dealing with excess acid is to excrete it. Carbonic acid is of course excreted by the lungs as carbon dioxide, and other acids are excreted by the kidney.

Now the pH of the urine can be varied by the kidney, according to the needs of the moment, over a wide range; urine may be as acid as pH 5 or as alkaline as pH 8, without any ill-effects. Usually, with

the average mixed diet containing an excess of inorganic acid radicles (chloride, sulphate, phosphate, etc.) over basic radicles (sodium, potassium, etc.) it is somewhat acid, having a pH in the neighbourhood of 6. Such a pH, however, in an unbuffered solution, would be produced by a minute trace of such an acid as hydrochloric or sulphuric, whereas urine contains considerable amounts both of chloride and sulphate. Evidently only a small part of these can be present as sulphate or chloride of hydrogen (*i.e.* as free acid) and by far the greater part must be present as salts. In other words, the kidney, though it excretes a little acid as such, excretes it mainly along with basic radicles, which either must have been ingested in sufficient amount or must originally have formed part of the base given up by the blood buffers to neutralise the acids. Ordinarily the amount of ingested base suffices, especially as the healthy kidney is able to supplement it to a considerable extent by forming ammonia (either from urea or from amino-acids) and using this to spare the more valuable sodium, potassium, etc. Nevertheless, the continued excretion of excessive amounts of acid neutralised in these ways may seriously deplete the available supplies of base—*i.e.* the buffers—and so damage to some extent the power of the blood to maintain the normal pH. In such a condition further excessive production of acid might easily overtax the resources of the blood and bring about a pH change great enough to be dangerous or even to cause death. In any case, such a lack of buffer salts seriously impairs the power of the blood to deal with the normal metabolic production of carbon dioxide.

It is to this decreased power of preserving the normal

pH on the addition of acid that the term *acidosis* is applied, and the term therefore does not necessarily imply an actual demonstrable change in pH. In acidosis the pH of the blood and tissues may be still within the normal range, and actually is so unless the acidosis be severe. Either through abnormal retention of acid, whereby the buffers have been partly used up, or through actual loss of buffer, as in the attempt to excrete the excess acid, the amount of buffer remaining available is decreased. The contrary condition, in which abnormally large amounts of base are available for the neutralisation of acid, is called *alkalosis*. Here again there is not necessarily any easily measurable increase in the pH—that is, any abnormal alkalinity of the blood, though such a change occurs more readily than in acidosis, since the blood buffers are more efficient against a decrease than an increase of pH.

Decrease in the amount of available buffer substances (*the alkali reserve*) does theoretically, of course, bring about some decrease in the blood pH, but the accurate measurement involves the use of expensive and not always accessible apparatus, together with great care to avoid loss of CO_2 during the obtaining of the blood sample. Direct measurement of the blood pH is not, therefore, a method of great use in routine examinations for acidosis or alkalosis.

It has been stated that the kidney can, and in health does, attempt to compensate for an alkalosis or acidosis by altering the pH of its secretion and by varying its production of ammonia. The amount of adaptation which can be obtained by these methods alone is, however, limited. In order to excrete excessive amounts of acid, the kidney can use still another

method—it can increase the total volume of urine. This involves either an increase in the water intake or an excessive withdrawal of water from the tissues. Since the clinical conditions resulting in acidosis frequently involve reduction of fluid intake or excessive fluid loss in other ways, it follows that dehydration is a frequent concomitant of disturbances of the neutrality regulation. Indeed, an adequate supply of fluid to make good the dehydration and to allow the kidney to do its share in adjusting the alkali reserve of the body is often an important factor (and one which is not seldom overlooked) in the treatment of such conditions. Since, further, an increased water excretion involves an increased output of sodium chloride, the body may be depleted of salt in either acidosis or alkalosis, and its administration also is necessary as part of a rational treatment.

MEASUREMENT OF THE ALKALI RESERVE

The defence of the body against acid thus lies essentially in a supply of available base, chiefly sodium and potassium, with which the acid may be neutralised. The available base is that part of the total base which corresponds to the weak acidic radicles, the bicarbonate, phosphate, and proteinate, which can combine with hydrogen ions to form molecules which are only slightly dissociated. Hence a measure of the available base, the so-called *alkali reserve*, affords a measure of the power of the body to resist acid. In order to estimate the alkali reserve Haldane introduced the method of determining the percentage of carbon dioxide in the alveolar air, a method based on the principle that in acidosis the power of the blood to

carry carbon dioxide is reduced, with a proportional reduction in the carbon-dioxide content of the alveolar air. This method, however, is by no means easy to carry out and is open to many objections, both theoretical and practical. Other indirect methods of calculating the blood pH (and so gauging its buffering power) indirectly by determining the ratio of free carbonic acid to combined bicarbonate ions have been evolved, but involve the use of arterial blood and are too difficult and laborious for routine use. Actually, since very considerable variations in buffering power from the normal mean are found in clinical conditions of acidosis and alkalosis, it is usually sufficient to determine the CO_2 content of a sample of venous blood, drawn with precautions to prevent loss of CO_2 , or even more simply, that of venous blood drawn and re-equilibrated with alveolar air. The latter method is founded on the fact that blood exposed to an atmosphere containing a definite amount of carbon dioxide absorbs that gas in proportion to its supply of available base. Thus this method, due to Van Slyke, measures the *carbon-dioxide combining power* of the blood, and not directly its alkali reserve. With certain reservations to be discussed later, however, the terms are interchangeable.

Although in experimental work, where very small alterations in the carbon-dioxide combining power of the blood may be of importance, a number of precautions in obtaining the sample must be observed, the requirements of clinical work are satisfied by the use of a fresh sample of oxalated venous blood obtained in the ordinary way. A thin layer of blood is exposed at room temperature in a suitable flask to an atmosphere containing a 5.5 per cent. concentration of

carbon dioxide, that being the normal concentration in alveolar air. Slight variation above or below this value has very little practical effect on the result. The amount of carbon dioxide present in the blood after this treatment is determined by acidifying a measured volume with lactic acid and extracting the gas in a vacuum. The result is expressed preferably in volumes of carbon dioxide taken up by 100 c.c. of blood, though often it is given as a plus or minus percentage of an arbitrary normal which is taken as being 54 vols. per 100 c.c.

INTERPRETATION OF RESULTS

Normal.—The carbon-dioxide combining power of normal healthy individuals actually varies within wide limits. We ourselves have obtained values as low as 55 vols. per cent. and as high as 70 vols. per cent. in individuals who were apparently perfectly healthy. This corresponds closely to the limits of carbon-dioxide combining capacity observed by Van Slyke, who gives the range 53 to 77 vols. per cent. as the normal. These variations are not due to experimental error, but represent actual differences between individuals, and variations in the same individual, due to purely physiological causes. We may therefore say that no clinical significance can be attached to a single analytical result falling within this range, and yet it is apparent that a change from 77 to 53, either of which alone must be considered normal, is one of considerable magnitude, and may be due to pathological as well as to physiological causes. Broadly speaking, however, an acidosis exists when the carbon-dioxide combining power falls below 53 vols. per cent., and an

alkalosis exists when it has risen above 77 vols. per cent.

It must be remembered, of course, that an acidosis due to excessive retention or accumulation of CO_2 in the blood, or an alkalosis due to excessive CO_2 loss (so-called "gaseous" acidosis or alkalosis) cannot be detected by determination of the CO_2 combining power of the blood. It can, however, be done by determination of the CO_2 content, or better by determination of the free carbonic acid. This holds since the acid causing the abnormality is the standard of reference and is removed from or taken up by the blood during the process of equilibration with alveolar air.

Acidosis

The alkali reserve may be lowered as the result of any one of three causes : (a) by an over-production of acid substances ; (b) by a failure to eliminate acid ; or (c) by an excessive loss of base ; and the pathological production of acidosis may be considered under these separate heads.

(a) **The Over-production of Acid.**—The most common cause of acidosis due to this factor in disease is the faulty oxidation of fat occurring in diabetes mellitus. β -hydroxybutyric acid and aceto-acetic acid may then accumulate to such an extent as to take up a very considerable part of the available base, and the continued production of these acids and their excretion by the kidney tend to a progressive diminution of the alkali reserve. It is quite commonly supposed that the mere appearance of acetone bodies in the urine is sufficient evidence to warrant a diagnosis of acidosis ; but this is entirely fallacious, since it is no uncommon experience to find diabetic patients

with considerable quantities of acetone in the urine, but with no diminution in the carbon-dioxide combining power of the blood. None the less such an appearance of ketone bodies in the urine must be treated as a danger-signal. Their continued production will in time lead to the development of an acidosis, and treatment should be directed, just as in the presence of actual acidosis, towards their oxidative removal. The administration of alkalis in these circumstances can at best be only the most temporary of expedients, since it does not greatly aid the elimination of the acid substances and does not at all prevent their continued production. A determination of the carbon-dioxide combining power, therefore, distinguishes between cases of ketonuria, since those cases in immediate danger of coma show a carbon-dioxide combining power below 53 vols. per cent., whereas less urgent cases do not show any significant variation from normal. Those cases which already exhibit symptoms of coma have a very considerable reduction in the carbon-dioxide combining power of the blood, and the severity of the condition may be assessed by determining the extent of this reduction. The approximate values of the carbon-dioxide combining power to be expected in these various conditions are shown below (Table V).

TABLE V

CARBON-DIOXIDE COMBINING POWER IN DIABETES MELLITUS

	<i>Vols. CO₂ per cent.</i>
Ketonuria, but no immediate danger of coma	50 or over
Ketonuria, with immediate danger of coma, but few or no symptoms	40-50
Ketonuria, with slight but definite symptoms of coma	35-40
Ketonuria, coma, but fair prognosis	20-35
Ketonuria, coma, bad prognosis	below 20

The carbon-dioxide combining power in hypoglycaemic as opposed to hyperglycaemic coma is of course normal.

The excessive production of acids, which brings about the state of diabetic coma, may also contribute to the production of acidosis in other pathological conditions. In starvation, where, the available glycogen supplies having been used up, carbohydrate metabolism is reduced to a minimum, the complete oxidation of fat is prevented. Hence the ketone acids are produced just as in diabetes, though to a less extent. This factor, combined with the loss of available base in the urine—a loss which is not being made good by the ingestion of salts—may cause such a lowering of the alkali reserve as to render it difficult for the patient to resist the extra strain thrown on the acid-base regulating mechanism by the acidosis of anaesthesia. The practice, therefore, which was once common, of starving the patient prior to operation is to be deprecated, and, where any cause predisposing to acidosis is suspected, a determination of the carbon-dioxide combining power should be made, in order that, if necessary, pre-operative treatment may be given to raise the alkali reserve to a safe level.

(b) **The Failure to eliminate Acid.**—We have already seen that the kidney plays a considerable part in the excretion of acids, either as such, or together with basic radicles such as sodium, potassium, and ammonium. When this function of the kidney fails, as it may do in cases of acute or subacute nephritis, or in the terminal stages of chronic nephritis, there is a retention of acid in the blood, and therefore a diminution of the available base and a lowering of the carbon-dioxide combining power.

This retention of acid, although it may at times be considerable, is only one factor, and perhaps not the most important, in the production of an acidosis due to impaired renal function. Probably the most important factor is the inability of the damaged kidney to manufacture ammonia and so spare the available bases of the body fluids. This failure of ammonia production is particularly marked in acute nephritis and in the terminal stages of chronic interstitial nephritis; it does not occur to any appreciable extent in nephrosis, a condition which is (probably in consequence) not characterised by lowering of the alkali reserve to any great extent.

Most cases of chronic interstitial nephritis show a carbon-dioxide combining power within the normal range, and it is only in the terminal stages of the disease, with commencing uraemic manifestations, that it is lowered much below 53 vols. per cent. The onset of chronic uraemia, however, is frequently accompanied by symptoms which are so varied, and which so often simulate those of many other conditions, that it may be difficult to decide whether they are due to uraemia or to other possible causes. In these circumstances the discovery of a lowered carbon-dioxide combining power may be of some significance. When uraemic coma supervenes the carbon-dioxide combining power of the blood is always markedly lowered. This may become a valuable diagnostic sign, for, even with coma present, the diagnosis of uraemia may not be perfectly obvious in those cases where the patient has not previously been under observation. For instance, some cases of cerebral haemorrhage, with albuminuria, may present many features in common with uraemic coma, but show

no lowering of the carbon-dioxide combining power. From the point of view of treatment, too, a knowledge of the alkali reserve may be useful, for the distressing breathlessness, which is one of the characteristic symptoms of one type of uraemia, is largely due to the acidosis, and may be alleviated by the therapeutic use of alkali. Indeed, this treatment may prolong life in cases of chronic uraemia and even preserve it in acute cases.

In acute nephritis and in hydraemic nephritis showing the nephrotic syndrome the carbon-dioxide combining power is usually lowered to some extent, and this forms the basis of the intensive alkali treatment recommended by some authorities for use in these conditions, and as a means of prophylaxis in such diseases as scarlet fever, in which nephritis is a common complication. It is now well known that the convulsions frequently seen in acute nephritis are due, not to true uraemia, but to cerebral oedema and high blood-pressure, and the immediate treatment is not the intravenous administration of alkali, but the relief of the cerebral symptoms by venesection and lumbar puncture. The differentiation of convulsions of this type from true uraemia is thus a matter of importance, and may be aided by a knowledge of the carbon-dioxide combining power of the blood, for whereas acute nephritis without uraemia rarely shows a carbon-dioxide combining power below 40 vols. per cent., true uraemia with convulsions rarely shows one above that figure.

Respiratory conditions, such as broncho-pneumonia, may be accompanied by some degree of acidosis owing to the retention of carbon dioxide, and, indeed, such a retention may occur in any condition involving de-

pression of the respiratory centre, inability of the lung itself to excrete carbon dioxide, or circulatory failure. This follows from the fact that carbon dioxide is present in the blood and tissues as carbonic acid, which, though a weak acid, combines with some of the available base and, when it cannot be removed rapidly, reduces the amount available for neutralising other acids. As has already been pointed out, determination of the carbon-dioxide combining power, however, cannot reveal this condition, since the retained carbon dioxide will be lost during the analytical procedure. Indeed, since the kidney may compensate for the presence of excessive amounts of carbon dioxide by excreting an equivalent amount of some other acid, the carbon-dioxide combining power of the blood may actually appear to be increased.

(c) **The Excessive Loss of Base.**—In the infectious diarrhoea so often seen in infancy, or in any prolonged or severe diarrhoea not accompanied by vomiting, an acidosis may occur. Such an acidosis is not brought about by failure in the elimination of acids or by their excessive production, though some ketone bodies may be produced by starvation and help to exacerbate the condition. It is due to loss in the diarrhoeal stools of material drawn from the blood for the construction of the alkaline digestive juices. Owing to the composition of the juices which are secreted into the intestine, there is a large withdrawal of fixed base from the blood, and hence a reduction in the alkali reserve and carbon-dioxide combining power. The characteristic hyperpnoea and drowsiness often observed in children suffering from severe diarrhoea is probably largely due to this acidosis and its accompanying dehydration. Administration of

sodium bicarbonate together with water to replace that lost in the motions will often abolish these symptoms. A determination of the carbon-dioxide combining power of the blood in these cases throws light on the condition and suggests the necessary treatment. A combining power of less than 30 vols. per cent. is a very grave prognostic sign.

Alkalosis

Just as a state of acidosis may be said to exist when the carbon-dioxide combining power of the blood falls below 53 vols. per cent., so an alkalosis exists when it is raised above 77 vols. per cent. Such an alkalosis may be due to excessive intake of alkali or to an excessive loss of acid.

(a) **Excessive Intake of Alkali.**—Though the carbon-dioxide combining power of the blood may undoubtedly be raised by the administration of alkalis such as sodium bicarbonate, it is usually raised only within normal limits, and a true alkalosis rarely occurs clinically from this cause. Occasionally, however, this does occur in patients undergoing the intensive alkali treatment for gastric or duodenal ulcer, especially when there is some degree of pyloric stenosis with vomiting and loss of gastric juice. Should symptoms of prostration, shallow breathing, headache, tetany, and vomiting supervene during the course of such treatment it is well to determine the carbon-dioxide combining power of the blood, and if this gives a reading over 77 vols. per cent. a reduction of the alkali intake should be advised.

(b) **Excessive Elimination of Acid.**—Excessive elimination of carbon dioxide, which, as we have seen, behaves as a weak acid, may cause an increase in the

alkali reserve of the blood. Such a washing out of carbon dioxide may be produced clinically by the hyperpnoea seen in light ether anaesthesia, or in surgical shock, when the loss of carbon dioxide may be so great that the patient may stop breathing. Treatment of the condition by prompt inhalation of carbon dioxide under artificial respiration is so efficacious that it may be said that no surgical theatre ought to be without its cylinder of carbon dioxide for emergency purposes. A similar alkalosis, due to washing out of carbon dioxide, is seen in mountain-sickness, carbon-monoxide poisoning, and in some cases of hysteria associated with hyperpnoea.

It must be remembered that gaseous alkalosis, like gaseous acidosis, cannot be detected by determination of the CO_2 combining power, though it may be shown by the much more complicated procedure of determining the free carbonic acid of the plasma.

The most important clinical manifestation of alkalosis, however, occurs in cases of excessive vomiting such as may be produced by a high intestinal obstruction or by pyloric stenosis. An examination of the carbon-dioxide combining power of the blood in such a case shows it to be greatly increased. Readings as high as 120 vols. per cent. have been obtained in cases of high obstruction just before death, and there is little doubt that this alkalosis, with the simultaneous dehydration, is the actual cause of death in such cases. The alkalosis is readily understandable when we remember that owing to the loss of acid stomach contents in the vomited matter the concentration of chloride ions, which is normally the chief acid factor in the plasma, may be reduced to about a third of its usual value. In consequence there is a considerable

increase in the concentration of bicarbonate ions in replacement of the loss of chloride ions, bringing about an extensive increase in the alkali reserve and carbon-dioxide combining power of the blood. By the simple expedient of supplying salt solution abundantly in such cases the bicarbonate concentration may be temporarily corrected (and the fluid loss made good) and the patient brought into a sufficiently satisfactory condition to stand an operation which might otherwise have proved fatal. A determination of the carbon-dioxide combining power is thus of service in verifying the presence and extent of such an alkalosis, and in those cases where the carbon-dioxide combining power is found to be significantly raised, operation should be deferred until the excessive alkalosis has been corrected by the supply of salts and water.

Co-existence of Alkalosis and Ketosis

Since the production of ketone bodies is the causative factor in the acidosis of diabetes and starvation, their frequent presence in the urine and occasional presence in the breath in cases of severe vomiting quite naturally lead many people to suppose that an acidosis must be present. Cases of cyclical vomiting in children, of severe gastro-enteritis, of hyperemesis gravidarum, and of post-operative vomiting frequently show such signs of acidosis. It is, therefore, something of a surprise to find that instead of a reduction there may sometimes be an increase in the carbon-dioxide combining power of the blood of such patients. There seems to be no doubt that there are two processes at work in these cases: a tendency to ketosis due to starvation, to an anaesthetic or to bacterial infection,

and a tendency to alkalosis due to excessive loss of acid in the vomited gastric contents (both processes leading to excessive water and chloride loss). The two antagonistic tendencies may cancel each other, so that the carbon-dioxide combining power of the blood is within normal limits. One or other factor may predominate, and where an alkalosis is present the accumulation of ketone bodies may limit its extent and indeed prevent one of fatal degree. Such a situation, therefore, presents an interesting therapeutic problem, for if the chlorides be restored faster than the ketones are removed an acidosis may be occasioned; but if the ketone acids be removed too rapidly a further and possibly dangerous exacerbation of the already existing alkalosis may occur. Without frequent determinations of the carbon-dioxide combining power we are, therefore, largely in the dark in dealing with those cases, but with such aid we immediately become acquainted with the exact state of affairs and are able to control the treatment indicated in a rational manner.

CHAPTER V

GLYCOSURIA

MODERN methods of micro-analysis have made it a relatively easy matter to differentiate true diabetes mellitus from other less serious causes of glycosuria, and in our opinion they should be applied to the thorough investigation of every case in which sugar is found to be present in the urine, and in which clinical examination does not make the diagnosis obvious. Examination of the blood may often show that a patient, though only occasionally and spasmodically showing glycosuria, is in reality suffering from slight diabetes, and control of the disease at this stage is, of course, a relatively simple matter. Although many cases of glycosuria are undoubtedly not diabetic, and though the passing of sugar is not in itself harmful, we believe that every instance of sugar in the urine demands complete investigation. If it is due merely to a leakage through the kidney, or to some emotional disturbance, so much the better ; but if it is a manifestation of diabetes the sooner proper treatment is commenced the better for both the patient and the physician.

It is, of course, true that the diagnosis is clear in the majority of cases of diabetes without recourse to complicated investigations. When a patient, who has lost weight rapidly, complains of severe thirst, polyuria and weakness, and his breath is found to be heavy

with the smell of acetone and his urine loaded with sugar, further investigation is unnecessary to establish the fact that he is suffering from diabetes mellitus. There remain, however, a large group of cases where symptoms are absent or equivocal—mostly cases of functional glycosuria, but a few with mild diabetes—where an accurate diagnosis is impossible without biochemical methods.

INVESTIGATION OF A CASE OF GLYCOSURIA BY THE CARBOHYDRATE TOLERANCE TEST

The detection of sugar in the urine by any one of the tests available should, as a general rule, be followed immediately by an examination of the blood. In symptomless glycosuria, this is of paramount importance in order to determine whether the patient is, or is not, suffering from diabetes. Further examination of urinary specimens while fasting and at varying times after a meal, which is recommended by some writers, is simply a waste of time. In some cases of mild diabetes, for instance, sugar is by no means invariably present in the urine, while certain cases of purely functional glycosuria may excrete sugar in every specimen of urine, not excluding the fasting specimen. The slight saving of trouble in avoiding the blood analysis is not worth the risk of missing a case of mild diabetes which, neglected, becomes more severe, but which, caught in time, is easily treated. On the other hand, it is well worth the very temporary inconvenience of blood examination to save a case of functional glycosuria from being regarded as one of diabetes with all the trouble and distress that such a diagnosis involves. 'Even where a condition of true

diabetes is obvious clinically, an examination of the blood is useful, not necessarily from the point of view of diagnosis, but rather as an aid to gauging the severity of the case and as a guide to the type of treatment to be adopted.

Amongst the circumstances in which the finding of glycosuria does not demand blood-sugar estimations as the next step are those in which the urine sugar may be, not glucose, but lactose—that is to say, in pregnancy or lactation. Excessive production of lactose during these periods may cause the appearance of that substance in the blood and so produce lactosuria, for which the kidney threshold is very low. Lactose responds to the usual qualitative tests in a manner similar to glucose, and therefore the two are readily confused. In theory, the best way of distinguishing them is by their reaction with phenyl hydrazine, whereby both form osazones which crystallise in rather different forms and can be distinguished by microscopical examination. In practice, however, it is difficult to prepare pure crystalline osazones from urine, and the difficulty is intensified when, as not uncommonly happens, the two sugars are found together. The presence of lactose can be shown by submitting the urine to the mucic acid test, but it is important to know, not merely whether lactose is present, but if glucose is also present. If the latter sugar is being excreted a positive response to the mucic acid test does not negative the diagnosis of possible diabetes. A more valuable method of differentiation, therefore, is the quantitative method (Appendix), which not only detects and estimates lactose, but shows whether or not glucose is present as well.

Glycosuria is a fairly common accompaniment of

hyperactivity of the thyroid and pituitary glands, so that in the presence of well-marked signs of these conditions the presence of sugar in the urine does not as a rule call for further investigation.

In an investigation of the blood sugar for the purpose of differentiating diabetes mellitus from other possible causes of glycosuria we believe it desirable, both as an ultimate saving of time to the physician and as a saving of inconvenience to the patient, to proceed at once with a carbohydrate tolerance test—that is, the construction of a blood-sugar curve. In this way information is obtained both as to the fasting-level of the blood sugar and as to the reaction of the blood towards glucose ingestion, and both in diagnosis and subsequent treatment the latter information may prove to be the more valuable.

The procedure to be adopted is as follows. The test should be begun early in the morning with the patient fasting since the preceding evening. The ingestion of glucose, and therefore of any carbohydrate-containing meal, raises the blood sugar above the fairly constant fasting-level, and though normally this increase disappears in the course of about two hours it sometimes lasts much longer, and a fast of twelve hours at least is necessary before one can be sure that the blood sugar has returned to the fasting-level. A sample of blood is withdrawn for determination of the fasting sugar content, and the bladder is emptied. Fifty grammes of glucose are then given in concentrated solution, flavoured, if desired, with lemon juice. Thereafter samples of blood and urine are obtained at intervals of thirty minutes for two hours. The urine samples are tested qualitatively for sugar, and the sugar content of the blood is deter-

mined by any suitable method, the analytical results being plotted graphically against time.

The Normal Response

The fact that blood normally contains sugar is due to the oxidation and storage of that substance by the tissues and the replacement of the lost sugar from the glycogen stores of the liver, the glycogen being hydrolysed and the resulting glucose carried by the blood to the various tissues. Normally, in a fasting subject, the rate of glycogen breakdown — glycolysis — and therefore the rate of entry of sugar into the blood so balances the rate of disappearance from the blood that the blood sugar remains remarkably constant. The true figure is probably, as recent exact methods have shown, about 80 mg. of sugar per 100 c.c. of blood. Some of the methods employed in clinical work, however, estimate other reducing substances also (which do not vary very much in comparison with the glucose), and with them the normal figure is about 100 mg. per 100 c.c. One of the methods most commonly used is that of Hagedorn and Jensen, and with it some of the non-sugar reducing substances are removed during the preliminary treatment of the blood. This method gives 80–90 mg. per 100 c.c. as the normal fasting blood sugar. Where other methods are employed it is advisable to ascertain from the analyst the actual normal range, when the figures given here and in the following charts can be suitably corrected.

The ingestion of glucose is followed by a rise in the sugar content of the blood, since the rate of absorption from the alimentary canal is at first greater than the rate at which the sugar is removed from the blood

by oxidation and—quantitatively the main factor—conversion to glycogen. This increase in the blood sugar is maximal about three-quarters of an hour after ingestion, by which time the level has reached, usually, 140 to 150 mg. per 100 c.c. By this time, however, oxidation and, particularly, synthesis of

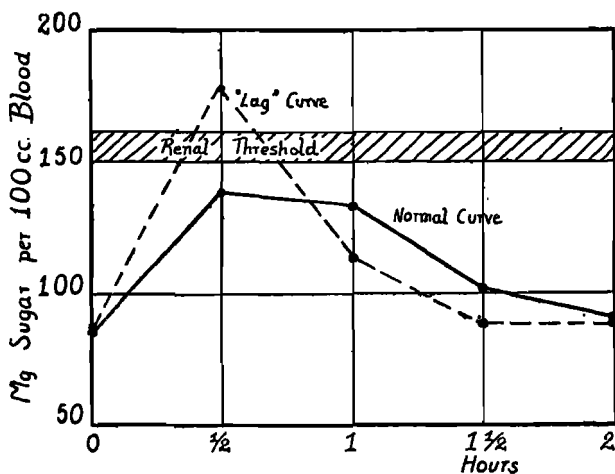


FIG. 4.—The blood sugar, after ingestion of 50 g. of glucose, in a normal healthy person, and in one showing a "lag" curve.

glycogen have become the predominating factors, so that the blood sugar begins to fall again, and reaches the fasting-level in one and a half or two hours. The normal blood-sugar curve is thus of the shape shown in Fig. 4.

Throughout the test the urine of a healthy person should remain sugar-free. The normal kidney excretes sugar only when the concentration of that substance in the blood exceeds 150 to 160 mg. per 100 c.c.—the normal threshold. It is very rarely indeed

that the blood sugar passes the normal threshold when a healthy person ingests 50 g. of glucose, so that if the sample of urine obtained at the end of the test contains more than a mere trace of glucose we are inclined to regard it as evidence of some lowering of the renal threshold, though this, if it is the sole abnormality, is of little or no importance.

The normal blood sugar curve varies somewhat according to the age of the patient. Young adults, and particularly children, show a greater sugar tolerance than old people. In consequence the normal blood sugar curve in youth is flatter and returns more rapidly to the fasting-level than in old age.

Abnormal Responses

The appearance of sugar in the urine may obviously be due either to the raising of the blood sugar above the threshold or to a lowering of the threshold—or, of course, to a combination of these causes. If even the fasting blood sugar is above the threshold, sugar will be present in all specimens of urine; otherwise it will be present only after the ingestion of sufficient carbohydrate to raise the blood sugar above the critical point. Similarly the lowering of the threshold may be slight, with consequent intermittent glycosuria, or it may be so great as to cause continuous excretion of sugar. It is this dual mechanism which renders it so difficult to tell from urine examination alone whether or not a glycosuria is an indication of diabetes mellitus.

Diabetes Mellitus.—In diabetes the glycosuria is due primarily to a raising of the blood sugar, though in some cases, particularly those of long duration, there may be either lowering or raising of the renal

threshold, a fact of which some account must be taken later. The raising of the blood sugar even during fasting, when the supply of insulin is deficient, is due to a combination of causes. The diabetic apparently cannot oxidise glucose as can the normal person, nor can he use it for synthesis of glycogen. Hence the two means by which glucose is ordinarily removed from the blood have failed to an extent which is seldom if ever complete, but is proportional to the severity of the diabetes. Besides failing to remove glucose from the blood, the diabetic organism, again to an extent depending on the severity of the condition, manufactures glucose from protein (and possibly fat) in a vain effort to supply the carbohydrate for which his tissues are starving, but which they cannot utilise. There is thus a tendency for sugar to accumulate in the blood even when no carbohydrate has been ingested for some time, and this accumulation may be great enough to raise the fasting blood sugar above the kidney threshold, in which case sugar will be found in all urine passed. Consideration of the factors involved makes it obvious that the height to which the fasting blood sugar is raised above the normal indicates to some extent the severity of the diabetes in an untreated case.

It is not unusual to obtain somewhat high values for the fasting blood sugar in non-diabetic persons, especially if they are of a nervous disposition or fear the slight pain of venepuncture. In these cases particularly the blood-sugar curve, which, of course, is normal, is valuable in leading to the correct diagnosis. Even when the increase in the fasting blood sugar is gross the curve is useful in affording further information for gauging the severity of the diabetes, and,

an aid in future treatment, in determining the renal threshold if this is higher than the fasting blood level.

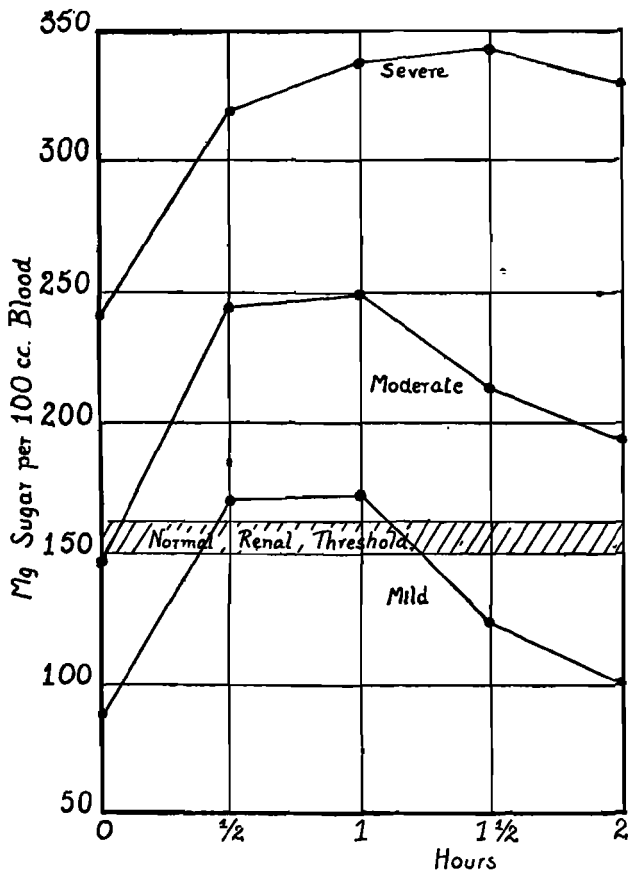


FIG. 5.—The blood sugar, in diabetes, after ingestion of 50 g. of glucose.

In diabetes, as in health, ingestion of glucose causes an increase in the blood sugar. The very factors

which raise the sugar-level in the blood of the fasting diabetic, however, prevent the removal of the absorbed glucose, and thus the rise is greater than that found in normal people. Moreover, the slow removal of glucose from the blood, and especially the failure in the glycogen-synthesising mechanism, means that absorption remains the more rapid of the processes for a longer time than normally. Consequently the maximum concentration in the blood is reached much later—at least an hour, and usually more, after the glucose ingestion—than in the healthy person. Oxidation and glycogen synthesis are reduced, and, though some sugar is excreted, this is not sufficient to cause a rapid fall in the blood sugar even after absorption has slackened. Hence the curve characteristic of diabetes, instead of showing a sharp return to the fasting-level, shows a considerable retention of sugar in the blood, and a slow fall, so that two hours after the glucose meal the blood still contains much more sugar than it did during fasting. The curve thus approximates to those shown in Fig. 5, which are from cases of mild, moderate, and severe diabetes respectively.

If one considers the whole day as a series of carbohydrate tolerance tests, the meals being substituted for the doses of glucose, the blood sugar is found to follow a course similar to that shown in Fig. 6, which is constructed from a case of mild diabetes. The interval between meals is not sufficient for the blood sugar to fall completely, and so there is a tendency for it to climb steadily throughout the day, both as regards maximal concentration after meals and minimal concentration immediately before meals. At night the blood sugar falls gradually to the lowest level of all, which is found early in the morning. The

curve also shows how it is possible, in a case of diabetes, for the early-morning urine to be sugar-free. It is interesting to compare this curve with that of a severe diabetic with a high fasting blood sugar and

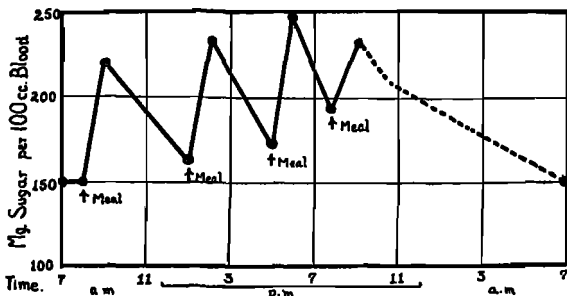


FIG. 6.—The blood sugar during twenty-four hours in an untreated case of diabetes. Note the rise after each meal, with inability to fall completely to the fasting-level before the next meal. At night the blood sugar is able to fall to the fasting-level.

undergoing insulin treatment (Fig. 7). Here the blood sugar is controlled during the day by insulin administration. It falls after each dose of insulin, but rises after the subsequent meal, and continues to rise towards the fasting-level until the next dose of insulin. During the day, however, the insulin is given with sufficient frequency to keep the blood sugar generally below the very high fasting-level, but during the night, without insulin, this is attained. Thus the highest blood sugar, instead of the lowest, is found early in the morning. This explains the finding of sugar in the early-morning urine of a patient, under insulin treatment, who may otherwise be sugar-free.

The considerable swing in the blood sugar is due to the rather evanescent effect of insulin. When insulin is combined with protamines, and still more when converted to a zinc-protamine insulin, it is liberated

slowly in the tissues, and the effect of a single injection is much more prolonged. Under these circumstances the blood sugar is kept much more constant, as is shown in Fig. 7.

Thyroid and Pituitary Dysfunction.—In over-

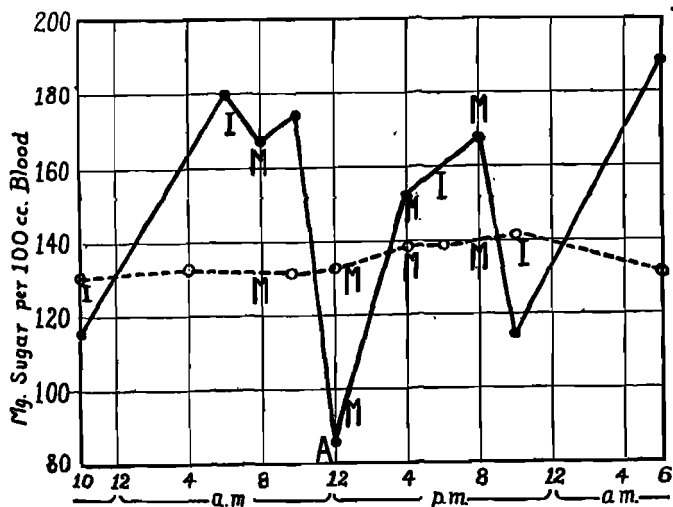


FIG. 7.—The dotted line shows the course of the blood sugar in a patient receiving two doses (each of 20 units) of insulin per day. The insulin was given at the points marked I, and meals were taken at the times M. On this particular day there was a hypoglycaemic reaction at A.

The continuous line shows the blood sugar in the same patient receiving, for the sixth successive day, 50 units of zinc-protamine insulinate once per 24 hours (I). Meals are marked as before.

activity of the thyroid and pituitary glands, both conditions in which glycosuria may occur, the blood picture may simulate that of mild diabetes. The fasting blood sugar is raised to some extent above the normal, and the response to glucose ingestion resembles that of diabetes in showing a rather greater

increase in the blood sugar than the normal, and a slow fall of the blood sugar from the maximal concentration, instead of the normal rapid return to the fasting-level. Usually, however, the maximum concentration of sugar in the blood is reached in very little more than the normal time, but in practice it is not easy to use this as a means of differentiating uncomplicated exophthalmic goitre or acromegaly from the same conditions complicated by the presence of mild diabetes. Those cases of diabetes which give fasting blood-sugar values of the order sometimes found in disturbance of thyroid or pituitary function (100 to 130 mg. per 100 c.c. of blood) are so mild that their blood-sugar curves do not show departures from the normal sufficiently marked for very fine distinctions to be drawn. In such cases the physician is thrown largely on his own clinical resources.

Although they are not accompanied by glycosuria, the opposite pathological condition of the thyroid and pituitary glands should perhaps be mentioned here for the sake of completeness. In myxoedema and hypopituitarism the fasting blood sugar may be abnormally low. There is also an increased tolerance of carbohydrate, which disappears from the blood almost as rapidly as it is absorbed and is followed by little or no increase in the blood sugar. The curve obtained from a sugar tolerance test in these conditions is thus much flatter than is normally the case, and in severe myxoedema it may approximate to a straight line parallel with the base-line. Typical curves from cases of thyroid and pituitary disturbances are given in Fig. 8.

Liver Deficiency.—It is held by some writers on the subject that the conversion of glucose to glycogen does not commence immediately the blood sugar

begins to rise after carbohydrate ingestion, but that a certain "head" of glucose is necessary before the reaction can proceed, at any rate with more than minimal velocity. This is the explanation given of the fact that in the normal person the blood sugar reaches its maximum so early as three-quarters of an hour after ingestion of glucose, when absorption cannot be completed. It is supposed that at this

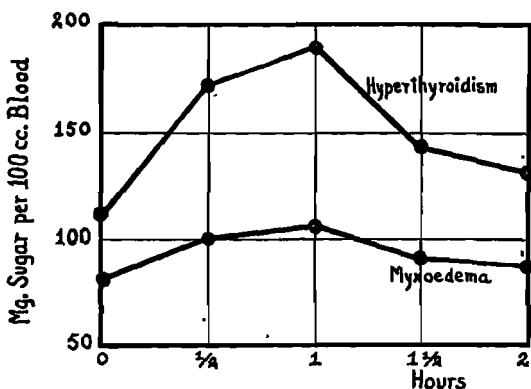


FIG. 8.—Blood-sugar curves in cases of hyper- and hypo-thyroidism. In pituitary dysfunction the curves are very similar.

time, when the blood-sugar concentration has reached about 140 mg. per 100 c.c., sufficient "head" of sugar is present to stimulate glycogen synthesis, and that this synthesis accordingly becomes the dominating factor, taking place at a much greater rate than absorption, so that the blood sugar begins to fall again. On this supposition it is easy to understand that a deficiency in the glycogen-storing mechanism, which is seated principally in the liver, may well have the effect of requiring an abnormally great "head" of glucose to be present before glycogen synthesis can proceed.

This is actually the explanation of a curious and rather unusual type of functional glycosuria. It is characterised by a normal fasting blood sugar, and then by an abnormal increase in the blood sugar after glucose ingestion. Once the maximum is reached, however, there is a rapid return to the normal fasting-level. In this type of curve, sometimes known as the "lag type," glucose synthesis does not begin until the blood sugar has risen over the renal threshold—perhaps to 200 or 210 mg. per 100 c.c. (see Fig. 4). Once initiated, however, it proceeds rapidly just as in the normal person, so that the blood sugar returns to its fasting-level within two hours. Thus, the abnormality in this type of curve does not lie in the kidney threshold for sugar, but simply in the fact that the liver seems to require an unusually high blood-sugar concentration to galvanise it into active glycogen synthesis. Thus sugar appears in the second, if not in the first, half-hourly specimen of urine obtained after glucose ingestion.

The kidney mechanism appears to resemble that of glycogen synthesis in that, though the excretion starts only when the blood-sugar content has been raised to the necessary height, it does not necessarily stop when the same point is reached during the later fall, and it may continue until the fasting-level has been reattained. Thus, contrary perhaps to what might be expected, the later samples of urine in a case of glycogen-storage deficiency may contain glucose, though the excretion may be, and often is, confined to the time when the blood-sugar concentration is high. The kind of curve to be expected in a case of this type is shown in Fig. 4. The condition is readily differentiated from one of true diabetes by the normal fasting

blood sugar, and by the rapid return to normal after the attainment of the unusually high maximum.

Renal Glycosuria.—We have seen that the excretion of sugar takes place only when the blood sugar rises above the renal threshold. If, however, in any individual case, this threshold happens to be lower than that which is found for the majority of healthy

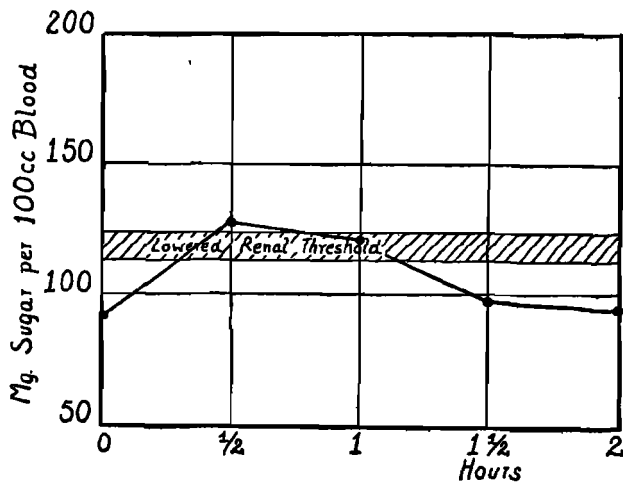


FIG. 9.—The blood-sugar curve in a case of renal glycosuria.

individuals, and which is therefore taken as the normal, there will be leakage of sugar into the urine with a much lower blood sugar than is usually required to give rise to glycosuria. In such a case the low threshold may be the sole abnormality. Utilisation of sugar is perfectly normal, both as regards direct oxidation and glycogen storage. Hence the fasting blood sugar is not raised above that found in healthy persons, and the response to glucose ingestion, as far as the blood

is concerned, is normal. The blood-sugar curve is thus of the shape seen in health, but sugar is found in the urine after glucose ingestion, and may be present in any number of the samples according to the extent to which the renal threshold is lowered.

The results of a glucose tolerance test carried out on a patient suffering from renal glycosuria are shown in Fig. 9, the renal threshold in this case standing at 120 mg. per 100 c.c. of blood. This condition of renal glycosuria is apparently harmless, and is much commoner than was supposed before the intensive investigations of the last few years. Unless the condition is accurately distinguished from true diabetes by means of the blood-sugar curve, it may give rise to much needless distress. Many patients suffering from this harmless condition are stigmatised as diabetics and condemned to unnecessary dietetic restrictions. Again, it is a common, if unjustifiable, cause for a life assurance proposal being rejected or heavily loaded.

BLOOD AND URINE TESTS DURING THE TREATMENT OF DIABETES

The effect of treatment on diabetes can be followed in two ways: (*a*) by making frequent estimations of the blood sugar; (*b*) by examining each specimen of urine passed. The former method is often impracticable, except when the patient is under observation in hospital. Even in hospital it is usually superfluous. Once the diagnosis of diabetes has been established beyond doubt by means of blood-sugar estimations, practically all the information required for treating a case may be obtained by frequent urine examinations, without undertaking further blood analyses, which

are distasteful to the patient, and which need be resorted to only in certain exceptional circumstances. The second plan, of examining each specimen of urine passed until the suitable diet, and possibly the suitable dose of insulin, for the patient has been established, has a wide application, since any intelligent patient, after he has been taught to test his urine with Fehling's solution, can make the observations by himself. This method of controlling treatment consists in examining any samples of urine passed during the day and a specimen collected after the hours of sleep. In this way it can be seen whether or not any meal contains an amount of carbohydrate with which the patient is unable to deal, and appropriate dietetic or insulin corrections can be made. In hospital, where quantitative as well as qualitative estimations of the amount of sugar passed may be performed, such corrections may be made with great accuracy. When a patient is taking insulin and his twenty-four hour specimen of urine shows a small amount of sugar, the time at which this leak of sugar occurs will be shown at once by this method of frequent urine sampling. Apart from the glycosuria being exacerbated by an infection or by mental excitement, such traces of sugar indicate that the carbohydrate in some meal is being inadequately covered by the insulin, or that the effect of the insulin has worn off too soon during the night.

Once the diet suitable for the patient has been established by this method, an occasional qualitative analysis of a twenty-four hour specimen is sufficient for practical purposes.

A possible objection to the method of controlling dietetic and insulin therapy by analysis of the urine

alone is that it yields information only that the blood sugar is below or above the renal threshold of the patient. In diabetics this threshold may be very considerably raised, so that some degree of hyperglycaemia may exist without any sugar, or with only a trace, being present in the urine.

We have repeatedly observed in old-standing diabetics fasting blood sugar values of as high as 300 mg. per 100 c.c. without glycosuria being present. It has not yet been definitely established whether hyperglycaemia moderate enough to show no glycosuria should be treated as a matter of great significance. The majority of workers believe that it should be, holding that hyperglycaemia of any severity causes further degeneration of the already diseased islet tissue in the pancreas, and that it may cause the arterial disease so commonly seen in the senile type of diabetic. On the other hand, it is possible that in diabetes of long standing the pancreas and kidneys have become acclimatised to working at their optimal efficiency with a slightly higher blood sugar level than normal, and that this hyperglycaemia may not, therefore, be entirely harmful. Certainly in many cases an attempt to keep the blood sugar of an old-standing diabetic within strictly normal limits causes hypoglycaemic symptoms to occur, and the patient feels less well than when a moderate hyperglycaemia is allowed to persist. For all practical purposes we believe that the diet and insulin should be so regulated that sugar does not appear in the urine, and that once a diagnosis of diabetes is definitely established treatment may be carried out perfectly efficiently by examination of the urine alone, except in exceptional circumstances, where a blood-sugar

estimation may be called for. One of those exceptional circumstances is the onset of coma.

Coma.—In diabetic coma the blood sugar is invariably greatly raised, acetone is present in large quantities in the breath and urine, and there is always, as we have seen in a previous chapter, some degree of acidosis. The diagnosis of the condition is rarely in doubt. The treatment consists in the administration of suitable quantities of glucose and insulin, together with large amounts of fluid to make good an invariable dehydration. The latter point is indeed almost as important as the former, and is frequently overlooked, with disastrous results. While it may be useful at the onset to determine the degree of acidosis present by an estimation of the carbon-dioxide combining power of the blood, a much more important estimation is that of the blood sugar; and it is ideal to control the case until no further danger exists by frequent readings of the level of the blood sugar. If this is not feasible, as it may not be in private practice, very frequent tests for sugar in the urine should be made. Insulin should never be administered without a covering dose of glucose, except in respect of the initial dose, when a large injection of insulin can usually be given alone without any risk of unduly lowering the blood sugar. The absence of sugar from the urine, or a rapidly diminishing blood sugar, should be regarded as a call to reduce the insulin dosage. Though the possibility of hypoglycaemia should always be kept in view, we believe that the danger of a patient passing imperceptibly from hyperglycaemic into hypoglycaemic coma is exaggerated, and is the frequent cause of inadequate insulin treatment in diabetic coma. The ideal to be aimed at is a slowly

falling blood sugar, rapid disappearance of ketone bodies from the urine, and some degree of glycosuria, until the symptoms of coma have disappeared. Only then may steps be taken to deal with the glycosuria itself.

Hypoglycaemia.—The diagnosis of hypoglycaemic coma, apart from that which may be produced during the treatment of true diabetic coma, is usually obvious from the history of the case, the absence of ketone bodies from the urine and breath, and the absence of glycosuria. In those rare emergency cases in which the diagnosis is in doubt, the examination of the sugar content of the blood or its carbon-dioxide combining power may be of service. Mild degrees of hypoglycaemia of a more chronic type may, however, exist in a patient under treatment and give rise to vague symptoms, the exact cause of which may be a matter of doubt. An estimation of the blood sugar while such symptoms are present may be exceedingly useful in differentiating such chronic hypoglycaemia from other causes of ill health. In this connection it must, however, be remembered that hypoglycaemic symptoms may occur in some old-standing case of diabetes when the blood sugar is apparently quite normal or even high. We have observed undoubted hypoglycaemic attacks in some diabetics while their blood sugar was as high as 140 mg. per 100 c.c. As has been said, these patients have become habituated to a higher blood-sugar level than normal, and so hypoglycaemia may occur at a much higher level of the blood sugar than would produce symptoms in the average case. The danger of hypoglycaemia is often exaggerated, and may become so much of a bugbear to patient and doctor that inadequate doses of insulin

are given. It must be remembered that thousands of patients die from hyperglycaemic coma, but death from hypoglycaemia is comparatively a clinical curiosity.

CHEMICAL TESTS AS A GUIDE TO PROGNOSIS IN DIABETES

Just as no clinical sign or symptom alone can furnish an indication of the severity of the disease, so no single laboratory test can by itself furnish a short cut to prognosis in diabetes. All tests fail to differentiate between severe uncomplicated diabetes, on the one hand, and slight diabetes on which are superimposed other complicating, though possibly temporary, factors on the other. It is only after keeping the patient under observation for some time, and noting his response to treatment, that a forecast of the future of the case can be given with any certainty. Some cases which, on first examination of the blood and urine, show a severely pathological picture do very well under treatment, while others, in which the picture is less marked, show a tendency for the pancreatic insufficiency to progress in spite of treatment. It is, therefore, recommended that no definite prognosis be given until the patient's response to treatment has been observed—unless, of course, the blood-sugar curves indicate quite definitely that the condition is not one of true diabetes, but one of the functional types of glycosuria. In these cases a favourable prognosis may be given at once.

In the blood, the most valuable points to investigate in regard to prognosis are, as we have seen, the fasting-level of the blood sugar and the response to the ingestion of 50 g. of glucose.

In regard to the urine, untreated cases of diabetes may show, in the twenty-four hour specimen, quantities of sugar varying in individual cases from a mere trace up to 10 per cent. An average case will show a loss of about 100 g. of sugar in the day, but as much as 300 g. may be lost in very severe cases. Again, however, it must be reiterated that such a single estimation only shows the severity of the condition at the moment, and is not necessarily an index of how the patient will respond to treatment. Large quantities of sugar may be excreted for some time owing to some temporary intercurrent infection, such as tonsillitis or a cold, and mere domestic worry or mental excitement may profoundly, though temporarily, influence the sugar excretion in diabetes.

In practice, then, the best guide to prognosis is the total amount of sugar lost in the urine over a period of twenty-four hours. The prognosis, however, should be made only after the effects of treatment in modifying the excretion have been determined, and after all external exciting factors, such as toxæmia or mental stress, have been excluded.

CHAPTER VI

ALBUMINURIA AND TESTS OF RENAL FUNCTION

IN this chapter it is proposed to consider first the general question of albuminuria, a condition which may exist quite independently of renal damage. Investigation of a case of albuminuria, however, often resolves itself into an investigation of kidney function, and therefore the chemical methods available for such an investigation have been discussed here. It is unfortunately not feasible to classify these methods in order of delicacy, since such a course would involve a great deal of repetition and possibly confusion. They have, therefore, been classified under the headings of blood analysis, urine analysis, and methods involving analysis of both blood and urine. An effort has been made, however, to indicate the relative delicacy of the tests described, and to give some idea of the ease with which they may be performed.

ALBUMINURIA

Disease of the kidney is accompanied, *inter alia*, by the passage of substances from the blood to the urine which the healthy kidney does not usually permit to pass. These substances are the proteins of the blood plasma, comprising albumins and globulins, of which the former are quantitatively, in nephritis, the most

important. A second factor in the production of protein in the urine in kidney disease may be found in the cells of the renal tubules themselves, which may proliferate and desquamate, or may swell and burst, thereby discharging some of their albuminous contents into the tubules. It is not difficult in these circumstances to understand the albuminuria which accompanies nephritis, where damage to the glomeruli and damage to the cells of the renal tubules may occur.

Just as, however, the discovery of sugar in the urine may be a matter of little importance or one of grave significance, so may the discovery of albumin in the urine be evidence of some trivial functional abnormality or of serious renal disease. Again, its absence from the urine, although it may exclude the possibility of certain types of kidney damage being present, is in itself no criterion of renal efficiency. Thus, many cases of grave, though chronic, kidney disease show no albumin in the urine, or only occasional traces, while numbers of people with perfectly healthy kidneys may occasionally show a considerable degree of albuminuria. The amount of albumin in the urine is therefore no criterion of the degree of renal damage. There is no doubt that in the past too much stress has been laid on the degree of albuminuria in coming to a conclusion as to a patient's renal condition, and we now know that the mere demonstration of albumin in the urine, apart from definite clinical signs and symptoms of kidney disease, is simply an indication that the case should be further investigated, and is not, taken by itself, of any diagnostic significance.

Protein in the urine frequently has its origin in

extra-renal sources. Thus blood, pus, or spermatozoa from the lower urinary passages, or albuminous discharges from the vagina or uterus, may give rise to it in moderate quantities. In this way a cystitis, a urethritis, or a cervicitis may occasion a proteinuria although the kidneys may be perfectly healthy. It is, therefore, essential to exclude such extra-renal causes before blaming the kidney for the condition. If it is suspected that the protein may be due to vaginal or uterine discharges a catheter specimen should be examined; or if a urethritis is thought to be the cause of it, in the male, he should be asked to pass his water into two glasses, and the contents of the second vessel should be examined for albumin, the urine in the first glass having washed out any albuminous secretion which may have been present in the urethra previously. This latter proceeding is safer in male urethritis than the passage of a catheter, which may cause an infection localised in the anterior urethra to be passed back into the posterior urethra and bladder. In all such cases the centrifuge should be used, and only the clear supernatant fluid tested for albumin. The microscopical examination of the centrifuged deposit itself may, of course, be of the greatest value in coming to a diagnosis. Indeed, the presence of pus, except in very large amount, can be demonstrated with certainty only by the microscope, since no reliance can be placed upon the liquor potassii reaction, and the guaiac test is not extremely delicate and is reliable only when the presence of blood can be excluded. Incidentally it may be remarked that a few leucocytes are always present in the centrifuged deposit from healthy urine, and it is only the presence of large numbers of pus cells in the deposit which is

of importance. This matter is further discussed in Appendix I, dealing with the microscopical examination of the urinary sediment.

Even when the source of the albumin is traceable to the kidney, it is not necessarily evidence of renal disease. For example, albuminuria is present in most young people after severe exercise, so that such albuminuria may really be called physiological. Again, protein may be present in the urine of many healthy individuals after a cold bath—possibly due to the driving of blood temporarily from the peripheral to the renal area, thereby causing congestion in the kidney. It may also occur after the use of certain drugs, such as arsenic, mercury, and other heavy metals, or irritants like cantharides, but in these cases the albuminuria cannot be looked upon as harmless, as such substances may cause renal damage.

A functional albuminuria is common in young men, between the ages of puberty and twenty-five, and frequently gives rise to trouble in securing life insurance. The subjects of such albuminuria are often weedy and neurotic youths, with clammy hands and a very variable blood-pressure. They are not uncommonly subject to fainting attacks when forced to stand in an upright posture for any length of time, as on parade in the army, or in early-morning chapel at school; and whereas, in normal individuals, a change from a recumbent to an upright posture is accompanied by a rise in blood-pressure of not more than 10 mm. of mercury, in such albuminurics the change is usually much more considerable, and may even be as much as 30 mm. A vasomotor centre, easily exhausted when the upright posture is maintained for some time, is probably the cause of this

albuminuria, so that cerebral anaemia may occur, causing fainting, and congestion in the kidney, which in turn causes albuminuria. This explanation is further suggested by the fact that albumin occurs in the urine of such individuals only when they are going about during the day, and is absent from the urine passed on rising from a recumbent posture the first thing in the morning. This absence of albumin from the early-morning specimen, the absence of casts other than hyaline, and the clinical examination of the patient with special reference to the blood-pressure, may be sufficient to establish a diagnosis of orthostatic albuminuria. There is no evidence to suggest that the subjects of orthostatic albuminuria are more apt to develop kidney disease in later life than other people, and the condition probably requires no specific treatment, except in so far as the general health of the individual may call for it. This albuminuria seldom persists into later life.

Albuminuria is present in uncompensated cardiac diseases, owing to a congestive state of the kidney produced by backward pressure. As the cardiac condition is improved, so the albumin usually clears from the urine. Owing to the fact, however, that true renal disease often accompanies cardiac lesions, it may be difficult during the stage of cardiac decompensation to assess how much of the urinary signs and symptoms are to be ascribed to the heart and how much to the kidney.

Some degree of albuminuria is frequently present in fevers and in the anaemias, owing, presumably, to cloudy swelling. Its presence alone cannot be taken as evidence of renal disease as such, unless further tests of the renal function are carried out,

and these are often most unsatisfactory in such conditions.

Although the orthostatic albuminuria of adolescence and early adult life, which has been described, is a well-known condition, it is not so well realised how common albuminuria is in older adults. This albuminuria may be due to a leaky kidney which has been slightly damaged by a previous disease but is not the seat of a progressive lesion. It is not of the orthostatic type—albumin being present in the early-morning specimen of urine—and the gravity or otherwise of its significance can be gauged only by further tests. There seems to be no doubt that a few adults show albumin in the urine persistently without any apparent upset of the renal function, and without developing any renal deficiency in the course of time. On the other hand, it must be remembered that progressive renal disease may be present with few or no clinical symptoms or signs except albuminuria, and it is therefore essential that in any adult case showing albuminuria the urinary deposit should be thoroughly examined and renal function tests should be performed before a favourable prognosis is given.

It is claimed that some help in differentiating the various types of albuminuria may be obtained from a determination of the relative amounts of albumin and globulin in the urine, though we ourselves are of opinion that no reliance can be placed on this test alone. In functional or orthostatic albuminuria the ratio of albumin to globulin is about 2 : 1 ; in albuminuria due to a leaky kidney it is about 1 : 2 ; but with actual renal damage it may be as much as 6 : 1.

The albuminurias of pregnancy, which present special

problems of their own, will be discussed in another place (Chapter XI).

To sum up, then, unless albuminuria is accompanied by definite symptoms of nephritis, it cannot by itself be taken as being of much significance, and further investigation of one kind or another is necessary.

DEFINITION OF TERMS USED

Before discussing the various tests of renal function, it is desirable to consider briefly the terms used to describe the different types of kidney disease. By the term **acute Bright's disease** we recognise that renal condition often encountered after scarlet fever or tonsillitis—particularly in young people. When the condition is severe, slight fever and general malaise occurs; a small amount of smoky blood-stained urine is passed and mild oedema of the nephritic type is present; the blood-pressure is temporarily raised and the heart enlarged. It is probable that only the severe types of the disease are commonly recognised. In the mild types a small shower of red blood cells occurs in the urine and there are no striking concomitant clinical features, so that the condition may escape medical attention altogether.

A few of the very severe cases may die in **acute uraemia**. This is, however, uncommon during the first attack, and, when death from acute uraemia occurs, it usually means that an acute lesion has been superimposed upon an already severely damaged kidney.

Most of the cases of acute Bright's disease recover entirely with complete restoration of kidney function and without any progressive renal deficiency resulting

in the future. In some cases, however, the acute symptoms subside, but small quantities of albumin and a few casts and red blood cells continue to be excreted in the urine. Such cases usually pass out of medical care, to return in a greater or smaller number of years suffering from advanced **chronic azotaemic Bright's disease**, called by some "chronic interstitial nephritis." Such cases are characterised by a raised blood-pressure, a hypertrophied heart and retinitis, and suffer from a great variety of clinical symptoms, among which polyuria is constantly noted.

A few cases of acute Bright's disease, instead of getting better or instead of developing chronic azotaemic Bright's disease, develop an hydraemic state in which the slight oedema which characterised the acute disease becomes exaggerated into a generalised anasarca; the red blood cells also, to a great extent, disappear from the urine, which, however, becomes very heavily laden with albumin and remains scanty. The increased blood-pressure falls and the heart becomes normal in size and any tendency to azotaemia which existed during the acute process subsides. Such a condition, characterised by gross oedema and heavy albuminuria, is termed the **nephrotic syndrome**, sometimes named "sub-acute parenchymatous nephritis." Again we do not suggest that all cases showing this nephrotic syndrome must necessarily have suffered from a previous attack of acute Bright's disease. It is possible that some cases develop an insidious degenerative lesion from the start without any previous acute renal inflammation.

A few cases exhibiting the nephrotic syndrome entirely recover in the course of time, with complete restoration of kidney function, or with it only very

slightly impaired. Others die from exhaustion or from an intercurrent infection to which their oedematous tissues are very susceptible. Should, however, the patient neither die nor completely recover, a tendency to azotaemia gradually becomes superimposed upon the hydraemic state. The oedema and albuminuria persist, though perhaps in a less marked form; but, in addition, the blood-pressure tends to rise, the heart to become hypertrophied and nitrogen retention to occur. These cases, thus, suffer from a mixed type of Bright's disease which we recognise by the name of "**secondary contracted kidney.**" Such cases may die eventually from **chronic uraemia** if this is not preceded by a cardio-vascular accident.

Chronic uraemia in these cases has, however, been reached by a road characterised by an obvious clinical picture, such as the nephrotic syndrome, instead of being reached by the path of chronic azotaemic Bright's disease, which is, particularly in its early stages, almost featureless from the purely clinical point of view. Biochemical tests of kidney function are thus more useful diagnostically and prognostically in azotaemic than in hydraemic Bright's disease.

One other kidney condition remains to be described, which we shall term the "**arteriosclerotic kidney.**" Here, any deficiency of renal function which occurs is the result of a primary vascular disease, whereas in Bright's disease any vascular changes which occur are secondary to the kidney condition. It is not very easy during life to separate the patient suffering from an advanced stage of arteriosclerotic kidney from one in an advanced stage of chronic azotaemic Bright's disease, since they present many clinical and biochemical features in common. In patients suffering

from the arteriosclerotic type of kidney, however, death is much more commonly due to heart failure or cerebral haemorrhage than to chronic uraemia.

When an individual is found to be suffering from a very high blood-pressure, an hypertrophied heart, albuminuric retinitis, to have a trace of albumin and some casts in the urine, and yet to have only a very

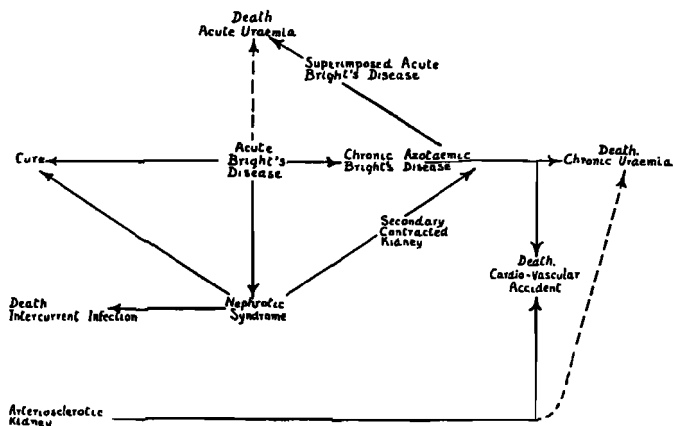


FIG. 10.—To illustrate the terms used in the discussion of kidney function tests.

slightly impaired renal function, we may safely assume that he is suffering from a primary vascular disease rather than from chronic azotaemic Bright's disease.

Reference to Fig. 10 will elucidate the terms used.

TESTS OF RENAL FUNCTION

Tests based on the Composition of the Blood

From the point of view of kidney function one of the most important groups of substances in the blood is that heterogeneous mixture of relatively simple

nitrogenous compounds which is collectively referred to under the title of the *non-protein nitrogen* of the blood. In this group are included the amino-acids, which the blood carries in pursuance of its function as provider of foodstuffs. The blood is also, however, the common sewer for the tissues, and as such it carries the end-products of protein metabolism, urea, uric acid, creatine, and creatinine. Of the total non-protein nitrogen normally present in the blood during fasting, some 25 to 35 mg. per 100 c.c. of blood, the nitrogen of the amino-acids accounts for 5 to 8, that of the urea for 12 to 18, that of the uric acid for about 1, that of creatinine for only 0.3 to 0.6, and that of creatine for 1 to 2. The remaining third or quarter of the total non-protein nitrogen of the blood is derived from a number of substances, some present only in very small amounts, and some—possibly many—as yet unidentified.

After a meal containing protein the amount of circulating amino-acid is somewhat increased for a while, as, indeed, is to be expected. Only a part of the ingested amino-acid, however, is used for the repair of tissue wastage, etc., and the remainder is at once oxidised—chiefly in the liver—to urea, carbon dioxide, and water, while any nucleoprotein yields uric acid in addition. Thus a meal is followed by an increase in the non-protein nitrogen of the blood owing to the increased amounts of these particular constituents. Creatine and creatinine do not participate in this increase to any appreciable extent, since they are derived almost entirely from the endogenous metabolism. The practical importance of these facts is, of course, that they necessitate the use of blood drawn during a fast for the investigation of the amount and

distribution of the non-protein nitrogen of the blood. In the absence of any knowledge of the time required, with a possibly damaged kidney, for the non-protein nitrogen to return to the fasting-level, it is usual, as a measure of safety, to obtain blood samples before breakfast, after a fast of twelve hours.

From the clinical point of view, and especially with regard to kidney function, it is those substances formed as the end-products of protein metabolism—substances which it is the province of the kidney to remove from the body—that are important. The amino-acids, during fasting, are remarkably constant in amount, and though they are said to be increased in such allergic conditions as asthma and urticaria, especially during the active phase, this fact is of little diagnostic value, and there is no evidence of any significant alteration associated with renal inefficiency. The end-products of protein metabolism, however, are in very different case. It is particularly in these constituents of the non-protein nitrogen that alteration is to be expected with deficient kidney function. Hence their separate estimation, or at least the estimation of the most important of them, is desirable in preference to the estimation simply of the total, composite, non-protein nitrogen. When only one estimation is to be made—and often that is all that is necessary—urea should be chosen : it is quantitatively the most important ; it is actually one of the substances which the kidney is called upon to excrete ; it shows, in disease, the widest range of variation ; and it is easy to estimate with a sufficient degree of accuracy. There are, of course, occasions on which it is desirable to know the concentration in the blood of other constituents than urea, and sometimes it is

desirable to know the value of the total non-protein nitrogen as well, but often either alone will suffice, and it is then that we recommend the choice of urea.

The blood urea is not increased in early chronic azotaemic Bright's disease, but it increases later as the kidney damage progresses, and with moderately advanced lesions figures of 25 to 40 mg. of urea nitrogen per 100 c.c. of blood are common. (Following a usual practice we give the figures for urea *nitrogen*,

which, multiplied by two, or, more accurately, by $\frac{60}{28}$,

give the actual amounts of urea itself. Analyses of uric acid and creatinine, on the other hand, are here, as usual, given in terms of the substance itself. There seems no real reason for this difference, but we have thought it advisable not to change to a more 'logical' mode of expression lest confusion arise in any comparison of the figures here and elsewhere.) In the later stages of severe azotaemia, and in some cases of acute Bright's disease, the retention of urea is very marked, amounts such as 60 to 100 mg. of urea nitrogen per 100 c.c. of blood being common, while even more—up to 250 or 300 mg. per cent.—may be found in uraemia. The actual daily output of urea, at least in the less severe cases, is not necessarily decreased very much, and may even be quite normal, but the damaged kidney cannot excrete urea properly at the normal blood-level; it requires a greater "head" of urea, which therefore accumulates in the blood until the requisite concentration is reached. It is thus not a question of any active retention of urea on the part of the kidney, but a mere passive inability to pass out the proper amount of urea until sufficient

has accumulated in the blood from protein breakdown to produce the necessary "head." As the kidney damage becomes progressively greater, so does the requisite "head" of urea, and so the concentration of urea in the blood increases. High concentrations of urea in the blood are thus to be expected in a variety of other conditions associated with impaired renal function — poisoning with heavy metals, such as mercury, lead, etc., arsenic poisoning, double polycystic kidney, prostatic obstruction, cardiac failure, and so on.

In the cases of poisoning accompanied by a high blood urea there is actual kidney damage, but with other conditions this is not necessarily the case. A retention of urine in the bladder, such as occurs in prostatic obstruction, may eventually lead to such a dilatation of the ureters, with consequent backward pressure on the kidneys, as to prevent proper excretion of urea until the "head" has been suitably increased. In such cases there may be no actual kidney damage, though some will undoubtedly be produced if the condition is allowed to persist. When the kidney is healthy, relief of the backward pressure by suitable means rapidly brings about a lowering of the blood urea to normal. Hence determination of the blood urea affords a valuable guide to surgical risk in cases of prostatic obstruction. With a normal value the prognosis may be regarded as good and a single-stage operation may be attempted. With a high blood urea only a simple suprapubic cystostomy should be performed. If after this procedure the blood urea returns to normal the radical prostatectomy may be carried out; if it does not, however, the outlook is bad.

The blood urea may be increased not only by a partial failure to excrete by a kidney damaged or subjected to backward pressure, but by an overloading of an actually healthy kidney owing to an over-production of urea in metabolic processes, or to a loss of water with consequent concentration of the blood. It is possible that these factors play a part in the moderate raising of the blood urea found in certain fevers. Dehydration is almost certainly concerned in the high blood urea seen in the terminal stages of high intestinal obstruction, where there has been excessive loss of fluid by vomiting, with consequent concentration of the blood. The kidneys, however, may still be working efficiently, as evidenced by a correspondingly high urea concentration in the urine. In this condition, too, there has been great loss of chloride in the vomitus, and the retention of urea may conceivably be in part a defensive mechanism—an attempt to maintain the osmotic pressure in spite of the loss of salts. The same mechanism is probably operative in producing the high blood urea observed in severe sweating, diabetic coma, and Addisonian crises. Conversely, a high chloride content of the blood is often accompanied, as in the nephrotic syndrome and eclampsia, by a low blood urea, and it may be suggested, without stressing the point, that this again may be due to an attempt to maintain the osmotic pressure at the optimal level in face of a deficiency or excess of one constituent.

Hence, *per se*, a high blood urea—or non-protein nitrogen—cannot be taken as evidence of renal damage. It is simply one more fact which, added to the clinical and other data, may aid in making a correct diagnosis. If the various other causes of a high blood

urea can be excluded, however, then such a discovery is of great diagnostic value as indicating the existence of gross kidney insufficiency. In the absence of acute Bright's disease such insufficiency must be due to sclerosis of the renal elements, the result either of renal or vascular disease.

Where it occurs in conjunction with the nephrotic syndrome, it is a sign that the degenerative lesion has resulted in destruction and sclerosis of the renal tissue, and that secondary contraction is taking place. If the blood urea is normal, though it by no means implies a properly functioning or undamaged kidney, it is at least evidence that renal sclerosis has not yet advanced so far as to render the organ hopelessly inadequate. In this connection it will be seen from Fig. 11 that practically all cases dying of chronic uraemia showed their blood urea nitrogen concentrations to be raised above normal within a year of death. A normal reading of the blood urea is, therefore, inconsistent with a very rapidly fatal issue from chronic azotaemic Bright's disease so long as fatal extraneous events do not occur, such as cardio-vascular catastrophes, intercurrent infections, or a superimposed acute kidney lesion.

It will also be seen from Fig. 11 that very high readings of the blood urea in chronic Bright's disease may furnish a rough guide to the immediate gravity of the prognosis. Thus no case with a blood urea nitrogen concentration of over 100 mgm. per cent. lived for more than five months, and no case with a reading of over 50 mgm. per cent. for more than fourteen months. The extent to which the blood urea is raised above normal, however, though invariably implying severe damage, is by no means always pro-

portional to the exact amount of the damage, and, unless the reading is very high, affords little accurate information as to the imminence of death from chronic uraemia. Thus it will be seen from Fig. 11 that moderately raised blood urea nitrogen readings, up

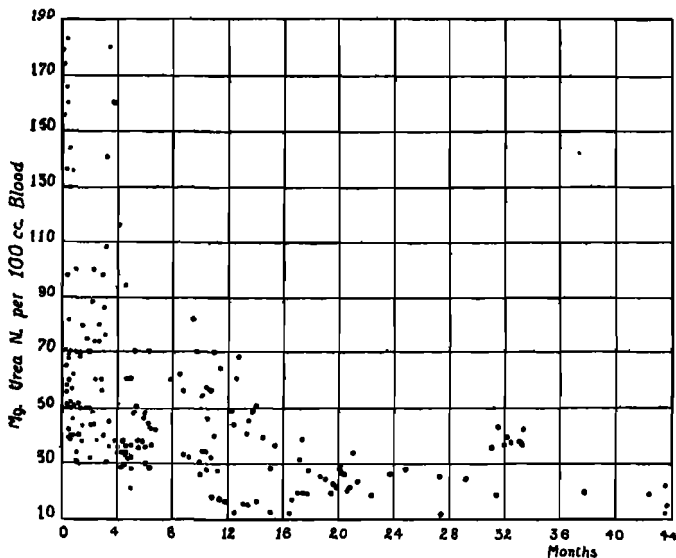


FIG. 11.—The relation between the height of the blood urea and the time of survival. Each point shows the blood urea and the time the patient survived after the observation was made.

to a concentration of 50 mgm. per cent., may be consistent either with survival for some years or almost immediate death from chronic uraemia.

An estimation of the blood creatinine may assist in making a prognosis as to the imminence of death in very severe cases. This substance the kidney finds easier to concentrate from the blood into the urine

than urea, and it is therefore only in very extensive kidney damage that the concentration of the blood creatinine is significantly raised. The estimation of this substance is, however, a somewhat inaccurate chemical procedure, and though the normal concentration is usually given as varying from 1 to 2.5 mgm. per cent., it is only when it rises to over 3.5 mgm. per

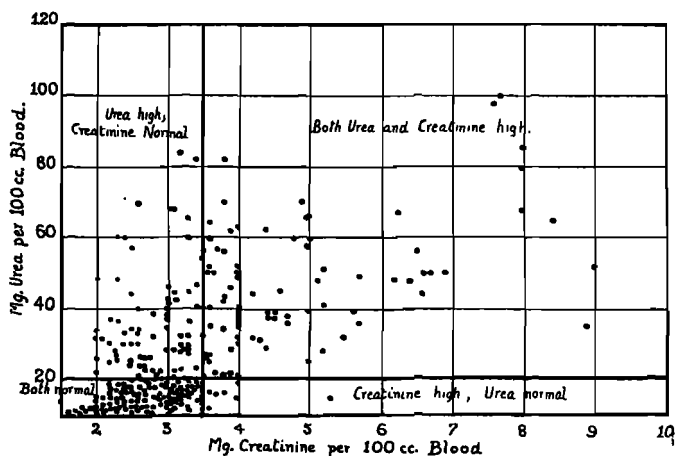


FIG. 12.—The relation between the blood urea and the creatinine. A high creatinine with a normal urea is very unusual.

cent. that much significance should be attached to the result, since perfectly healthy people give, on occasion, readings up to that figure. Fig. 12 represents some hundreds of simultaneous readings of the blood urea nitrogen and creatinine concentrations in cases of chronic azotaemic Bright's disease. It will be seen that whereas the blood urea nitrogen is almost invariably greatly increased when the creatinine concentration is above

3.5 mgm. per cent., yet the creatinine concentration is frequently normal when the blood urea nitrogen concentration is significantly raised. It is apparent, therefore, that just as other tests to be described later, such as urea concentration and urea range tests, are more delicate indications of impaired kidney function than the simple estimation of the blood urea, so the blood urea figure is a more delicate index than that of the blood creatinine. In very severe cases, however, the latter may be of service in estimating the immediate gravity of the prognosis. Fig. 13 represents the creatinine concentration at varying periods before death in a considerable number of cases of fatal chronic azotaemic Bright's disease. It will be seen that all cases with readings above 7 mgm. per cent. died within a month. Similarly, all cases with concentrations above 5 mgm. per cent. died within six months. Once, therefore, the concentration of this substance in the blood is significantly raised in chronic Bright's disease, a fatal issue must be looked for within a very short time, and this is true in spite of any lowering of the blood urea which may be brought about by treatment, such as the administration of a low-protein diet or of alkalies. Diet has little effect on the blood creatinine, which is produced almost entirely endogenously from the breakdown of the body tissues, and once its concentration has been significantly raised owing to extensive renal insufficiency, no lightening of the exogenous load can bring it back to normal. Fig. 13 also shows, however, that, unlike the blood urea, a normal creatinine concentration is not inconsistent with immediate death from chronic uraemia.

Estimations of the blood uric acid, though occasionally useful in other conditions, such as gout or

leukaemia, are of little service in determining the prognosis, or in following the course of treatment, in Bright's disease. Uric acid, formed by oxidation of the purines derived from the breakdown of nucleoprotein, is the most difficult of the main end-products of protein metabolism to excrete. This is shown by the ratio of its concentration in the urine to its con-

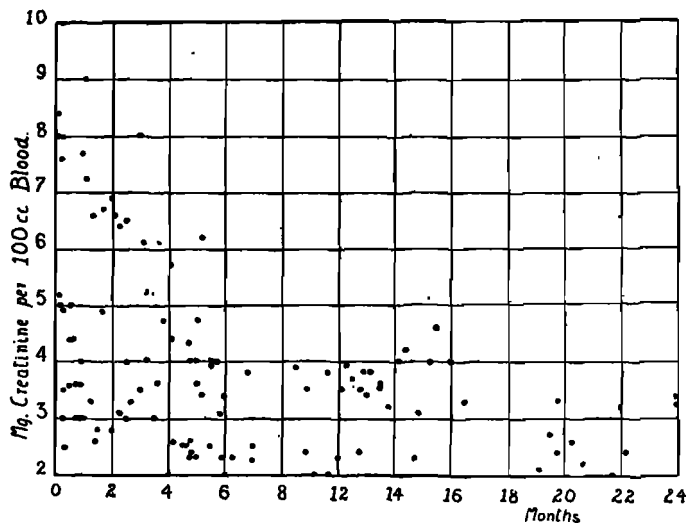


FIG. 13.—The relation between the blood creatinine and the time of survival after the observation was made.

centration in the blood, a ratio which is normally about 20, as against 60–80 for urea and 100 for creatinine. Hence, so far as the blood is concerned, failure of the kidney to eliminate nitrogen normally is shown first in an increase in the uric-acid content, and a fairly early case of chronic azotaemic Bright's disease may show this without any increase in the blood urea. It must be remembered, however, that a

considerable amount of kidney damage has taken place before even the blood uric acid shows any increase above the normal value, and the mere fact that the blood chemistry shows no abnormality is no criterion of renal efficiency. More delicate tests are necessary to demonstrate all but gross lesions.

Those types of kidney damage which are characterised by an accumulation of nitrogenous waste products in the blood show also an increase in the inorganic phosphate of the blood, and it has been suggested that estimation of this substance affords a more delicate test of renal function than does estimation of the uric acid, urea, and creatinine. In our experience this is not the case, but the inorganic phosphate has about the same significance as the blood creatinine, provided that any increase found can be ascribed to kidney damage. This is shown in Table VI. It is interesting to note that a retention of phosphate, sometimes associated with a high blood non-protein nitrogen, probably plays a part in the somewhat rare and curious condition known as renal rickets (renal dwarfism).

The nephrotic syndrome is characterised by oedema which may be very marked indeed. When azotaemia is superimposed, as in secondary contracted kidney, this oedema may become progressively less marked. The cause of renal oedema is a matter of considerable debate, and numerous hypotheses have been advanced to explain the phenomenon. It may be the inability of the kidney to excrete water, with, as a secondary effect, the retention by the body of sufficient chloride to keep the concentration at the normal 0·6 per cent. in spite of the increased water-content of the tissues; it may be the exact opposite of this, a difficulty in

TABLE VI

NON-PROTEIN NITROGEN, URIC ACID, UREA NITROGEN, CREATININE,
AND INORGANIC PHOSPHORUS OF THE BLOOD IN CHRONIC
AZOTAEMIC BRIGHT'S DISEASE

Case	mg. per 100 c.c.				
	N.P.N.	Uric Acid	Urea N.	Creatinine	Inorganic Phosphorus
Normal (Also in Early Chronic Azotaemic Bright's Disease.)	25-35	2-3.5	12-18	1-3	3-4
Moderately Advanced					
(1)	37	9.1	20	2.4	3.7
(2)	41	7.3	24	2.0	4.2
(3)	45	6.9	29	2.7	3.9
Severe					
(4)	69	8.3	44	3.7	6.4
(5)	65	7.6	36	3.2	5.4
(6)	91	9.5		4.0	8.3
Very Severe. (Died shortly afterwards.)					
(7)	245	15.0	195	8.6	13.6
(8)	152	8.6	90	7.5	9.8
(9)	161	12.3	110	9.1	12.2

excreting chloride, and hence a retention of water to dilute the chloride content to the normal level; it may be bound up with the loss of albumin in the urine, a loss which is characteristically large in the condition; or it may be due to a generalised alteration of capillary permeability. We believe there is not sufficient evidence to allow any of these hypotheses to be definitely accepted, though some at least can be excluded. As in many biological processes, however, more than one causative mechanism may be at work.

Whatever the cause of the oedema, there is no doubt that in acute Bright's disease it is accompanied by a reduction in the concentration and total output of chloride in the urine. It might be thought that this would be reflected in an increase in the blood chloride. This, however, is not the case, since the simultaneous retention of water keeps the tissue chloride and the blood chloride at a level so near the normal as to be indistinguishable from it.

The oedema itself affords a sufficient criterion of the severity of the condition, but if a chemical means of assessment is desired the estimation of the blood cholesterol is probably one of the most satisfactory, since it seems to be raised in proportion to the severity of the disease. The clinical data leave no doubt as to the diagnosis of the nephrotic syndrome, so that the fact that the blood cholesterol is raised in many other conditions need cause no confusion. Typical results from the estimation of the blood cholesterol in Bright's disease are given in Table VII.

TABLE VII

THE BLOOD CHOLESTEROL IN BRIGHT'S DISEASE

	<i>Cholesterol mg. per 100 c.c. blood</i>
Normal	140-180
Acute Bright's Disease	150-500
Chronic Azotaemic Bright's Disease	100-450
Nephrotic Syndrome	250-1000

The other striking feature of the blood chemistry in the nephrotic syndrome is to be found in a fall in the plasma protein concentration. The plasma proteins consist of albumin, globulin, and fibrinogen, and together they normally total from 6.8 to 8.8 gm. per 100 c.c. of plasma. The normal plasma albumin

concentration is 4·6 to 6·7 gm. per cent. and of globulin 2·0 to 2·5 gm. per cent. The fibrinogen is, of course, removed from the blood in clotting, and since fibrinogen is included in the globulin fraction of the plasma protein, the serum globulin is about 0·2 per cent. lower than that of the plasma globulin. In the nephrotic syndrome the total plasma protein concentration usually falls below 5 gm. per cent., and in very severe cases may even fall below 3·5 gm. per cent. The diminution in the total plasma proteins is at the expense of the albumin rather than the globulin fraction. Hence in severe cases of the nephrotic syndrome the concentration of the plasma albumin may actually be the same as, or even less than, the plasma globulin—the so-called inversion of the albumin-globulin ratio.

When such cases develop secondary contraction a diminished plasma protein concentration continues, though it may be less striking, and a moderate fall in the plasma proteins also occurs in acute Bright's disease. Such a fall is not seen, however, either in chronic azotaemic Bright's disease nor in those cases of renal insufficiency due to primary vascular disease.

Changes in plasma protein concentration are by no means pathognomonic of hydraemic Bright's disease, since they may occur in many other conditions. A decreased concentration is observed in famine oedema, in pernicious anaemia, in severe haemorrhage, and in congestive heart failure; whereas an increased concentration occurs, as might be expected, in all conditions giving rise to dehydration, such as severe sweating, vomiting, and diarrhoea. An increased concentration also occurs in cases of myeloma, and most strikingly in kala-azar. In the two latter con-

ditions the increase in the total protein is due mainly to an increase in the globulin and fibrinogen fractions.

It is significant that the plasma proteins are decreased in conditions which give rise to oedema, and increased in those associated with dehydration. In the nephrotic syndrome, for instance, oedema invariably occurs when the total protein concentration falls below 4.5 gm. per cent., and is never present when the concentration is over 5.5 gm. per cent. If the serum albumin level is taken alone, it is found that, with few exceptions, nephrotic oedema does not occur till this has fallen below 2.5 gm. per cent. Again, nephrotic oedema rarely occurs unless an average of at least 6 gm. of albumen is being lost in the urine in the course of twenty-four hours. These observations have suggested that nephrotic oedema is due to the fact that damage to the glomerulus allows the albumen molecules, normally retained in the blood, to fall through into the urine, but the globulin molecules, being larger, are retained in the bloodstream. There is no doubt that the urine albumin in Bright's disease is identical with the plasma albumin. It is therefore suggested that this long-continued loss of albumin leads eventually to a depletion of the plasma protein concentration, and a tendency to inversion of the albumen-globulin ratio. In consequence of this fall in protein concentration the osmotic pressure due to these proteins (oncotic pressure) falls, and oedema results.

This attractive and simple theory is difficult to accept for many reasons. A significant fall in plasma protein concentration, for instance, may occur during the first few days of acute Bright's disease, long before a loss of albumin in the urine has occurred sufficient

to cause a significant depletion in the plasma albumin. Again, when recovery occurs in the nephrotic syndrome, the oedema disappears first, next the plasma protein concentration rises, and, lastly, the albuminuria subsides. This is manifestly just the reverse order to what would occur if the above explanation of nephrotic oedema were the true one. Lastly, in individual cases, observed for a sufficient time under controlled conditions, no accurate correlation can be observed between fluctuations in the intensity of the albuminuria, the height of the serum protein concentration, or the extent of the oedema.

Just as an increased concentration of plasma proteins is present in dehydration due to a concentration of the blood, so it might be thought that a diminished concentration is found in hydraemic conditions simply on account of hydraemic plethora. Such an explanation, however, cannot be the true one, since all careful estimations of blood volume in patients suffering from the nephrotic syndrome have gone to show that the blood volume is certainly not increased and may indeed be diminished.

Lastly, it has been suggested that the diminished plasma protein concentration seen in hydraemic Bright's disease is due to a loss of albumin in the oedema fluids. This theory is in its turn disproved by the discovery that the oedema fluids in question contain only a trace of protein, quite insufficient to account for the phenomenon under discussion.

We ourselves incline to the view that the low plasma protein concentration observed in hydraemic Bright's disease is due to some deficiency in the synthesis of plasma albumin, rather than to the actual loss of this substance from the body, though this latter may be a

subsidiary cause. If a simple loss of albumin were in itself a sufficient explanation of the phenomenon, such a loss could readily be made good—as it is in famine oedema—by feeding a high protein diet. Though this measure is advocated in the nephrotic syndrome, and may indeed be beneficial in certain respects, yet it usually has little effect in raising the depleted concentration of plasma proteins to normal. Where and how the synthesis of plasma proteins occurs is, however, still largely a matter for conjecture.

Estimation of the blood urea is of some value in indicating whether secondary contraction is occurring. In the pure nephrotic syndrome there is no difficulty whatever in excreting the end-products of protein metabolism, so that all renal function tests based on the retention or excretion of nitrogen yield normal results. Secondary contracted kidney, however, is accompanied by a gradually progressing azotaemia, so that a heightened blood urea would indicate that this stage had been reached.

Tests depending on the Composition of the Urine

The function of the kidney is to secrete a solution containing those water-soluble substances which at the time of secretion are superfluous. These substances include the end-products of protein and of nucleic acid metabolism, the excess of acids, bases of salts ingested with the food, and so on. The secretion of such a solution naturally implies the loss to the body of water which may originate largely in the ingested water, but partly in the oxidation of foods in the body. It is obvious that though the end-products of metabolism are always superfluous, this is not true

of other substances. Chloride, for example, or even water itself, may at one time be present in excessive amounts and may, therefore, be superfluous, but at another time may be a substance which it is desirable or even necessary to retain. The properly functioning kidney, in fact, is charged with the double office of removing waste or superfluous substances from the body, and at the same time of maintaining a fairly constant composition of the body fluids. Hence one outstanding characteristic of the healthy kidney is its power of adaptability, of varying the volume and composition of the urine to accord with the needs of the moment. Whether this adaptability is achieved by varying the number of secretory units in action or by varying the amount of work done by each unit is really immaterial to our present discussion; in either case the position can be expressed by saying that under average conditions the kidney possesses a large reserve of power for emergency purposes. This appears to be true, and, indeed, it is only under very exceptional circumstances that the excretory capacity of the healthy kidney is taxed to the utmost. Yet it does not express adequately the whole state of affairs, for the fact is that the normal kidney not only can, but does, vary both the amount and composition of its secretion.

Even the grossly damaged kidney can contrive to remove all or nearly all of the waste substances from the body during the course of twenty-four hours, although to do so it may be forced to permit certain abnormalities in the composition of the body fluids. It does so, however, in a manner very different from that of the healthy organ. The power of adaptability has largely been lost, through functional, or anatomical

destruction of many of the secretory units, and it is now working at or near its maximum capacity all the time. So marked is the loss of adaptability associated with very severe renal damage that one might expect a similar though less marked loss to be evident in cases where the actual kidney damage is relatively slight. In considering whether or not this expectation is realised in actual fact, much depends on the value to be placed on the term "slight." It is almost certainly true that kidney damage may occur without any detectable limitation in the power of adaptability; it is equally true, however, that a considerable failure in adaptability has occurred before any evidence of renal insufficiency can be detected by chemical examination of the blood.

From these considerations it follows that little useful information as to kidney function can be derived from chemical examination of a single sample of urine. Examination of a twenty-four hour specimen may be more useful, though not much. It is true that, on the average, the total output over the twenty-four hours tends to be greater in chronic azotaemic Bright's disease, and less in the nephrotic syndrome than the normal average of 1200-1400 c.c.; it is equally true that the greater volume is associated, as a rule, with a lowered specific gravity, and *vice versa*. The range of variation in individual cases, however, is so great that these averages are of little or no diagnostic value.

Tests of very considerable value have been based on the loss of adaptability associated with deficient kidney function. The simplest of all consists simply in collecting all samples of urine passed over a period of 24 or 48 hours, noting the time of each, and measuring the volume and specific gravity. The kind of

result to be expected from a normal healthy person is shown in Fig. 14 (A), and the loss of adaptability found in a case with damaged kidneys in Fig. 14 (B).

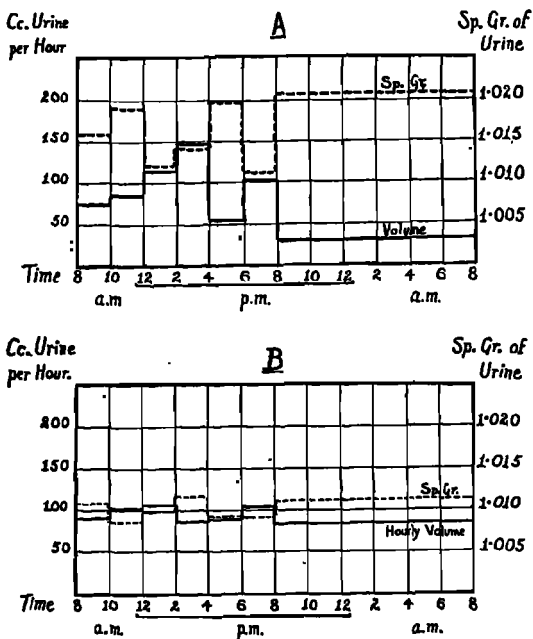


FIG. 14.—The specific gravity and hourly volume of urine (A) in a normal healthy person, and (B) in a case of early chronic interstitial nephritis. The night urine is collected in one sample from 8 P.M. to 8 A.M.

A point particularly to be noted is the failure of the damaged kidney to produce a scanty concentrated urine during the night; instead it continues secreting at a steady rate as if to overtake arrears of work. If results obtained by this simple procedure are plotted logarithmically, the loss of adaptability which

appears as a change in the slope of the curve and by a clumping of the points is even more striking (Fig. 15),

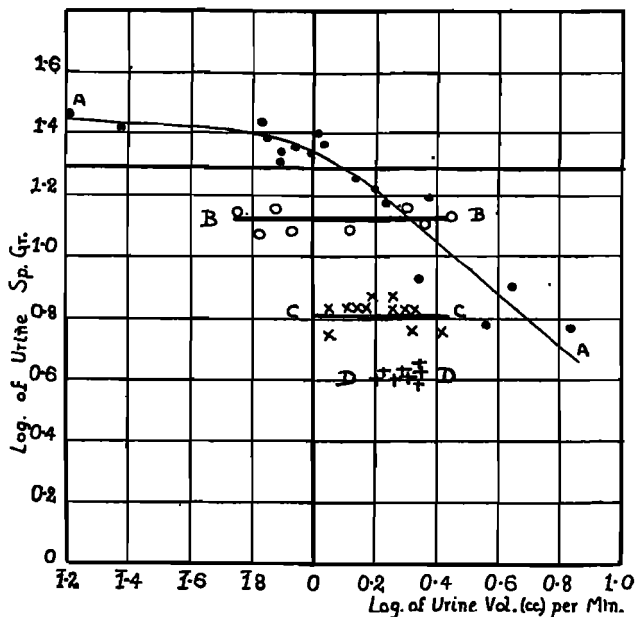


FIG. 15.—The relation between the specific gravity and the rate of secretion of urine. A : normal; the slope of the curve and the wide range both of secretion rate and specific gravity show the adaptability of the kidney to varying loads.

B, C, and D: kidney deficiency; the horizontal line shows the loss of power to vary the concentration of the urine, and the clumping of the points shows the relative inability to vary the rate of water secretion. D is an extreme case.

and this is an excellent method of showing up relatively small deficiencies in kidney function.

The Water Concentration Test.—A very similar test, but one of which the result is obtained in a shorter

time, consists in obtaining one-hour samples of urine before, and for three hours after giving the patient a pint of water to drink. The time must be measured with reasonable accuracy and the patient must be instructed to empty the bladder as completely as possible on each occasion. The healthy kidney quickly responds by a diuresis which eliminates all or, at any rate, most of the extra water within the period of three hours. The diuresis is usually greatest even during the first hour. The damaged kidney, however, less able to adapt itself to the extra work of removing the superfluous water, fails to respond in this way, and the rate of water excretion increases relatively little above that obtaining before the water was imbibed (Fig. 16). Specific gravity determinations on the venous samples show the same thing; relative constancy in the presence of the damaged kidney, but a sudden drop in specific gravity in the normal case immediately after drinking water.

The Urea Concentration Test.—The fundamentally important property of the healthy kidney, its adaptability to varying circumstances with, as a corollary, its power of responding to an extra load by the performance of extra work, can be tested in a variety of ways. The essence of all tests is the same, the test substance and the details of the technique may vary, and just as the excretion of added water has been used as a test of renal function, so may the excretion of added urea. The patient, for the urea-concentration test, must be fasting since the previous evening, and after the collection of a one-hour sample of urine he is given 15 g. of urea in a pint of water, flavoured in order to disguise the disagreeable metallic taste of the solution. It is as well not to tell the patient the

actual constituents of the draught, as sensitive persons are occasionally nauseated by the association of urea with urine. After the ingestion of the urea the urine

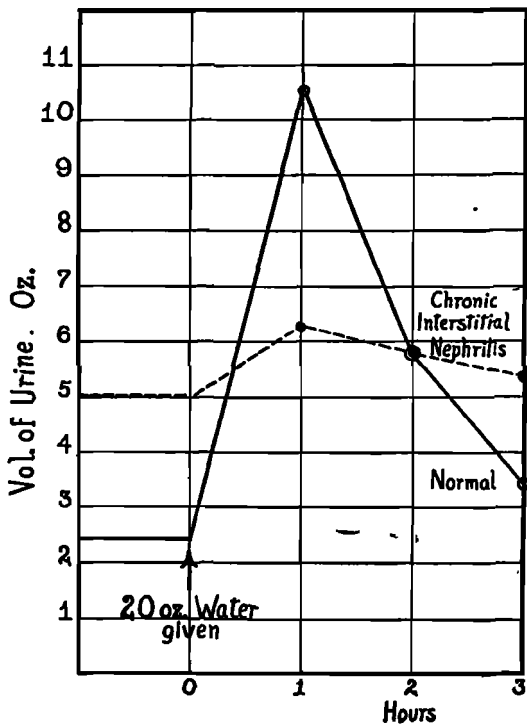


FIG. 16.—Results of the water-concentration test in normal and nephritic persons.

is collected at the end of the first and second hours. If there is reason to suppose that there is incomplete emptying of the bladder, accuracy demands the use of a catheter. If the diuresis—as shown by the volume—has not subsided by that time a third

sample is collected at the end of another hour. The volume, specific gravity, and urea concentration are determined in each specimen. The normal kidney, besides showing the phenomena noted in the water-excretion test (*vide supra*), usually excretes 75 per cent. or more of the added urea in the two hours, giving a urea percentage in the urine of at least 2.5. In the first sample, where the excretion of water is the predominating factor, this percentage of urea may not be attained, but in the second—the diuresis having largely subsided—a percentage of about 3 is almost invariable in healthy young persons. With advancing years the concentration of urea may be slightly decreased, but any concentration below 2.5 per cent. is diagnostic of some degree of renal inefficiency. When the concentration does not reach 1.5 per cent. in either specimen the damage is very definite, and a concentration never exceeding 1 per cent. is of very serious import. Besides showing these relatively low concentrations of urea in the urine, the damaged kidney fails to excrete more than a small amount of the added urea, and, as in the water test, shows very little increase in the volume of urine secreted as compared with the rate prior to administration of the urea (Table VIII).

The Urea Range Test.—The urea range test is a rather more elaborate form of urea concentration test, and combines measurement of the kidney's power to produce a concentrated urine along with its power of producing a very dilute one. That is, it tests the kidney's range of adaptability.

From noon on the day of the test the patient is given the minimal amount of fluid. At 9 P.M. the bladder is emptied, and a dose of 15 g. of urea dis-

TABLE VIII
THE UREA CONCENTRATION TEST

Case	1st Sample One hour after Urea		2nd Sample Two hours after Urea		3rd Sample Three hours after Urea	
	Vol. in c.c. 300	Gms. Urea per cent. 1.80	Vol. in c.c. 200	Gms. Urea per cent. 3.00	Vol. in c.c. 100	Gms. Urea per cent. 2.50
Normal						
Acute Bright's Disease : Convalescent stage showing recovery :						
(1)	64	0.90	84	1.33	35	1.37
(2)	74	1.20	74	1.54	65	1.80
(3)	120	1.50	90	1.90	60	2.60
Chronic Azo- taemic Nephritis :						
Early	200	1.50	180	1.60	160	1.60
Severe	150	0.89	130	0.94	—	—

solved in 100 c.c. of water is given. At 10 P.M. the bladder is again emptied and the specimen kept. These three specimens (or two if no urine has been passed during the night) have been obtained at a time when the kidney has been forced, by a reduced fluid intake and a heavy dose of urea, to exert its maximum powers of concentration; at least one of them should normally contain not less than 3.5 g. of urea per 100 c.c. At 6 A.M. the second part of the test begins, the response to an intake of two pints of water (given as such, as weak tea, or flavoured with lemon, lime juice, etc.) Urine is then collected at 7 A.M., 8 A.M. (and at 9 A.M. if no diuresis has previously occurred).

In one of these samples the urea concentration will ordinarily fall below 0.5 per cent. It is sometimes difficult, particularly in the case of females, to persuade the patient to drink as much as two pints of water. One has then to be content with inducing the patient to drink as much as possible. In evaluating the results of the test, both parts are taken into account, the attainment of a maximum of at least 3.5 per cent. urea in one of the night specimens, and a fall to below 0.5 per cent. in one of the morning specimens. Impairment of renal function is indicated by a narrower range of variation than this. It is found that as a rule the highest concentration in any sample is 2.3 per cent. (or less), when the damage has reached the stage at which the blood urea is just beginning to rise, and in terminal cases adaptability has been so far lost that all the samples contain about the same concentration of urea.

In the nephrotic syndrome tests involving urea are, naturally, not employed. The diagnosis is clinically obvious, and, since in this condition there is no difficulty in excreting urea, the tests are valueless in prognosis. Such tests are harmless, and if, owing to increasing blood-pressure, suspicion arises that secondary contraction is occurring, they may yield confirmatory evidence. Again, they are not employed in acute Bright's disease nor in advanced chronic azotaemic nephritis, where the blood urea is already high and furnishes sufficient information. Their main use lies in supplying information concerning those cases of albuminuria which, from an examination of the urinary sediment, are apparently not functional, but do not show any alteration in the blood chemistry. Here their value is considerable, for they usually de-

monstrate the presence of a kidney lesion much earlier than does estimation of the non-protein nitrogenous constituents of the blood.

Tests based on Examination of a Single Sample of Urine.—It has already been pointed out that little help is provided by clinical analysis of single samples of urine—even of twenty-four hour samples. There are, however, occasions when, *faute de mieux*, such analyses may be of slight service. Thus in a very advanced nephritis the urea concentration of the urine (and also, of course, the specific gravity) tend to fall well below the normal average even for a similar protein intake. Thus in the absence of any obvious cause of diuresis, a urea concentration over 2 per cent. or even 1·5 per cent. will exclude the possibility of true chronic uraemia.

The terminal stages of chronic azotaemic Bright's disease are accompanied by the development of an acidosis, and, as we have seen, one of the means by which the alkali reserve is conserved is the production of ammonia in the kidney. The ammonia is then excreted with acid as an ammonium salt, while sodium and potassium are retained to be utilised again. This mechanism can ordinarily be adapted to circumstances, more ammonia than usual being produced and excreted as the alkali reserve of the blood falls. Hence, in the terminal stages of chronic azotaemic nephritis, in acute Bright's disease, and in the nephrotic syndrome we may expect some increase in the ammonia excretion with a corresponding diminution in the urea excretion. In other words, we may expect the ammonia coefficient, the ratio ammonia-nitrogen \times 100/urea nitrogen, to rise above the normal figure of 3 to 5. The alteration of this ratio, however,

must be expected to be proportional, not to the amount of kidney damage, but to the degree of acidosis which is the real cause of the alteration. Moreover, damage to the kidney may, and usually does, involve damage to the ammonia-producing mechanism. Consequently, in kidney disease with acidosis the increase in the ammonia coefficient is much less than is found in cases with a comparable lowering of the alkali reserve, but with healthy kidneys.

Just as in chronic azotaemic nephritis the urine urea concentration may be low, so in the nephrotic syndrome the urine chloride is usually low and, in very severe cases, may be nearly non-existent. This is not diagnostic of the condition (though it may supply useful confirmatory evidence at times), since it is a common finding whenever chloride becomes of outstanding value to the body tissues—*e.g.* in the state of chloride lack following the severe vomiting of high intestinal obstruction, in other conditions of alkalosis, or in pneumonia.

The presence of diastase (amylase) in the urine has been made the basis of another test for renal function. Diastase reaches the urine by a rather roundabout route. It is secreted into the intestine by the pancreas, absorbed thence by the blood, and excreted by the kidney. Diastase is, of course, a starch-splitting enzyme, and its quantity in the urine is measured by the number of cubic centimetres of 0·1 per cent. solution of starch which is digested in a given time by 1 c.c. of urine. Each cubic centimetre of starch solution so digested is called a *diastatic unit*. In subjects with healthy kidneys the diastatic index in the urine varies within wide limits. There are seldom, however, more than twenty-five units in 1 c.c. of healthy urine,

and almost never less than six. In subjects with renal insufficiency the diastatic index may be decreased, but the test is not an accurate one, since it may be influenced by a number of factors, such as the acidity or alkalinity of the urine. It is, indeed, not uncommon for the most misleading and anomalous results to be obtained by it, in that some grossly diseased kidneys occasionally seem to excrete a urine with a diastatic index in the region of twenty.

Tests based on the Excretion of Foreign Substances.

—The main value of tests based upon the excretion of foreign substances is that they lend themselves to the easy detection of gross inefficiency in one kidney, since by passage of a ureteral catheter the secretion of each kidney can be collected separately. Without catheterisation they can, of course, be used to gauge the total kidney efficiency, though in the medical case the tests previously described are probably to be preferred.

The substance chosen must be non-irritant and harmless when injected, must be excreted freely by the healthy kidney, and must be readily detected in the urine. These criteria of suitability are fulfilled by a number of dye-stuffs, of which phenol-sulphone-phthalein is most commonly used. In order to ensure an adequate volume of urine, the patient is given 300 c.c. of water to drink, and, the bladder having been completely emptied, 1 c.c. of the saline solution of the dye (containing 6 mg.) is injected intramuscularly. If the technique of ureteral catheterisation is being employed, the urine from the catheters is allowed to drop into separate test-tubes, each containing a little alkali. The appearance of the dye in the urine is heralded by a red colour in the test-tube, since the dye produces a deep red colour in alkaline solution. With

a healthy kidney this will occur within ten minutes of injection. In addition, information may be obtained by measuring the rate of excretion of the dye during the two hours after its injection, and this method is usually employed when catheterisation is not being used. Two one-hour samples of urine are collected, the time beginning ten minutes after the injection. In each sample the amount of dye is determined by making the urine alkaline and comparing its colour with that of a standard solution. Normally, 40 to 60 per cent. of the dye (*i.e.* 2.4 to 3.6 mg.) will be excreted in the first hour, and 20 to 25 per cent. (1.2 to 1.5 mg.) in the second. A total excretion of only 50 per cent. (3 mg.) of the dye within the two hours, and a corresponding decrease in the hourly rate, indicates definite renal inefficiency, and with increasing severity of the lesion less and less of the dye is excreted. It will be observed that when the kidneys are being tested separately, use of this quantitative method is capable of showing not only that one kidney is damaged (which is done by the time method), but also whether or not the other is compensating for its deficiency.

Other substances than phenol-sulphone-phthalein may, of course, be used in the same way, provided they satisfy the conditions laid down above. Thus uroselectan, injected for purposes of pyelography, may be used at the same time for comparing the efficiencies of left and right kidneys. As in the case of the dye, the secretion of each kidney may be collected separately by ureteral catheterisation, two or three one-hour samples being obtained. Since uroselectan markedly increases the specific gravity of the urine, hydrometer readings alone suffice to show

whether it is being excreted. It can, however, be estimated chemically by determining the iodine content of the urine, or less accurately by weighing the amount precipitated by strong acid. Uroselectan is not used for the sole purpose of testing the functional efficiency of the kidneys, it merely affords the possibility of making such a test when a pyelographic examination is, in any case, being carried out.

Occasionally patients suffering from amyloid disease of the kidney (waxy or lardaceous kidney) may bear some clinical resemblance to those exhibiting the nephrotic syndrome. In both conditions albumin is found in the urine in large quantities, all varieties of casts appear, there may be no tendency to azotaemia, and the patient is pale and may have considerable dropsy. In amyloid disease, however, the quantity of urine passed is usually greater than in the nephrotic syndrome and the oedema correspondingly less. In addition there is always a history of syphilis, or of some chronic tuberculous or other suppuration to account for the amyloid condition, and enlargement of the spleen or liver and intractable diarrhoea are frequently present. Thus, differential diagnosis is rarely a matter of difficulty. Where doubt still exists, however, a test based upon the absorption of Congo red by amyloid tissue may be used.

The technique is very similar to that used with phenol-sulphone-phthalein (without catheterisation): 15 c.c. of sterile 1·5 per cent. aqueous solution of Congo red are injected intravenously, and one-hour samples of urine are, thereafter, collected for two hours; 10 c.c. of blood are withdrawn (from the other arm) five minutes and one hour after the injection. The presence of the dye is indicated by a strong red colour,

which may appear more clearly on addition of HCl; a pale pink tinge shows the presence of an insignificant trace. The plasma from the five-minutes sample is taken as the standard, and the other plasma is compared with it colorometrically.

When Congo red is injected intravenously into a healthy person or into one suffering from chronic azotaemic nephritis, it remains in the blood-stream, deeply staining the plasma for a considerable time. Less than 40 per cent. of the dye disappears from the blood in the course of an hour and no significant quantity of it appears in the urine. In the nephrotic syndrome, and especially in amyloid disease, the dye tends to disappear from the blood-stream more rapidly—in the former case because, like albumin, it falls through the damaged kidney into the urine, and in the latter because it is absorbed by the amyloid tissues, for which it has an affinity. Thus in the nephrotic syndrome a loss of from 40 to 60 per cent. of the dye occurs from the blood in the course of an hour, and the urine contains considerable quantities of Congo red. In severe amyloid disease a loss of over 60 per cent. of the dye may occur from the blood in the course of an hour, but none of it appears in the urine.

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Tests founded on Analysis of both Blood and Urine

Various methods have from time to time been propounded for detecting and estimating renal inefficiency of the azotaemic type, by studying the concentration of urea simultaneously in the blood and urine. Many of them are open to criticism both from the theoretical standpoint and on account of the practical difficulties involved in determining satisfactory con-

ditions. Nevertheless, in view of the fact that normally the kidney does secrete a fluid in which the urea concentration is much greater than that of the blood, there are *a priori* reasons for believing that, provided all the factors involved in this differentiation are determined, a completely satisfactory test may, some day, be devised.

As we have seen, the normal kidney is capable of concentrating the blood urea sixty to eighty times. In other words, the ratio :

$$\frac{\text{mg. urea per 100 c.c. of urine}}{\text{mg. urea per 100 c.c. of blood}} = 60 \text{ to } 80.$$

In azotaemic Bright's disease, where the kidney has lost much of its concentrating power, this ratio is usually lower, and in very severe cases may be much lower, so that figures of under 10 may be found, and may be of grave significance. This simplest of formulae connecting blood and urine, which involves analysis of a twenty-four hour specimen of urine and a single, fasting, blood sample, may be quite fallacious. It fails to take into account a number of factors which may affect the concentration of urea both in the blood and urine. In health the ratio may vary over quite a wide range and may occasionally fall well below the so-called normal range, which, in reality, is merely an average.

In the past, many attempts, mainly empirical, have been made to devise formulae which would take into account such factors as might affect the urine urea/blood urea ratio, the most obvious of these being the water excretion and the body weight. These older formulae were cumbersome, insensitive, and tended to give markedly abnormal values only when the blood

urea was already raised and when, therefore, they were really unnecessary. They have consequently fallen into desuetude.

One more recent improvement of the simple ratio is, however, in use, and is capable of yielding valuable information in cases of azotaemic Bright's disease before the blood urea has begun to rise. This is based on the idea that one can imagine part of the blood losing the whole of its urea during its passage through the kidney. This urea then appears in the urine. Suppose, then, that in one minute 2 c.c. of urine are produced, and that this urine contains 1.5 per cent. of urea. The amount of urea excreted in the one minute will then be 0.03 g., *i.e.* 30 mg. Suppose, further, that while this urine is being secreted the blood reaching the kidney contains 40 mg. of urea per 100 c.c.; then 30 mg. of urea will be present in 75 c.c. of blood, and therefore this volume of blood must be cleared of urea in one minute to provide the urea found in the urine. Van Slyke found that, under certain conditions, the "*urea clearance*," the volume of blood which must be cleared of urea per minute to provide the observed urine urea concentration, was normally about 75, and that kidney inefficiency of the azotaemic type was accompanied by a marked decrease in the figure before any abnormality of blood urea could be detected. This held only when the volume of urea produced was more than 2 c.c. per minute, and since this is at least twice the normal rate, Van Slyke termed it the *maximum urea clearance*. It

is given by the formula : $C = \frac{U}{B} V$, where C = maximum

urea clearance, U = urine urea concentration in grams

per 100 c.c., B=blood urea concentration in grams per 100 c.c., and V=volume of urine per minute (in c.c.). The maximum urea clearance is usually attained only under the stimulus of a large intake of water or urea (or both). The normal figure for the maximum urea clearance is found to be 75 (± 7) whatever the rate of urine secretion, provided it is over 2 c.c. per minute.

If, however, the rate falls below 2 c.c. per minute, the urea clearance depends upon the volume of urine, and, experimentally, was found to be very nearly proportional to the square root of the volume of urine excreted per minute. This means that when less than 2 c.c. of urine are excreted per minute, either a table must be constructed giving the normal values for all possible excretion rates or it must be made possible to calculate, for every excretion rate, the value of the urea clearance at some arbitrarily chosen rate. (A third, theoretical, but practically impossible, method would be to measure the urea clearance at the chosen rate of urine secretion.) The second of these alternatives is the one adopted in practice.

If C_s =standard urea clearance; *i.e.* the urea clearance at the arbitrarily chosen rate of urine section, V_s :

And C=observed urea clearance at the observed rate of urine secretion V (V being less than 2 c.c. per minute),

Then, since the clearance is found experimentally to be proportional to the square root of the urine secretion rate,

$$\frac{C_s}{C} = \frac{\sqrt{V_s}}{\sqrt{V}}$$

$$\text{Hence, } C_s = C \frac{V_s}{V}$$

V_s is chosen as 1 c.c. per minute, partly because that simplifies calculation, and partly because it is the normal average rate.

In that case,

$$C_s = C \frac{\sqrt{1}}{\sqrt{V}}$$

But C , as we have seen, is represented by the value,

$$\frac{U}{B} V.$$

$$\text{Therefore, } C_s = \frac{U}{B} V \frac{1}{\sqrt{V}}$$

$$\text{i.e. standard urea clearance} = \frac{U}{B} \sqrt{V}.$$

Determination of the standard urea clearance, which is normally 54 ± 10 , presents the advantage that it does not necessitate special stimulation of the kidney to diuresis. If diuresis is present at the time of the test, the observed figures are, of course, used to calculate either the maximum or the standard urea clearance according to whether " V ," the volume of urea per minute, is found to be greater or less than 2 c.c.

The technique of the urea clearance test is simple. Urine is collected over a measured period, of one or two hours, with or without stimulation of the kidney by administration of urea, and a sample of blood is drawn at the middle point of the collection period. From the volume of the urine and the time over which it was collected, the volume per minute is calculated, and urea is estimated in both urine and blood samples.

The test can readily be combined with any of the others involving urine collection over definite periods (the urea concentration test, the urea range test, etc.), simply by obtaining a sample of blood half-way through any one of the collection periods.

The urea clearance (maximum or standard) is usually lowered in acute Bright's disease to less than half the normal, and increases again with clinical improvement. In chronic azotaemic Bright's disease the urea clearance is generally normal during the latent period following recovery from the acute phase (though albuminuria may persist), but later falls and reaches a value half (or less) the normal before the blood urea begins to rise. With values below 20 per cent. of the normal, the survival time rarely exceeds two years, and death has occurred within a year in more than half the cases studied. Chronic uraemia is invariably present when the urea clearance falls to about 5 per cent. of the normal values. In the nephrotic syndrome the urea clearance is normal, but the onset of secondary contraction produces the same changes as in chronic azotaemic Bright's disease. The arterio-sclerotic kidney is accompanied by a normal urea clearance usually maintained over a long period, but finally giving place to a slow, steady fall, or to a relatively sudden drop to the uraemic level within a short time. It is worth remembering that, with increasing renal inefficiency of the azotaemic type, the urea clearance is said to fall well below the normal some time before abnormality becomes detectable by the phenol-sulphone-phthalein test, and, conversely, with improving kidney function, recovery is shown earlier by a better excretion of the dye.

SUMMARY

The value of renal function tests in the various types of Bright's disease may conveniently be summarised as in Fig. 17 and Table IX. The indications given as to the relative delicacy of the tests must, of course, be taken as merely approximate.

Abnormal = + Normal = -	Amount of Kidney Damage	Acute	Chronic											
Albumin in urine		+++	±	+	+	+	+	+	+	+	+	+	+	+
Constancy of urine volume and sp-gr. } Water concentration test Urea concentration test Urea range test Urea clearance test		+	-	±	+	+	+	+	+	+	+	+	+	+
Phenol-sulphone-phthalein test		+	-	-	±	+	+	+	+	+	+	+	+	+
Blood N.F.N.		+	-	-	-	-	-	±	+	+	+	+	+	+
Blood Urea N.		+	-	-	-	-	-	±	+	+	+	+	+	+
Blood creatinine Blood phosphate		+	-	-	-	-	-	-	-	+	+	+	+	+
Urine ammonia		+	-	-	-	-	-	-	-	-	-	-	+	+
Blood CO ₂ combining power		+	-	-	-	-	-	-	-	-	-	-	-	+

FIG. 17.—The response to kidney function tests at various stages of chronic azotaemic Bright's disease. The curve showing the amount of kidney damage is purely hypothetical. The initial acute stage may or may not occur in a given case. The period during which all tests are negative is, of course, of very variable length. The stage at which the patient usually seeks medical advice is marked "B," but cases are sometimes seen at "A"—*e.g.* after examination for life insurance. Similar findings appear in secondary contracted kidney, but are there associated with the changes which characterise the nephrotic syndrome.

TABLE IX

THE RESPONSE TO RENAL FUNCTION TESTS IN
THE NEPHROTIC SYNDROME

Volume of Urine . . .	Low daily output, hourly volume tends to keep constant.
Specific Gravity of Urine . . .	High, and varies little.
Water Concentration Test . . .	Little or no response.
Urea Concentration Test . . .	Normal.
N.P.N. of the Blood . . .	Normal.
Blood Urea Nitrogen . . .	Normal or slightly low.
Blood Creatinine . . .	Normal.
Ammonia Coefficient . . .	Somewhat raised.
CO ₂ Combining Power . . .	Tends to be low.
Blood Cholesterol . . .	Markedly raised.
Plasma Proteins . . .	Low.
Albumin-Globulin ratio . . .	Lowered.

CHAPTER VII

THE EXAMINATION OF STOMACH CONTENTS

WHEN Rehfuss introduced the method of analysing the gastric contents by the withdrawal of samples at short intervals of time throughout the digestive cycle, it was hoped by many that the diagnosis of gastric disorders had been placed on a concrete and scientific basis. These expectations have unfortunately not been fully realised, for there are many factors which may modify the gastric secretion and motility and of which our knowledge is still inexact. The method, therefore, cannot be regarded as giving a chemically exact picture of the progress of gastric digestion, but it is nevertheless capable of affording, in certain cases, valuable information.

TECHNIQUE

The Preparation of the Patient.—The day before the test the patient receives no drugs; the nature of the test and its purpose are explained to him, and at 11 P.M. he is given a cup of milk containing two teaspoonfuls of charcoal, or two large charcoal biscuits. Charcoal is an easily recognisable substance in the gastric contents, and if it is found in the fasting juice the next morning it indicates a gross delay in the emptying of the stomach. It is usual to ask the patient not to brush his teeth on the morning of the

test, lest blood be introduced into the stomach contents through bleeding of the gums.

The Passage of the Tube and the Withdrawal of Samples.—The passage of the tube is commenced in hospital usually at 6.30 A.M., no food or drink having been taken previously. If the tube is passed later in the morning the taking of the charcoal the previous night must be correspondingly later. The tube used is the narrow No. 8 French rubber tube devised by Ryle. It is about 105 cm. long, having a bulbous blind end into which is inserted an oval lead weight. At a distance of about 2 cm. from the tip it is perforated by several holes of about 2 mm. diameter. It is marked by transverse lines at 40 cm. and 57 cm. distance from the tip to give an approximate indication of the cardiac orifice and pylorus respectively. The tube is boiled and kept lying in the water till required for use, when the end is lubricated with glycerine or milk.

The patient sits upright in bed, and is told to take the weighted end of the tube into his mouth, together with two or three inches of tubing so that there is plenty of "slack" in the mouth. He retains this till it is thoroughly moistened with saliva, and is then told to work the bulbous tip towards the back of the tongue by pressing his lips firmly round the tubing. He is then told to say "Ah!" and the operator drops the end of the tube well down into the pharynx. This immediately produces a swallowing reflex which forces the bulb past the constriction corresponding to the cricoid cartilage, the point at which obstruction to the passage of the tube is most apt to occur. When he gets it past this point he should take a few breaths, to reassure himself that he can breathe perfectly easily,

and then should continue to swallow the tube, quietly and taking his own time, till the mark on the tube indicating the pylorus is just reached, when the passage of the tube is stopped. If the patient feels any inclination to retch during the proceedings he should close his lips on the tube and take several long breaths through the nose before continuing to swallow.

The difficulties in swallowing the tube are almost entirely psychical, since, success having once been achieved, repetition on future occasions becomes almost as easy as swallowing a pill. Success largely depends on the personality of the operator, who should be able to impress the patient with the ease of the procedure, or, better still, when dealing with hysterical people, to demonstrate how easy it is by swallowing a tube himself in front of the patient. Many patients have to make two or three attempts before getting the bulb past the constriction of the cricoid, but total failure to pass the tube should be very rare.

When the tube has been swallowed as far as the pyloric mark, withdrawal of the fasting juice is commenced by suction with a record syringe attached to the open end of the tube. Suction is applied with the patient sitting up, lying on his back, lying on his right side, then on his left side, and, finally, on his face. No change in position is made until suction in that particular position produces only a little froth. While the patient is in each position the tube is raised and lowered a little, so as to search the stomach thoroughly. This complete emptying of the stomach of fasting juice is of considerable importance, and is frequently neglected. We believe that many of the indifferent results obtained with the fractional method of gastric analysis are due to neglect of this essential

point. When the stomach has been completely emptied, and the aspirated juice has been transferred to a measuring cylinder, the patient is asked to maintain the tube so that the pyloric mark is just at the level of his lips, and thereafter, throughout the test, to spit out any saliva which accumulates in his mouth rather than to swallow it, so as to prevent an artificial hypo-acidity of the stomach contents owing to excessive salivation.

The patient, with the tube *in situ*, then eats his "test-meal." This consists of thin gruel, made by boiling two tablespoonfuls of oatmeal in two pints of water until the total bulk is reduced to one pint, and then straining through muslin. No salt should be added to the meal, because chloride estimations would thereby be rendered valueless, but the gruel can be flavoured with a little sugar. This meal, though not epicurean, has the advantage of giving a colourless specimen when aspirated from the stomach, so that, when it is examined later, titration end-points and other colour reactions can be determined with great ease. After taking the meal the patient lies down again and remains in that position for the remainder of the test. The tube is closed by a clip to prevent any leakage of gastric contents between the aspiration of samples.

Samples of about 10 c.c. each are withdrawn from the stomach at intervals of a quarter of an hour, reckoned from the time the patient began to take the meal, the syringe being thoroughly washed out after each aspiration and the tube cleared by forcing a little air down it. If this latter point is neglected the first one or two c.c. of gastric contents to be aspirated on the next occasion may consist of the previous sample.

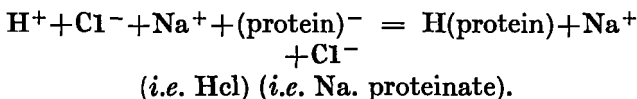
Occasionally some difficulty is experienced in withdrawing the samples, owing to the orifices at the lower end of the tube becoming blocked, but this again can be overcome by the injection of a little air. Samples are withdrawn in this way for two and a half hours, so that eleven samples, including the resting juice, are obtained, and are placed in numbered test-tubes. The stomach is completely emptied when the last sample is obtained by a similar procedure to that already described for procuring the resting juice, and this specimen is also measured.

The tube is withdrawn at the end of the test by steady, gentle traction. It usually comes away perfectly freely, but occasionally the bulbous end may show a tendency to stick at the level of the cricoid cartilage, in which case the patient should be asked to swallow while the traction is continued, and the end will then slip past the constriction quite easily.

Each sample of gastric contents is examined with respect to odour ; colour ; presence of bile, starch, and mucus ; and titratable acidity. In certain cases, to be discussed later, the total chloride content is determined. The fasting sample is also examined for charcoal, blood, pus, and (if the titratable acidity is low) lactic acid.

The titratable acidity is determined by titrating a measured volume of the sample with N/10 NaOH, using first Töpfer's reagent and second phenolphthalein as indicator. Töpfer's reagent (dimethyl-amino-azo-benzene) is an indicator which changes colour at about pH 4.5, and as any acidity greater than that is due either to a strong acid such as hydrochloric or to a very high concentration of a weaker organic acid, it is regarded as indicating the amount of free hydrochloric

acid present. If, however, the amount of acidity shown by Töpfer's reagent is small, it is necessary to prove that it is really due to hydrochloric acid by some more specific test, such as that of Gunzberg. Phenolphthalein, colourless in acid solution, turns pink at about pH 8, and titration with this indicator includes in the estimation all weak organic acids, and therefore gives the total titratable acidity. Even if such acids as lactic and butyric are absent, the total acid and the free (hydrochloric) acid are not equal, and the difference between them is generally termed the combined acid. The explanation is that both the gastric juice and the test-meal contain salts of protein—mucin in the former and oatmeal or other protein in the latter. Hence the following reaction takes place:—



Some of the hydrochloric acid is thus replaced by the very weak acid, free protein. This acid can be titrated, but only when phenolphthalein is the indicator, and it may vary from sample to sample in accordance with the amount of protein present. The so-called combined acid, however, will include, besides the free protein derived from this reaction between protein salts and hydrochloric acid, any organic or other weak acids which may be present. The term is, therefore, not strictly accurate.

THE RESULTS IN HEALTHY SUBJECTS

The results obtained from such a fractional analysis in healthy individuals vary within very wide limits.

The volume of the resting juice is usually about 50 c.c., but is frequently considerably less, or as much as 100 c.c. may be obtained without being of much significance. When the volume of the resting juice, however, is much more than 100 c.c. there is a strong suggestion of gastric stasis due to pyloric spasm or stenosis, or to a very atonic condition of the musculature of the stomach.

No charcoal should be present in the fasting juice of healthy individuals, except possibly a few specks which may have adhered to the gastric mucosa. The finding of any quantity of charcoal in the specimen, so long after its ingestion as seven and a half hours, is again strong evidence of gastric stasis. The only substance with which charcoal might conceivably be confused in the stomach contents is the dirty grey sputum which miners sometimes swallow.

The volume of gastric juice removed at the end of the two and a half hours' test is small in healthy people, and, as has been said, it is often impossible to obtain a sample at all. A volume of 30 c.c. or over suggests that there is delay in emptying the stomach or, more rarely, hypersecretion.

Lastly, the meal itself, as recognised by the iodine test, or by the simple inspection of the sample, has usually disappeared from the stomach contents some two hours after the ingestion of the meal. Small quantities of starch may sometimes be demonstrated in apparently healthy stomachs so long as two and a half hours after the taking of the meal. If a large quantity of the meal is still present in the last specimen, however, it may be considered, along with other signs, as evidence of delay in emptying. The disappearance of starch from the samples earlier than an hour and

a half after the meal has been taken suggests hypermotility.

Bile is frequently discovered in the fasting juice of healthy persons, and, though it often appears again when the stomach is nearly empty, does not usually occur in the samples obtained early in the test. When it occurs in these early samples it indicates a premature patency or relaxation of the pylorus which may be pathological. The presence or absence of bile may thus give some indication as to the state of the pylorus. It must be remembered, however, that under the conditions of the test, at least, the alkaline pancreatic juices may be regurgitated into the stomach independently of the presence of bile (but see p. 161).

In health none of the samples should have a foul odour, and should contain no demonstrable quantity of lactic acid.

It is usual to find a few leucocytes on microscopical examination of the stomach contents of healthy people, but no recognisable quantity of pus should be present. If pus is found, it is essential to exclude extra-gastric sources, such as swallowed purulent sputum, before attributing its presence to a gastric lesion, which only seldom gives rise to any quantity of pus in the stomach.

Blood should not be found, though it must be borne in mind that the presence of small streaks of blood in the fasting juice is usually evidence of trauma due to the passage of the tube and not to disease of the stomach. The presence of a considerable quantity of blood or of "coffee-ground" material is definitely pathological.

The presence of mucus in any of the samples is

shown readily by their behaviour while they are being aspirated and while they are being analysed. There is, unfortunately, no satisfactory method of estimating mucus quantitatively, and its amount can therefore be gauged only roughly. In addition to gastric mucus, oesophageal secretion and mucus from other parts of the upper alimentary and respiratory tracts may also find their way into the stomach. So long as any accurate investigation of gastric mucus remains difficult or impossible, the significance to be attached to varying amounts of mucus lacks precision. In the healthy stomach mucus is not present in very large amounts, and when it is found in excessive quantities it is usually an index of one of the gastric conditions associated with achlorhydria, which will be referred to later, and is particularly abundant in gastritis. There seems usually to be a rough inverse relationship between the mucus content of the stomach and its acidity.

The secretory response of normal stomachs to the stimulus of a test-meal is again most variable, an average response being shown in Fig. 18. When there is a fair concentration of free acid in the fasting juice, the diluting and neutralising effects of the meal cause a fall. The concentration then gradually rises owing to the secretion of gastric juice, being at its maximum usually in an hour or an hour and a half, but rarely exceeding 40 c.c. of N/10 NaOH per cent. Towards the end of the test it tends to diminish, owing to a variety of causes, such as the cessation of gastric secretion and the possible regurgitation of alkaline duodenal contents. Some apparently healthy people, however, show curves of free hydrochloric acid which rise to a much higher maximum than that shown to be

the limit for the majority, and a few show no free acid whatsoever throughout the test. Considerable variations in the acid curves obtained from consecutive tests, performed at short intervals of time, in the same healthy subject, may be observed. The general conformation of the curve is, however, maintained, and

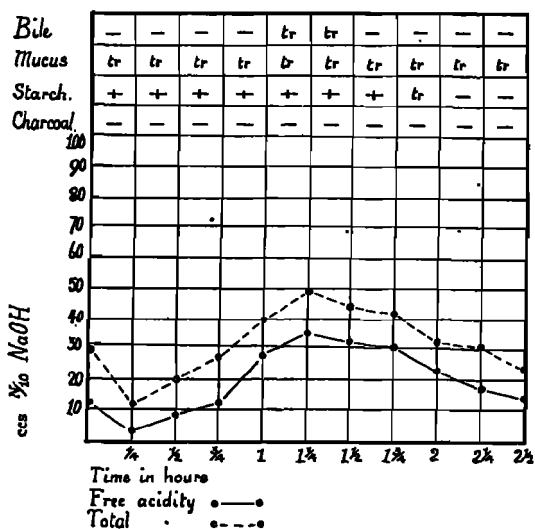


FIG. 18.—Results of a fractional test-meal in a normal healthy person.

Fasting juice, 45 c.c., no blood or charcoal. Final specimen, 13 c.c., starch absent.

the rate of emptying, as judged by the disappearance of starch, is very constant. The extent of emotional stress occasioned by the test does not seem to exert a significant effect upon secretion. There is a tendency for secretion to diminish with advancing years, but on the whole the sex and age variations may be said to be negligible. The curve of total acidity is usually

some 10 c.c. above that of the free hydrochloric acid.

A considerable amount of experimental evidence has accumulated which suggests that the stomach contents do not mix so rapidly or so completely as might be supposed, at all events during the first hour of digestion. It is, therefore, probable that the apparent acidity of the stomach contents varies quite considerably according to the region of the stomach from which the sample is removed. Hence it becomes a matter of importance to ensure that all samples throughout a fractional test-meal are drawn from the same part of the stomach, and it has been suggested that this is a practical impossibility owing to movements of the tip of the tube being constantly produced by the stomach contractions. It is probable, however, that actually the tip of the tube does *not* move about the stomach to any great extent, provided the mark on the tube indicating the distance of the pylorus is kept constantly in position at the patient's lips. Under such circumstances X-ray examination shows that the tip of the tube lies like a sinker on the lowest point of the greater curvature of the stomach and does not move appreciably. None the less, even if it be granted that the tip of the tube remains approximately still during any one test-meal, it is plain that there may be considerable differences in its position in different stomachs, since the distance between the lips and the lowest point of the stomach is subject to individual variations, and cannot be accurately gauged. On the whole, however, although this may account for some of the variation in gastric acidity from person to person, it is probable that the experimental error so introduced is not very great.

A more serious criticism is based upon the contention of some authorities that the regurgitation of alkaline duodenal contents into the stomach is not a physiological process, and it seems to us that further work is needed before such a regurgitation can be definitely accepted as taking place in health. The mere fact that bile is often found in the stomach contents of apparently healthy people, when samples are removed through a stomach tube, is no evidence of physiological regurgitation, since an artificial reflux of duodenal contents may have been occasioned by retching due to the presence of the foreign solid body, or by suction at the tube when the sample is withdrawn by the syringe. It is therefore quite possible that those patients who are more prone to retch than others may show an artificial hypo-acidity of the stomach contents, owing to neutralisation, solely during the test, by alkaline duodenal contents. The whole question of duodenal regurgitation is still very debatable, and its occurrence is certainly one of the factors which, at present, renders gastric examination by fractional test-meals most open to criticism.

ABNORMAL RESULTS

Hyperchlorhydria

Simple hyperchlorhydria is probably the commonest form of digestive disturbance. A specimen of the high acid curves given in such cases is shown in Fig. 19, but except for such hyperacidity there is little unusual in the analysis. No abnormal constituents are found, and the gastric motility is usually either normal or a little rapid, as demonstrated by the quantity of juice

found at the end of the test, and the disappearance of starch from the gastric contents.

A second type of hyperchlorhydria is found, however, which is not primarily due to the excessive quantity of hydrochloric acid secreted, but rather to

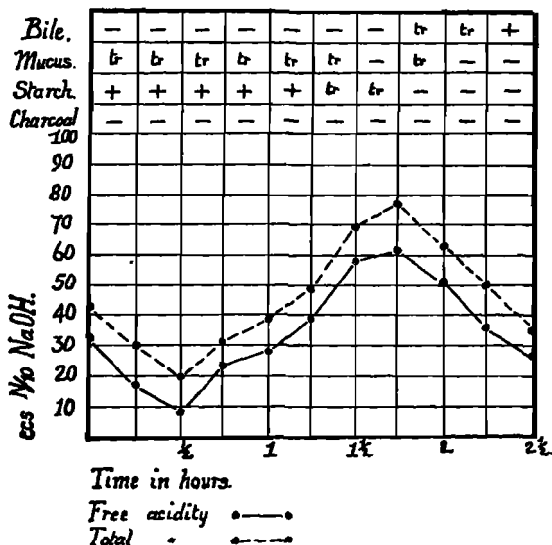


FIG. 19.—Results of a fractional test-meal in a case of simple hyperchlorhydria.

Fasting juice, 15 c.c. ; juice recovered at the end of test, 8 c.c. Curves show hyperchlorhydria, and the early disappearance of starch shows rather rapid emptying.

a spasm of the pylorus, associated with some unknown central nervous stimulation or with a reflex irritation from some other organ, such as the appendix. Owing to such a spasm no neutralisation of the stomach contents by alkaline regurgitation from the duodenum can occur, and the lengthy presence of food in the

stomach induces a prolonged secretion of acid. In such a case the quantity of juice recovered at the end of the test will be fairly large. Starch will not have disappeared from the stomach contents at the end of

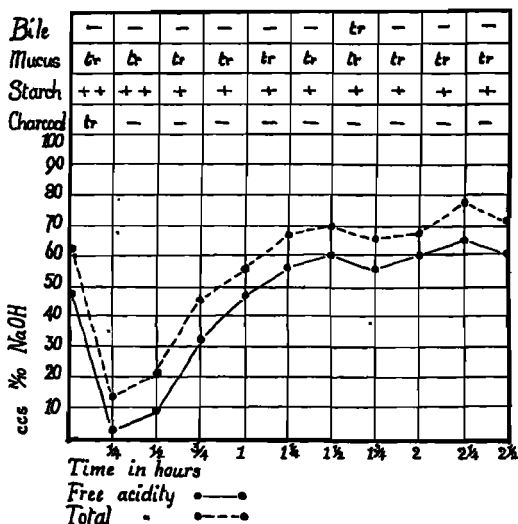


FIG. 20.—Results of a fractional test-meal in a case of hyperchlorhydria with pyloric spasm.

The fasting stomach contained 90 c.c. of fluid, with a trace of charcoal. The acidity curves climb to a high level and show no tendency to fall at the end of two and a half hours. 32 c.c. of fluid were recovered at the end of the test, and starch was still present. Hence the hyperchlorhydria was associated with slow emptying of the stomach.

two and a half hours and no bile will be found in the samples (*vide* Fig. 20).

Duodenal Ulcer.—In the vast majority of cases a duodenal ulcer is associated with marked hyperchlorhydria. Beyond this fact, however, the test-meal

seldom offers further help in the differential diagnosis of the condition. A duodenal ulcer may cause excessive gastric motility, and give rise to a picture very similar to that shown in Fig. 19. Motility, on the other hand, may be normal, or there may be slight gastric stasis due to pyloric spasm (*vide* Fig. 20). In some cases, when the ulcer is situated just beyond the pylorus, definite pyloric stenosis may eventually be brought about. This condition gives a very typical picture, a specimen of which is shown in Fig. 21. The quantity of fasting juice recovered is large, sometimes being about 200 c.c. or over, and considerable quantities of charcoal are found. Again, the quantity of gastric contents recovered at the end of the two-and-a-half-hour test is plentiful, and much starch is present. Thus the final sample may be as much as 200 or 300 c.c., the quantity depending on the severity of the stenosis. The acid-secretion curve is high and shows no tendency to fall, but may even rise continuously throughout the test. It is unusual to find any abnormal constituents.

Slight degrees of organic stenosis, however, cannot be differentiated by the test-meal findings alone from functional pyloric spasm, since the pictures given by the two conditions may be almost identical.

Gastric Ulcer.—Just as it is usually impossible to make a diagnosis of duodenal ulceration from test-meal findings alone, so is it usually impossible to diagnose a gastric ulcer with certainty by such means. The presence of “coffee-ground” blood in the fasting juice, however, is usually pathognomonic of gastric ulceration of some sort, and when found is a very strong point in differential diagnosis. A gastric ulcer may give rise to any of the types of picture illustrated

in Figs. 19–21. On the other hand, it is quite common for a gastric ulcer to be present with no sign of hyperchlorhydria on analysis of the stomach contents, especially when the ulcer is situated on the lesser

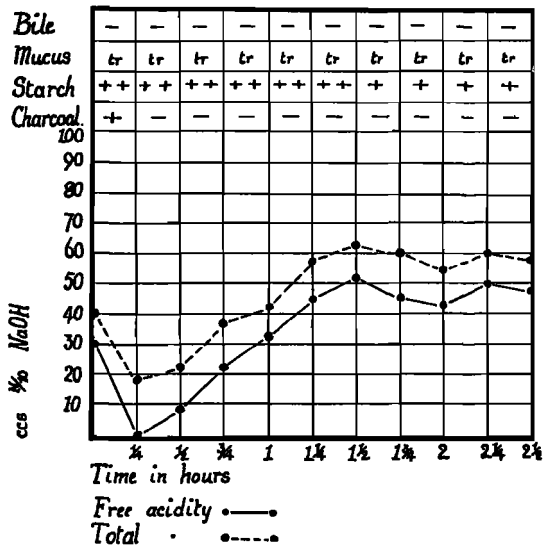


FIG. 21.—Results of a fractional test-meal in a case of duodenal ulcer with pyloric stenosis.

The fasting stomach contained 120 c.c. of fluid mixed with a considerable amount of charcoal. The acidity curves climb to a moderately high plateau. At the end of the test as much as 160 c.c. of fluid were recovered, still containing a quantity of the gruel meal, and giving a strong starch reaction. Blood was absent from all samples, and no abnormal quantities of mucus were found.

curvature. A condition of pyloric stenosis is, as we have seen, easily diagnosed by the test-meal findings, but results obtained in this way cannot differentiate between a stenosis caused by a duodenal ulcer and one

caused by a gastric ulcer, unless blood is discovered in the stomach contents, when there will be strong evidence of a gastric rather than a duodenal lesion.

To sum up, the symptoms of hyperchlorhydria are usually very typical, and the symptoms and signs of pyloric stenosis are generally clear. The fractional analysis of the stomach contents in those conditions is thus generally of service merely as a verification of a clinical diagnosis. As a more subtle diagnostic test, in distinguishing between simple hyperchlorhydria and organic ulceration, or between pyloric spasm and the lesser degrees of true stenosis—which are just the conditions in which the clinician may require help—fractional analysis is, unfortunately, often, though not always, of very little service.

Test-meal Findings after Gastro-enterostomy and Gastrectomy

Considerable information can be obtained as to the success or otherwise of operations on the stomach by fractional analyses.

A successful gastro-enterostomy causes complete neutralisation of the stomach contents by regurgitation through the stoma. In consequence, free acid is absent throughout the test, and the curve of total acidity is low. Bile is usually abundantly present in all the samples. In addition, very little fasting juice can be obtained and the stomach empties rapidly (Fig. 22). If the stoma is working inefficiently free acid occurs to a greater or less extent, and bile is less abundant, but if it ceases to work altogether a hyperchlorhydric climbing curve, indistinguishable from pyloric spasm or stenosis, is obtained.

After a successful partial gastrectomy, the curve of

free acidity is very low and sometimes—though infrequently—complete achlorhydria exists. Again there is a very small quantity of fasting juice, the stomach empties rapidly and bile is present in most of the samples (Fig. 23). In unsuccessful cases the acid secretion continues to be high.

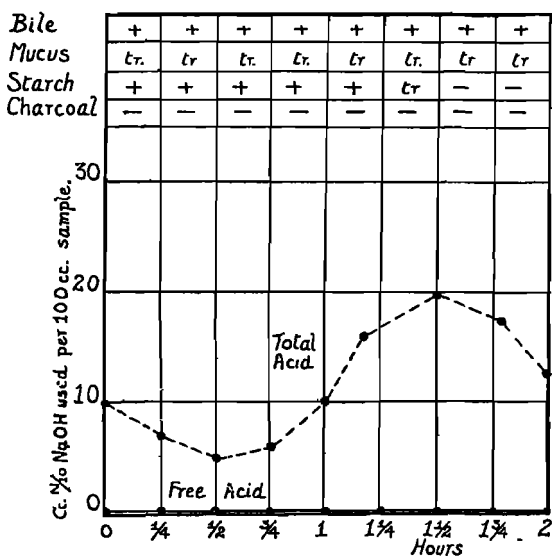


FIG. 22.—Results of a fractional test-meal in a successful case of gastro-enterostomy.

Fasting juice, 10 c.c.; only 8 c.c. recovered at the end of two hours. Bile present in all specimens.

It can hardly be urged too strongly that a routine surgical practice should be established of conducting a fractional analysis within a month of such operations. In this way it is possible to ascertain whether or not the operation has been successful in combating the previous hyperchlorhydria and in assuring free

emptying of the stomach. In those cases where free hydrochloric acid is still found to be present in significant quantities more stringent measures as regards diet and alkalis should be adopted in an attempt to

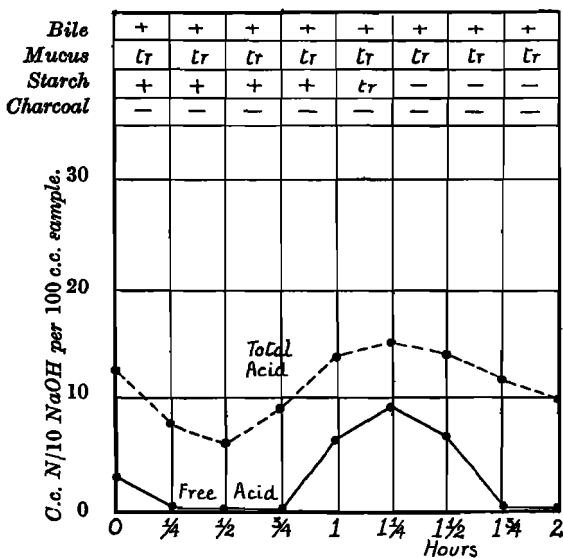


FIG. 23.—Results of a fractional test-meal after partial gastrectomy
Fasting juice, 9 c.c.; 6 c.c. recovered at the end of two hours. Bile present in all specimens.

prevent the occurrence of the far too common sequelae of operations such as jejunal ulceration.

Hypochlorhydria

Whatever may be thought as to the importance of gastric analysis in the diagnosis of hyperchlorhydric conditions, there can, nowadays, be no dubiety as to the importance of the recognition of achlorhydria.

For many years achlorhydria was known to occur as the result of, or in the course of, a number of diseases—particularly in association with chronic gastritis, gastric carcinoma, and pernicious anaemia. This achlorhydria was usually looked upon as a rather unimportant incident in such diseases, which was often, however, of some slight diagnostic value. The incidence of achlorhydria is now recognised to be extremely high in ill-health generally, and, though it may occur as the result of certain diseases, there can now be no doubt that a number of morbid processes are direct sequels of achlorhydria. These may be due to the lack of hydrochloric acid, to the absence of peptic activity, or to the inadequate absorption or utilisation of fundamental nutritional factors of which the achlorhydria may be a cause or an expression.

Achlorhydria is a common accompaniment of various wasting or febrile diseases. Thus it is frequently present in tuberculosis, in the convalescent stage of influenza, and in the terminal stages of malignant diseases in any part of the body. In such conditions as these achlorhydria is simply an indication of the general debility, chloride starvation, or lack of tone, known to be present.

Again achlorhydria is a fairly constant concomitant of chronic gastritis. Here it is again obviously secondary to the disease. It is due either to the gastric mucosa being infiltrated with inflammatory cells and covered with mucus, or to the eventual complete atrophy of the secreting cells themselves, the result of a long-continued inflammatory process.

On the other hand, we know that diseases such as pernicious anaemia and subacute combined degeneration of the cord are definitely secondary to a deficient

gastric condition characterised by achlorhydria. It is quite possible, though by no means certain, that a somewhat similar primary defect expresses itself in many other morbid pictures. Thus, achlorhydria is often found in patients suffering from colitis, cholecystitis, rheumatoid arthritis, microcytic anaemia, rosacea, sprue, neuritis, neurasthenia, and in certain allergic conditions such as asthma and urticaria. It may well be that the loss of the bactericidal action of the hydrochloric acid, the deficient absorption of iron and vitamins, or the improper digestion of proteins—all the results of an atrophic alimentary mucous membrane of which achlorhydria is an expression—may be important aetiological factors in the causation of such conditions.

These numerous associations of deficient hydrochloric acid secretion with disorders of health indicate the importance of detecting achlorhydria, which necessitates analysis of the gastric contents. In hyperchlorhydria the symptoms are usually so typical that, as we have seen, fractional analysis usually does little more than furnish corroborative diagnostic evidence. The symptoms and signs usually encountered in achlorhydria, on the other hand, are much less definite and may present a great variety of different clinical pictures. The most common are loss of appetite, a sense of fullness and discomfort in the epigastrium, constipation or diarrhoea, neurasthenia, anaemia, and glossitis. It is apparent that a fractional test-meal carried out under such circumstances may reveal the underlying cause of a puzzling condition of affairs and indicate the lines of rational treatment.

Carcinoma of the Stomach.—Gastric carcinoma is associated, in the majority of cases, though not in-

variably, with absence of free hydrochloric acid from the stomach contents in all the samples of a fractional test-meal. Many explanations have been put forward to explain this phenomenon, but its true cause has not yet been definitely established. It has been suggested that the achlorhydria is simply due to the fact that all cachetic conditions, including malignant disease anywhere, tend to cause achlorhydria; gastric carcinoma, however, causes achlorhydria much more frequently than carcinoma in other parts of the body. Again, since gastric carcinoma involves the pyloric part of the stomach more frequently than other parts, and since the pyloric portion is responsible for the secretory mechanism, it has been thought possible that the destruction of the pyloric mucosa might explain the achlorhydria of gastric carcinoma. This cannot, however, explain the very frequent occurrence of achlorhydria even when the growth involves portions of the stomach other than the pylorus, and, indeed, it is now known that the secretion of hydrochloric acid is not a function solely of the pyloric portion of the stomach. Thirdly, gastritis no doubt invariably occurs as the result of gastric carcinoma with its associated outpouring of excessive mucus and a consequent tendency to achlorhydria; yet it is well known that a carcinomatous stomach can often be washed out till the return is quite clear and yet no free hydrochloric acid can even then be obtained. Lastly, it has been suggested that gastric carcinoma, like some carcinomata in other regions, is due to chronic irritation, and thus usually occurs in patients who have suffered for a long time from chronic gastritis, of which achlorhydria is a sequel. Such an explanation, however, does not accord with the well-known

fact that the majority of patients suffering from gastric carcinoma give a very short, rather than a long, history of stomach trouble.

While none of these explanations is thus, in themselves, sufficient to account for the approximately 80 per cent. of patients with gastric carcinoma who show concomitant achlorhydria, it may well be that one or other operate in certain cases and, taken together, they may account for the fact that achlorhydria is a common finding in this disease.

Complete achlorhydria in gastric carcinoma is almost invariably associated with the presence of lactic acid in the stomach contents. This lactic acid is usually caused by the fermentation of gastric contents in an achlorhydric stomach, but it is quite possible that it may be produced in another way, since we have observed its presence in the contents of several carcinomatous stomachs, where the growths were situated so as not to interfere in any way with the emptying of the stomach, and even in cases where the motility of the gastric contents was unusually rapid.

Where free hydrochloric acid does occur in this disease, it is usually present in small amount. Cases of gastric carcinoma have been reported in which large quantities of free hydrochloric acid have been discovered, but it is very probable that Gunzberg's test was not applied in those cases, and what was estimated was not in reality free hydrochloric acid, but lactic acid, since, as we have seen, when very large concentrations of this acid are present, it may behave to Congo-red paper and to Töpfer's reagent in the same way as does hydrochloric acid.

The absence of free hydrochloric acid and the presence of lactic acid are not necessarily, as is

commonly supposed, evidence of an advanced stage of the carcinoma, since some of the earliest cases may show such results on analysis of the gastric contents. Conversely, the presence of free hydrochloric acid is not necessarily evidence of an early stage of the growth.

Owing to the presence of lactic acid, and sometimes other organic acids, the curve of total acidity is often moderately high. Blood is frequently present in an altered state, and mucus is excessive.

Other results depend on the presence or absence of pyloric stenosis. Since the pylorus is the commonest site for gastric carcinoma, signs of stasis in the gastric contents are common in this disease. Consequently, an excessive quantity of fasting juice may be obtained, stained black with charcoal and altered blood, and foul smelling owing to the presence of lactic and butyric acids. Yeasts, sarcinae, and numerous leucocytes may be present, and decomposing foodstuffs which may have been ingested twenty-four hours previously. The subsequent specimens are thick in consistency, and nearly the whole of the meal may be recovered at the end of the test period. A typical result from a case of gastric carcinoma with pyloric stenosis is shown in Fig. 24.

We believe, contrary to many authorities, that gastric carcinoma, even in its early stages, can be diagnosed or excluded, in the majority of cases, by the examination of the gastric contents, particularly of the fasting juice. The macroscopic examination of the fasting juice as a method of diagnosis in gastric carcinoma is, indeed, insufficiently stressed. It is usually thought that achlorhydria is the most important test-meal finding in this respect, whereas the

presence or absence of foul fasting juice is probably a more valuable diagnostic indication. If, on aspiration of the fasting juice, the syringe is filled with a

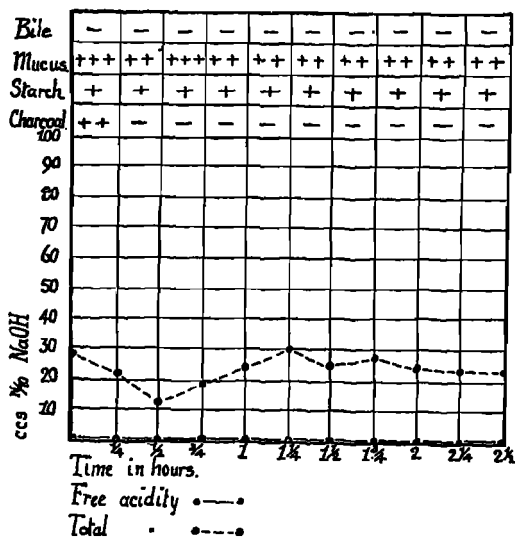


FIG. 24.—Results of a fractional test-meal in a case of gastric carcinoma with pyloric stenosis.

The fasting stomach contained 130 c.c. of foul-smelling fluid, with much mucus, charcoal, and altered blood. No free HCl was present, but a relatively large amount of "combined" acid. Lactic acid was present. Later specimens showed free HCl to be constantly absent, and varying amounts of "combined" acid. Gastric motility was much impaired, 180 c.c. of fluid, mixed with gruel and blood, being recovered at the end of the test.

dark-coloured, evil-smelling material, then a diagnosis of gastric carcinoma can be made with considerable assurance, especially when this appearance is associated with achlorhydria on biochemical examination. If,

on the other hand, a clear, inoffensive juice is obtained, then a diagnosis of gastric carcinoma is most unlikely whether achlorhydria be present or not.

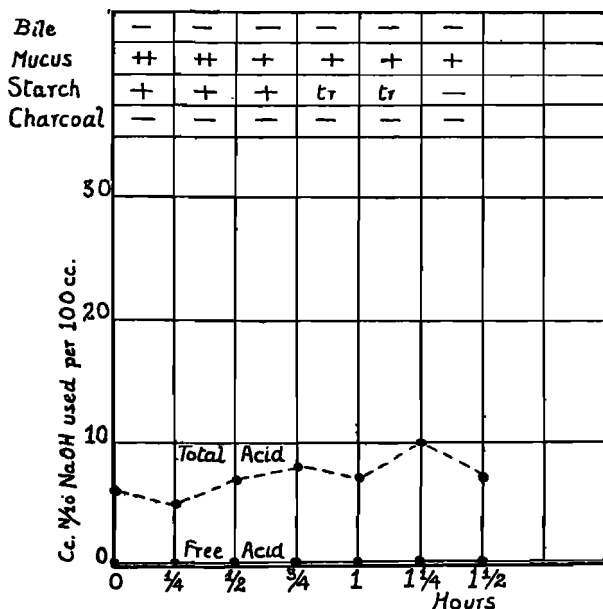


FIG. 25.—Results of a fractional test-meal in a case of pernicious anaemia.

The fasting stomach contained only 5 c.c. of juice, no free HCl, and only a very low concentration of total acid. Lactic acid and blood were absent. Free HCl was absent throughout, and all specimens gave an almost identical low reading for the total acid. Mucus was present in excess, and motility, as shown by the rapid disappearance of starch, was increased. Only a few cubic centimetres of starch-free gastric contents could be recovered with difficulty at the end of one-and-a-half hour.

Pernicious Anaemia.—The results obtained from fractional analysis of the stomach contents in pernicious anaemia are exceedingly typical. They may,

indeed, be said to be invariable in their main features, an example of which is given in Fig. 25, and there can be little doubt that the gastric condition shown precedes the development of pernicious anaemia by many years, and is, indeed, probably congenital in origin.

The most typical and striking feature of the curve is the complete absence of free hydrochloric acid from the stomach. One or two cases of anaemia whose blood picture was typically that of the pernicious type have been described in which free acid was found in the stomach contents, but such a finding is so exceptional that it may be said that the discovery of free hydrochloric acid in any specimen is very strong evidence against a diagnosis of true pernicious anaemia. Again, the curve of total acidity is uniformly low, and there is a narrow interval and close parallelism between it and the base-line. This curve, therefore, differs considerably from the curve of total acidity observed in the secondary achlorhydria of gastric carcinoma, where, owing to the presence of organic acids, the curve of total acidity may be fairly high, and does not show the parallelism with the base-line observed in pernicious anaemia.

The stomach usually empties very rapidly in this condition and, except for occasional excessive quantities of mucus, no abnormal constituents are found.

ACHLORHYDRIA AND ACHYLIA

From what has been said, it is apparent that achlorhydria may be due to either of two main causes: an actual absence of secretion of hydrochloric acid—which may be temporary or permanent—or the neutralisa-

tion of whatever hydrochloric acid is produced, whether normal or reduced in amount.

The first of these causes operates in the true achylia characteristic of the complete atrophy of the gastric glands found in cases of very long-standing chronic gastritis, in pernicious anaemia, and occasionally as a congenital abnormality in apparently healthy persons. In such conditions the achylia is permanent, but there are, no doubt, some cases of less long-standing gastritis where the cessation of gastric secretion is still susceptible to treatment.

The second main cause of achlorhydria accounts for cases in which a normal, or even increased, acid secretion is neutralised by alkaline regurgitation—as in a successful gastro-enterostomy—or in which a reduced secretion is completely neutralised by either slight duodenal regurgitation, increased gastric mucous secretion, or possibly, as in some cases of pulmonary tuberculosis, by swallowed sputum.

It is obviously important in many cases to be able to differentiate between an atrophic and irrecoverable achylia gastrica and a secondary or functional achlorhydria. There is no doubt that in the ordinary curve of total acidity there is, as we have seen, in true achylia gastrica a very narrow interval and close parallelism with the base-line, whereas a wide interval and a lack of parallelism are more likely to be found in secondary achlorhydria. It is not possible, however, to distinguish all cases with certainty in this way, and it is certainly impossible, by this method, to distinguish a temporary recoverable achlorhydria from one which is due to complete atrophy of the secreting glands.

The importance of a complete chloride analysis is often stressed for the purpose of differentiating

between the two types. It is pointed out, and quite rightly, that the ordinary acid estimation is incomplete as a measure of secretory activity, since it takes no account of the hydrochloric acid which has been neutralised and is present as inorganic chloride. When the gruel meal is taken the total concentration of chloride (*i.e.* free hydrochloric acid plus combined hydrochloric acid plus inorganic chloride) represents as nearly as possible the concentration of the total hydrochloric acid secreted, and the curve of the total chlorides is, therefore, the real secretory curve as nearly as can be obtained. It is claimed that by means of such total chloride estimations, achlorhydria, due to excessive neutralisation of the gastric juice, may be distinguished from simple diminution in the amount of gastric juice secreted, or from true achlorhydria (achylia), in which no hydrochloric acid is secreted at all. Unfortunately, though such chloride estimations are eminently possible, they are somewhat laborious and require considerable technical skill, while the results are not sufficiently helpful to warrant the procedure becoming general. They have been largely displaced for practical purposes by the much simpler and more accurate "histamine test-meal."

Histamine is the best known stimulant for the secretion of true hydrochloric acid, and it has been suggested that it is the gastric secretory hormone, just as secretin is the pancreatic secretory hormone. Many stomachs, apparently achlorhydric by the ordinary methods of fractional analysis, may be demonstrated to be capable of acid secretion after its injection.

The test is applied after the fasting juice has been withdrawn and the stomach has been thoroughly washed out with water. Instead of the ordinary

meal being fed, 0.4 mg. of histamine is injected hypodermically. Half an hour later the stomach contents are again aspirated and examined for free hydrochloric acid. The volume of juice produced by a normal individual to such stimulus varies from 10 to 50 c.c. and the sample is highly acid, requiring from 50 to 150 c.c. of $\frac{N}{10}$ NaOH per 100 c.c. of gastric contents to neutralise it. Complete absence of free hydrochloric acid in the sample indicates atrophic achylia gastrica.

Although the injection of such a quantity of histamine is not usually dangerous, the patient should be warned previously that somewhat unpleasant, though very transient, effects are sometimes produced—such as headache, flushing, and palpitation. Since symptoms of collapse appear very occasionally, the injection should always be given by the physician himself.

THE EWALD TEST-MEAL

A second method of investigating the gastric function has been practised for many years, but has now been largely superseded by the fractional method already described. It consists of the analysis of a single specimen of stomach contents, removed one hour after the ingestion of a meal. It is usually called the Ewald or, in contradistinction to the fractional method, the one-hour test-meal.

The preparations made for the test are identical with those for the fractional test, but the constitution of the meal is different. At 6.30 A.M. a cup of tea and a small slice of dry toast are given, and an hour later

an ordinary large-size stomach-tube is passed and as much fluid as possible siphoned off from the stomach. The volume of this specimen is measured, and after filtration through muslin it is examined by the methods already described for the fractional samples.

The only advantage of this method is that it takes up less of the time of the doctor or nurse in charge of the case. The disadvantages, on the other hand, are numerous. To begin with, the swallowing of a large-size stomach-tube is excessively unpleasant for the patient, and the stomach contents obtained for analysis are so coloured that it is difficult to detect small quantities of blood or charcoal by naked-eye examination. The test takes no account of the volume of the fasting juice, which is included along with the response to the test-meal. Hence no indication of gastric motility is furnished unless it is very grossly impaired, when the damage is usually quite obvious clinically. Again, it gives very little information about the acid secretion of the stomach. It is quite true that this is usually at its maximum about an hour after the taking of a meal, but we have already seen that in many cases the free-acid concentration goes on rising long after this, and may, indeed, still be rising at the end of two and a half hours. The estimation of free acid in the one-hour specimen will therefore give, in such cases, but a poor idea of the extent of the hyperacidity. On the other hand, the analysis of a single specimen may give rise to an utterly unjustifiable diagnosis of achlorhydria, for in fractional test-meals we have not infrequently found the first four specimens to contain no free hydrochloric acid, while later specimens contained free acid in fair amount. Some of these disadvantages may, of

course, be overcome by substituting oatmeal gruel for the tea and toast of the original Ewald test-meal, by passing a Ryle's stomach-tube instead of the ordinary large-size tube, and by including a separate examination of the fasting juice. Even then many of the disadvantages, already enumerated, of the single sample remain in full force, and, in addition, we have seen in studying fractional analyses that the actual shape of the free or total acid curves may be of diagnostic value, so that a method which does not permit of a curve being plotted at all is to be deprecated. Considering these disadvantages, it would appear all but impossible to obtain reliable and valuable information from the one-hour method, and there can be little doubt that, if time does not allow of a complete fractional test-meal, the simple examination of the fasting juice removed by means of a Ryle's tube will give all the information, and more, that can be obtained from a one-hour meal, besides being infinitely less disagreeable for the patient.

FAECAL BLOOD

A test which should not be omitted in any case in which gastric or duodenal ulceration is suspected is the examination of the stools for blood. Its presence in the stools is, of course, simply an indication of bleeding in some part of the alimentary tract, but bleeding from the lower part usually gives rise to blood stains which are apparent in the naked-eye inspection, whereas bleeding in the stomach or duodenum usually causes the presence of occult blood which can be detected only by chemical means. (See Appendix I.)

This test is exceedingly delicate. When it is negative it is definite evidence that bleeding is not occurring in the alimentary tract. A strongly positive result indicates bleeding, but a faintly positive reaction is frequently obtained when meat is included in the diet. When a faint reaction is observed, therefore, meat should be excluded from the diet for two days and the test repeated.

CHAPTER VIII

TESTS OF PANCREATIC FUNCTION

THE internal secretory function of the pancreas has been discussed already in Chapter V, during the consideration of glycosuria. Failure of its other function, the supply of an external secretion which contains the most important digestive enzymes in the body, has still to be studied. Disturbances of this second function of the pancreas have been supposed to be comparatively rare, probably because they are seldom diagnosed rather than because they are actually infrequent. Few cases of pancreatic disease, affecting the external secretion, give rise to any typical syndrome which points definitely to the pancreas as the seat of the trouble, and in consequence many cases must escape recognition. With a view to placing the diagnosis of such conditions upon a sounder basis, a number of pancreatic function tests has been suggested at one time or another. Many of them are laborious, and, except in certain cases, they usually give rather unsatisfactory results.

In order to understand the rationale of these tests—of which only the more important are referred to here—it is necessary to note the enzymes which the pancreatic secretion contains. These are :—

(1) Diastase (amylase), the starch-splitting enzyme, which continues the work begun by the ptyalin of the saliva and hydrolyses starch into maltose ; the work

is completed by the enzyme maltase, which further hydrolyses maltose to glucose.

(2) Lipase (steapsin), which splits the fats into glycerol and fatty acids.

(3) Trypsin, which becomes active trypsin on coming into contact with the enterokinase of the succus entericus, and plays a great part in the hydrolysis of proteins, proteoses, and peptones to amino-acids.

Pancreatic function tests are, therefore, based upon the presence of the three chief enzymes here enumerated.

TESTS BASED UPON THE PRESENCE OF DIASTASE

As we have already seen (p. 138), some of the diastase which the pancreas secretes into the intestine is absorbed into the blood, and is thence excreted by the healthy kidney in amounts varying roughly from ten to twenty-five diastatic units, this unit being the number of cubic centimetres of 0.1 per cent. starch solution digested by 1 c.c. of the urine. In cases of acute pancreatitis very large amounts of diastase pass into the blood, and hence a very high diastatic index is found in the urine. The exact cause of this great escape of diastase into the blood in this condition is debatable. In biliary obstruction the bile pigment, failing to get into the intestine, is removed by the blood and eventually appears in the urine. Similarly it is possible that in the pancreatic obstruction following severe inflammation and swelling of the cells, the diastase escapes through the damaged epithelium into the blood-stream and is excreted in high concentration by the kidney. This explanation is, however, not an entirely satisfactory one, since

diastase is found in the urine when no pancreatic obstruction exists, and in experimental animals it is found that the diastatic index is greatly increased after the complete extirpation of the pancreas. It is obvious, therefore, that the pancreas is not the only gland in the body which is responsible for the supply of diastase, and it is almost certain that the liver also is responsible to some extent. The high urinary diastase found in acute inflammation, or after extirpation, of the pancreas has thus been explained by some authorities as due to the liver taking on the production of diastase in greatly increased amount when the supply from the pancreas fails. Neither of these explanations is perfectly satisfactory, nor is any other of those which have been advanced; but, whatever be the explanation, there is no doubt that in acute pancreatitis, instead of a normal urinary index of 6-25 diastatic units, there is usually one of over 100, and often of much over 200.

Where acute pancreatitis is suspected this test is, therefore, of very considerable value, since the acute abdominal signs and symptoms encountered are often of such a nature as to make an accurate diagnosis a matter of the greatest difficulty without chemical aid. Again, the test has the advantage over many of the other chemical tests used in medicine of being exceedingly definite, the results of the analysis showing a gross change from normal quite outside the bounds of experimental error or of physiological variation, and which is given by no other condition to the same extent. A diastatic index of over 100, when accompanied by symptoms of acute abdominal disease, is significant, and one of over 150 is practically diagnostic of acute pancreatitis.

The test, which is otherwise performed in the same way as the diastase test for renal function (p. 138), is carried out on the first specimen of urine which can be obtained, since it would, obviously, be ridiculous to wait for a twenty-four hour specimen before making a diagnosis of such an acute malady. The small variations in the diastatic index which occur in the specimens passed throughout the twenty-four hours—variations which may be of importance in the renal function test—are, of course, of no significance in this condition, where only large abnormalities are taken into account.

Cases of acute pancreatitis are, however, comparatively uncommon, and are seldom encountered under circumstances where the diastase test can be performed. The test, therefore, has a necessarily limited application, and it is only in acute pancreatitis that it is of any service as a pancreatic function test. It is quite useless as an index of chronic pancreatitis, where readings range from the rather low values obtained in cases of slight renal deficiency to somewhat above the normal maximum. If a chronic disease of the pancreas is suspected it is necessary to have recourse to other tests.

It would seem logical to test the faeces for the presence of diastase, or of one of the other enzymes, so that an index of pancreatic efficiency might be obtained in this way. Several tests for the presence of diastase and trypsin in the faeces have been described, but they have all proved most unsatisfactory. Consequently the pancreatic function is usually investigated by the less direct method of examining the faeces chemically or microscopically for evidence of incomplete digestion of foodstuffs, which

would result from diminution or absence of the pancreatic enzymes.

TESTS BASED ON THE ACTION OF LIPASE

In the normal subject most of the fats taken in the food are absorbed from the intestine after being acted on by the lipase from the pancreas and the bile from the liver. A small part of the ingested food, however, escapes absorption, and is excreted in the faeces. Normally, about 15–25 per cent. of the dried stool consists of fat and fatty acids, but it seems probable that this is by no means entirely derived from unabsorbed residues. Part, it has been suggested, is due to actual re-excretion through the intestinal mucosa, and a little, conceivably, is due to bacterial synthesis. However this may be, it is an undoubted fact that animals fed on a fat-free diet for considerable periods still produce faeces containing little less than the normal amount of fat.

Fat digestion may possibly begin in the stomach, through the action of the gastric lipase and of the hydrochloric acid. This digestion is, however, of little importance, and by far the greatest amount of hydrolysis occurs in the small intestine, where the fat meets the pancreatic lipase. Intestinal digestion is greatly aided by the bile acids, which, by emulsifying the fat, accelerate the action of the enzyme. The fatty acids liberated during the hydrolysis are absorbed in loose combination with bile acids. Since the reaction of the intestinal contents is normally acid, soluble soaps are not formed, though a small amount of the fatty acids may combine with calcium (or magnesium) to form insoluble compounds which escape absorption. In-

gested fat is usually absorbed almost completely, but the fat re-excreted into the intestine is also subjected to the action of lipase and, if it remains in the alimentary tract for the normal length of time, is nearly completely hydrolysed. It is evident that two main factors are concerned in the hydrolysis and absorption of fat—pancreatic lipase and bile acids. Deficiency in either can cause decreased hydrolysis and absorption, but lipase must be expected to have the greater effect on hydrolysis, while the bile acids have the greater effect on absorption.

The excreted fat, then, has normally been largely split by the action of the lipase into fatty acids, some of which have been converted to insoluble (calcium) soaps, but a little of the fat has escaped the fat-splitting enzyme and is present in the stool as neutral fat. Thus the total fat in the stool should not, in health, form more than 25 per cent. by weight of the dried faeces, and should be made up approximately of 80 per cent. of "split fats" (fatty acids and soaps) and not more than 20 per cent. of neutral or unsplit fat. These figures are subject to variation from day to day even in health, and only gross variations from the average should be taken as significant.

When the external secretion of the pancreas is deficient there is a tendency for the total fat in the stools to increase above 25 per cent. This tendency is considerably exaggerated when the pancreatic lesion is associated, as it so often is, with a simultaneous biliary deficiency. In such cases large, bulky, pale stools are passed in which fat droplets can be seen—the fat forming up to 80 per cent. of the dried faeces. The characteristic feature of the faeces, how-

ever, in pure pancreatic deficiency is not so much the increase in total fat as the increase in the percentage of unsplit fat, which, in a severe case, may form from 80-90 per cent. of the total fat in the stool instead of the normal 20 per cent. or less. On microscopical examination, therefore, oily globules, but not an excess of fatty acid crystals or of soap plaques, may be found. A small quantity of split fat is nearly always present, even in the worst cases in which no lipase, possibly, is being secreted at all, since a certain amount of fat-splitting is carried on in the stomach and by the intestinal bacteria. A characteristic analysis of the faeces in a case of pure pancreatic deficiency is shown in the following Table (Table X (b)). Table X (c)

TABLE X

THE FAECES FATS

	<i>Total Fat</i>	<i>Split Fat</i>	<i>Unsplit Fat</i>
	<i>Percentage of Dried Faeces</i>	<i>Percentage of Total Fat</i>	
(a) Normal	19	80	20
(b) Pancreatic insufficiency	40	25	75
(c) Pancreatic insufficiency with obstructive Jaundice	76	19	81
(d) Jaundice alone, Coeliac Disease or Sprue	60	76	24
(e) Diarrhoea, small intestine	46	60	40

shows the results of an analysis in a case in which the pancreatic deficiency is associated with obstructive jaundice. Here it will be seen that fat-absorption is much more deficient than it was when the pancreas was alone responsible, and fat-splitting is as poor as

ever. Table X (*d*) supplies the figures of an analysis of the stool from a case of obstructive jaundice unassociated with pancreatic deficiency. It will be noted that though fat-absorption is inefficient, fat-splitting is going on perfectly well.

It is apparent, then, that a considerable amount of information may be obtained by the analysis of the faecal fat content, provided the results obtained all tend to show the same features, and a single examination is not relied upon. In this way some evidence may be obtained as to the true cause of certain very obscure disorders, such as emaciation associated with nervous symptoms, and some cases of infantilism. It may also give an index of chronic pancreatitis, pancreatic calculus blocking the main duct, and carcinoma of the pancreas. A source of fallacy lies in the fact that occlusion of the main duct may be partially compensated for by the existence of the subsidiary duct of Santorini. Again, carcinoma of the pancreas may or may not give rise to deficiency in fat-splitting. In fact, it is probable that no deficiency arises till the growth is very far advanced, or unless complete occlusion of the duct has taken place. Lastly, there is no doubt that the work of the pancreas in fat-splitting may be taken on to some extent by the bacteria of the intestine and possibly also by the gastric juice. This may account for the fact that cases are not unknown in which the pancreatic function is markedly deficient, but which show relatively little abnormality in the faecal fat. From these considerations, therefore, a negative finding does not exclude the possibility of pancreatic disease, and only a positive result is of importance in diagnosis.

It must be remembered, further that, a high total fat in the stools, and even some deficiency in fat-splitting, may be occasioned by other conditions besides disease of the pancreas. Thus diarrhoea due to irritation of the small intestine may give rise to such results, owing to the fact that the food does not remain long enough in the small intestine for the fat to be efficiently absorbed, or, indeed, in the alimentary canal as a whole for the enzyme to exert its full action. Thus it is not unusual for children with abdominal tuberculosis, causing irritation and hurry in the small intestine, to have stools with a high fat content, and occasionally with some increase in the unsplit fat. A bulky, fatty stool may also be found where mesenteric tuberculosis blocks the lacteals. Diarrhoea affecting only the large intestine—that is, colitis—will not cause any upset in the total fat found in the faeces, nor usually in the proportion of split and unsplit fat which the stool contains. The test may thus, on occasion, be of some service in differentiating between a diarrhoea due to a pathological condition of the large intestine only and one involving the small intestine as well. It must be remembered, as a practical corollary of this, that the application of the test as a guide to the diagnosis of pancreatic disease is useless if diarrhoea is present or is induced by the administration of purgatives.

Lastly, the total amount of fat in the faeces may be greatly increased in coeliac disease. The aetiology of this condition is still somewhat obscure. It is associated with markedly deficient absorption of fat, but with less marked deficiency in fat-splitting and is possibly due to some abnormality in the bile salts. Consequently, while the total fat is greatly increased

in the stools, the ratio of split to unsplit fat may be nearly normal. The inspection of the very typical bulky, greasy stool passed in coeliac disease, combined with the characteristic clinical appearance of the child, usually makes the diagnosis definite enough, without any need of confirming it by chemical methods. In less definite cases, however, the analysis of the stool differentiates the condition clearly from that of chronic pancreatitis. In sprue a bulky, fatty stool is found, but the fat-splitting tends to be even better than in the normal case.

It must be remembered that the estimation and analysis of the faecal fat is a laborious procedure, and, therefore, it is desirable to avoid it, if the same information can be obtained by the simpler methods of naked-eye and microscopical examination. Inspection methods, however, cannot yield satisfactory evidence of the relative amounts of split and unsplit fat.

TESTS BASED ON THE ACTION OF TRYPSIN

Many tests of pancreatic function have been based on the tryptic action of the pancreatic juice. Of these there is probably only one which is of any practical importance, and this consists in the examination of the faeces for undigested muscle fibres. A few meat fibres are usually found in the healthy stool when meat is included in the diet, but, owing to the action of the pancreatic juice, they show practically no striations, and the ends of the fibres are regular. It would appear that the trypsin secreted by the pancreas is essential for the digestion of meat fibres to advance to the stage at which their striations disappear. If protein digestion be inefficient owing to lack of this

enzyme, meat fibres will be present in the faeces in an excessive quantity, and the great majority will be striated, and will have irregular ends. It is well to bear in mind that the same effect is produced by diarrhoea, particularly when it affects the small intestine, for in this case the meat will not be in contact with the pancreatic enzyme for a sufficient length of time to ensure proper digestion, even though the pancreatic secretion may itself be perfectly normal. Conversely, if pancreatic secretion is deficient, constipation may so lengthen the period of digestion as to cause an apparently normal disappearance of striated muscle fibres. The discovery in the stool of numerous well-striated meat fibres with irregular ends thus points strongly to a pancreatic deficiency where constipation can be excluded. Their absence, however, does not exclude pancreatic disease.

Some emphasis has been laid on the presence of nuclei in the animal cells of the faecal matter, since it is supposed that the pancreatic juice is entirely responsible for their digestion. Thus if the pancreas is functioning efficiently, no such nuclei should be present in the stool. Several tests involving somewhat complex histological procedures have been based on this point. The results obtained are usually unsatisfactory, and are hardly worth the labour involved.

It would seem that some information as to the pancreatic juice might be obtained from its direct withdrawal by the passage of a Ryle's tube into the duodenum. This has indeed been attempted, but again the results obtained are not of sufficient value to warrant this somewhat difficult and laborious procedure being undertaken. The withdrawal of the duodenal contents is probably of more use in the

diagnosis of inflammatory and infective conditions of the biliary tracts than as a pancreatic function test.

SUMMARY

In summary, then, acute pancreatitis can be diagnosed almost certainly by the application of the diastatic test, which is, however, of no service in more chronic pancreatic lesions. Some light on the latter may be shed by the microscopical examination of the stool, and by an analysis of its faecal fat content.

CHAPTER IX

TESTS OF HEPATIC FUNCTION

THE multifarious activities of the liver include the secretion of bile and the conversion of glucose and certain other sugars to glycogen, and on these two activities are founded the most important tests of hepatic function. It is not that these are necessarily the most important of the liver functions, which include the obscure mechanism by which the liver participates in the production of red blood cells; the oxidation of amino-acids not required for immediate tissue repair; the synthesis, from certain poisonous substances, of other harmless compounds which can be excreted; the replacement, if need arise, of the pancreas in the production of digestive enzymes; and the excretion, in the bile, of certain foreign substances. Obviously these various activities cannot be ranged in order of importance, but many of them do not lend themselves readily to measurement.

It is proposed, in this account of the more important of the hepatic tests, to deal first with the formation of bile pigment, and this for two reasons. Firstly, an abnormality in the formation of bile pigment, whatever its cause, invariably manifests itself in the pigmentation of the skin, and since this single clinical sign of jaundice may be due to a number of causes, it is convenient to consider it separately. Secondly, while functional deficiency, as shown by

any other of the tests, implies some damage or destruction of the liver cells, this is by no means the case with disturbance of the bile-pigment production, since such a disturbance may be extra-hepatic in origin, whatever its *ultimate* effect on the liver.

THE PRODUCTION OF BILE PIGMENT: JAUNDICE

The van den Bergh Test

During the last two decades our views of the origin of jaundice have been revolutionised, and the revolution has involved a return, in part, to the old classical division of jaundice into two types, hepatogenous and anhepatogenous.

This, of course, is not the place for a full discussion of the modern theory of jaundice—which, be it remembered, is still a theory, and is by no means completely proved, though it rests on a good deal of experimental evidence. It is necessary, however, to consider very briefly the theory and its implications with respect to the production and fate of bile pigment. It is based on the view that bile pigment—mainly bilirubin—is elaborated from the haemoglobin of the red blood corpuscles in the cells of the reticulo-endothelial system—possibly those of the spleen, possibly the Küpffer cells of the liver. The bile pigment formed in these cells is taken up by the polygonal cells lining the bile channels, and, after slight modification, is transferred to these channels. According to this view there are two slightly different forms of bile pigment, one which has passed through the polygonal cells, and one which has not. The existence of these two forms of bilirubin is evidenced

from the application of van den Bergh's test, to be discussed shortly. Moreover, in spite of some failures, there have been enough claims to the isolation of two slightly different bile pigments to make their existence at least probable.

If this theory of pigment formation is correct, it is evident that jaundice, which is essentially an accumulation of bilirubin in the blood, with deposition of the pigment in the tissues as a secondary consequence, may arise in several ways. Thus :—

(1) It may be caused by obstruction to the escape of bile from the fine bile channels, the pigment, which has passed through the polygonal cells, being reabsorbed into the blood-stream.

(2) It may be due to an inability of the polygonal cells to allow entrance of the pigment manufactured by the reticulo-endothelial cells, the pigment, therefore, passing direct into the hepatic vein. The blood, in jaundice so produced, contains an excess of bilirubin which has not passed through, and been modified by, the polygonal cells.

(3) It may be due to the production of an excessive amount of bilirubin—more than the polygonal cells, though functioning normally, could deal with. The result, so far as the blood is concerned, is the same as that of damage to the polygonal cells.

(4) It may be due to a combination of obstruction and damage to the polygonal cells or over-production of pigment. In this case both types of bilirubin would accumulate in the blood.

Van den Bergh, applying the diazo-benzene sulphionate reagent of Ehrlich to icteric sera, found that in cases of frankly obstructive jaundice a colour developed with great rapidity—a result obtained also

with bile from the gall-bladder. He called this the *immediate direct reaction*. Normal serum contains a little bilirubin, though insufficient to react under the conditions of the test. On the other hand, sera from cases of haemolytic jaundice, or from haemorrhagic effusions into the chest or abdomen, gave a colour only after a long delay (or not at all)—*delayed direct reaction*. Hence an immediate direct reaction is taken to indicate the presence in excess of bilirubin which has passed through the polygonal cells, and therefore is to be expected when the first of the above-mentioned causes of jaundice is operating. A delayed direct reaction indicates the presence of pigment which has not passed through these cells, and is therefore to be expected in jaundice due to the second and third theoretical causes. It has later been found that in some cases of jaundice the serum gives a direct reaction, the *biphasic reaction*, which is a mixture of van den Bergh's two types, and consists in an immediate slight coloration, gradually increasing in depth. It is taken as indicating the presence of both forms of bilirubin, and corresponds, therefore, to the fourth theoretical type of jaundice mentioned above—that is, in practice, to the toxic and infective jaundices.

All sera which give a positive direct direction, whatever the type of reaction, behave similarly when protein is precipitated by alcohol and the diazo-reagent is added to the filtrate. This is called the *indirect reaction*, and is of particular importance, because, being more sensitive than the direct reaction, it can detect hyperbilirubinaemia, which would pass unnoticed if only the direct reaction were employed. Indeed, if the test is carefully carried out under

optimal conditions, a faint, but recognisable (pink) colour is given even by normal sera. It is of importance, too, in that it is not obscured, as is the direct reaction, by the presence of haemoglobin in the serum, since this pigment is precipitated along with the other proteins by alcohol.

The indirect van den Bergh reaction may be used as a means of estimating quantitatively the amount of bilirubin in the blood. Normally the values appear to lie between 0.1 and 0.3 mg. per 100 c.c. of serum, or, taking van den Bergh's "unit" of 0.5 mg. per 100 c.c., between 0.2 and 0.5 units. The original quantitative method is not very convenient, however, for clinical work, and a simpler technique is that of Meulengracht, who compares the colour of serum, suitably diluted, with that of a standard solution of potassium bichromate, equality of colour representing unity, as the "icteric index." Using this technique, the normal "icteric index" lies, in our experience, between 4 and 8, though some others prefer the lower range of 1 to 5. Though simple, the test is not specific and a high icteric index is found, for example, in carotinaemia. It is, moreover, impossible to obtain a colour comparison if haemolysis has occurred during collection of the sample.

The findings in the various types of jaundice may be summarised as follows :—

Obstructive Hepatic Jaundice.—In jaundice of this type an immediate direct reaction is obtained, and quantitatively the amount of bilirubin in the serum may be very large, as much as 50 van den Bergh units having been reported—an amount equal to that found in bile from a gall-bladder fistula. The serum

bilirubin bears no relation to the depth of skin pigmentation, which is relatively slow in appearing, so that a high serum bilirubin, or icteric index, may be found with little or no pigmentation. Pigmentation of the skin is equally slow in disappearing, so that it persists after removal of the obstruction, when the icteric index may have fallen. In the later stages of obstructive jaundice, too, there is a tendency for the manufacture of bilirubin to diminish, or even cease; so that the icteric index may be quite low, even though the skin is still markedly pigmented. Bilirubin of the type present in this condition appears in the urine only when the serum content reaches about 4 van den Bergh units or the icteric index reaches about 50. Jaundice of the obstructive type may be due, for example, to occlusion of the common bile duct by gall-stones, which, however, produces little or no increase in the icteric index unless the occlusion is complete. It may be caused also by carcinoma of the head of the pancreas involving the orifice of the common duct, or by carcinoma of the liver itself completely occluding one or more of the groups of bile capillaries.

In practice, unfortunately, the immediate direct reaction is obtained only in the early stages of an obstructive jaundice. The accumulation of bilirubin in the blood tends to cause damage to the liver cells; the obstruction to the bile flow causes an accumulation of bilirubin in the polygonal cells and so prevents them from obtaining and dealing with the fresh supplies of bilirubin which the reticulo-endothelial cells continue, for a while, to produce. Both of these processes lead to an increased concentration in the blood of bilirubin which has not passed through

the liver, and so to a van den Bergh reaction of the biphasic type. It may still approximate to the immediate type of reaction, but so may that obtained in a jaundice of frankly infective origin (*vide infra*).

Haemolytic Jaundice.—In haemolytic jaundice the direct reaction is either of the delayed type (as it is usually) or negative, but the indirect reaction is positive, and shows quantitatively that the serum content of bilirubin is high. The very excessive amounts of bilirubin occasionally found in obstructive jaundice do not occur here, where the value is rarely above eleven units, or, in terms of icteric index, about 100. Even at this level, biliuria is not present, though the urine usually contains urobilin, formed, possibly, from the bilirubin which has not passed through the polygonal cells. Apart from following the course of the jaundice—for which purpose, as has been explained, pigmentation is of little value—and from detecting latent jaundice (*vide infra*), possibly the chief clinical value of a van den Bergh test in this type of jaundice lies in distinguishing pernicious or splenic from other types of anaemia. In haemolytic anaemias the bilirubin content of the blood—whether skin pigmentation is present or not—is invariably sufficient to give an indirect reaction with the van den Bergh technique.

A toxic jaundice due solely to hepatic damage ought also to give a van den Bergh reaction of the delayed direct type—*e.g.* salvarsan jaundice. Secondary effects, however, again tend to produce a mixture of the two types of bilirubin with, in consequence, a biphasic reaction.

Toxic and Infective Jaundice.—This group includes the jaundice which complicates acute fevers (pneu-

monia, typhus, spirochaetal jaundice, etc.); which follows administration of such drugs as chloroform, arsenicals and phosphorus; and which accompanies certain types of liver damage such as yellow atrophy, or such general infective or toxic conditions as acute sepsis. Here, again, the van den Bergh is unfortunately—for here a reliable test would be of great service—of little clinical value. Typically, a biphasic direct reaction is obtained, but, according to whether the cell damage or the obstruction is the predominating factor, the result of the test may approximate so closely either to the immediate or to the delayed reaction as to be indistinguishable from one or other of them. Thus catarrhal jaundice, which belongs to this class, cannot be distinguished by the van den Bergh test from obstructive jaundice, with which it is most liable to be confused. In catarrhal jaundice the obstruction is often the main factor, and the van den Bergh reaction, though strictly biphasic, may consequently simulate the immediate direct reaction very closely, and may even be indistinguishable from it.

Latent Jaundice.—It is obvious that jaundice, in the sense of bilirubinaemia, may be present without biliuria, which appears only when the serum bilirubin reaches some four units with obstructive jaundice, and not at all with haemolytic jaundice. Equally it may be present without skin pigmentation, which develops slowly. Such a jaundice is said to be latent. With the van den Bergh reaction it is readily detected; without it, it is not so easily found, though it is frequently accompanied by the presence of urobilin and bile salts in the urine. Latent jaundice occurs quite often in chronic liver disease, such as cirrhosis, when

an immediate direct reaction (often, however, faint) is obtained. Similarly, an indirect reaction, with, at times, a delayed direct reaction, is found in all cases of pernicious anaemia, though there is not always pigmentation of the skin. The detection of latent jaundice giving an indirect or biphasic direct reaction may be a useful warning in cases treated with salvarsan, the continued use of which is not infrequently followed by severe, or even fatal, jaundice.

The van den Bergh Test in Pathological Fluids.—Pathological fluids, such as may be drawn from the peritoneal or pleural cavities, may contain haematoidin, which is probably isomeric or identical with bilirubin, and which responds to the van den Bergh test. Haematoidin is produced by the breakdown of haemoglobin *in situ*, and consequently occurs only in fluids which have contained blood for some time. The test may therefore be used to decide whether in a haemorrhagic effusion, or in aspirated fluid containing blood, the blood has been present for some time, or was introduced at the time of puncture. In the former case a positive indirect reaction is obtained, in the latter none. This holds, of course, only if blood drawn direct from a vein is normal. Similarly, the test may be used to decide whether the yellow colour of a sample of cerebro-spinal fluid is due to a lipochrome or to a haemorrhage of one or two days' standing.

Other Tests in Jaundice

It has to be confessed that the van den Bergh reaction, while of considerable academic interest for the understanding of the etiology of jaundice, is of little or no value in differentiating the various types of jaundice. Its main use, as has been pointed out, is

in detecting latent jaundice and, in the quantitative form (which may be replaced by Meulengracht's icteric index determination), for following the course of a jaundice.

Examination of the urine and faeces will often yield information of greater value for a differential diagnosis than will the van den Bergh reaction, though here, too, caution is required in interpreting the results in view of the possible secondary changes which may be superimposed on any primary type of jaundice.

Excretion of Bile Pigments.—The bilirubin excreted by the liver is reduced by bacteria to hydrobilirubinogen, and this is further converted to the brown pigment hydrobilirubin, which is mainly lost in the faeces. A small amount of the hydrobilirubinogen, however, is reabsorbed, and of this some is reconverted to bilirubin by the liver and returned as such to the intestine, while a little (not detectable by the ordinary tests) is excreted by the kidney as urobilinogen, which is probably identical with hydrobilirubinogen. This, on exposure of the urine to light and air, is oxidised to urobilin (not identical with hydrobilirubin).

With excessive breakdown of haemoglobin the rate of formation of all these substances is increased, so that in haemolytic jaundice we expect, and usually find, very dark stools containing excessive amounts of hydrobilirubin, and much increased urinary excretion of urobilinogen. The urine may even contain bilirubin itself, though seldom more than a trace.

Damage to the liver cells without obstruction to the flow of bile, on the other hand, will not produce excessively dark stools (and indeed they are often pale, since the formation of bile is decreased). Nor, in consequence, is there increased excretion of uro-

bilinogen (or urobilin), though there may be traces of bilirubin.

Partial obstruction of the bile passages causes a decreased excretion of bile with, therefore, pale stools and no increased urobilinogen in the urine. On the other hand, the accumulation of bilirubin in the blood may, if it is sufficiently large, lead to the appearance of that pigment in the urine. These findings are, of course, accentuated in complete obstruction.

Jaundice of the infective or catarrhal type is usually the result of functional destruction of the liver cells in association with some degree of obstruction. Hence the stools contain a diminished amount of pigment, the urine contains only the normal amount of urobilinogen (or little more), though it may contain considerable amounts of bilirubin.¹

Of the various functions performed by the polygonal cells of the liver, it seems that the most difficult is the conversion of hydrobilirubinogen to bilirubin. Consequently the earliest sign of liver damage involving these cells is the excessive excretion of urobilinogen in the urine. As the damage to the cells increases, the excretion of bile pigment into the intestine decreases. Hence even less hydrobilirubinogen than normal is formed, and therefore urobilinogen disappears from the urine, being replaced by bilirubin.

Excretion of Bile Salts.—The bile acids, which are condensation products of glycine (amino-acetic acid) or taurine (ethylamine sulphonic acid), with a variety of hydroxy-acids closely related to the sterols, are

¹ Urobilinuria is present in many conditions not regarded as involving liver damage—*e.g.* fevers, severe constipation, pernicious anaemia—and during the absorption of large extravasations of blood.

normally excreted as salts with the bile and so completely reabsorbed that they are not detectable in the faeces. Obviously, therefore, obstruction to the flow of bile cannot be detected by examination of faeces for bile salts, and, further, they cannot be expected to be affected by alterations in the rate of haemoglobin breakdown. On the other hand, obstruction to the excretion of bile must cause the accumulation of bile acids, and therefore some leakage into the urine is to be expected. A similar state of affairs will arise when there is damage to the liver cells, and we consequently find urinary excretion of bile salts in obstructive jaundice, jaundice of hepatic origin, but not in haemolytic jaundice (which is thus an example of "dissociated jaundice"). In catarrhal jaundice bile salts are said to appear in the urine before the pigment, disappearing as bilirubinuria develops and reappearing later. The available tests for bile acids, however, are not very good.

THE GLYCOGENIC FUNCTION OF THE LIVER

The liver, as has been mentioned in a previous chapter, is the great storehouse of glycogen, which it synthesises from ingested glucose. It can, however, utilise for this purpose other sugars, such as galactose, which it normally gets from milk-sugar, and laevulose, which it normally gets from cane-sugar.

As long ago as 1901 Strauss noted that ingestion of fructose was followed by fructosuria in the great majority of his patients with liver disorders, but not, as a rule, in healthy persons. As a test for deficient liver function this was soon shown to be unreliable, since fructosuria after fructose ingestion is quite a

common occurrence. But in 1920 McLean and De Wesselow found that practically no hyperglycaemia followed the ingestion of 100 g. of fructose. When Spence and Brett found that under similar circumstances patients suffering from various types of liver disease showed definite hyperglycaemia, the **laevulose¹ tolerance test** in its more modern form was founded. It was soon realised, however, that the small number of normal persons studied by Spence and Brett had given too perfect a picture. It was therefore necessary to regard as normal (though actually it might not be so) any case in which the blood sugar, an hour after the ingestion of fructose, had risen less than 30 mg. per 100 c.c.

During the succeeding decade the laevulose tolerance test was severely attacked on both theoretical and practical grounds. Theoretically it was objected that no proof existed that the liver was primarily concerned in fructose metabolism, and that the pancreas was probably a more important factor. Practically it was objected, on the basis of an experiment by Mann, that an enormous amount of liver destruction must occur before the damage was revealed by the test; that since the test was invariably positive in diabetes, that condition must always be excluded, and that there was considerable dubiety as to the normal result.

Thus, although the test retained a number of supporters, it gradually fell into disuse if not disrepute.

Evidence has, however, accumulated in favour of the view that the liver is the main site of the conversion of fructose to glycogen. Glucose, on the other hand, is largely dealt with by extra-hepatic tissues. Moreover, the conversion of fructose to glucose, which

¹ The terms "laevulose" and "fructose" are synonymous.

appears to be the first step in glycogen formation, seems to be independent of insulin. Hence the fructose tolerance test is fundamentally sound. The recent evolution of methods enabling fructose to be estimated separately even in the presence of glucose has removed most of the practical obstacles, and in its newest form the test seems likely to be of some value. It cannot differentiate one type of liver disease from another, but an abnormal result with the new fructose tolerance test does seem to indicate liver damage, and the test is much more sensitive than Mann's experiments would suggest (see, however, the experiments of Rosenthal, quoted on page 214). A normal result, of course, does not indicate a healthy liver any more than a normal blood urea indicates perfectly healthy kidneys.

The test is carried out by a method very similar to that used in applying the glucose tolerance test for diabetes. The patient, fasting since the preceding evening, is given 50 g. of laevulose after a sample of blood has been obtained, and four further blood samples are withdrawn at intervals of half an hour. If the laboratory is equipped with a micro-colorimeter 0.2 c.c. of blood suffices; otherwise 2 c.c. are required. The samples are oxalated. Fructose is estimated in each sample. Normally the fructose concentration never rises above 20 mg. per 100 c.c. of blood (at half-hour or one hour after fructose) and falls to about 5 mg. per 100 after two hours. Figures greater than these, an increased maximum being the more important point, are indicative of hepatic insufficiency. The test can be successfully used in cases of diabetes (Fig. 26).

It is a matter of great practical importance to obtain a pure sample of laevulose and to give it in as palatable

a form as possible. With impure laevulose, nausea and vomiting frequently occur, owing, probably, to the presence of some irritating impurity. We ourselves give 50 g. of pure laevulose, dissolved in about 150 c.c. of water and liberally flavoured with lemon juice, and have had no trouble.

A galactose tolerance test has been used, but is not

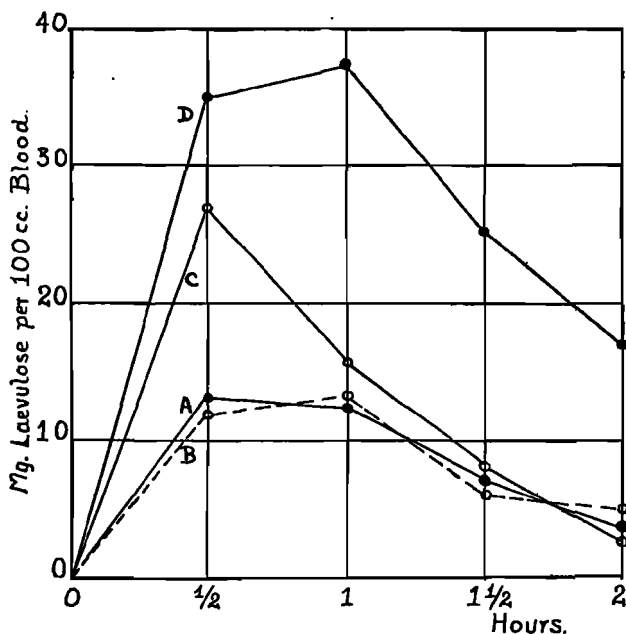


FIG. 26.—The blood laevulose after the ingestion of 50 g. of laevulose. A, normal; B, uncomplicated diabetes; C, cirrhosis of the liver; D, diabetes, with carcinoma of the liver.

of much value, since there is no satisfactory method of estimating galactose and the available evidence

does not sufficiently support the assumption that the liver is the main site of galactose metabolism.

THE LIVER AND AMINO-ACID METABOLISM

A great part of the amino-acids taken into the body is not used for the repair of tissue wastage, but is oxidised and used as a source of energy. This oxidation, which takes place to a large extent in the liver, involves, as a first step, the removal of the amino group in the form of ammonia. Ordinarily, this ammonia, along with carbon dioxide, is converted—again in the liver—to urea, which of course is excreted by the kidney. The synthesis of urea is so complete that the blood contains only mere traces of ammonia.

Although the blood contains so little ammonia, the urine contains quite considerable amounts. This ammonia is believed to be produced by the kidney, possibly, though not certainly, from urea. In health the ammonia excretion amounts to about 3 per cent. of the urea excretion. In other words, the "ammonia coefficient" = $\frac{(\text{ammonia nitrogen} \times 100)}{\text{urea nitrogen}}$

of the urine is about 3, with a range of 2 to 5. It has been suggested that liver damage may lead to deficient urea formation with a correspondingly increased ammonia concentration in the blood and an increased ammonia excretion. This would mean an increased ammonia coefficient. Such an increased coefficient is quite often, though not always, found when the liver is grossly damaged. Since, however, the ammonia coefficient is very sensitive to changes in the acid-base balance (see Chapter IV), it is at best difficult to inter-

pret. An equal lack of success attends other proposed methods for detecting insufficiency of liver function in relation to protein metabolism—*e.g.* concentration of amino-acids in blood or urine, blood urea and ammonia concentrations following amino-acids or protein meals, etc.

The urinary excretion of tyrosine usually, though not invariably, along with leucine, occurs somewhat rarely. It is usually associated with acute yellow atrophy, but may occur in other (non-hepatic) conditions in which there is very excessive breakdown of tissues. Tyrosinuria is, in fact, an indication simply of such an excessive breakdown, and not of an inability on the part of the liver to deal with amino-acids.

THE DETOXICATING POWER OF THE LIVER

The liver is probably the chief organ concerned in detoxicating a variety of poisonous substances which may be formed by putrefactive processes in the intestine or may be administered as drugs. The detoxication consists in combining ("conjugating") the poisonous substances with some other substance already present or manufactured for the purpose, so as to form a harmless compound which can be excreted. For combination with many toxic alcohols, phenols, or aromatic acids, glycuronic acid is used, and is probably obtained by oxidation of glucose; for combination with some of these toxic substances (and with other poisons not included in these classes) sulphuric acid is used. Thus indoxyl, formed from tryptophane by bacterial action, is excreted partly as a glycuronate, and partly as indoxyl sulphuric acid (urinary indican); benzoic acid appears in the urine to some slight extent

as a glycuronic acid derivative, but mainly combined with glycine (to form hippuric acid) or glutaminic acid.

The presence in the urine of these conjugation products formed for detoxication purposes depends upon the capacity of the liver and also upon the supply to it of the toxic substances. Hence, for example, absence of indican from the urine is as likely to be a sign of absence of the right kind of bacterial action on tryptophane as of deficient liver function. Attempts to abolish the intestinal factor by administering indoxyl or indole have not been very successful.

A similar state of affairs holds for glycuronates. Normally they may or may not be found in the urine in readily appreciable amounts, but administration of a substance such as aspirin (5 to 10 grains orally) or camphor (5 grains subcutaneously), which is detoxicated by conjugation with glycuronic acid, stimulates their production and excretion. In liver disease with very gross damage, the conjugation often fails to occur, and the urine remains free from glycuronic acid. Often, however, even a badly damaged liver produces satisfactory amounts of glycuronates (or else its function can be taken over by other tissues). Hence a failure to excrete glycuronates after aspirin or camphor is a sign of gross liver damage, but the sign is by no means sensitive, the amount of glycuronates excretion is not proportional to the liver damage, and a satisfactory excretion does not remove suspicion from the liver.

THE EXCRETION OF FOREIGN SUBSTANCES IN THE BILE

A number of foreign substances are, normally, excreted exclusively in the bile, and with hepatic

deficiency their removal from the body is more or less delayed. Any test founded on this excretory function—which is, of course, the basis of the S.T.I.P.P. (sodium tetra-iodo-phenolphthaleinate) method of radiological examination of the gall-bladder and bile ducts—requires the use of a substance which is easily recognised and estimated. Such a substance exists in phenoltetrachlorphthalein, which can be injected intravenously, is normally excreted to a very large extent by the liver, and can readily be detected, since it gives a reddish colour on addition of alkali. A similarly convenient dye is the closely related phenoltetra-brom-phthalein sulphonic acid, which is used as the sodium salt (“brom sulphalein”). In some cases of hepatic disease a little of the dye appears in the urine, but the amount is small, and excretion by the kidney is too irregular to be of service as a test of hepatic efficiency.

In the past the dye excretion has been estimated by examination of the faeces or of the duodenal contents withdrawn by duodenal intubation.

A much more convenient method of applying the dye excretion test consists in determining the amount of dye remaining in the plasma at various times after its injection. Normally, it is found, phenoltetrachlorphthalein disappears from the plasma rapidly and at a remarkably uniform rate, so that under the conditions of the test no more than 6 per cent. of the amount injected remains at the end of fifteen minutes, while the whole of the dye is removed within an hour. When the liver is damaged, however, considerable quantities of the dye are retained in the plasma for several hours, and improvement in the condition of the liver is accompanied by an approach to normal

in the rate of removal of the dye. Rosenthal, who introduced this modification of the phenoltetrachlorophthalein test, states that the method gives results that are quantitative, in that the degree of divergence from the normal of the curve of disappearance of the dye affords an index of the amount of functional damage. In rabbits he was able to detect the removal of as little as 12 per cent. of the liver, and the removal of 20 per cent. gave frankly abnormal curves.

After first drawing about 8 c.c. of blood from a superficial vein of the arm, 5 mg. of phenoltetrachlorophthalein per kilogramme of body weight are injected into the same vein. For injection, the required amount of dye, which is obtained in ampoules containing 5 mg. per 0.1 c.c., is drawn into a sterile syringe and diluted to about 25 c.c. with sterile physiological saline. The time of completion of the injection is noted, and the vein wall is then washed free of dye by injecting a further 20 c.c. of saline through the same needle. At the end of fifteen minutes, counting from the completion of the injection of the dye, 2 c.c. of blood are drawn from a vein of the other arm, and a second sample is obtained at the end of an hour. Later samples may be drawn if the second sample, on analysis, shows the presence of the dye.

Though this modification of the phenoltetrachlorophthalein test appears to be more delicate than the older forms, and to give some idea of the amount of functioning liver tissue remaining, it, like other hepatic function tests, fails to differentiate the various types of liver damage. Rosenthal notes, however, that the degree of abnormality as shown by this test does not parallel the degree of jaundice or hepatic enlargement, and that in early jaundice due to mechanical

obstruction, before extensive damage to the liver cells had been produced, the disappearance curves were practically normal.

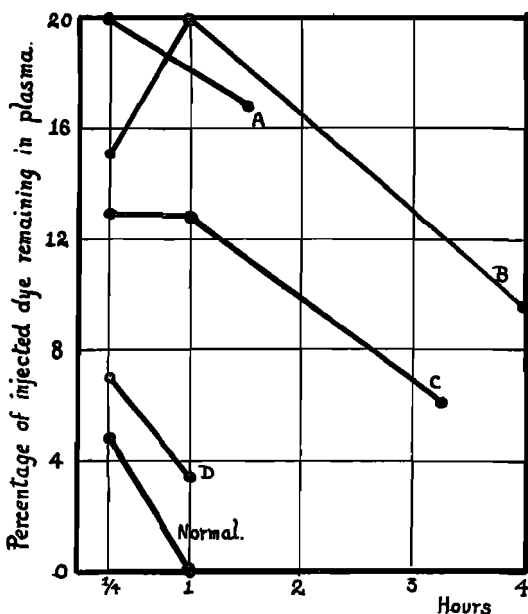


FIG. 27.—Curves showing the disappearance of phenol-tetrachlorophthalein from the blood in various conditions. (After Rosenthal.)

- A—Acute hepatitis after arsphenamine.
- B—Atrophic cirrhosis.
- C—Carcinoma of the liver.
- D—Acute cholecystitis.

Brom sulphalein is used in the same way as phenol-tetrachlorophthalein and gives similar results. The amount to be injected is rather less—2 mg. per kilo body weight, given in 5 per cent. solution. Normally,

no dye (or only a trace) remains in the plasma at the end of 30 minutes, though in non-hepatic diseases amounts up to 5 per cent. of the initial concentration may be found. More than that indicates liver deficiency.

CHAPTER X

THE CEREBRO-SPINAL FLUID

THE examination of the cerebro-spinal fluid is a factor of the greatest importance in the diagnosis of diseases of the central nervous system. In the early stages of many of the acute forms of such diseases it is usually impossible to make an accurate diagnosis, and, therefore, prognosis, without recourse to such an examination. Even in the more chronic forms of central nervous system disease the cerebro-spinal fluid often provides diagnostic evidence of the greatest value.

The information which the examination of the cerebro-spinal fluid yields is based largely upon cytological and bacteriological evidence, and is, therefore, outside the scope of this book ; but a chemical analysis is by no means to be despised, and may, on occasion, give diagnostic results after the cytological and bacteriological examinations have failed to be of service. It is necessary, however, before discussing the changes met with in pathological cerebro-spinal fluid, to refer briefly to the characteristics and more important chemical constituents of the healthy fluid.

THE NORMAL CEREBRO-SPINAL FLUID

The methods by which cerebro-spinal fluid may be withdrawn have been discussed in Chapter II, and, provided no contamination by blood has occurred

during the process, the fluid is found, in health, to be perfectly clear and colourless, and to remain so on standing, provided it is protected from bacteria by stoppering the test-tube containing it with cotton-wool. In addition, no sedimentation of any kind occurs, owing to the fact that the normal fluid contains only one or two cells per cu. mm. and very little protein.

The chemical constituents of the cerebro-spinal fluid are similar to those of blood, though they are not always present in the two fluids in similar concentration. Thus, while some are present in approximately the same concentration in blood and spinal fluid, others are present in much lower concentration in the cerebro-spinal fluid than in blood, and one, at least, is in much greater concentration.

Protein Content.—In the healthy fluid protein may be present in quantities ranging from 14 to 50 mg. per 100 c.c., with an average of about 28 mg. per 100 c.c. The protein content is not influenced to any appreciable extent by the age of the patient, but if the fluid be removed from the ventricle, instead of from the cistern or lumbar regions, the protein content is usually found to be considerably diminished. A reading of over 50 mg. per cent. for the total protein may be taken to be definitely pathological. The total protein in the healthy fluid is made up almost entirely of albumin and pseudo-globulin. In pathological fluids euglobulin and fibrinogen make their appearance, while the normal proteins are increased in amount. The amount of globulin never exceeds that of albumin, but insufficient work has been done on the determination of their relative proportions in health and disease to make their separate estimation

of value. All the information required may be obtained from an estimation of the total protein, which is preferable to the more cumbersome and less reliable method of estimating the globulin alone. A less satisfactory, but simple, substitute for the estimation of total protein is the qualitative test for globulin, the technique of which is so arranged that the small amount of globulin present in healthy fluid fails to give a positive result.

Sugar Content.—The relationship of the sugar content of the cerebro-spinal fluid to that of the blood is to some extent controversial, probably owing to the fact that different methods of estimation have been used by various investigators. The consensus of opinion appears to be that the normal concentration of sugar in the cerebro-spinal fluid varies from 45 to 85 mg. per 100 c.c. Thus it is probably not much, if at all, less than the concentration of glucose in the blood, for, as has been explained (p. 82), the blood contains reducing substances other than glucose, and these are included in estimations by some methods, so that the figures obtained by their use are too high. Recent accurate methods for estimation of the blood sugar suggest that the normal range, instead of the 90–110 formerly taken, is actually from 70 to 90 mg. per 100 c.c. The sugar in the ventricular fluid is usually said to be higher than that in the fluid obtained by lumbar or cistern puncture.

Chloride Content.—The chloride content of the cerebro-spinal fluid is higher than that of the blood, and is found, in adults, to vary within the range 720–750 mg. per cent. (calculated as sodium chloride). Any reading above or below these amounts must therefore be looked upon, in adults,

with suspicion; but in young children a somewhat greater physiological variation may be observed, so that readings between 700 and 760 mg. per cent. may be consistent with health. The chloride content of the fluid seems to be similar in the ventricles, the cisterna magna, and the lumbar region.

Urea Content.—Owing to the fact that urea is a very easily diffusible substance, it is not surprising that the concentration in the cerebro-spinal fluid should usually be almost identical with that found in the blood, though it may sometimes be a little lower. Its estimation is, therefore, of value only when the blood urea is not being examined. Except in cases in which the blood urea is also raised, the urea nitrogen in the cerebro-spinal fluid varies from 6 to 25 mg. per cent., and any reading much above this figure must be regarded as pathological.

PATHOLOGICAL VARIATIONS

A chemical change in the cerebro-spinal fluid, besides being brought about by an acute or chronic infection of the meninges, as in the acute and syphilitic meningitides, or by new growths of the meninges, may also, on occasion, be caused by simple meningeal irritation, though to a less extent. Such irritation may be produced by an increase in the blood—and therefore in the cerebro-spinal fluid—of certain chemical substances, as in diabetes and nephritis. It may also be caused by bacterial toxins, such as those of influenza and pneumonia; by inflammatory changes in the brain, as in encephalitis; by increased intracranial pressure, such as may result from a cerebral tumour; or by an inflamed state of the structures adjacent

to the cranial cavity, as in some cases of otitis media.

Changes in Pressure.—Changes in the pressure of the cerebro-spinal fluid, though undoubtedly common in many diseases of the central nervous system, are difficult to gauge accurately and, unless they are gross, are of little diagnostic value. Opinions as to the cerebro-spinal fluid pressure based on the rate of flow through the lumbar puncture needle are apt to be very misleading, and it is most desirable that a manometer should be used. The best is probably a simple vertical glass tube graduated in millimetres with a special attachment to the lumbar puncture needle and a three-way tap. Before introducing the needle the tap is opened, and whenever fluid begins to drip out it is turned so as to allow the fluid to rise in the tube. Here the pressure will fluctuate slightly, being rather greater during expiration than during inspiration. In normal patients in the recumbent posture the fluid in the lumbar theca is found by such manometric readings to be under a pressure of 60–150 mm. When the puncture is performed in the sitting posture this pressure is approximately doubled. Even slight variations in the posture of the patient may, however, profoundly affect the pressure of the fluid, and crying, coughing, holding the breath or struggling—all of which may occur, especially when a child is the subject of the examination—may materially affect the result, any one of them being enough to send up the pressure by 100 mm. or more. The statement, therefore, that “The fluid is under pressure” must always be accepted with reserve. When the above fallacies can be excluded, however, a reading of 175–250 mm. is suspiciously

high, and a reading of over 250 mm. is definitely abnormal.

The pressure of the cerebro-spinal fluid, depending as it does on the intracranial venous pressure, is raised in such conditions as meningitis, encephalitis, cerebral haemorrhage, cerebral tumour, or any irritation of the meninges causing an increase in the amount of fluid in the subarachnoid space. The extent of the pressure increase is determined by the character and acuteness of the underlying disease.

The pressure is lowered when there is some obstruction to the flow of the fluid into the lumbar theca, as in tumours of the cord, in advanced spinal caries, or in cases in which adhesions of the arachnoid have occurred as the result of meningitis and have produced an obliteration of the subarachnoid space. The pressure is lowered also when there is an obstruction between the ventricular and spinal fluid. Thus a tumour causing a blocking of the foramen magnum, or an obstructive hydrocephalus, will cause a lowering of the pressure of the cerebro-spinal fluid. Some information as to the site of the lesion may be obtained in such cases by simultaneous cistern and lumbar puncture, the pressure of the fluid in each case being measured by means of a manometer. If the pressure is normal in the cistern space, but decreased in the lumbar theca, then the inference is that the obstruction is spinal in origin; if, however, the pressure is decreased in both spaces, the obstruction must be between the cistern and ventricular spaces.

Pressure on the neck over the jugular causes a distension of the veins in the brain and in normal individuals an instant rise in the spinal fluid pressure of from 30 to 50 mm.; this increased pressure dis-

appears as the jugular compression is removed. This is known as the Queckensted phenomenon. If compressing the jugular causes no such rise in the manometric reading, which, however, may still be increased by coughing or straining, the inference is that a complete block of the spinal subarachnoid spaces is present, such as may be caused by adhesions, tumours of the cerebellar fossa, or double and complete lateral sinus thrombosis. If, however, the spinal block is incomplete, very slow rise in pressure with an unusually slow fall takes place. A rapid rise in pressure followed by a very slow fall is suggestive of a meningeal tumour situated so as to exert a ball valve effect. If no rise in pressure takes place on compressing both jugulars, however, the possibility of the needle opening having become blocked by a nerve root floating against it must always be kept in mind, and the needle should be moved and rotated slightly in order to free it from such a possible obstruction.

After the removal of about 10 c.c. of fluid the cerebro-spinal fluid pressure normally falls about 50 mm. When, however, the fluid is under great pressure, as in meningitis, the fall is usually less than half this amount after a similar quantity of fluid has been withdrawn. When, after the removal of a small amount of fluid, a high initial pressure is greatly reduced, there is probably an obstruction around the tentorium in the space between the spinal cord and dura.

Changes in Appearance.—Turbidity of the usually clear cerebro-spinal fluid is always pathological unless it has been artificially produced by contact of the fluid with alcohol or some other disinfectant used for

sterilising the puncture needle, or by accidental contamination with blood during its withdrawal. Provided these possible fallacies have been excluded, any turbidity is due to an increase in cells or organisms (or both). Such a turbidity may be so slight as to be barely perceptible, as in acute syphilitic meningitis, or may be so gross, as in cases of pyogenic meningitis, that the fluid is frankly purulent. When turbidity is present there are usually over 400 polymorphonuclear cells per cu. mm. A similar lymphocytic increase does not cause turbidity.

A turbidity together with a colour varying from bright red to a mere pink may be occasioned by the presence of blood, due either to recent ventricular or subarachnoid haemorrhage or to the traumatism of a vein during the process of puncture. In doubtful cases a brown deposit on centrifuging the fluid will indicate the presence of blood definitely. It is usually possible to distinguish between a haemorrhagic fluid due to a ventricular or subarachnoid haemorrhage and one due to accidental injury to a vein, since in the former cases the fluid is uniformly coloured, while in the latter the fluid is heavily contaminated at first, but becomes clearer after the first few cubic centimetres have been withdrawn. As was mentioned in Chapter IX, the van den Bergh reaction can be used in cases of doubt to distinguish blood due to a haemorrhage of one or two days' standing from fresh blood, since the former has been partly broken down to haematoidin and gives a reddish violet colour with the van den Bergh reagent.

If the haemorrhage is not of quite recent date the fluid is found to possess a yellowish colour, which gradually deepens to a maximum about ten days after

the haemorrhage, when very few red blood cells are to be seen. After this the yellow colour slowly fades, and it has usually disappeared completely at the end of three weeks, provided no further haemorrhage has occurred. This yellow coloration, or xanthochromia, is not significant if a previous lumbar puncture has been performed, since it may occasionally be caused by blood artificially introduced into the cerebro-spinal fluid.

A xanthochromia associated with rapid coagulation of the fluid has been described by Froin, and the syndrome bears his name. Froin's syndrome may be due to syphilitic arachnoiditis, or to compression of the cord, though it may more rarely be found in other conditions. The yellow tinge of the fluid in Froin's syndrome is due to extravasated blood pigment from congested meningeal vessels, and the spontaneous clotting to a great increase in the fibrinogen content of the fluid.

A yellow fluid is obtained also in cases in which there is a blockage in the spinal subarachnoid space, such as may be caused by tumours of the cord or by Pott's disease. It may also occur occasionally in cerebral thrombosis.

Lastly, in severe jaundice of long standing the cerebro-spinal fluid may eventually become yellow owing to the presence of bile. In all these xanthochromias the van den Bergh reaction is positive, and the pigment is, presumably, haematoidin.

Normal cerebro-spinal fluid, like normal urine, froths on being thoroughly shaken in a test-tube, but this froth quickly settles down and disappears in a few minutes. An increased protein content of the fluid causes the froth to be greater in amount and to

last very much longer, so that any condition which markedly increases the protein content of the fluid may give rise to this phenomenon.

The presence of fibrinogen, which is not a constituent of normal cerebro-spinal fluid, is strikingly indicated by the formation of a fibrin coagulum or pellicle on allowing the fluid to stand at room temperature without being disturbed. This delicate test is very frequently missed, as most fluids are analysed immediately and are not left undisturbed for any length of time. Again, the coagulum, though it may form quickly, may appear only slowly and may require as much as twenty-four hours to make its presence obvious. It is therefore wise, while going on with the rest of the analyses, to set aside a little of the fluid to see whether or not a fibrin coagulum will form. The coagulum may be seen as a very fine web or meshwork, taking a long time to form—a type seen typically in tuberculous meningitis—or may form very quickly and be exceedingly gross, as in suppurative meningitis. On prolonged standing the coagulum may be destroyed by autolysis, so that the examination for the presence of a coagulum should take place not more than twenty-four hours after withdrawal of the fluid. The appearance of a fibrin coagulum indicates that the total protein is considerably increased, usually to 100 mg. per cent. or more, though occasionally it is found with a protein content slightly less than this. Owing to the fact that its formation from fibrinogen depends on the presence of an excessive number of cells in the fluid, a coagulum will not form if the cells are not increased, even in a highly albuminous fluid. A practical corollary of this, of course, is that the fluid to be examined for coagulation must not be cleared

by centrifuging, but must be observed in its untreated state.

Changes in Cells.—Although the cell count in the cerebro-spinal fluid of healthy people is seldom more than 5 per cu. mm., no real pathological significance can be attached to such a count unless it totals over 10 per cu. mm. A slight lymphocytic cell increase is occasionally found in such chronic conditions as herpes zoster, chronic encephalitis, polyneuritis, and disseminated sclerosis. These conditions, however, are not usually associated with any abnormality in the cell count. Abnormal cell counts are also rare in cases of cerebral tumour, though high counts have occasionally been recorded in patients with degenerating gliomata in relation to the walls of the ventricles. A moderate lymphocyte increase up to 100 cells per cu. mm., associated with a positive Wassermann reaction, is, of course, pathognomonic of syphilis of the central nervous system. In the late chronic stages of tabes dorsalis and general paralysis of the insane the cell counts may, however, show few abnormalities. A high lymphocytic count of two hundred or more cells is highly suggestive of tuberculous meningitis, though of recent years a condition of benign lymphocytic meningitis has also been described in which the fluid may contain several hundred lymphocytes. In this latter disease, however, which is sometimes associated with influenza and mumps, and is probably due to a virus infection, no chemical changes occur in the cerebro-spinal fluid and no organisms are present. Tuberculous meningitis, on the other hand, is, as we shall see, associated with very characteristic chemical changes in the fluid, in which the tubercle bacillus is also frequently found. Large

numbers of polymorphonuclear cells are found in the cerebro-spinal fluid in the invasive stage of acute anterior poliomyelitis. In the course of a week these are replaced by lymphocytes, which may persist for a few weeks longer. Otherwise a high polymorphonuclear count of from 500 to 2000 cells is almost invariably diagnostic of an acute coccal infection of the meninges.

Changes in Protein.—The commonest pathological change in the composition of the cerebro-spinal fluid is an increase in its protein content, but the very universality of this alteration makes it of little use in the differential diagnosis of the conditions in which it occurs. Usually the increase in protein content is not very marked, readings up to 100 mg. per cent. being commonest, while values of over that figure are much less usual, and values over 500 mg. per cent. are very rare. As has already been explained, the protein in pathological cerebro-spinal fluids consists of a mixture of albumin with globulins—pseudoglobulin and euglobulin—and, sometimes, fibrinogen. The albumin is always present in greatest amount, but the ratio of albumin to globulin is too variable to make the separate estimation of globulin a matter of importance in differential diagnosis. Hence, though the qualitative test for globulin does indicate an increase in the protein content of the fluid, it does no more, and for quantitative work it is desirable to estimate directly the total protein. An increase in the protein content of the cerebro-spinal fluid may or may not be accompanied by an increase in the number of cells. It is found without increase in cells in such conditions as spinal tumour or compression of the cord, cerebral arteriosclerosis, polyneuritis, and usually, though not invariably, in tumours of the brain. When the pro-

tein increase is associated with a cellular increase, as is most commonly the case, it is usually an indication of a meningeal inflammation, and is therefore found in all cases of meningitis, whatever their cause. This is true even though the meningeal affection is secondary to some such condition as encephalitis or poliomyelitis. An increase of protein with a slight cellular increase, however, may occasionally be present without any meningeal involvement, as in a few cases of disseminated sclerosis.

Changes in Glucose.—An increase in the glucose content of the cerebro-spinal fluid is an index of an increased glucose content of the blood, and is thus usually an expression of diabetes mellitus. It may be slightly increased in certain other conditions, especially in uraemia, but, except in diabetes, the increase is insignificant and variable, and has little or no diagnostic importance.

A diminished glucose content is, on the other hand, a matter of great significance, from both a diagnostic and a prognostic point of view. Owing to the fact that the organisms invading the meninges use glucose as a food, sugar is reduced or absent in all acute meningeal infections due to glucose-fermenting organisms. Thus, in pneumococcal, streptococcal, and meningococcal meningitis and, to a less extent, in tuberculous meningitis, the glucose content of the cerebro-spinal fluid is markedly diminished, and this is all the more striking since the blood sugar is frequently increased in these conditions. In meningococcal meningitis the return of sugar in the cerebro-spinal fluid may be the first indication of improvement under treatment, and is, therefore, an important prognostic sign. Besides the meningitic conditions, the glucose of the cerebro-

spinal fluid is decreased in cases of localised cerebral abscess. It is worthy of note that the sugar of the cerebro-spinal fluid is never decreased in encephalitis and may be increased, a useful diagnostic point in differentiating the condition from other acute diseases of the central nervous system.

Changes in Chloride.—The chloride content of the cerebro-spinal fluid is increased in some cases of hydraemic and acute nephritis, and in uraemia. Such an increase in the chloride content of the fluid may occasionally be of diagnostic aid when a lumbar puncture has been performed on a case of coma or convulsions due to uraemia, in the belief that the symptoms were primarily due to disease of the central nervous system.

The chloride content is decreased in cases of acute meningitis, especially in tuberculous meningitis, where readings as low as 500–600 mg. per cent. may be obtained. Any reading below 550 mg. per cent. is practically diagnostic of tuberculous meningitis, since no other condition (except, possibly, acute intestinal obstruction, with which it cannot be confused) causes a reduction of such magnitude in the chloride content of the cerebro-spinal fluid. The other acute meningitides cause a more moderate reduction, usually giving readings of between 600 and 700 mg. per cent. Unfortunately, from a diagnostic point of view, cases of simple meningism are also frequently associated with a fall in the chloride content of the cerebro-spinal fluid. Symptoms of meningism may occur, particularly in children, in pneumonia, typhoid, vomiting, or diarrhoea—all conditions which may cause a rapid fall in the blood chlorides, with consequent secondary effects on the chloride content of the cerebro-spinal

fluid. In meningism, however, as opposed to meningitis, the cell count in the cerebro-spinal fluid is within normal limits and its protein content low.

The discovery of a lowered chloride content of the cerebro-spinal fluid is of considerable importance in differentiating an acute meningitis from such a condition as a cerebral abscess, where, provided the inflammatory condition remains localised and does not spread to the meninges, no reduction in the chloride content of the cerebro-spinal fluid occurs. A slight decrease in the chloride content may be the first indication that such a localised inflammatory process is becoming generalised.

Just as the presence or absence of sugar in the cerebro-spinal fluid may be used as an important prognostic sign in cases of meningococcal meningitis, so may the extent of chloride reduction be used as an aid to prognosis. As the inflammatory process becomes more widespread, so the chlorides in the cerebro-spinal fluid continue to diminish; as it retrogresses and improves, so the chloride content tends to return to normal.

Changes in Urea.—The urea content of the cerebro-spinal fluid is increased almost proportionately with an increase in the blood urea. It is usually, therefore, an expression of nephritis, but its separate estimation in the cerebro-spinal fluid offers no further information beyond that which can be obtained by an estimation of the blood urea. Occasionally in young children with acute nephritis it may be easier to obtain a specimen of cerebro-spinal fluid than a specimen of blood on which to estimate the urea, and in cases of coma or convulsions, when a lumbar puncture is being performed in any case, an estimation of the

cerebro-spinal fluid urea makes a separate estimation of the blood urea superfluous. Apart from cases of nephritis the estimation of urea in the cerebro-spinal fluid is, however, of little or no importance.

The variations in the composition of the cerebro-spinal fluid dealt with in this chapter are summarised under separate diseases in Table XI. (pp. 234–235).

LANGE'S COLLOIDAL REACTION

In certain pathological conditions the cerebro-spinal fluid has the power of precipitating gold from a colloidal solution. This power seems to depend on the presence of globulin, and to be prevented, wholly or in part, by the albumin. The precipitation or otherwise of a gold sol, however, does not depend entirely on the relative amounts of albumin and globulin, but appears to be affected by some change in the physical or chemical condition of the globulin, the exact nature of which is not known. Normal cerebro-spinal fluid, which contains little globulin, fails to precipitate the gold.

Method.—The test is made by adding a definite amount of the gold sol to a series of tubes containing cerebro-spinal fluid in increasing dilutions, and, after mixing, leaving the tubes for twenty-four hours before examining them. It is usual to employ a series of numbers to indicate changes of colour—which correspond to different degrees of precipitation. Thus slight precipitation is shown by a deepening of the red colour, and is indicated by the figure 1, while complete precipitation, which gives a colourless solution, is indicated by 5. The numbers showing the degree of precipitation are then plotted graphically against the

dilution, as shown in Fig. 28. Alternatively, the result is given as a series of numerals, and the curves in Fig. 28, expressed in this way, would read thus:—

Normal	0000000000
Paretic	55555431000
Tabetic	01231000000
Meningitic	00013442100

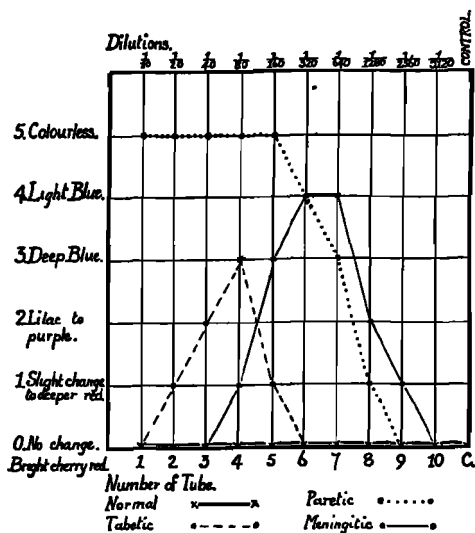


FIG. 28.—Curves illustrating Lange's Colloidal Gold Reaction.

The tabetic and paretic types of curve are not diagnostic of syphilis, since they may be obtained from cases of disseminated sclerosis and less commonly in other conditions. In presence of a positive Wassermann reaction, however, the colloidal gold reaction may be used to differentiate between the different

TABLE XI.
THE CEREBRO-SPINAL FLUID IN VARIOUS DISEASES

Disease	Mgm. per 100 c.c.		Globulin Test	Lange's Test	Colour and Appearance	Remarks
	Total Protein	Chloride (as NaCl) Adults Children				
Normal	14-50 Average 28	720-750 700-760	Negative	Negative	Clear & colourless	2-5 lymphocytes per cu. mm.
Meningitis, T.B.	30-400	500-650	Positive	Meningitic	Clear; fine fibrin, coagulum	Increased pressure. Lymphocytic cellular increase
" Meningococcal	100-400	800-700	Positive	Meningitic	Slightly turbid, coagulum	Increased pressure. Polymorphonuclear cellular increase. Fluid becomes purulent after injection of anti-serum
" Pneumococcal " Streptococcal	100-250	600-700	Positive	Meningitic	Turbid; often purulent and coloured; coagulum	Increased pressure. Polymorphonuclear cellular increase
Encephalitis	14-50	700-760	Negative	Occasionally tabetic	Clear & colourless	Cells increased.
Poliomyelitis Pre-ataxic	14-50	700-760	Negative	Negative	Clear & colourless	Polymorphonuclear cellular increase.
First week	30-60	700-760	Negative	Weak, tabetic	Clear & colourless	Lymphocytic cellular increase.
Subsequent weeks	50-300	700-760	Negative, rarely positive	Weak, tabetic	Clear & colourless	Cells normal

Cerebral Tumour	14-50 +	700-760	60-100	Negative	Negative	Clear	Occasionally very abnormal fluids are found
Cerebral Abscess	30-60	700-760	60-90	Positive or negative	Usually negative	Clear	When abscess ceases to be localised there is a fall in sugar and chloride
Cerebral Haemorrhage or Thrombosis	Usually high	700-760	60-90	Negative	Negative	Colourless, yellow, or blood-stained	
Meningeal Haemorrhage	14-100	700-760	80-100	Negative	Negative	Bloody or yellow	
Spinal Tumour	30-2000	700-760	60-90	Negative or positive	Negative	Xanthochromia; often Froin's syndrome	Reduced pressure on lumbar puncture
Disseminated Sclerosis	14-50 +	700-760	60-90	Negative or faint positive	Negative or tabetic or paretic	Clear & colourless	Negative Wassermann
Acute Polyneuritis } Landry's Paralysis }	14-350 +	700-760	60-90	Positive or negative		Sometimes yellow; occasionally Froin's syndrome	
G.P.I.	40-100	700-760	25-62	Strong, positive	Paretic	Clear & colourless	Increased pressure. Lymphocytic cellular increase in acute stages
Tabes	25-70	700-760	60-90	Positive	Usually tabetic	Clear & colourless	Lymphocytic cellular increase in acute stages
Acute Tertiary Syphilis	50-100 +	700-760	60-90	Strong, positive	Usually tabetic, sometimes paretic	Clear or slight coagulum; sometimes xanthochromia, with Froin's syndrome	Lymphocytic cellular increase

types of neuro-syphilis, since general paralysis of the insane tends to give the paretic type of curve, whereas locomotor ataxia tends to give the tabetic type. The paretic type is sometimes given by cases of tabes without mental symptoms, and by cases of syphilitic meningo-myelitis, but these tend to revert to a tabetic curve under treatment, while cases of general paralysis present the paretic type throughout.

CHAPTER XI

CHEMICAL TESTS IN PREGNANCY

THE main abnormalities of pregnancy in which chemical examination may prove of service are those characterised by the appearance in the urine of reducing substances or of albumin. These urinary constituents may signify some entirely harmless abnormality which requires no treatment, or they may be indicative of grave complications which must be treated at once and may need the premature termination of the pregnancy if the life of the mother is to be saved. Hence they necessitate full investigation by clinical and, in many cases, by chemical means. As usual, however, the chemical findings must not be taken alone, but must always be considered together with the results of thorough clinical examination.

THE GLYCOSURIAS OF PREGNANCY

The appearance of reducing substances in the urine is a commoner phenomenon among pregnant than among non-pregnant women. When it is an accompaniment of pregnancy it may, just as in the non-pregnant, be intermittent, occurring only after a meal rich in carbohydrate, or it may occur in all specimens of urine passed. This constancy or otherwise of the glycosuria depends upon the factors discussed in Chapter V—the level of the blood sugar and the

height of the kidney threshold—and the fact that glycosuria occurs only after a meal does not mean that the condition can be neglected. Even though clinical signs of diabetes are absent, the condition is not necessarily due merely to a lowering of the threshold.

In a proportion of cases the reducing power of the urine is due entirely to lactose, and in these no treatment is necessary, as the condition appears to be quite harmless. Even when lactose is present, however, glucose may be present as well, and it is desirable to know if this is the case, since the glycosuria, even in the absence of clinical signs, may be due to diabetes mellitus. The simultaneous presence of the two sugars should, therefore, be tested for, using the quantitative method (see Appendix). If glucose is found, with or without lactose, the case should be further investigated, with the possibility of diabetes in mind, and for this purpose the glucose tolerance test (p. 82) should be applied.

The great majority of glycosurias of pregnancy give normal blood-sugar curves or curves showing a rather small rise in the blood sugar after glucose ingestion; for the cause of most of these glycosurias is simply a lowered threshold for the excretion of sugar by the kidney—the so-called renal diabetes. This lowering of the threshold is usually purely temporary and disappears when the pregnancy is terminated. Lactosuria, too, is a temporary abnormality, and disappears at the end of lactation, if not earlier. Even when the glucose tolerance test reveals a true diabetic type of curve, it does not necessarily mean that the diabetic tendency will be permanent, though proper treatment may have to be given with the modifications

made necessary by the presence of the foetus, and precautions taken during labour to prevent the occurrence of acidosis and coma. We have, ourselves, observed several cases of glycosuria of pregnancy associated with diabetic symptoms and diabetic sugar curves—in one case requiring as much as 90 units of insulin a day to control the condition—whose carbohydrate metabolism returned to normal as soon as the child was born. This abrupt return to normal carbohydrate metabolism after delivery is not infrequently attended by symptoms of hypoglycaemia which are sometimes alarming or even dangerous. No doubt such cases are due to a temporary over-action of the diabetogenic hormone of the pituitary, which may occur during pregnancy, and which may so antagonise the internal secretion of the pancreas as to produce a definite, though temporary, diabetic condition. It is important, therefore, when diabetes is noted for the first time in pregnancy to give a guarded prognosis as to the permanence of the condition.

THE ALBUMINURIAS OF PREGNANCY

The albuminuria of pregnancy may be, and most often is, purely functional. The presence of albumin in the urine is due, possibly, to some temporary alteration in the permeability of the glomeruli, or to the extra strain thrown by the pregnancy on a kidney which, though apparently normal, has less than the usual large reserve of power. This type of case is often called the "low reserve kidney of pregnancy." The albuminuria is frequently the only sign, though there may sometimes be a slightly increased blood pressure. The blood non-protein nitrogen is not raised in total

amount and its distribution among the various constituent substances is normal. Indeed, since in normal pregnancy the blood urea is somewhat lower than usual, the non-protein nitrogen of the blood, even with a functional albuminuria, is slightly below the normal for non-pregnant women. Figures for the blood urea nitrogen in cases of "low reserve kidney," as in normal pregnancy, range from 5-15 mg. per 100 c.c., while the total non-protein nitrogen varies between 20 and 35 mg. per 100 c.c. The reason for this lowered blood urea is not at all clear, though it may be due partly to increased utilisation of amino-acids for tissue growth of the foetus and uterus, with, in consequence, a diminished utilisation of these substances for the direct production of energy with formation of urea. In the "low reserve kidney of pregnancy" there is no abnormality of the urine other than the presence of albumin; the variation in volume and specific gravity is normal, as is the relative concentration of urea and ammonia, and the response to the urea-concentration test, or to any other renal function test, fails to show the presence of any kidney damage. Similarly, there is no sign, as determined by functional tests, of any liver inefficiency. Although there is no definite evidence that women with functional albuminuria are more liable than others to develop any of the graver complications of pregnancy—and, indeed, the condition may not recur in future pregnancies—yet we feel that the incomplete state of our knowledge demands that they should be watched more closely than others.

In a second type of albuminuria the pregnancy is complicated by a definite chronic azotaemic Bright's disease. The disease may have existed prior to the pregnancy and have been exacerbated by it, or it may

have developed subsequently to conception. In either case the blood-picture and the response to kidney function tests are similar to those given by an uncomplicated case of chronic azotaemic Bright's disease. In interpreting the results of blood analysis it is, however, necessary to take into account the lower figures found in normal pregnancy. The extent of kidney damage, as determined by such tests in conjunction with careful clinical examination, will determine the course to be adopted. With slight damage it may be possible to allow the case to go to term, but more extensive damage calls for the immediate emptying of the uterus. It may be said that a blood urea nitrogen above 20 mg. per 100 c.c. is definitely pathological (although values below this do not, of course, exclude the possibility of some renal damage), and that values above 40 mg. per 100 c.c. indicate the need for the immediate termination of the pregnancy. Whether the pregnancy is terminated normally or artificially, in this type of case special care is needed during the puerperium, since, as in so many other diseases (*e.g.* tuberculosis and cardiac disease), there is a great tendency towards rapid progression of the lesion during this period. Immediately after delivery, too, the amino-acid metabolism resumes its normal course, so that the production of urea becomes greater than during the pregnancy. Hence there is a greater tendency to nitrogen retention in the blood.

A third type of albuminuria of pregnancy can often be distinguished from the nephritic type by examination of the blood, since it is not accompanied by an increase in the blood urea. In drawing this distinction chemical analysis may be of service, since the clinical pictures may be somewhat similar. This third type of

albuminuria of pregnancy, the pre-eclamptic type, is associated with a large quantity of albumin in the urine, casts, and some degree of oliguria. The only other urinary abnormality which is at all commonly present is an increase in the ammonia content, with a corresponding slight decrease in the urea content, and this is due usually to an acidosis. In contradistinction to the true nephritic type of albuminuria, the urea-concentration test gives, in pre-eclampsia, results within the normal limits. In pre-eclampsia, as in eclampsia, the blood has an increased uric-acid content, with, consequently, a slight increase in the total non-protein nitrogen, but there is no marked nitrogen retention, as is evidenced by the absence of any rise in the blood urea. The carbon-dioxide combining power usually tends to be lowered, in some cases to a considerable degree, and the blood cholesterol is considerably increased. Some slight increase in the blood cholesterol is indeed a common finding in pregnancy, and has no clinical significance. In pre-eclampsia, however, the increase is very marked. The whole blood and urine picture thus corresponds in many respects with that seen in the nephrotic syndrome.

The pre-eclamptic state, which is characterised by certain fairly typical symptoms, may come on insidiously or abruptly, usually during the last two months of pregnancy. It differs in no important particular as regards the chemical findings from true eclampsia, being simply a potential state of the latter, from which it can hardly be differentiated except by the absence of convulsions. Chemical analysis is of little, if any, service in estimating the imminence of true eclampsia. The acidosis usually becomes more marked with the

onset of convulsions, as does the retention of uric acid; the serum calcium is occasionally lowered still further than it is in the later stages of a normal pregnancy; and chemical methods may show the presence of bilirubinaemia with excretion of bile pigments, together with other evidence of hepatic insufficiency, none of which liver changes is marked in early pre-eclampsia. The chemical changes are, however, not distinct enough to form a foundation for the prophecy of imminent eclamptic convulsions, and when the convulsions have occurred, by which time the chemical changes may have become distinct, there is no need to have recourse to chemical tests. The real service of chemical tests in the albuminurias of pregnancy lies in the differentiation of an albuminuria due to a complicating chronic azotaemic nephritis from a mere functional albuminuria or one due to pre-eclampsia. Possibly, in addition, the discovery of a lowered carbon-dioxide combining power may give a useful indication of the need for anti-acidotic treatment.

THE VOMITING OF PREGNANCY

Though vomiting is a very common accompaniment of pregnancy in the early months, the type known as the pernicious vomiting of pregnancy is only a very occasional complication, occurring in about one case per thousand. The vomiting of pregnancy has been divided by some observers into three types: the reflex, the neurotic, and the toxæmic or pernicious type. Whether these three types really exist, or whether all cases are neurotic or all toxæmic, as others have suggested, the chemical picture in severe cases is almost invariably that of a dehydration, together

with a lack of carbohydrate utilisation. It is hardly fair, perhaps, to suggest that lack of carbohydrate is a causative factor in the disease (though this has been done), for it would seem equally possible that the vomiting might be the cause of the carbohydrate lack. Since the same thing is equally true of the dehydration, it seems that we must admit ignorance of the true cause of vomiting in pregnancy.

The loss of water and of hydrochloric acid in the vomitus leads eventually to dehydration and a depletion of the blood and tissue chloride. Hence in severe cases the blood chloride is low, and there is no need to postulate kidney damage in order to explain the high blood non-protein nitrogen, uric acid, and urea which are so often found. The retention of these substances may be due to a mere general concentration of the blood, or it may, as has been suggested of a similar increase in other conditions (p. 115), be an attempt to maintain the blood osmotic pressure in face of a falling chloride content. In high intestinal obstruction a similar chloride loss with dehydration leads to a very severe alkalosis, but in severe vomiting of pregnancy, though the blood chloride may be as low as 250 or 300 mg. per 100 c.c., it is generally agreed that the carbon-dioxide combining power is generally normal or low—*i.e.* there is rather a tendency to acidosis. This is explained by the superimposed lack of carbohydrate oxidation (whatever the cause of this may be), which leads to incomplete oxidation of fatty acids, with, therefore, production of aceto-acetic acid and hydroxy-butyric acid. There is, in fact, a very considerable ketosis in this condition, and figures of 100–150 mg. per 100 c.c. of blood for the concentration of acetone bodies in the blood are not uncommon

in severe cases. There is a corresponding ketonuria (unaccompanied, of course, by glycosuria), and, in severe cases at all events, the customary response of the kidney to a demand for the continued excretion of large quantities of acid—a production and excretion of ammonia at the expense of urea. Hence the ratio of ammonia nitrogen to urea nitrogen in the urine is high, and figures of over 10 may be obtained for the ammonia coefficient. This, together with the ketonuria, is the main chemical fact of prognostic importance, for the opposing tendencies to alkalosis (caused by the chloride loss) and acidosis (caused by the continued ketosis) cancel each other and most frequently give a normal carbon-dioxide combining power. Treatment is usually directed—with good results—towards the removal of the ketosis by the administration of glucose, insulin being given as well if necessary. In addition it would seem desirable to pay attention to the chloride loss and to correct this by administration of salt and water, lest the removal of the ketosis leave the patient in a state of alkalosis. It seems to us that hitherto insufficient attention has been paid to this point.

ACUTE YELLOW ATROPHY OF THE LIVER

The rare condition of acute yellow atrophy of the liver, or icterus gravis—of which pregnancy seems to be a predisposing cause, since 60 per cent. of the reported cases have been associated with pregnancy—is not one which usually requires chemical data as an additional aid in diagnosis. It is, of course, accompanied by a positive van den Bergh reaction and a high icteric index, even before the skin pigmentation

occurs; and it is usually possible to demonstrate liver damage by any of the hepatic function tests. The most characteristic chemical abnormality is the excretion in the urine of leucine and tyrosine. Though there is no possibility of confusing the condition with that of chronic interstitial nephritis accompanying pregnancy, it is perhaps as well to note that in acute yellow atrophy of the liver the blood non-protein nitrogen and urea are usually increased. With a badly damaged liver there may be failure of the glycogenic function, with consequent ketosis, ketonuria, and a slightly lowered carbon-dioxide combining power.

TESTS FOR PREGNANCY

Several chemical tests have been proposed for determining the existence of pregnancy in doubtful cases. Some, such as the ninhydrin reaction, are frankly useless; others, such as the histidine test, are liable to be fallacious, though giving correct results in a considerable proportion of cases. The only tests which can be recommended are the Asheim-Zondek and those based on the same principle—the urinary excretion, during pregnancy, of the pituitary gonadotropic hormone.

CHAPTER XII

THE BLOOD CALCIUM AND PHOSPHORUS

THE calcium of the blood is confined to the plasma, which, in normal persons, contains from 9·5 to 10·5 mg. per 100 c.c. Figures below 9 and over 11 mg. per cent. may be taken as definitely abnormal. For a variety of reasons, including the variability, in disease, of the cell volume, and the relative difficulty of estimation, it is not advisable to rely on determinations of calcium in whole blood, and it is most usual to use the serum for analysis. Citrated plasma is often recommended, but its use is undesirable, since the amounts of sodium citrate required to prevent coagulation may precipitate a little of the calcium.

Of the total serum calcium about 20 per cent., or 2 mg. per 100 c.c., is present in the form of free ions, and this is included in the 60 per cent. which is normally capable of diffusing through a collodion membrane. The remaining 40 per cent., the non-diffusible calcium, is apparently combined with protein and possibly lipoids in some non-ionised form. Though the distribution of calcium amongst these various forms is often altered in disease, insufficient is as yet known to make their separate estimation of value, and, indeed, there is no really accurate method for the determination of the ionic calcium.

The total phosphorus in the blood amounts to about 30 mg. per 100 c.c. Of this, about a third is

combined with protein, and about a third is present in phospholipins (the lipid phosphorus). The remainder consists of inorganic phosphates, which account for 2-3 mg. phosphorus per 100 c.c. of blood in adults and up to 5 mg. per 100 c.c. in children, and a number of esters which are soluble in dilute acid (the acid-soluble phosphorus).

FUNCTIONS OF CALCIUM AND PHOSPHORUS

The most obvious function of calcium is, of course, the formation of bone, and in this phosphate is equally important. The mechanism of bone calcification is not yet completely understood, but it seems to be established that an important part of it hinges on the enzyme phosphatase. Phosphatase is widely distributed, and is, for example, present in blood, but the amount in bone far transcends that in other tissues. Moreover, it is present in greatest amounts when and where calcification is most rapid, and in the embryo bone it appears only when calcification begins. The action of phosphatase consists in the hydrolysis of organic phosphates with liberation of phosphate ions. This action, occurring locally, so raises the concentration of phosphate as to raise the product $\text{Ca} \times \text{PO}$ above the level at which precipitation of calcium phosphate occurs. It does not, however, account entirely for the calcification of bone, since the inorganic part of bone does not consist of pure calcium phosphate, but contains considerable amounts of calcium carbonate.

Calcium is essential for the clotting of shed blood, and there is evidence to show that in this process some complex non-diffusible calcium compound is con-

cerned, while free calcium ions may actually inhibit the coagulation. There is an equilibrium amongst the various calcium compounds in the blood, and alteration in the concentration of any one causes a readjustment of the equilibrium so that the change is distributed amongst them all. It is, consequently, rare to find any deficiency in blood-coagulability which can be attributed directly to an abnormal blood calcium. Even after parathyroidectomy, when the total serum calcium may be as low as 5 mg. per 100 c.c., the blood still clots normally. The therapeutic use of calcium salts to expedite blood clotting, in haemorrhagic conditions is, therefore, usually irrational.

The conductivity and irritability of nervous tissue and the contractility of muscle are profoundly affected by variations in their calcium content and that of the fluids bathing them. In certain forms of tetany, accompanied by a 40 per cent. reduction in the serum calcium, the symptoms are due to hyperexcitability of the peripheral nerves and of the striped muscle, and disappear when the serum calcium is raised to the normal level.

Without a supply of calcium in the perfusing fluid the heart cannot continue to contract, and it is apparently free calcium ions that are needed. A large excess of calcium, however, is toxic to the heart, and causes its stoppage in systole.

It has been suggested that calcium is concerned in the maintenance of the acid-base equilibrium of the body, but though the amount in the blood is often altered in alkalosis or acidosis, there does not appear to be sufficient evidence to show that calcium has any function in this respect different from that of the other inorganic elements.

Phosphoric esters and inorganic phosphates are, as has been mentioned, concerned in bone calcification. Certain esters are also formed as obligate steps in the metabolism of carbohydrate, in which another phosphorus compound, adenylic acid pyrophosphate, functions as a co-enzyme. Phosphoric acid is also present, of course, in phosphocreatine, which is intimately concerned with muscular contraction; in nucleic acids; in such phosphoproteins as caseinogen; and in the phospholipins, which, besides being structurally important, are probably involved in the transport and utilisation of fats.

VARIATIONS OF THE SERUM CALCIUM

It appears, from the vast amount of work that has been done in recent years, that the serum calcium may vary widely from the normal in a great number of pathological conditions. In some conditions individual cases seem to vary both in the degree and direction of the abnormality without reference to the severity of the disease. In others, however, the variation is always in the same direction, a finding which would seem to indicate some connection, direct or indirect, between the disease and the abnormality of calcium metabolism. Thus in various acute fevers, such as pneumonia, both hyper- and hypo-calcaemia have been reported; in a number of dermatological disorders the serum calcium has been found to be high, low, or normal in individual cases without any apparent correlation between the calcium level and the disease; and similar inconstant abnormalities have been found in syphilis, sprue, epilepsy, arthritis, diabetes, and many others.

Acidosis and Alkalosis.—In some conditions there seems to be a relationship between the level of the blood calcium and the carbon-dioxide combining power. An acidosis causes, at first, an increase in the calcium content of the blood, but the resulting increased excretion of calcium may, if the acidosis continues, eventually lead to a depletion of the body stores and so to an ultimate lowering of the blood calcium. This seems to be a feasible explanation of the low serum calcium so often found in diabetes accompanied by ketonuria, and in nephritis with acidosis. On the other hand, an alkalosis produced by excessive dosage with sodium bicarbonate lowers the blood calcium, and such low values may accompany the alkalosis produced by overdosage with alkali in the treatment of gastric and duodenal ulcer.

Pregnancy.—The serum calcium is usually at or slightly below the lower limit of the normal range during the later months of pregnancy, a state of affairs which is due most probably to the heavy and growing demand of the foetus—a demand which amounts finally to about 0·5 g. per day, or about a third of the calcium content of the ordinary daily food. Attempts have been made to correlate this tendency to a lowering of the calcium stores with the occurrence of eclamptic convulsions, but, although in eclampsia the serum calcium is slightly lowered, there seems to be no definite causative connection between the two. It is, however, probable that the drain on the maternal calcium is responsible for the dental affections which so often accompany pregnancy, and for the osteomalacia which is, fortunately, rare in Occidental countries, though common in India and China, where the diet is so frequently deficient in

vitamin D. The expectant mother should be given a diet rich in calcium and containing adequate supplies of vitamin D. Thus the food should include plenty of milk and green vegetables, and may profitably be supplemented by cod-liver oil.

Haemophilia.—Among the important functions of calcium is the conversion of fibrinogen to fibrin in shed blood. Calcium plays, therefore, an essential part in the clotting of blood, and its precipitation by oxalates, or conversion to an inactive form by citrates, prevents the formation of fibrin. It is, however, not the only factor concerned in blood coagulation, and in the most striking clinical condition in which blood clotting is deficient—haemophilia—the blood calcium is normal and the condition is not improved by attempts to increase it. It is said that the deficient clotting in haemolytic jaundice is improved by injections of calcium salts, especially when, as is often the case, the blood calcium is somewhat low. A very efficient method of controlling post-operative oozing in this condition, however, is the intramuscular injection of sodium citrate (30 c.c. of 10 per cent. solution into each buttock). It seems probable that the citrate acts by combining with, and removing, the free calcium ions which seem to have an inhibitory effect on blood coagulation.

Rickets.—A fact which has an important bearing on the use of estimations of the blood calcium is that an error of calcium metabolism is by no means necessarily accompanied by an appreciable alteration in the calcium content of the blood. This is well illustrated in rickets. In this disease there is, of course, failure to deposit calcium phosphate in the bones, and there seems no doubt that it is due, in part, to a failure

to absorb calcium or phosphate (or both) from the intestine. Other factors may be concerned, but that this is one appears probable from the lowering of the intestinal pH which follows administration of vitamin D to the rachitic animal, a change which is in the direction favouring absorption of calcium and phosphate. In the rachitic child, however, the blood calcium may be present in normal amount, and it is uncommon to find figures below the normal minimum unless the condition is complicated by the presence of spasmophilia. Taking the normal range for the serum calcium as 9·5–10·5 mg. per 100 c.c., simple rickets may give results from 8·5–10, with the majority about 9–10, while in spasmophilia normal results are very rare, and values as low as 5·8 may be obtained. It is commoner, in fact, to find a low inorganic phosphate content of the blood in simple rickets than to find a markedly lowered serum calcium. The frequent existence of a low serum phosphate with a normal serum calcium has led to the recognition of two types of rickets—the low phosphorus and the low calcium types. They may be ascribed to different dietetic errors, but are clinically identical except that tetany (spasmophilia) is associated only with the low calcium type. The clinical signs of rickets are so obvious, however, and the treatment—a diet well balanced as regards calcium and phosphate, and containing adequate amounts of vitamin D—is so well known and reliable that estimation of the serum calcium is never more than a matter of interest in this condition.

Renal Rickets.—Renal rickets appears to be due, not to inability to absorb calcium and phosphorus, but to renal insufficiency. The serum calcium and inorganic phosphate may be normal, but as the

disease progresses the phosphate usually rises along with the blood urea. The serum calcium, as usual in late chronic azotaemic Bright's disease, tends to fall as the phosphate rises, and in consequence tetany becomes a frequent concomitant of renal rickets. As in true rickets, the plasma phosphatase is high.

Osteomalacia (adult rickets).—Osteomalacia, so far as its chemistry is concerned, resembles, and is due to the same causes as, low calcium rickets in children. The causes are a diet poor in calcium and vitamin D, together with insufficient exposure to sunlight. The condition is consequently common in the Orient, and more particularly among women whose calcium metabolism is further strained by repeated pregnancies and (often prolonged) periods of lactation. The serum calcium is almost invariably low, and often so low (below 7.5 mg. per 100 c.c.) that tetany is present in active or latent form. The inorganic phosphate is usually normal and the plasma phosphatase may be somewhat raised. Calcium balance experiments, in which a low calcium diet is given and the total calcium excretion is compared over a period of some days with the intake, give very varying results, though there is rarely more than a slight net loss of calcium. The usual anti-rachitic treatment, of course, brings about marked retention of calcium, with increase of the serum calcium, abolition of tetany, and recalcification of the bones.

A somewhat similar condition of osteoporosis occurs in sprue, coeliac disease, and congenital steatorrhoea. In these diseases the bone abnormality is due (in part, at least) to faulty calcium absorption resulting from the excessive formation in the intestine of insoluble calcium soaps.

Generalised osteitis fibrosa (von Recklinghausen's disease).—This disease is apparently due to over-activity of the parathyroids, and the chemical picture is, therefore, that given by normal animals after injection of the parathyroid hormone. The serum calcium is markedly raised, occasionally to double the normal level, though more usually to 12·5–16·0 mg. per 100 c.c. The inorganic phosphate, as frequently happens, changes in the opposite direction (unless there is also renal deficiency, when it may be raised); figures of 1·0–2·7 mg. phosphorus per 100 c.c. blood have been reported. In most conditions associated with either active decalcification of bone or inefficient calcification, there seems to be some leakage of phosphatase from the bones, and consequently an increase in the plasma phosphatase; such an increase usually is found in generalised osteitis fibrosa. The active withdrawal of calcium phosphate from the bones and the resulting high serum calcium lead to an increased excretion of calcium, mainly in the urine. This is most easily observed by measuring the excretion over a period of three or four days while the patient is receiving a diet containing the minimum of calcium; under such conditions the excretion is markedly greater than the intake.

Focal osteitis fibrosa, affecting as a rule only a few bones, and developing slowly, is probably of entirely different, and at present unknown, origin, and not due to a parathyroid tumour. The blood chemistry, with respect to calcium, inorganic phosphate, and phosphatase, is normal, as also is the calcium balance.

Osteitis deformans (Paget's disease). — Paget's disease differs essentially from von Recklinghausen's disease in being characterised by excessive apposition

as well as resorption of bone, whereas in the latter condition there is little excessive apposition, and that merely focal. In Paget's disease the chemical picture depends to some extent on whether, at the time of examination, apposition or resorption happens to be the predominating process. Thus the calcium intake may exceed the output (positive balance), the two may be equal (correct balance), or the output may exceed the intake (negative balance). The balance, however, is never so markedly negative as in generalised osteitis fibrosa. The serum calcium and inorganic phosphate are usually normal, but the plasma phosphatase is constantly and markedly increased, the increase being roughly proportional to the severity of the condition.

TABLE XII

<i>Condition</i>	<i>Serum</i>		<i>Plasma Phosphatase</i>	<i>Calcium Balance</i>
	<i>Calcium</i>	<i>Inorganic Phosphate</i>		
Rickets	Normal or low	Normal or low	Often raised	Not positive
Renal Rickets	Normal or low	Normal or high	Raised	Not positive
Osteomalacia	Low	Normal or low	Raised	May be negative
Generalized Osteitis fibrosa	High	Low	Raised	Markedly negative
Focal osteitis fibrosa	Normal	Normal	Normal	Normal
Osteitis deformans	Normal	Normal	Markedly raised	Positive, normal, or negative

Tetany.—Tetany is to be regarded, not as a single disease, but as a group of diseases, all characterised by increased excitability of the peripheral nerves with spasmodic contractions of the muscles of the extremities; but, though these symptoms are very suggestive of a calcium deficiency, they may or may not be accompanied by a low serum calcium. Nevertheless, it is an attractive, though admittedly unproved, hypothesis that all these tetanies, whatever the level of the blood calcium, are due to a deficiency of calcium ions in the tissues and in the fluid bathing the peripheral nerves.

It has long been recognised that gastric disorders accompanied by vomiting may be associated with symptoms of tetany, yet in these conditions there is no decrease in the serum calcium. Long-continued vomiting, as we have seen, results in an alkalosis (which may be very severe) and a marked lowering of the blood chloride. This state of affairs is seen in pyloric obstruction, in occlusion of the duodenum or upper jejunum, in the pernicious vomiting of pregnancy (where, however, the alkalosis may be masked by ketosis), and also in uraemic vomiting, all conditions which may be accompanied by tetany. It is surely more than coincidence that in two other conditions characterised by alkalosis tetanic convulsions are liable to occur. Of these the first, and commonest, is overdosage with alkali in the treatment of gastric or duodenal ulcer, and here, as in vomiting, the tetany is not accompanied by any lowering of the serum calcium. The second is hyperpnoea (rarely, in clinical practice, accompanied by active tetany), in which the excessive elimination of carbon dioxide has produced an alkalosis with, however, a normal or slightly raised serum calcium.

The common factor in all these conditions is the alkalosis, and it has been shown that an increase in the blood pH leads to a decrease in the amount of calcium existing as free ions. Even with the pH remaining constant it has been found, *in vitro*, that an increase in the concentration of bicarbonate ions (such as occurs in vomiting or overdosage with alkali) is accompanied by a decrease in the ionic calcium. Hence it may well be that in these alkalotic tetanies the cause of the hyperexcitability of the peripheral nerves is the secondary decrease in the concentration of ionised calcium. Administration of calcium, however, serves no useful purpose, since the body stores are not depleted. Treatment should be directed towards the correction of the alkalosis, whereby the distribution of the calcium will be restored to normal and the tetany banished.

In the idiopathic tetany of childhood, on the other hand, the serum calcium is actually decreased, not infrequently to as low a level as 6 mg. per 100 c.c., and improvement in the condition coincides with an increase in the serum calcium. Actual estimation of the serum calcium is rarely of value, however, since the presence of rickets makes the cause of the tetany sufficiently obvious. Treatment is directed towards the underlying rickets. In urgent cases the serum calcium may be raised, and the symptoms of tetany abolished, by administration of calcium chloride intravenously, or parathormone intramuscularly. Though such medication is rapidly effective, its action is purely temporary, and permanent relief is to be sought in improvement of the normal assimilation process by administration of vitamin D and a diet rich in calcium.

Complete removal of the parathyroids is followed by a rapid fall in the serum calcium to some 5 or 6 mg. per 100 c.c., and acute tetany usually appears within three or four days of the operation. Little harm results, however, from the partial removal of the glands, so that, although tetania parathyreopriva occasionally follows thyroidectomy or destruction of the parathyroids by tubercle, it is very rare. Intravenous administration of parathormone has been used successfully to remove the symptoms of tetany with simultaneous raising of the serum calcium. The parathyroid hormone does not aid absorption or diminish excretion of calcium, its function, apparently, being to maintain the balance between the calcium content of the blood and soft tissues and that of the bones. Excess of the hormone produces hypercalcaemia, which, if sufficiently severe and prolonged, is dangerous, and may even prove fatal. Hence treatment with parathormone should always be controlled by frequent estimations of the serum calcium.

A point of practical importance arising from these considerations is that parathormone is of no service except as an emergency measure in tetany. Indeed, it is obviously contra-indicated in those conditions in which the object of treatment is to bring about deposition of calcium in the bones, and not merely to raise the serum calcium. When parathyroid therapy is employed our experience shows that parathormone, injected subcutaneously or intramuscularly, regularly produces an increase in the serum calcium. Given by the mouth, however, it has no effect on the serum calcium, and dried preparations of the gland are useless.

It is mainly in cases of deficient parathyroid

function that regular administration of the hormone may be useful, though it can be of service in combating the tetany which sometimes results from grossly deficient calcium absorption in sprue. As an emergency measure, a single dose or a short series of doses may be of use in dealing with other tetanies, *e.g.* the spasmophilia associated with low calcium rickets. Absorption of calcium may be assisted by increasing both the amount of vitamin D available (either by direct administration of some source of the vitamin, or by exposure to sunlight or ultra-violet light) and the amount of calcium in the diet (either by increasing the calcium-rich foods, such as milk, or by direct administration of calcium salts). In attempting to raise the serum calcium by administration of calcium salts it seems to matter little what salt is used, provided it is given in sufficient quantity, but the lactate is most often used on account of its solubility and palatability. At best, much of the calcium ingested escapes absorption, and to produce a rapid rise in the serum calcium small doses are useless. Thus cases of parathyroid tetany have been found to respond well to treatment with a high calcium diet supplemented by vitamin D and a daily intake of 300 grains of calcium lactate. The dose of calcium lactate can usually be reduced gradually, and often finally abolished. It has to be remembered, of course, that just as a deficiency in calcium metabolism is not necessarily reflected in a decrease in the serum calcium, so calcium administration may produce a beneficial effect without causing an appreciable increase in the serum calcium. It would seem that a low serum calcium is readily raised to normal by ingestion of vitamin D and calcium salts, but that it is

relatively difficult to produce a hypercalcaemia by such means. With large doses of calcium salts, or grossly excessive amounts of vitamin D, however, even this can be accomplished.

LEAD POISONING

The conditions which govern the deposition of calcium phosphate in, and its resorption from, bone, may be expected to affect similarly any other insoluble phosphate. Under conditions which favour calcification there has been shown to be a tendency for various metals (*e.g.* arsenic, silver, mercury, strontium, barium, radium, lead) to be deposited in bone. The case of lead has been most fully studied, but the results obtained in researches with that metal seem likely to apply to others. Lead is removed from the circulation and deposited in the bones when calcification is active—*i.e.* when the patient is receiving a high calcium diet rich in vitamin D. Since the symptoms of lead poisoning are due to the circulating lead, and since the metal is deposited in the bones more easily than it is excreted, this affords a rapid means of abolishing the more dangerous results of lead poisoning. Excretion from the body must be the final aim of treatment, however, and therefore the deposition of the lead in the bones is followed by its resorption at a rate so controlled that the excretory mechanism can prevent a dangerous accumulation in the blood and soft tissues. This can be accomplished by giving a low calcium diet, poor in vitamin D, and supplemented either by careful dosage with parathyroid hormone or by an acidosis-producing salt, such as ammonium chloride. Should symptoms

of active lead poisoning reappear, the process is immediately reversed. It is usual, indeed, to alternate the periods of high and low calcium diets, each period lasting about a week. The conditions which favour resorption of calcium and lead from the bones can also be used diagnostically in cases of possible lead poisoning, for whereas the amount of lead ordinarily present in the urine may be insufficient to establish the diagnosis, an increased amount will usually be found, in cases of lead poisoning, after administration of parathyroid hormone or of ammonium chloride.

CHAPTER XIII

THE BLOOD SEDIMENTATION RATE

WHEN blood is prevented from clotting, it separates on standing into an upper layer of plasma and a lower layer of erythrocytes. This erythrocyte sedimentation is more rapid in pregnancy, in acute infective conditions, and in certain diseases associated with toxæmia and tissue destruction. In health, however, the sedimentation rate is fairly constant, though it is slightly more rapid in women than in men. The cause of an abnormal sedimentation rate is not well understood and involves complicated questions relating to the physics of the blood, such as its suspension—stability, and its albumen-globulin ratio. It is commonly supposed that tissue breakdown releases an increased amount of fibrinogen, which in its turn induces an increased clumping or larger rouleaux formation of the red cells. The larger clumps settle more rapidly than smaller clumps or single cells, and an increased rate of sedimentation thus occurs.

Methods

There are, unfortunately, several methods in common use for performing the blood sedimentation test, and there are also several ways of recording the results. This often makes it difficult to make an accurate comparison between the results obtained by one observer with those obtained by another.

The method which is, perhaps, in most common use is that of Westergren. One c.c. of a 3.8 per cent. solution of sodium citrate is drawn into a 5 c.c. syringe, or 2 c.c. into a 10 c.c. syringe. The needle is then attached and by venepuncture the syringe is completely filled with blood. The citrated blood is then emptied into a test-tube, and thoroughly mixed by inversion. The actual test is performed in a special pipette, which is graduated in millimetres and has a mark on it 200 mm. from its lower end. The mixture of citrated blood is sucked into the pipette several times to ensure complete mixing, and having eventually sucked it up accurately to the 200 mm. mark, the pipette is set to stand vertically in a special rack with its lower end embedded in plasticine or in a rubber cork. The rate of sedimentation of the red cells is read off in an hour's time—the measurement taken being the difference between the lower edge of the meniscus of the plasma and the upper free surface of the red cells. In healthy persons the erythrocytes have usually fallen from 3 to 5 mm. in an hour's time. A reading above 8 mm. is suspicious, and above 12 mm. is certainly pathological.

A frequently used modification of this method consists in expelling the whole 10 c.c. of citrated blood, obtained as above, into a special tube which is graduated from 0 to 10 c.c. in increments of 0.1 c.c. The blood thus fills the tube to the top mark. After thorough mixing by inversion, the tube is stood up vertically and the height of the column of sedimented erythrocytes is read off in an hour's time. The method of recording the result, using this method, thus differs from that of the Westergren technique. In a healthy individual, after an hour's sedimentation, the column

of red cells will extend up to and beyond the 9 c.c. mark. Any reading below 8.5 c.c. must be regarded as definitely pathological.

Various micro-methods are also employed, using blood which is obtained from a finger prick, which is then mixed with a proper proportion of citrate and drawn up into a thin pipette. These micro-methods require only the simplest of apparatus and dispense with venepuncture. They require, however, rather more skill in technique, and the results are perhaps not quite so accurate. In addition, they are not in such common use, and thus the results so obtained are not so easy to compare with those recorded by the majority of other workers.

Value of the Test

It must be clearly understood that an increased sedimentation rate is not diagnostic of any particular disease, since it is definitely non-specific, being influenced by a large variety of pathological conditions. A normal result may sometimes, however, be of diagnostic service, since it may help to exclude some suspected disease usually associated with an increased sedimentation rate. Conversely when such a disease is suspected, the findings of an increased sedimentation rate may furnish another small link in the diagnostic chain of evidence.

In acute inflammations the increased rate of sedimentation is roughly proportional to the severity and extent of the inflammatory process. Thus a mild upper respiratory tract catarrh will only affect the rate very slightly, while a suppurating appendix will have a greater effect, and a generalised pelvic cellulitis

or peritonitis will cause a very rapid sedimentation indeed. The test is of no value as an assessment of the infections of pregnancy, since a considerably increased rate of sedimentation is observed normally after the third or fourth month of pregnancy, and persists for a few weeks into the puerperium. An ectopic gestation, however, which practically never continues beyond the first few months, does not significantly affect the sedimentation rate. The test has thus been used in distinguishing between an ectopic gestation and salpingitis, which latter causes a markedly increased sedimentation rate. It has been claimed that in pelvic inflammations it is dangerous to perform an extensive operation if the sedimentation rate is over 18 mm. (Westergren), and that when such readings are obtained it is wise to treat the case conservatively or, when an abscess is present, to be content with a simple drainage operation.

An increased sedimentation rate is observed in malignant disease; and where the tumour is fungating or extensive, the rate is seldom lower than 18 mm. Early localised malignant tumours or slow-growing scirrhus carcinomata do not cause such rapid rates, and benign tumours give normal results. It is claimed that benign fibroid or ovarian tumours may thus be differentiated from an abdominal swelling due to pregnancy, provided that such tumours are not undergoing torsion or degenerative changes. The clinical signs of pregnancy and the Asheim-Zondek reaction are, however, much more reliable diagnostic criteria.

The sedimentation rate is greatly increased in rheumatic fever, as in other febrile conditions, readings of from 60–120 mm. being obtained during the acute stages of the disease. The rate falls as the

temperature and severe symptoms decline, but may still remain considerably increased long after the temperature and pulse rate have become normal and the physical signs and symptoms have completely disappeared. We believe that the repeated application of the test to a patient in the convalescent stage of acute rheumatism gives a most valuable index of the latent activity of the rheumatic process, and it is probably unwise to allow such patients out of bed till the sedimentation rate has returned to normal.

A normal rate of sedimentation is probably never found in active tuberculous disease. This applies to tuberculosis generally and not only to the pulmonary variety. By repeating the test at intervals of a month a conception of the progress of the disease can be obtained which is often a more delicate expression of the activity and extent of the tuberculous process than any other single clinical finding, such as the pulse rate, temperature, or weight; and it is in tuberculosis that the test has received its widest application as a prognostic index. In an afebrile ambulant case, showing little systemic or local disturbance, some additional aid in the estimation of prognosis is most useful, so that the patient's exercise or work may be regulated with greater accuracy, and it is in such cases that the test is of particular value. Further, when serial readings are being made on a patient, a suddenly increased rate of sedimentation not infrequently gives warning earlier than clinical signs or symptoms of an oncoming exacerbation of the disease or of incipient complications, and the test thus forms a guiding factor of real value in the treatment and control of a tuberculous case.

In anaemias the sedimentation rate is increased

proportionately to the degree of anaemia. Owing to this fact it has been held by some workers that a blood count should always be performed at the same time as the test is carried out, and an appropriate correction made when anaemia is present. Unless the anaemia is very profound, however, when the sedimentation test will obviously be inaccurate as a prognostic guide in an associated disease, we do not believe that such a correction is necessary, nor in our experience are the results more accurate when it is made. In polycythaemia and in cyanotic conditions the sedimentation rate is, as might be expected, slower than normal.

In summary, the blood sedimentation rate may be of some slight use in diagnosis, but its main value lies in prognosis, especially in tuberculous and rheumatic conditions. In these diseases it is particularly useful as an assessment of the degree of active disease which may still be present when other signs and symptoms of activity have disappeared.

APPENDIX I

THE EXAMINATION OF THE URINE

Volume

THE normal twenty-four hour volume of urine may be taken as 1200–1400 c.c., but is greatly influenced by diet and environmental temperature. Marked increases are found characteristically in diabetes insipidus, diabetes mellitus, certain types of kidney disease, and in a few diseases of the nervous system, etc. Oliguria is usually present in febrile conditions, in dehydration caused by diarrhoea or vomiting, acute nephritis, etc.

Normally, the night urine collected from 10 p.m. to 6 a.m. forms not more than a quarter to a third of the total daily output. In cases of renal disease, the proportion of the urine secreted at night is increased, and its specific gravity approximates more closely than usual to that of the whole twenty-four hour sample.

Colour

The normal yellow tint of urine is due principally to urochrome, but traces of haematoporphyrin, urobilinogen, uroerythrin, and other pigments are present; its intensity varies with the concentration of solids in general, *i.e.* with the specific gravity of the urine, which, in turn, depends very largely on the

daily volume. Hence the straw-like colour of the average urine becomes a pale lemon in the dilute urine of diabetes mellitus (and disappears almost entirely in that of diabetes insipidus), while it deepens to amber when the urine is scanty.

The colour of the urine may be altered in a variety of ways—by certain inborn errors of metabolism which may be harmless; by pathological processes, or by drugs. The more important of these are summarised in Table XIII.

Tests for Urinary Pigments

Haemoglobin.—The presence of erythrocytes must be determined by use of the microscope to differentiate passage of the cells into the urine (haematuria) from excretion of previously liberated pigment (haemoglobinuria). The pigment may be detected spectroscopically (p. 340) or by tests based on its peroxidase-like action—an action which, unlike true enzymes, it retains after boiling. In the presence of hydrogen peroxide, haemoglobin (or its derivatives, methaemoglobin, haematin, etc.) brings about the oxidation of a variety of substances, *e.g.* guaiacol, benzidine, reduced phenol-phthalein.

Guaiac Test.—If the urine is alkaline, acidify it with dilute acetic acid (the test only works satisfactorily in an acid medium); boil for two minutes to destroy any peroxidases present (*e.g.* in pus); cool somewhat; add enough alcoholic solution of guaiac (1.5 per cent.) to produce a turbidity; then add a few drops of hydrogen peroxide solution, avoiding excess. A blue or green colour indicates the presence of haemoglobin or its iron-containing derivatives.

TABLE XIII

ABNORMAL URINARY APPEARANCES.

<i>Appearance</i>	<i>Cause</i>
<i>Reddish.</i>	<p><i>Haemoglobin</i>—disintegration of red cells in alkaline urine ; excretion of pigment released by intravascular haemolysis—scurvy, typhus, severe burning, various poisons, after blood transfusion, etc.</p> <p><i>Haemotoporphyrin</i>—principally after such drugs as quinine, trional, tetronal, sulphonal.</p> <p><i>Increased Uroerythrin</i>—after severe exercise, in acute fevers, etc. (i.e. when urates are abundantly present and a red sediment is produced).</p> <p><i>Drugs and Food Pigments</i>—e.g. santonin, pyrazolone (e.g. pyramidon), senna, rhubarb, madder, logwood, etc. (in some cases the colour is intensified if the urine is made alkaline).</p>
<i>Brown or brownish-black.</i>	<p><i>Methaemoglobin</i>—blackwater fever, etc. ; after various drugs, e.g. prontosil, chlorates, nitrites, acetanilide.</p> <p><i>Homogentisic Acid</i>—the rare condition, alcaptonuria, a failure to oxidise tyrosine normally.</p> <p><i>Dihydroxy-phenols</i>—after various aromatic drugs and poisons, e.g. carbolic acid, lysol.</p> <p><i>Melanin precursors</i>—melano-sarcoma.</p>
<i>Yellow or brown with greenish tinge.</i>	<p><i>Bile Pigments.</i></p> <p><i>Urobilin.</i></p>
<i>Dirty green.</i>	<p><i>Drugs</i>—e.g. methylene blue.</p> <p><i>Excess of Urinary Indican</i>—cholera, typhus, gangrene, etc.</p>
<i>Milky.</i>	<p><i>Fat</i> (chyluria).</p> <p><i>Pus.</i></p>

The test is more delicate when applied to the ether extract prepared as described for the benzidine test.

Benzidine Test.—A saturated solution of benzidine in glacial acetic acid can replace guaiac, but is not very satisfactory in testing urine, though it is very delicate with aqueous solutions of haemoglobin. The following modification, however, is reliable. Boil a few c.c. of urine for half a minute, acidify with acetic acid, and extract with 5–10 c.c. of ether. Evaporate the ether extract on a hot water-bath, and to the residue add a few drops of water, a drop or two of benzidine solution, and a drop of hydrogen peroxide. Haemoglobin, or any of its iron-containing derivatives, will give a green or blue colour.

As a control, the benzidine (or guaiac) solution should be tested with hydrogen peroxide alone. Some samples may give a blue colour and are then useless.

Haematoporphyrin and Methaemoglobin are detected by means of the spectroscope (pp. 339, *et seq.*).

Uroerythrin forms a pink deposit with urates, from which it is extracted by amyl alcohol. It gives a green colour with caustic soda or potash.

Homogentisic Acid.—Urine containing this acid may be normal in colour when fresh, but darkens on exposure to air, especially rapidly when alkaline. It reduces Fehling's solution (alkaline copper), but not Nylander's (alkaline bismuth).

Melanin Precursors.—In this case also the freshly voided urine may be yellow, darkening on exposure to air. Addition of a few drops of ferric chloride gives a gray colour; more of the reagent produces a dark precipitate which dissolves in excess.

Indican.—To a few c.c. of urine add an equal volume of concentrated hydrochloric acid, 2–3 c.c. of chloro-

form and a few drops of freshly prepared calcium hypochlorite (bleaching powder) or potassium chlorate solution. Closing the mouth of the test-tube by the thumb, invert the tube ten or twelve times without shaking violently. Indican is indoxyl potassium sulphate, and the indoxyl is oxidised by the bleaching powder to indigo blue (or sometimes indigo red), which dissolves in and colours the chloroform. If the colour is not a pure blue, the chloroform may be shaken with sodium thiosulphate, when interfering colours which are due to iodides (violet) or bromides (yellow-red) will be removed.

Bile Pigments.—The many tests for bile pigments are based on the oxidation of bilirubin to a series of coloured substances, green (biliverdin), blue (bili-cyanin), yellow (choletelin), etc.

Trousseau's Test.—The urine is acidified with acetic acid, and a dilute alcoholic solution of iodine (the oxidising agent) is added so as to form a separate upper layer. The presence of bile pigment is indicated by the development within a minute or so of a green ring at the junction of the two layers.

Huppert-Cole Test.—Boil about 10 c.c. of the urine, add two drops of saturated magnesium sulphate solution, and then a 10 per cent. barium chloride solution, drop by drop (boiling after each addition) until no further precipitation occurs. The precipitate absorbs the bile pigments. Allow it to settle, pour off the supernatant fluid, and to the precipitate add 3–5 c.c. of alcohol, two drops of concentrated sulphuric acid, and a single drop of a 5 per cent. solution of potassium chlorate. Boil for half a minute and allow the precipitate to settle. If the alcoholic liquid becomes coloured a greenish-blue bile pigment is

present. If the test is negative, add a second drop of potassium chlorate and repeat the boiling. Confirmation of the identity of the pigment may be obtained by adding an equal volume of water and 1–2 c.c. of chloroform. Bile pigment will be transferred, on shaking, to the chloroform layer which settles out at the bottom of the test-tube. Urobilin, if present, gives a reddish-brown colour.

Foam Test.—The two tests just described are very delicate. Urines containing a relatively large amount of bile pigment form, on shaking, a yellow foam; the sediment (if any) is stained yellow; and paper through which the urine is filtered is similarly coloured.

Urobilin.—The tests depend either on spectroscopic examination, or on the fact that solutions of urobilin appear yellow by transmitted light, but green by reflected light (*i.e.* have a green fluorescence). Other fluorescent substances (*e.g.* eosin) must be absent. To convert all urobilinogen (the form in which the pigment is mainly present in fresh urine) to urobilin, a mild oxidising agent is required. About 10 c.c. of the urine are acidified with acetic acid, and a few drops of iodine solution are added. To remove interfering pigments, an equal volume of a saturated alcoholic solution of zinc acetate is added, and the precipitate is removed by filtration. The filtrate is then examined for green fluorescence against a dark background. It may also be examined spectroscopically—urobilin, in acid solution, gives a single absorption band in the green-blue region of the spectrum, from a little to the right of *b* to slightly beyond *F*.

Fat.—The extraction of the urine with ether causes complete or partial clarification, and the fat may be

identified as such by separating and evaporating the ether layer.

Pus.—The microscope affords the best means of identifying pus (see p. 311). When present in relatively large amounts, pus gives a blue colour in the guaiac or benzidine test, provided the urine is not boiled.

Specific Gravity

The specific gravity of the urine varies, normally, from 1.010 to 1.025, for the twenty-four hour specimen, but in exceptional cases figures beyond this range may be given by urine from healthy persons. The specific gravity is low in diabetes insipidus, later stages of chronic azotaemic Bright's disease, etc., and high in fevers, the nephrotic syndrome, profuse glycosuria, etc. An approximate estimate of the total dissolved solids may be obtained by multiplying the last two figures of the specific gravity by 2.33 (Häser's coefficient). Thus if the specific gravity is 1.015, the total solids dissolved in the urine amount to 15×2.33 (=3.495) grams per litre. The figure obtained is only a rough approximation, and may be fallacious if the specific gravity is far removed from the normal range.

Measurement of the specific gravity is made by means of a hydrometer. The instrument sold for the purpose and graduated to cover the expected range of specific gravity in urine is commonly termed a *urinometer*. It consists of a suitably weighted glass cylinder surmounted by a stem so graduated that when the instrument is floating freely the graduation mark at the surface gives the specific gravity of the liquid. The urine is poured into a cylinder of adequate

size, and in sufficient amount (about 200 c.c.) to allow the urinometer to float freely. Foam, or even occasional bubbles, on the surface must be removed either

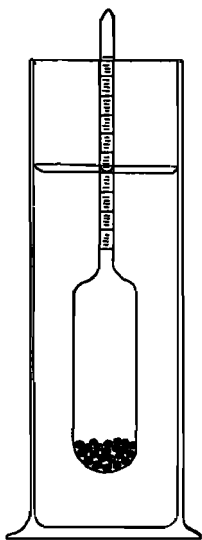


Fig. 29.
Urinometer.

with filter paper or by addition of a single drop of ether. The urinometer is then allowed to float freely—*i.e.* without contact with the walls of the cylinder. The reading is made by looking up obliquely through the urine and raising the eye until it is just level with the bottom of the curved surface of the urine. (Fig. 29.)

The urinometer is usually calibrated for a temperature of 15° C. Small differences of temperature can be ignored for ordinary purposes, but with larger ones it is desirable to correct by adding .0001 for each 3° C. above 15° C., or subtracting a similar amount for each 3° C. below 15° C. Thus at a temperature of 21° C., and an observed value of 1.018, the corrected specific gravity at 15° C. is 1.018, *i.e.* 1.020.

Reaction

Usually a twenty-four hour sample of urine is slightly acid (pH about 6), but so many factors, dietary and other, affect the urinary pH that a considerable difference must be expected and is, indeed, found. It is no uncommon thing to find urine from perfectly healthy persons with a pH as low as 5.5 or as high as 8.0; exceptional figures outside even this range have been reported. Since, however, the pH

of urine may be altered after collection by the action of organisms or by loss of CO_2 , excessively low or high values for the pH must be regarded with some suspicion unless it is shown that such factors have been non-operative. It follows that only fresh urine is useful for measurement of pH or of titratable acidity.

The reaction of urine is the result of equilibrium among a great many acidic and basic constituents both organic and inorganic, though of these the phosphates (X_2HPO_4 and XH_2PO_4) are from a quantitative standpoint the most important. The phosphates are, of course, buffer substances, since phosphoric acid is a weak acid. The monohydrogen phosphates are alkaline in solution, while the dihydrogen phosphates are acid, and although their concentration in blood is not very great, a considerable part of the effect of the kidneys in regulating tissue reaction consists in adjusting the absolute and relative amounts of these salts in its secretion.

Individual urine samples from the same person vary considerably in reaction as in other properties. There is a tendency to a regular swing; a more acid urine being excreted early in the morning after a fast (*acid tide*) and a relatively less acid urine after a meal and especially after breakfast (*alkaline tide*). There is no satisfactory explanation of this phenomenon, but the usual one, that it is due to the gastric secretion of hydrochloric acid, is certainly incorrect. Diet has a great effect, however, on the individual samples as well as on the mixed twenty-four hour sample. Cereals and meats contain an excess of acid radicles and tend to produce an acid urine; whereas fruits produce a tendency to an alkaline urine, since the acid radicles are largely organic and are burnt to CO_2 in the body.

Ordinarily; urine becomes more alkaline on standing owing to the production of ammonia from urea by bacteria which gain access to it from the air (*alkaline or ammoniacal fermentation*). Such a fermentation may begin within the bladder if the urine is already infected; *e.g.* in cystitis, and is then of some diagnostic importance. Occasionally urine becomes somewhat more acid (and simultaneously darker in colour) when allowed to stand (*acid fermentation*), usually owing to the presence of fungi. Eventually, even here, ammoniacal fermentation occurs, and the acidity then decreases as usual.

Urinary acidity is more often increased than decreased in disease. In cardiac and renal conditions in particular there is a marked tendency to a lower average pH.

Determination of Urinary pH.—For some purposes it is desirable to know the reaction of the urine with greater accuracy than is attained by the simple procedure of finding whether the sample is acid, neutral, or alkaline to litmus paper. The exact measurement of pH is by no means easy and is unnecessary for clinical purposes, but measurement to within 0.1 or 0.2, which is a relatively simple matter, is adequate for such purposes as control of mandelic acid therapy. The colour given by the urine on addition of an indicator is compared with that given by the same indicator added in the same amount to a series of solutions of known pH. Such a series of standard solutions, if kept in sealed tubes, will remain unchanged for several months, and can be obtained commercially from such firms as British Drug Houses. They are supplied with instructions as to the strength of the indicator solution and the amount to be used.

It is useless to give these instructions here therefore; especially as they vary somewhat with the source of the standard tubes. Very convenient (and more permanent) glass standards can be obtained, the principle involved being exactly the same as when liquid standards are used. When the pH of urine is being determined a complication is introduced by the fact that the urine is itself coloured. To overcome the difficulty a sample of urine without indicator is placed in front of the standard tube, and then, to equalise the depth of liquid which is being viewed, a tube of plain water is placed in front of the tube containing urine plus indicator. The examination is very conveniently made in a comparator, a box with six holes in the lid to accommodate six test-tubes, and with ground glass sides. The standard tubes are changed until the colour of the urine tube is identical with one or is midway between two.

Protein and Protein Derivatives

Albumin and Globulin.—There is no single test capable of detecting infallibly the presence of protein under all conditions. The proteins which at times appear in urine, however, are usually albumins and globulins, which are distinguished by being first altered (denatured) and then precipitated from solution (coagulated) by heat. This coagulation is greatest from a weakly acid solution, and may not occur at all if the solution is markedly alkaline. With proper precautions this property of albumins and globulins forms the best single test for the presence of these substances in urine. Other tests, depending on the precipitation of proteins by various reagents, are

available, but are, on the whole, more subject to fallacy.

The Heat-Coagulation Test.—Fill a test-tube about three-quarters full of urine (filtered if not already clear) and heat the upper part to boiling. Any turbidity is due to albumin or phosphates or both. Even if the urine remains clear, add a few drops of dilute acetic acid, avoiding excess of the acid, however, since excess of acid dissolves the coagulated albumin. If the turbidity is due to albumin (and globulin) alone, it will persist and may even increase; if phosphates are also present in the precipitate the turbidity will decrease, but will not disappear; if phosphates alone have been precipitated, then the urine will become quite clear. If acidification produces a precipitate, then the urine has been too alkaline to allow of coagulation, and the precipitate indicates the presence of protein. The object of heating the upper part of the tube only is to aid in the detection of small traces of protein, since a faint cloud is readily seen by comparison with the clear, unboiled urine below. The examination is best made by holding the tube against a dark background. Certain substances simulate albumin and globulin in this test—protein from the genito-urinary tract; the excretion products of certain drugs such as cubebs and turpentine; albumoses, Bence-Jones protein; bile salts in excessive amounts, etc. Protein from the genito-urinary tract is precipitated by acetic acid in the cold, without boiling, and so are bile salts. Turpentine, etc., produce resinous precipitates which, unlike albumin, are soluble in petrol-ether, alcohol, etc. Albumoses usually dissolve on boiling and reappear on cooling.

The Sulphosalicylic (Salicyl-sulphonic) Acid Test.—

To the urine add an equal volume of a 5 per cent. solution of sulphosalicylic acid in 20 per cent. sodium sulphate. Albumin gives a precipitate. The Bence-Jones protein also gives a precipitate, which, however, disappears on further heating, while the albumin precipitate remains. Alternatively, a 20 per cent. aqueous solution of the reagent may be added, drop by drop, to the urine.

The Nitric Acid Ring Test.—To about 5 c.c. of concentrated nitric acid in a test-tube add a few c.c. of urine, which has been diluted with two or three times its own volume of water, so that it forms a separate layer above the acid. Stratification of liquids is most easily accomplished by adding the lighter one from a pipette held so that the liquid flows slowly down the side of the test-tube. A white ring at the zone of contact indicates the presence of protein. The dilution of the urine will usually prevent precipitation of urates or of urea nitrate which might otherwise, in the case of concentrated urines, interfere with the test. Protein from the genito-urinary tract produces a ring, usually a little above the zone of contact of the two layers; Bence-Jones protein and albumoses give a ring at the zone of contact, but it disappears on heating; bile salts in high concentration give a precipitate which slowly spreads throughout the upper layer; resinous acids, thymol, etc., give precipitates, but can be removed from the urine by extraction with petrol-ether before the test is repeated.

Distinction between Albumin and Globulin.—It is not, as a rule, worth while to distinguish between albumin and globulin. It is, however, possible to do so. When urine is mixed with an equal volume of

saturated ammonium sulphate, the globulin is precipitated (usually along with ammonium urate, etc.), and the albumin, which remains in solution, can then be precipitated by further addition of solid ammonium sulphate.

Quantitative Estimation of Protein.—Although for accurate work much better methods are available, a rough, and perhaps sufficiently close, approximation to the quantity of protein present can be obtained by use of the Esbach albuminometer. The graduated tube (Fig. 30) is filled to the mark “U” with the urine to be tested, reagent (10 g. of picric acid and 20 g. of citric acid in a litre of water) is added to the mark “R,” the tube is stoppered, and, after mixing by inverting once or twice, it is allowed to stand undisturbed for twenty-four hours. The height of the precipitate in the tube is then read, and the reading, divided by ten, gives the grammes of protein in 100 c.c. of the urine. To obtain the relative amounts of albumin and globulin this procedure may be carried out with the untreated urine and also after the globulin has been precipitated with ammonium sulphate and filtered off. In the calculation, of course, it has to be remembered that in determining the albumin alone the urine has been diluted to twice its original volume by the addition of saturated ammonium sulphate, and the result has therefore to be multiplied by two. The difference between the total protein and the albumin gives the globulin.

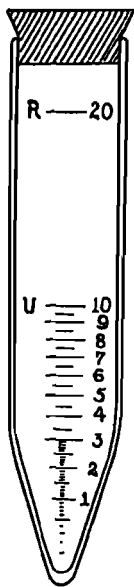


FIG. 30.
The Esbach
Albumino-
meter.

albumin alone the urine has been diluted to twice its original volume by the addition of saturated ammonium sulphate, and the result has therefore to be multiplied by two. The difference between the total protein and the albumin gives the globulin.

Bence-Jones Protein.—This substance, which is probably a proteose, has been found in the urine from cases of multiple myeloma, occasionally in that from cases of malignant disease involving the bone marrow, and of leukaemia. Its occurrence is uncommon, and since other proteoses (which are of relatively frequent occurrence) resemble it in many ways, its identification with certainty is difficult. Positive results with the usual tests indicate that it is probably present.

Heat Coagulation Test.—(a) The urine must be faintly acid to litmus, acetic acid being added if necessary. It is then heated gently in a test-tube with a thermometer in the liquid. Bence-Jones protein gives a precipitate which appears about 40° C., is maximal about 60° C., and disappears as the temperature rises further. The disappearance may be incomplete, since albumin is often present as well. In that case the boiling urine is filtered rapidly to remove the albumin. Bence-Jones protein reappears as the filtrate cools. The precipitate flocculates and sticks to the sides of the test-tube.

(b) Make 10 c.c. of the urine acid by adding 2 c.c. of 50 per cent. acetic acid. Filter off any precipitate (urates, resin acids, bile salts, etc.). To 5 c.c. of the filtrate add 3 c.c. of saturated sodium chloride. Bence-Jones protein gives a precipitate which disappears on warming (globulin in high concentrations also gives a precipitate which, however, persists when the urine is heated). Albumin (and globulin in lower concentrations) gives a precipitate only after heating.

Estimation of Urea in Urine.—Urea is, of course, the chief nitrogenous end-product of protein metabolism, and the amount of urea excreted thus depends on the

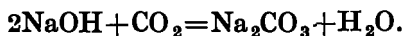
amount of protein metabolised and therefore on the protein content of the diet. Urea containing 1 g. of nitrogen is produced from rather more than 6 g. of protein. Hence with a daily protein intake of 80 g. (an average amount) the daily output of nitrogen in the form of urea (*plus* ammonia which may have escaped urea formation or may have been re-formed from urea) is about 13 g., which corresponds to about 26 g. of urea. Taking the average daily volume of urine as 1800 c.c., this gives the average concentration of urea plus ammonia nitrogen in the urine as 1 per cent.

There are two methods of estimating urea, both of which include the ammonia. Ordinarily the two are not differentiated, since it is their sum which indicates the amount of protein metabolism. The ammonia can, however, readily be estimated separately and the urea found by difference.

The Hypobromite Method.—Urea is decomposed by sodium hypobromite, with liberation of nitrogen and carbon dioxide :



if the reacting mixture is kept alkaline, the CO_2 is absorbed and only the nitrogen appears as a gas.



Hence 1 gram molecule (=22,400 c.c. at N.T.P.) of nitrogen are obtained from 1 gram molecule (=60 g.) of urea. Obviously, by measuring the gas evolved in such an experiment, the amount of urea present can readily be calculated. For clinical purposes, the small error involved in collecting the nitrogen at room temperature and pressure without correcting to N.T.P.

can be ignored, and the apparatus can be constructed with the gas-measuring part graduated in grams of urea instead of c.c. of nitrogen. The method, though sufficient for many purposes, is in any case inaccurate, since hypobromite reacts with ammonia, uric acid, creatinine, etc., in the same way as with urea, yielding nitrogen, and even with pure urea does not give a quantitative yield.

The apparatus used is termed a *Ureometer*. One convenient type is shown in Fig. 31. The instrument is filled with the reagent, the tap communicating with the side-tube being kept closed. Care must be taken that all air is removed, especially from the small space below the side-tube tap. The urine

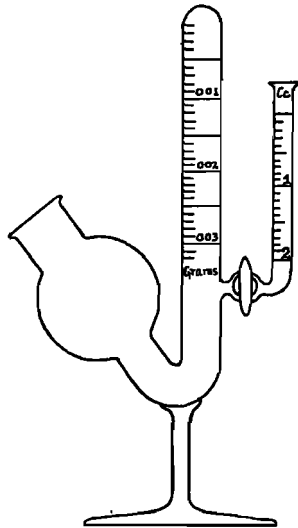


FIG. 31.—A Ureometer.

to be tested, which must be clear, and protein-free, is introduced into the graduated side-tube from a pipette, and a measured volume (usually 1 c.c.) is admitted through the tap. Fifteen minutes are allowed for the reaction to proceed, and the volume of nitrogen evolved is read in terms of grams of urea per volume of urine used. If 1 c.c. of urine has been used, this figure multiplied by 100 gives the percentage of urea in the urine.

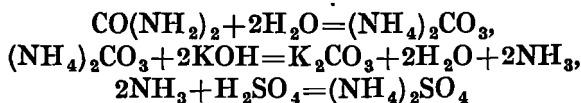
The reagent should be freshly prepared by adding one volume of bromine to ten volumes of 40 per cent. sodium hydroxide. The mixture is immediately stoppered, thoroughly shaken, and allowed to cool

before being used. About 30 c.c. of reagent are required for each estimation, the exact amount depending on the capacity of the particular ureometer tube used.

The Urease Method.—Urea, being the amide of carbonic acid, can be hydrolysed to ammonium carbonate. This hydrolysis is enormously accelerated by the specific enzyme *urease*, which is present in soya and Jack beans. Since the enzyme causes the hydrolysis of urea only, all the ammonia present in urine submitted to its action will consist of that formed from urea plus pre-existing ammonia. The total amount of ammonia can then be estimated in various ways. Usually the urine, after the enzyme has acted, is made alkaline, and the ammonia, thereby set free, is drawn off by a current of air and absorbed by a known volume of standard sulphuric acid. Some of the acid is neutralised in combining with the ammonia to form ammonium sulphate, and the amount left over can be estimated by titration with sodium hydroxide.

Suppose the original amount of acid was 10 c.c. N/70 H_2SO_4 , and the amount of sodium hydroxide used in the titration was x c.c. N/70 NaOH. Since x c.c. N/70 NaOH neutralise x c.c. N/70 H_2SO_4 , this amount of acid was left un-neutralised by the ammonia. Hence the amount neutralised by ammonia was $(10 - x)$ c.c. N/70 H_2SO_4 .

From the equations :



it is evident that 1 g. mol. of H_2SO_4 neutralises the ammonia from 1 g. mol. (=60 g.) of urea which con-

tains 28 g. of nitrogen. Now 1 g. mol. of H_2SO_4 forms 2000 c.c. of N solution, or 140,000 c.c. of N/70 H_2SO_4 solution.

Therefore, 140,000 c.c. N/70 H_2SO_4 used up would indicate the presence of 60 g. of urea or 28 g. of urea nitrogen.

Therefore $(10-x)$ c.c. N/70 H_2SO_4 used up indicate the presence of $\frac{60(10-x)}{140,000}$ g. of urea or $\frac{28(10-x)}{140,000}$ g. of urea nitrogen.

If, in the analysis, v c.c. of urine were used, the percentage of urea is evidently $\frac{60(10-x) \times 100}{140,000 xv}$, *i.e.* $\frac{3(10-x)}{70 xv}$; that of urea nitrogen is $\frac{28(10-x) \times 100}{140,000 xv} = \frac{(10-x)}{50 xv}$.

Usually the result is expressed as urea nitrogen, and v is made 0.1 c.c. In that case the percentage of urea nitrogen is $\frac{10-x}{5}$. It is in order to simplify the final calculation in this way that N/70 acid and alkali are used.

The apparatus required is shown in Fig. 32. Any number of estimations can be carried out simultaneously by connecting the exit-tube of the tube "C" of the first set to the entrance-tube of the tube "B" of the second set, and so on. The tube "A" contains dilute sulphuric acid to remove any ammonia present in the air, and only one is required, however many sets are connected in the series.

For suction a glass filter-pump attached to the ordinary water-tap suffices.

Dilute the urine accurately to ten times its own volume with distilled water, and measure exactly 1.0 c.c. of this diluted mixture. Into "B," a large test-tube of about 1 in. diameter and 8 in. length, add about half a gramme of soya-bean meal and two

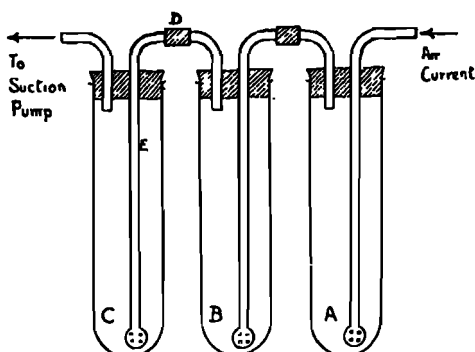


FIG. 32.—Apparatus for the estimation of urea or ammonia in blood or urine. The tube "A" contains dilute sulphuric acid to absorb any traces of ammonia from the air; "B" contains the blood or urine and reagents, and "C" contains the standard sulphuric acid.

or three drops of activator. The activator is a solution of 14 g. of sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$) in enough 0.5 normal phosphoric acid to make 100 c.c. Instead of soya-bean meal, commercial preparations already containing the activator may be obtained. The tube is then incubated at 50°C . for fifteen minutes, most simply by immersion in a large beaker of water kept near that temperature. During this incubation the urea is hydrolysed. The apparatus is then connected, as shown in the diagram, with 10 c.c. of a N/70 solution of sulphuric acid in the tube "C." Into

tube "B" is then poured 5–10 c.c. of distilled water, a drop or two of caprylic alcohol, the function of which is merely to prevent frothing, and about 5 c.c. of approximately 20 per cent. sodium carbonate, to make the mixture alkaline and so allow the ammonia to be drawn off by the current of air. The addition of the alkali and the replacement of the stopper must be carried out rapidly to avoid loss of ammonia. The current of air is then started, slowly at first, and gradually increased until it is as rapid as is possible without dangerous splashing of the liquids. It is not possible to lay down any hard-and-fast rule for the time of aeration, which will depend, amongst other factors, on the efficiency of the pump and on the water-pressure, but in our own laboratory we find it necessary to aerate for an hour and a half in order to remove the ammonia completely. The ammonia is carried by the air current into the tube "C," where it is absorbed by the sulphuric acid, some of which is accordingly neutralised. The excess of acid present is determined by titration with N/70 sodium hydroxide, using methyl-red as indicator. Before titration (disconnect at "D") the tube "E" must be washed, both inside and outside, with distilled water.

Estimation of Ammonia in Urine.—The method is very similar to that employed for estimation of urea plus ammonia, the only difference being that the preliminary digestion with urease—the object of which is simply to convert the urea to an ammonium salt—is omitted. Usually about 2 c.c. of urine are required, though with very dilute urines 5 c.c., and with urine from a case of acidosis only 1 c.c., should be used. The calculation, as before, is simple, the number of

milligrammes of ammonia nitrogen per 100 c.c. of urine being given by the formula :

$$\frac{20 \times (10 - x)}{\text{vol. of urine used}},$$

and the amount of ammonia itself being obtained by multiplying this figure by $\frac{17}{14}$.

Sugar

Normally, urine contains traces of carbohydrates (mainly glucose), but the tests ordinarily used are not sufficiently sensitive to detect such small amounts. The usual qualitative tests are really tests for reducing substances and are not specific for reducing sugars ; considerable quantities of non-carbohydrate reducing substances, however, are present in urine only very exceptionally. In practice, therefore, a clear-cut positive result with these tests properly performed indicates the presence of reducing sugar with reasonable certainty. Generally, any reducing sugar found in urine is glucose, but at times other sugars may be present—lactose, fructose, pentoses—and it may be necessary to use more specific tests to identify the sugar.

Lactosuria may occur during pregnancy and lactation ; fructosuria in a normal person after ingestion of fructose (or cane sugar) in considerable amount, more easily in diabetes and perhaps in cases of liver disease ; pentosuria is found very occasionally as a personal idiosyncrasy, but may occur after the ingestion of large amounts of vegetables or fruits containing pentose derivatives (pentosans).

Detection of Sugar.

Fehling's Test.—Fehling's solution is a mixture (freshly made when required) of equal parts of solutions A and B. A contains 69·3 g. of copper sulphate per litre; B contains 250 g. of potassium hydroxide and 346 g. of Rochelle salt per litre. It is essentially an alkaline solution of cupric hydroxide which is reduced by glucose, etc., to yellow-red insoluble cuprous oxide on heating.

In separate test-tubes boil about 5 c.c. of urine and the reagent. The urine should be clear (filter if necessary) and not excessively acid, as the final mixture *must* be alkaline. As soon as the fluids boil, mix them and *do not heat further*. In the presence of sugar a precipitate appears (at once or on standing for a minute or two) varying in colour from green to yellow, red or reddish-brown. No very definite deduction as to the amount of sugar can be drawn from the colour. A clear greenish-blue or purple colour may be ignored; it is usually due to excessive amounts of urobilin or protein. A dirty green precipitate may be due to traces of sugar or to excessive amounts of interfering substances such as glycuronates, creatininc, urates, and added preservatives such as formalin and chloroform. If such a precipitate appears, dilute the urine and repeat the test or use other tests.

Benedict's Test.—Benedict's solution contains 17·3 g. of copper sulphate, 173 g. of sodium or potassium citrate, and 100 g. of anhydrous sodium carbonate per litre. To 5 c.c. of the reagent add not more than eight drops of urine and boil the mixture vigorously for two minutes. Allow it to cool spontaneously—*i.e.* without immersion in cold water. A precipitate

—usually yellow, but sometimes greenish or red—appearing during the boiling denotes the presence of over 0·3 per cent. of sugar ; one which appears during the subsequent cooling denotes the presence of 0·1 to 0·3 per cent. of sugar. Benedict's test is much less sensitive than Fehling's to creatinine, urates, formalin, and chloroform, but gives positive results with excessive amounts of glycuronates.

Nylander's Test.—The reagent is prepared by saturating with bismuth oxynitrate a solution containing 4 per cent. Rochelle salt and 10 per cent. sodium hydroxide. The final solution is then filtered. The urine, freed from albumin by boiling and acidification with acetic acid, is mixed with one-tenth of its volume of the reagent, and boiled for five minutes (not longer). Sugar is indicated by the appearance of a dark coloration or precipitate of metallic bismuth. Creatinine and urates do not give a positive result, but glycuronates, chloroform, and formaldehyde do.

Mucic Acid Test for Lactose and Galactose.—Evaporate in a porcelain dish a mixture of 100 c.c. of urine and 20 c.c. of concentrated nitric acid. When the volume of the mixture has been reduced to 20 c.c. (when it should still be quite clear), allow it to cool. Lactose or galactose will have yielded mucic acid through the oxidising action of the nitric acid, and this will form a white precipitate when the solution is allowed to cool and, possibly, to stand for some time.

Seliwanoff's Test for Fructose.—To 5 c.c. of a solution containing 0·05 g. of resorcinol dissolved in 100 c.c. of dilute hydrochloric acid (30 c.c. of concentrated acid diluted to 100 c.c. with water) add a few drops of urine, heat to boiling, and allow to boil for *not more than thirty seconds*. If fructose is present,

a red colour followed, perhaps, by a red or reddish-brown precipitate will appear. The precipitate dissolves in alcohol, giving a red solution. Prolonged boiling must be avoided, since glucose, if present in considerable amounts, reacts slowly with the reagent. Certain drugs, such as santonin and rhubarb, also give a positive reaction.

Tests for Pentoses.

(a) *The Phloroglucinol Test (Tollens).*—To a mixture of equal volumes of urine and concentrated hydrochloric acid (about 5 c.c. of each) add a little solid phloroglucinol and heat on a boiling water-bath. Pentose, galactose, lactose, and glycuronic acid all give a red colour, followed by a dark precipitate if the substance is present in large quantity. Examine the red solution spectroscopically; in the presence of pentoses and glycuronic acid there will be an absorption band midway between D and E.

(b) *The Orcin Test (Bial's Modification).*—Bial's reagent consists of a 0.3 per cent. solution of orcin in concentrated hydrochloric acid, with addition of five drops per 100 c.c. of 10 per cent. ferric chloride. Heat about 5 c.c. of the reagent to boiling, and add a few drops (not more than 1 c.c.) of urine without further heating. A green colour, appearing at once or developing in a few seconds as the solution cools, indicates the presence of pentose. The coloured substance is soluble in amyl alcohol. Glycuronic acid does not interfere.

Glycuronic Acid.—Urine normally contains no free glycuronic acid, but a trace of "conjugated glycuronates"—compounds of glycuronic acid with phenols,

alcohols, etc., produced in the liver for purposes of detoxication. The amount of glycuronate excreted is, normally, increased after ingestion of phenols, alcohols, etc., such as menthol, camphor, antipyrin, salicylic acid derivatives, chloral hydrate.

The Naphthoresorcinol Test (Tollens).—To about 5 c.c. of urine add about 1 c.c. of a 1 per cent. alcoholic solution of naphthoresorcinol (or an equivalent amount of the solid substances) and about 5 c.c. of concentrated hydrochloric acid. Boil the mixture for one minute, allow it to stand for four minutes, and then cool rapidly in running water. Extract with about 10 c.c. of ether (addition of a few drops of alcohol may help the separation into water and ether layers). If glycuronates are present in large amounts the ether layer will be coloured dark blue or violet; reddish-purple or faintly blue with smaller amounts. Examined *immediately* by the spectroscope, a single absorption band near the D line will be seen. The amount of glycuronates normally present in urine is not detected; other substances (*e.g.* pentoses) produce pigments which, however, are insoluble in ether.

Identification of Carbohydrates by Preparation of Osazones.—All reducing sugars react with phenyl hydrazine, giving crystalline osazones. The osazones of glucose and fructose are identical, but differ in crystalline shape, melting-point, and rotation from those of lactose, mallosi, pentoses, and glycuronates. Unfortunately, though easy enough to prepare from pure solutions, osazones frequently fail to crystallise readily from urine. This is especially true of concentrated urines, which seem to prevent their proper precipitation. It is often advisable to dilute the urine with water, but even so, in our experience, a con-

siderable amount of practice is necessary before satisfactory results are obtained.

A suitable reagent is prepared by mixing equal weights of phenylhydrazine hydrochloride and anhydrous sodium acetate. To enough of this mixture to fill a test-tube to a depth of about half an inch, add 5 c.c. of urine, shake, and heat on a boiling water-bath for forty-five minutes. Add sufficient boiling water to restore the volume to its original 5 c.c. (If the urine is known to be concentrated with respect to urea, dilute it with an equal volume of water before making the experiment.) Allow the test-tube to cool slowly, and when quite cold examine any crystals microscopically. If they are present in sufficient quantity, they may be filtered off and re-crystallised from 60 per cent. of alcohol.

The osazones of glucose and fructose are identical, crystallising in sheaves of long, straight, thin, yellow needles, or in "thorn apple" bundles, melting-point 205° C.; the osazone of maltose forms rosettes of thick stumpy yellow needles, melting-point 207° C.; that of lactose forms rosettes or clumps of hair-like, curved yellow needles, melting-point 200° C.; that of arabinose (the commonest urinary pentose) forms long, straight, thin needles, melting at 168° C.

Quantitative Estimation of Glucose and Lactose.—Ordinarily the total sugar of the urine is estimated and assumed to be glucose. If the presence of lactose is known or suspected it is possible, by quantitative analysis, to show whether it is alone or whether glucose is also present. Of the various methods available for estimating sugar in urine, that of Benedict is a good example of the titration method in which the object is to find what volume of urine contains a

definite amount of sugar, equivalent to a measured volume of reagent. That of Cole (which we prefer for lactose estimation) depends on the idea that the greater the concentration of sugar, the more quickly will the reagent be reduced.

Benedict's Method.—The reagent is prepared by dissolving 100 g. of anhydrous sodium carbonate, 200 g. of sodium citrate, and 125 g. of potassium thiocyanate in about 800 c.c. of warm distilled water, filtering, adding slowly with constant stirring exactly 18 g. of copper sulphate dissolved in 100 c.c. water, then adding 5 c.c. of 5 per cent. potassium ferrocyanide solution, and finally (after cooling) making the volume up to 1000 c.c. with distilled water. This is, in effect, an alkaline solution of cupric thiocyanate which is reduced by sugar to the white insoluble cuprous thiocyanate.

Twenty-five c.c. of this reagent, which will be reduced by 50 mg. of glucose, are measured into a porcelain basin, 5–10 g. of anhydrous sodium carbonate (or twice as much of the crystalline salt) are added and a little powdered pumice or broken glass (to prevent bumping). The mixture is boiled, and *kept boiling freely* while the urine is run from a 50 c.c. burette. The urine is added fairly rapidly, but not so as to stop the mixture from boiling, until, with the formation of a chalky precipitate, the mixture has become perceptibly paler. Thereafter it is added more cautiously until the last trace of blue has disappeared, a point which marks the end of the titration. It is advisable to allow an interval of 15–30 seconds between successive additions of urine, but the process should not be unduly prolonged. Water may be added if the mixture becomes very concentrated,

but the mixture must be allowed to boil again before the titration is continued. Usually the end point is a little difficult to see, as the colour of the urine causes the final mixture to be a dirty yellow. In order to improve the end point we have found it advantageous to decolorize the urine before using it in the titration by shaking 50 c.c. of it with about a teaspoonful of Merck's medicinal charcoal, and filtering.

If the volume of urine used in the titration has been x c.c., then that is the volume which contains 50 mg. of glucose. Hence 100 c.c. of urine contains $\frac{50 \times 100 \text{ mg.}}{x}$

In other words, the urine contains $\frac{5}{x}$ per cent. of glucose.

If the volume of urine used is very small, the titration will not be accurate. It is then necessary to dilute the urine with distilled water to, say, ten times its original volume, and to repeat the titration with the diluted, shaken urine. In that case, assuming the urine to have been diluted tenfold, and the volume used in the titration to have been x c.c., the percentage of sugar in the original urine will be $\frac{50}{x}$. In general, if the urine is diluted to a times its original volume, the urine sugar percentage is $\frac{5a}{x}$.

Cole's Method.—This is a rapid and convenient method which may be used for the differentiation of glucose and lactose. About 10 c.c. of the urine are shaken with about 0.3 g. (a small teaspoonful) of Merck's medicinal charcoal, and the mixture is filtered. This treatment removes the pigment and certain interfering substances, such as creatinine. Exactly

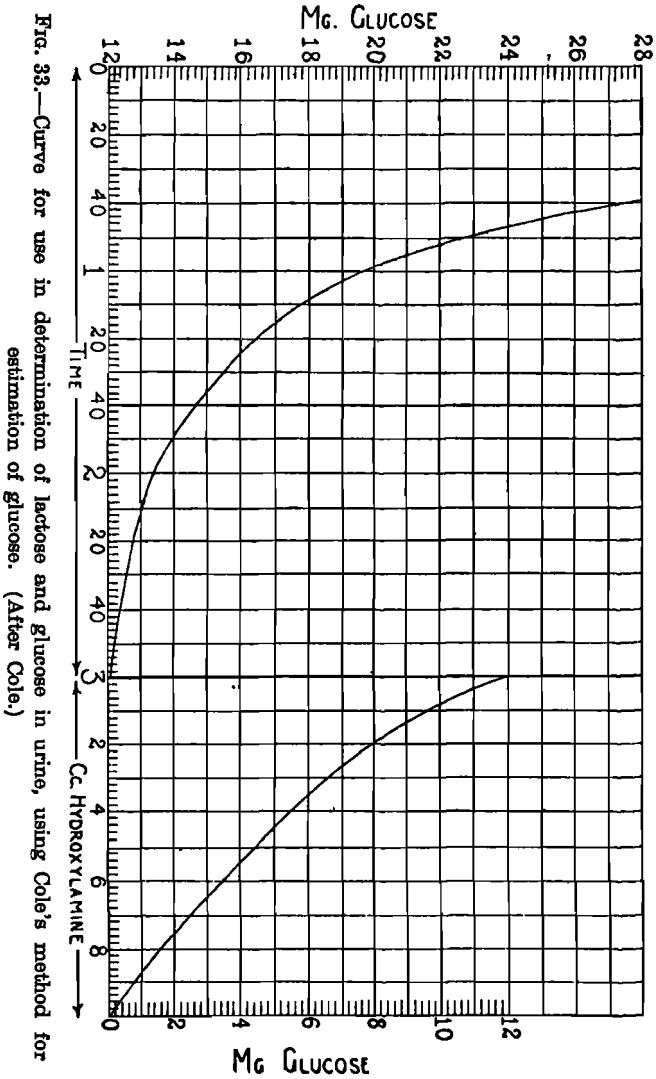


FIG. 33.—Curve for use in determination of lactose and glucose in urine, using Cole's method for estimation of glucose. (After Cole.)

3 c.c. of the filtrate are added to 5 c.c. of the special reagent¹ in a boiling tube (one having a diameter of 1 inch is best), and the mixture is heated with the flame so adjusted that the boiling-point is reached in thirty to forty seconds. This adjustment is readily found by one or two trials, and thereafter gives no trouble. A gas flame of about an inch in length is required. The beginning of boiling being taken as zero, the time required for the blue colour of the solution to disappear is noted. From the time, the first part of the curve reproduced in Fig. 33 gives the amount of glucose present in the 3 c.c. of urine. If the colour disappears too rapidly the process is repeated with less than 3 c.c. of urine filtrate, the volume, however, being made up to 3 c.c. with distilled water. If, on the other hand, the colour persists at the end of three minutes' boiling, which would mean the presence of very little glucose, it is discharged by titration with hydroxylamine sulphate, and the second part of the curve then gives the amount of sugar corresponding to the volume of hydroxylamine solution used. The hydroxylamine solution is prepared by dissolving 0.5 g. hydroxylamine sulphate in 200 c.c. of 4 per cent. potassium thiocyanate.

The application of this method to the estimation of lactose in urine with or without the simultaneous presence of glucose depends on the facts that, under the conditions of the analysis, (a) $2x$ mg. of lactose are required to decolorise the solution in the time taken by x mg. of glucose, and (b) after boiling with

¹ *Cole's Reagent*: (1) 100 g. KHCO_3 ; (2) 250 g. K_2CO_3 ; (3) 40 g. KCNS ; (4) 24 g. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. Dissolve separately and mix the solutions, adding (2) to (1), (3) to this mixture, and adding (4) slowly. Make up the volume to 1000 c.c., allow to stand two or three days, and then filter if necessary.

hydrochloric acid lactose is split into glucose and galactose, which reduce the copper solution with equal velocity, so that after hydrolysis the $2x$ mg. of lactose behave as $2x$ mg. of glucose. Suppose, then, that a urine contains, in the 3 c.c. taken for analysis, x mg. of glucose and y mg. of lactose. The y mg. of lactose behave as $\frac{y}{2}$ mg. of glucose, so that the analysis gives the result as $\frac{x+y}{2}$. After hydrolysis, if the analysis be repeated, the result will be $x+y$, and the two equations can be solved :

$$\left. \begin{array}{l} x + \frac{y}{2} = A \\ x + y = B \end{array} \right\} \text{determined by the analysis,}$$

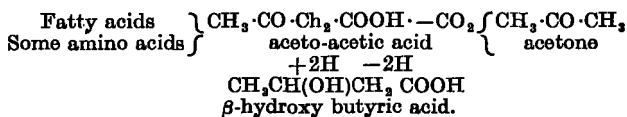
whence $\frac{y}{2} = B - A$, and $x = 2A - B$.

To carry out the estimation the urine is treated with charcoal and put through the procedure already described. Meanwhile 20 c.c. of the urine are boiled for fifteen minutes with 2 c.c. of concentrated hydrochloric acid, without any attempt to prevent evaporation. The solution is then cooled, an amount of sodium hydroxide equivalent to the hydrochloric acid (*i.e.* 2 c.c. of 40 per cent.) is added, and the volume is made up to 20 c.c. Ten c.c. of this solution are used for the estimation of sugar exactly as in the case of the original urine.

The Ketone (or acetone) Bodies

The "ketone bodies" consist of aceto-acetic acid, β -hydroxy butyric acid, and acetone. The first

of these is an intermediate in the oxidation of fatty acids and of certain amino-acids; the second, which is not a ketone, is probably formed from aceto-acetic acid by reduction (and can be re-oxidised to it), and acetone is formed from aceto-acetic acid by decarboxylation.



Normally, 5–15 mg. of acetone and aceto-acetic acid are excreted per day (of this about a quarter consists of acetone) and 20–30 mg. of hydroxy butyric acid. These amounts are vastly increased when carbohydrate oxidation is impaired or deficient, and in the severe acidosis of diabetes amounts up to 70 or more grams per day of the acetone bodies may be excreted, with β -hydroxy butyric acid forming 50–80 per cent. of the total.

Quantitative estimation of the acetone bodies, separately or together, is quite possible, but is not worth while for clinical purposes. Indications of the severity of the acidosis they produce are more easily and more reliably obtained by estimation of the blood CO_2 combining power. Qualitative tests are useful, however, since the presence of increased amounts of these substances in the urine indicates the existence or imminence of acidosis and, in any case, a derangement of carbohydrate metabolism. The tests do not respond to the amounts normally present.

There is no simple test for β -hydroxy butyric acid, and aceto-acetic acid is so easily decomposed to acetone that it responds to the tests for acetone.

The Ferric Chloride Test.—This test is given by aceto-acetic acid, but not by acetone. It is less sensitive than the tests described later, and a positive result indicates the presence of at least 0.04 per cent. of aceto-acetic acid (*i.e.* 40 or 50 times the normal amount).

To a few c.c. of urine add ferric chloride solution, drop by drop, until no more precipitate forms. A wine-red colour is produced if aceto-acetic acid is present, but it may be necessary to filter off the precipitate (ferric phosphate, etc.) before the colour is clearly seen. Boil half of the solution for 2–3 minutes; if the colour is due to aceto-acetic acid there will be a perceptible fading. Many substances may give a similar colour, which is usually stable on boiling—acetates, formates, thiocyanates, phenacetin, anti-pyrin, etc.; salicylic acid, aspirin, phenol, and others give a violet colour, which may be reduced to a reddish-purple by the yellow colour of the urine itself.

The Nitroprusside Test.—Both acetone and aceto-acetic acid give a red-purple colour with sodium nitroprusside in alkaline solution, but the test detects much smaller amounts of aceto-acetic acid than of acetone. When the interpretation of the test is doubtful the urine may be distilled, and the test performed on the distillate which contains pre-formed acetone along with that produced by decomposition of the aceto-acetic acid, while interfering substances remain behind. The sodium nitroprusside solution must be freshly prepared, and must be used sparingly to avoid difficulties of interpretation due to its own colour. There are various ways of performing the test.

(1) Saturate 10 c.c. of urine with ammonium

sulphate, add a few drops of concentrated ammonia and two to three drops of sodium nitroprusside solution. A ruby-red colour, which gradually deepens, is a positive result; a yellow or brown colour may be ignored. Substances containing the group $-SH$ (hydrogen sulphide, cysteine, glutathione, etc.) give a similar colour. A deep colour appearing immediately indicates the presence of about 0.2 per cent. of aceto-acetic acid, but a faint colour appears on standing with as little as 0.005 per cent. of the acid.

(2) To 2 or 3 c.c. of urine add a drop of nitroprusside solution and make alkaline with sodium hydroxide. A red colour is given by aceto-acetic acid, acetone, creatinine in high concentration, melanin precursors, etc. That given by the ketone bodies and by creatinine fades rapidly to yellow. If glacial acetic acid is added while the colour is still red, the colour persists or deepens in the case of the ketone bodies, but disappears or is replaced by green in the case of the interfering substances.

(3) Add a drop or two of sodium nitroprusside solution to the urine (preferably saturated with ammonium sulphate), mix, and superimpose a layer of concentrated ("0.880") ammonia solution. A purple ring developing at the junction of the two layers and spreading downwards has the same significance as the first of these tests.

Estimation of Chloride in Urine

The following simple method is sufficiently accurate for clinical work, and is more rapid than the more complicated procedure necessary for complete accuracy. Pipette 5 c.c. of the urine into a porcelain

evaporating dish (or a beaker standing on a white paper), dilute with about 20 c.c. of distilled water, add exactly 10 c.c. of silver nitrate solution (29.06 g. of silver nitrate per litre, the solution to be made with distilled water), and then about 2 c.c. of the acidified indicator (100 g. of ferric ammonium sulphate dissolved in 100 c.c. of 25 per cent. nitric acid). Standard ammonium thiocyanate solution is then run in from a burette until the first trace of yellow shows throughout the mixture, which must be kept stirred during the titration. The ammonium thiocyanate used is equivalent to the excess of silver nitrate, so that if x c.c. are required in the titration, the amount of silver nitrate used in precipitating the urinary chloride is $10-x$ c.c. Since each cubic centimetre of the silver nitrate is equivalent to 0.01 g. of sodium chloride, and 5 c.c. of urine were used, the percentage of sodium chloride in the urine is equal to

$$\frac{100 \times 0.01 \times (10-x)}{5}$$

The thiocyanate solution is prepared by dissolving about 13 g. of the salt in 800 c.c. of distilled water, and using this solution to titrate 10 c.c. of the silver nitrate solution. From the result of this titration the thiocyanate solution can be diluted so that exactly 10 c.c. of it are needed to give the yellow colour with 10 c.c. of the silver nitrate and 2 c.c. of indicator. Thus if, in the trial titration, y c.c. are used, each y c.c. of the thiocyanate solution must be diluted to 10 c.c.—*i.e.* to 500 c.c. of the solution it is necessary to add $\frac{500 \times (10-y)}{y}$ c.c. of water.

Determination of the Diastatic Index

Prepare a series of mixtures of urine and normal saline according to Table XIV, and to each add 2 c.c. of 0·1 per cent. starch solution (Table X).

Shake quickly and incubate in a water-bath at 37° C. for exactly thirty minutes. Immediately after removal from the water-bath fill each tube to within about an inch of the top with distilled water, to slow down the digestion of the starch, and add to each a drop or two of iodine solution (approximately decinormal iodine in potassium iodide). It will be found that somewhere in the series one tube shows a faint blue colour (the preceding ones showing a stronger colour as a rule),

TABLE XIV

PREPARATION OF TEST-TUBES FOR ESTIMATION OF DIASTATIC INDEX IN URINE

<i>Test-Tube</i>	<i>Urine</i>	<i>Saline</i>	<i>Unit Value of Tube</i>
	<i>c.c.</i>	<i>c.c.</i>	
1	1·0	0·0	2·0
2	0·6	0·4	3·3
3	0·4	0·6	5·0
4	0·3	0·7	6·6
5	0·2	0·8	10·0
6	0·1	0·9	20·0

To each tube add 2 c.c. of 0·1 per cent. starch solution.

while the next (and all subsequent ones) shows none at all. The diastatic index of the urine lies between the values of these two tubes.

In testing for pancreatic function in cases of suspected acute pancreatitis (see Chapter VIII) the technique is the same, except that, as the values to be

expected are over 100, the urine should be diluted to ten times its volume with normal saline. Test-tubes are set up as described, using this diluted urine, and the values of the tubes are then ten times those given in the above Table.

Bile Acids

The chief bile acids found in human urine are taurocholic and glycocholic acids. In acid urine they are almost insoluble, and therefore produce a turbidity; in neutral or alkaline urine they exist as salts which are soluble. Normally, the amounts present in urine are so small as to be undetectable by ordinary means; they are increased in certain types of jaundice, in cirrhosis of the liver, and (often) in pernicious anaemia.

They may form a white ring in the nitric acid ring test for proteins.

The most sensitive tests depend upon their power of lowering surface tension, but, since they share this property with other substances, these tests are not specific. There is actually no simple specific test for them.

Hay's Test.—Sprinkle a little finely divided sulphur (flowers of sulphur) on the surface of the cold urine contained in a glass cylinder. Use a pepper-box or similar sprinkler held an inch or two above the surface of the urine. Normally, the sulphur will float indefinitely. If bile salts are present it will sink immediately or within a few minutes, according to the amount of bile salt present. The test is said to detect as little as a milligram of bile acid in 100 c.c. of urine.

Oliver's Test.—This test is less delicate than Hay's, but will detect about 5 mg. of bile acid per 100 c.c. of

urine. To 5 c.c. of a filtered 1 per cent. solution of Witte's peptone, or of a filtered 2 per cent. solution of egg albumen weakly acidified with acetic acid, add the urine, drop by drop. In the presence of bile salts a white precipitate is formed—a loose compound of the bile acid with the peptone or protein.

Urinary Calculi

Urinary calculi almost invariably contain uric acid (or urates), though usually only as a minor constituent. Those containing one main constituent are termed *simple*; those with two or more are *compound*. Calculi usually consist of a nucleus surrounded by a series of concentric layers; the nucleus and the outer layers may be of different composition. Thus a fibrin nucleus (formed by coagulation of blood in the urinary tract) may be surrounded by layers of calcium and magnesium phosphates.

Uric Acid Calculi.—Burn without flame when heated on porcelain or platinum foil. A small amount of ash usually remains. The murexide test for uric acid is strongly positive. Ammonia may be evolved on heating with sodium hydroxide (ammonium urate).

Murexide Test.—Evaporate to dryness with a few drops of concentrated nitric acid, using a water-bath or a very low flame to avoid overheating. Uric acid gives a red or yellow residue which, after cooling (a) turns reddish-purple on addition of a drop or two of dilute ammonia solution, or (b) turns reddish-violet on addition of dilute KOH solution. These colours disappear on warming.

Xanthine Calculi are very rare. They burn like

urate calculi. With the murexide test they give an orange colour on addition of ammonia, and this becomes red on warming.

Phosphate Calculi do not burn (though a small amount of uric acid may be present and will burn). They dissolve in hydrochloric acid without effervescence, and the solution gives the tests for phosphoric acid (ammonium molybdate test) and for any metallic radicles present (calcium, magnesium, ammonium).

Molybdate Test.—To about 1 c.c. of the solution add 1 c.c. of concentrated nitric acid and 2 c.c. of ammonium molybdate solution. Warm. A yellow precipitate is given by phosphoric acid.

Ammonium.—Heat with sodium hydroxide solution; ammonia is evolved.

Calcium.—(a) Ammonium oxalate gives a white precipitate. (The solution must first be made nearly neutral by ammonia, and then made slightly acid with acetic acid.) The precipitate is insoluble in acetic acid, but soluble in hydrochloric acid. (b) Sulphuric acid gives a white precipitate of calcium sulphate.

Magnesium.—After removal of calcium by addition of excess ammonium oxalate, filter, and to the filtrate add ammonium chloride, ammonium hydroxide, and sodium phosphate solutions. Magnesium gives a white crystalline precipitate of magnesium ammonium phosphate. (Since phosphoric acid is already present, the precipitate usually appears before the addition of the sodium phosphate.)

Calcium Oxalate Calculi are rare. They do not burn, and dissolve in hydrochloric acid. If they are warmed with dilute sulphuric acid they form oxalic acid (which dissolves) and calcium sulphate (which is

only slightly soluble). The oxalic acid solution in sulphuric acid, heated to 60° C., rapidly decolorizes potassium permanganate solution.

Cystine Calculi occur very rarely (cystinuria). They burn with a bluish flame and peculiar odour. They dissolve in hydrochloric acid or in sodium hydroxide. The solution in alkali, on boiling, decomposes with production of sodium sulphide, which, on addition of lead acetate, precipitates black lead sulphide.

Urostealiths are extremely rare. They burn with a steady yellow flame. Being composed mainly of fat and fatty acids, they dissolve in alcohol, ether, chloroform, etc.

The Examination of the Urinary Deposit

The urinary deposit may consist of cells, casts, crystals, or amorphous debris. In addition, microorganisms, strands of fabric, hairs, mucous threads, spermatozoa, starch granules, and parasites may occasionally be found. If examination of the deposit is to be satisfactory it is of the utmost importance that it should be carried out as soon as possible after the urine has been passed. Cells and casts rapidly disintegrate in urine on standing and may quickly become unrecognisable. This is particularly apt to occur in alkaline urine, but even if the urine is acid when passed, it may become alkaline on standing owing to bacterial action. Again, the detection of crystals in the deposit, at no time a matter of very great diagnostic importance, becomes practically worthless when the specimen is not quite fresh, as their presence under such circumstances is usually due simply to the changing reaction of the urine which

takes place on standing. Lastly, red blood corpuscles become quickly haemolysed in urine unless it is a very concentrated specimen. Hence they may be undetectable when in small numbers, unless looked for immediately. Even in fresh specimens, if the urine is dilute and therefore hypotonic, the erythrocytes haemolyse so readily that they may not be discovered unless a special technique is adopted. This no doubt accounts for the fact that red cells are usually reported as being absent from the urine in cases of chronic azotaemic Bright's disease, as they lyse so quickly in the dilute urine which characterises the condition. In cases, therefore, in which the discovery of small numbers of erythrocytes in the urinary deposit is likely to be of diagnostic importance, it is wise to examine a fresh early morning specimen procured on waking, the patient having been warned to drink very little the previous day and to take no fluid at all from 8 P.M. the previous evening.

The urinary deposit may be allowed to settle out in a conical glass by force of gravity—a drop of it being procured by a pipette and transferred to a slide for microscopic examination. It saves time, however, and it is preferable to centrifuge the urine for three or four minutes. The supernatant fluid is then poured off, and after the deposit has been mixed by shaking, a drop is put upon a slide and covered with a cover glass. Whatever chemical test is applied to the deposit, its microscopic examination should never be omitted. Deposits are so frequently of a mixed nature that a chemical test for one constituent will fail to reveal the presence of others, and is often inconclusive even for the special constituent examined for, as for example, in the detection of blood or pus

when these are present in small quantities. It should be remembered also that significant quantities of red blood corpuscles or pus cells may be present in the urine without being in sufficient quantity to cause a positive reaction for albumen.

Cells.—The cellular content of the urinary deposit may consist of red blood cells, leucocytes, or epithelial cells. The latter, which occur more frequently in the urine of women than of men, are of little significance and frequently come from the genital passages.

The number of leucocytes excreted in healthy urine varies, and their microscopical enumeration will, of course, depend on the amount of centrifugalisation employed. In a well-centrifuged specimen there should not be more than two to three leucocytes discovered per high-power field. Any considerable number of pus cells in a single microscopic field must be regarded as pathological. Such pus may arise from a urethritis, prostatitis, or cervicitis. If a male patient is suspected of the two former he should pass the urine for the examination into two glasses. The urine in the first glass will under such circumstances contain far more numerous pus cells than the urine in the second glass. When the pus arises from a pyelitis or cystitis it will be more uniformly distributed. Pus from the genital passages in the female may be excluded from the urine by obtaining the specimen by catheter. Apart from these conditions the presence of pus in the urine in significant amounts indicates an infection of the bladder or kidney, and the infecting organism can usually be discovered. A pyuria without easily demonstrable organisms should lead one to suspect the possibility of a renal tuberculosis. It is usually fairly easy to distinguish pus cells from

erythrocytes, especially when the urine is concentrated. In such specimens the red cells are only about half the size of the leucocytes and are frequently crenated. In hypotonic urines the red cells may become bloated and swollen and not unlike pus cells. The latter have, however, a granular centre which can usually be distinguished from the clear smooth centre of the red cells.

Just as occasional leucocytes are found in health in the centrifuged urinary deposit, so occasional erythrocytes are also found, but not more than one or two per high-power field. If each field of the microscope contains more than five red cells this must be regarded as pathological. It must be remembered, however, that the urine of female patients is frequently contaminated by blood from the menstrual flow or from bleeding haemorrhoids. Small numbers of red cells may be found in the urine in chronic azotaemic Bright's disease, if the precautions referred to above are taken to prevent their haemolysis by a hypotonic urine. Large quantities are excreted in acute Bright's disease and may also be found in infections of the urinary tract, such as tuberculosis, pyelonephritis, cystitis, and prostatitis. Calculi and malignant disease of the bladder and prostate are again frequent causes of haematuria. More rarely it is due to malignant disease of the kidney, to blood diseases, such as purpura, or to renal infarcts such as may occur during the course of bacterial endocarditis. Essential haematuria may occasionally occur, but care should be taken to avoid making such a diagnosis till all other causes of haematuria have been excluded.

Casts.—Casts in the urine are small cylindrical bodies derived from diseased renal tubules. Several

varieties are distinguished—hyaline, epithelial, granular, blood, pus, fatty, and waxy. More than one of these types may be found in the same specimen, but one type usually predominates. They must not be confused with cylindroids, scratches on the slide, threads of mucus, or fibres of cotton or hair. The sides of a cast are parallel to one another, and the ends are rounded and do not taper to a pointed tail as do the ends of cylindroids. In hunting for casts a subdued light is necessary with the shutter of the microscope partially closed, and its focus should be continually adjusted.

Hyaline casts are due to a coagulation of albumen inside a renal tubule. This is eventually cast off and appears in the urine as a mould of the particular part of the tubule in which the cast was formed. They appear as highly refractile cylindrical tubes, quite clear in the centre and with clear-cut edges. The discovery of a very occasional hyaline cast may be ignored, and after very severe exercise a temporary trace of albumen in the urine, associated with a few hyaline casts, is of no serious pathological importance. The constant presence of such casts in significant numbers is always indicative of kidney damage, and hyaline casts may be found in all forms of Bright's disease.

Epithelial casts are due to the shedding of the tubal epithelium, the cells of which stick on to a hyaline cast, thereby forming an aggregation of epithelial cells in a tube-like form. Their edges are thus not so clean cut as those of the hyaline type, and the outlines of the individual cells forming the cast as well as their nuclei can be distinguished. They are found mostly in acute and nephrotic Bright's disease and in the acute

or sub-acute exacerbations of the chronic azotaemic type.

Granular casts are formed initially in the same way as epithelial casts. When epithelial casts are not shed quickly from the tube, however, the epithelial cells composing it undergo a degenerative change. Thus the individual outlines of the cells can no longer be made out and the cast has merely a coarsely granular appearance.

On the whole, the predominance of granular and epithelial casts indicate a more active and diffuse inflammatory or degenerative process in the kidney than when hyaline casts alone are present. Very broad epithelial and granular casts are sometimes seen, which have been formed in the larger connecting tubules, and their discovery is of very bad prognostic significance. They are sometimes referred to as "renal failure casts" and are usually associated with a greatly raised blood urea and creatinine.

Blood and pus casts are also due to the implantation of these cells on a hyaline cast. They appear as a conglomeration of blood or pus cells bound together in tubular form. Blood casts may occur whenever albuminuria and bleeding from the kidney are associated, and are therefore found in such conditions as acute Bright's disease and in severe congestive heart failure. A great predominance of pus casts is evidence of pyelonephritis.

Sometimes epithelial or granular casts, if retained for a very long time, in degenerating tubules, undergo fatty or waxy degeneration. The *fatty cast* appears coated with fat globules, while the waxy cast is highly refractile with characteristic indentations. Such casts are uncommon, but are sometimes found in patients

suffering from degenerative Bright's disease exhibiting the nephrotic syndrome.

Crystals.—Amorphous or crystalline deposits of various kinds are usually precipitated in the urine after it has been left to stand for any length of time, especially when the urinary specimen is concentrated. They are seldom of any diagnostic or clinical importance, and there is no doubt that in the past too much stress has been laid upon their discovery and recognition. They consist usually of urates, phosphates, or oxalates, and common examples of their crystalline forms are shown in Fig. 35.

Urates are formed in acid urine, with the exception of ammonium urate, which occurs in alkaline urine. They may be amorphous or crystalline, and form a characteristic pink deposit in the urine, which goes into solution on heating. They are particularly abundant in the highly concentrated urines obtained from febrile cases. Uric acid appears in the deposit as small reddish granules, which have been compared to particles of cayenne pepper. Though quite common in normal urine, uric acid crystals are particularly abundant in the urines of patients suffering from gout during the stage of remission or when under treatment with colchicum. They are also common in cases of leukaemia, acute yellow atrophy of the liver, and in persons consuming a diet particularly rich in nucleoproteins. Uric acid crystals exist in a considerable variety of forms—mostly as squares or wedges—but are all characteristically yellowish-brown in colour. Ammonium biurate appears as yellowish or brownish spheres, sometimes studded with spicules, so that they have the appearance down the microscope of small crab apples.

Phosphatic deposits are found in alkaline urine, with the exception of calcium hydrogen phosphate, which may occur when the urine is slightly acid. Phosphates are thus commonly found in urine which has been allowed to stand for a considerable time and to undergo bacterial decomposition. They are also common in cases of urinary infection due to streptococci, staphylococci, and bacillus proteus, which all tend to render the urine alkaline. Phosphates are often abundant in the urine of patients suffering from wasting diseases with much tissue breakdown. They are thus common in cases of advanced active tuberculosis. For some unexplained reason phosphaturia is common in neurasthenics. A phosphatic deposit may be amorphous or crystalline, and appears as a heavy white precipitate which may be mistaken for pus. Unlike urates, phosphates do not dissolve on heating, but are augmented by it. They dissolve on adding dilute acetic acid, which distinguishes a phosphatic cloud obtained by heating urine from the cloud due to albumen. Crystals of ammonium magnesium phosphate (triple phosphate) are very common and appear as the well-known "coffin lid" or "knife-rest" crystals or more rarely in feathery forms. Calcium hydrogen phosphate (stellar phosphates) appear as long crystals converging to a point in the centre.

Oxalates never exist in an amorphous, but always in a crystalline form. They may be found in either acid, alkaline, or neutral urine. Their occurrence in large quantities in freshly passed urine may occasionally be of some diagnostic significance, since a simple oxaluria is sometimes associated with symptoms of lumbar pain, depression, and general ill-health. Cal-

cium oxalate crystals appear in "envelope" and "dumb-bell" forms. They do not dissolve on heating nor on addition of acetic acid, but are soluble in hydrochloric acid.

Occasionally deposits of *calcium carbonate* are found, distinguished by the fact that addition of acid to the urine causes them to dissolve with the evolution of bubbles of carbon dioxide. The crystals of calcium carbonate have the appearance of small dumb-bells.

Several types of rare crystals may sometimes occur in the urine, including *cystin*. Owing to a very rare hereditary inborn error of metabolism, some persons are unable to oxidise this cleavage product of protein digestion, and its crystals are excreted in large numbers in their urine as thin transparent hexagonal plates.

Crystals of *cholesterol* are very rarely found in urinary sediments. They form flat, transparent, colourless plates, often with notched corners. They may sometimes be associated with degenerative renal disease, such as nephrosis.

Leucine and tyrosine have been detected in urinary sediments or neutral or acid urine, separately or together. When they are present the urine is dark and shows a heavy deposit. Usually if one is present the other is present also. Their appearance indicates very excessive and rapid tissue breakdown, and the fact that they crystallise is due to their relative insolubility in water or dilute acid. (They dissolve in strongly acid or in alkaline solutions.) They have been found, therefore, in the urine from cases of acute yellow atrophy of the liver, cirrhosis of the liver, phosphorus poisoning, severe typhoid fever, smallpox, leukaemia, etc. Leucine is usually found in a spherical form

resembling a worm cast ; tyrosine in sheaves of fine needles.

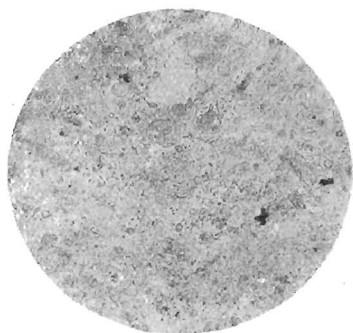
Identification of Leucine and Tyrosine in Urinary Deposit.—Centrifuge off the deposit from the urine (acidified slightly and allowed to stand for some hours if necessary) ; wash with 30 per cent. acetic acid to remove phosphates (this will also remove leucine) ; wash again with acetone to remove colouring matter. Centrifuge after each washing and reject the supernatant fluid. Examine the washed deposit microscopically, looking for the characteristic tyrosine crystals, and noting their solubility in hydrochloric acid (which differentiates them from uric acid).

For leucine, examine the deposit without washing, and to any crystals suspected of being leucine add a drop of 30 per cent. acetic acid. Leucine dissolves rapidly ; uric acid (with which it may be confused) does not.

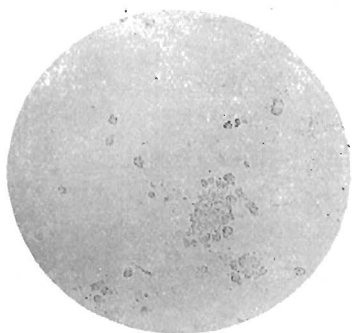
The appearance of a number of urinary deposits is shown in Figs. 34 and 35.

THE EXAMINATION OF GASTRIC CONTENTS

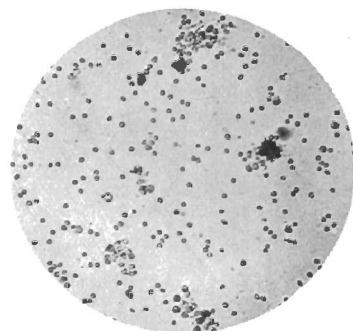
In the analysis of test-meal samples, each sample is examined for the presence of starch, bile, blood (either fresh or in the “ coffee ground ” state), and excessive quantities of mucus. In addition, the titratable acidity, both “ free ” and “ total ” is determined by titration with standard sodium hydroxide solution. The fasting sample is submitted to the same examination, and is also examined for odour, the presence of charcoal (or other evidence of



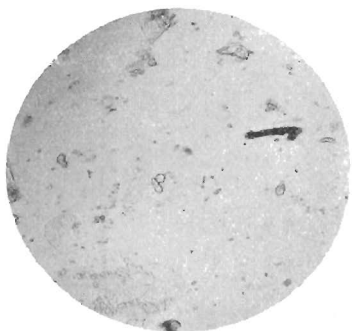
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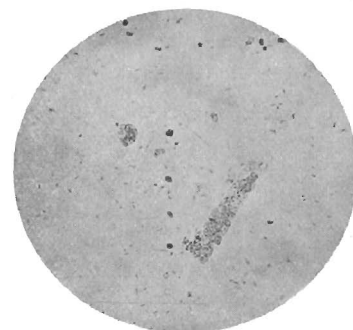
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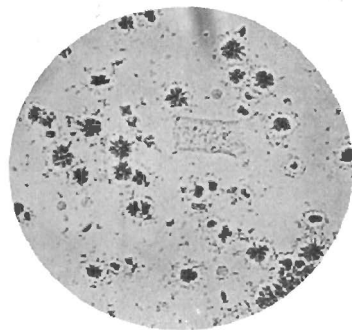
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4



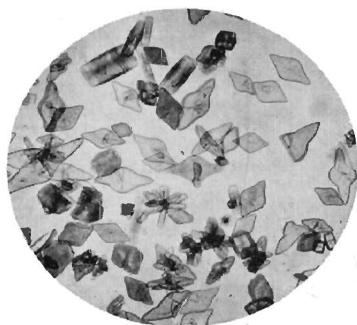
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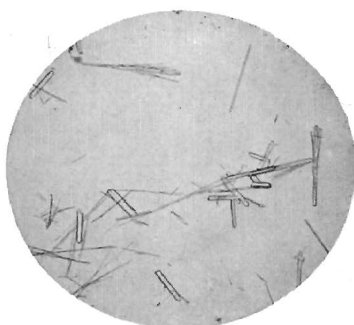
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FIG. 34.

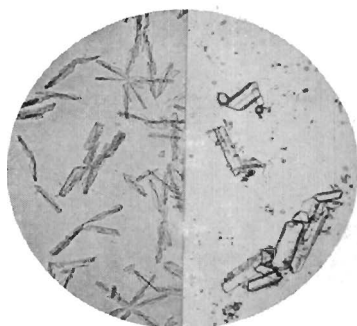
1. Granular debris with pus and epithelial cells. $\times 90$.
2. Pus cells. $\times 90$.
3. Blood. $\times 90$.
4. Epithelial cells from bladder. $\times 90$.
5. Granular cast. $\times 75$.
6. Ammonium urate (Thorn-apple crystals) with ammonium phosphate simulating cast, and crystal of calcium oxalate. $\times 150$.



1

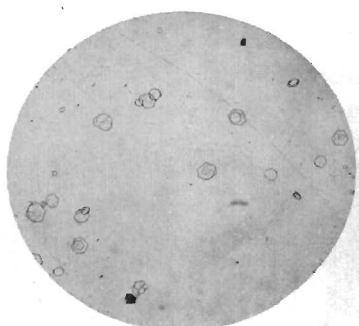


2

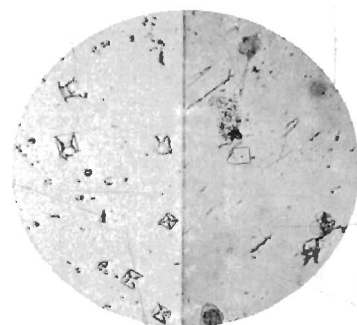


3a

3b

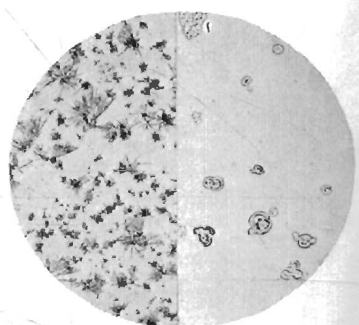


4



5a

5b



6a

6b

FIG. 35.

1. Uric acid. $\times 35$.

2. Stellar phosphate. $\times 35$.

3a and 3b. Triple phosphate, various forms. $\times 35$.

4. Cystine. $\times 35$.

5a. Calcium oxalate. $\times 70$.

5b. Cholesterol. $\times 35$.

6a. Tyrosine. $\times 35$. 6b. Leucine. $\times 70$.

stagnation) and pus. If the amount of "free" acid in the fasting juice is small (or if none appears to be present), qualitative tests for free hydrochloric acid are applied, and lactic acid is sought.

The test for starch, of course, is used only when a starch-containing meal has been given (and not, therefore, in the histamine test-meal). Since starch is little hydrolysed in the stomach, the disappearance of starch from the samples coincides approximately with the disappearance of the test-meal from the stomach; it thus gives an index of the rate of emptying of the stomach.

The fasting juice and the last sample obtained (which should have emptied the stomach completely) are measured, since their volume provides evidence as to whether the stomach is emptying properly or not.

Odour.—The foul, rancid odour of such organic acids as butyric is almost diagnostic of gastric carcinoma. A sample of fasting juice with such an odour will probably be dark in colour owing to the presence of altered blood, and will probably contain little or no free acid.

Starch.—To a drop or two of the unfiltered sample (or to the residue after the other tests have been made) add a drop of a dilute solution of iodine in potassium iodide. Tincture of iodine is less satisfactory, since the presence of at least a little potassium iodide is necessary. Starch gives an intense blue colour.

Bile.—The yellow colour of bile is a good index of its presence when uncoloured test-meals have been given. Any of the tests for bile pigment previously described (p. 273) may be used as confirmatory evidence.

Blood.—Blood is tested for by naked-eye and, in the case of the fasting juice, by microscopic examina-

tion. The appearance of blood in cases of gastric ulcer is very characteristic, and both in colour and texture closely resembles that of coffee grounds. Traces of fresh blood may be due to trauma during the passage of the tube.

In case of doubt—*e.g.* when the amount of blood is small—chemical tests may be used. Mere traces of blood, however, are of doubtful significance. We prefer the guaiac test, performed in the following way. As material it is convenient and economical to use the solid residue obtained in filtering the sample for lactic acid testing. Otherwise a small volume of the unfiltered sample may be used. To the material add a few drops of glacial acetic acid, boil for two minutes, shake and cool and add 2 or 3 c.c. of ether. Shake thoroughly and allow the two layers to separate; if separation is slow, add water without further shaking. Put about 0.5 c.c. of alcoholic solution of guaiacum and 2 c.c. of 3 per cent. ("10 volume") hydrogen peroxide in each of two test-tubes. To one of these tubes add the clear ether extract, keeping the other as a "control," which should remain almost colourless. A blue colour appearing in the tube containing the ether extract indicates the presence of blood. Interfering substances, such as pus, oxidising enzymes, salts of iron and copper, are either destroyed by the boiling or are insoluble in the ether.

Mucus.—There is no satisfactory method of determining the amount of mucus present, and it becomes necessary, on the basis of experience, to judge this from the consistency and appearance of the specimen. Very little is present normally, provided saliva has not been swallowed during the test-meal.

Free Hydrochloric Acid (Qualitative).—Of the follow-

ing usual tests only Gunzberg's can be regarded as specific. Even it is not really so, since it is given by mineral (*i.e.* inorganic) acids other than hydrochloric. Except in those rare cases (which will be known beforehand) when such acids as sulphuric have been ingested, it does indicate the presence of hydrochloric acid. The other tests, however, simply indicate the existence of a hydrogen ion concentration so high as to be given only by a strong acid (such as hydrochloric) or by a weak (organic) acid such as lactic acid in exceptionally high concentration.

(1) *Congo Red*.—Congo red, employed usually as a test-paper, turns blue in strongly acid solution (approximately pH 4). *Thymol blue*, used as a solution of which a few drops are added to the test-sample, turns red in the presence of free hydrochloric acid (approximately at pH 2).

(2) *Töpfer's Test*.—Töpfer's reagent, a 0·5 per cent. alcoholic solution of dimethylamino-azo-benzene, is another indicator which changes colour at a moderately low pH (about 4). In strongly acid solution (below pH 4) it turns red; above pH 4 it is yellow.

(3) *Gunzberg's Test*.—Two drops of Gunzberg's reagent are added to five drops of the gastric contents (filtered through muslin) in a porcelain dish. The reagent should be freshly prepared every fortnight by dissolving 1 g. of vanillin and 2 g. of phloroglucinol in 30 c.c. of alcohol. Evaporate the mixture of reagent and test-sample to dryness on a water-bath (or over a small flame, taking care to avoid over-heating with consequent charring). A red coloration appears if free hydrochloric acid is present. If the amount of acid present is very small this may be confined to the edge of the residue.

Lactic Acid.—These tests need to be performed only if free hydrochloric acid is absent or present merely in traces. They should be made soon after the sample has been withdrawn. In a fractional test-meal only the fasting sample need, in any case, be tested.

(1) *Uffelmann's Test.*—About 5 c.c. of reagent (5 per cent. carbolic acid, 10 c.c.; *distilled* water, 20 c.c.; 10 per cent. ferric chloride, 1 drop) are placed in a test-tube and a few drops of the filtered specimen are added to it. A change in colour of the solution from purple to yellow is taken to be a positive result. Such a positive reaction is, unfortunately, obtained with many other substances which may be found in the stomach besides lactic acid. A negative reaction is, therefore, informative, but a positive reaction is only slightly suggestive. The reagent must be freshly prepared, and used during the few minutes it retains its purple colour.

(2) *Maclean's Test.*—In its original form this test consists in placing half to three-quarters of an inch of carefully filtered gastric specimen in one tube, and an equal amount of tap water in another. To each two drops of a 5 per cent. solution of ferric chloride are added. The tube with the gastric contents then shows a definite reddish coloration. Each mixture is then treated with five or six drops of a freshly prepared saturated solution of mercuric chloride. If the colour in the tube of gastric contents disappears a negative reaction has been obtained; but if the reddish appearance gives place to a distinct yellow colour the reaction is positive. Comparison with the control tube enables one to determine more easily the difference between a positive and a negative reaction.

This test, although sufficiently delicate for all practical purposes, often gives reactions which are neither clear-cut negative nor clear-cut positive. The presence of bile in the gastric contents also obscures the result.

Difficulty due to colour or opalescence in the test-sample may be overcome to some extent by extracting the lactic acid with ether before carrying out the test. For this the sample is acidified with hydrochloric acid, shaken with an equal volume of ether, and the ethereal layer is pipetted into another test-tube containing about 3 c.c. of dilute sodium carbonate, where, after thorough shaking and neutralising with dilute hydrochloric acid (avoid excess of acid), the test is performed as already described. This modification, although it makes the result of the test more definite in many cases, involves some loss of delicacy, since the extraction of lactic acid by ether and its re-extraction by water are never quite complete.

Free and Total Acidity.—If the qualitative tests for hydrochloric acid are negative, the “free” acidity is, of course, *nil*.

Free and total acidity are determined by titration with decinormal sodium hydroxide, using for the first an indicator whose colour change occurs at pH 2 to 4, and for the second one which changes about pH 8. Thus Töpfer's reagent is not very sharp, and a better indicator is thymol blue. This, indeed, serves both purposes, for it changes from red to yellow-orange when the free acid is neutralised, later becomes pale yellow, and then turns to green and finally to blue when the total acid is neutralised.

It is advisable to filter the sample through muslin before titration, or to clear it by forcing, down the test-tube containing it, a wad of cotton-wool wrapped

round a glass rod. No attempt, however, should be made to filter it through paper. For the titration 5 c.c. of gastric contents are pipetted into a small flask containing 5 to 10 c.c. of distilled water. A drop of Töpfer's reagent (or thymol blue) is introduced, turning the specimen red in the range of pH corresponding to the presence of free hydrochloric acid. Decinormal soda is then run in from a burette, the titration flask being shaken the while, until the red colour just changes to yellow, and the volume of soda used is noted. A drop of phenol-phthalein is then added (if Töpfer's reagent has been used), and more alkali is run in, until a faint pink colour (or blue in the case of thymol blue) is just perceptible throughout the liquid, when the volume of soda used is again noted. The volume of alkali given by the sum of the two readings represents the total acid present, the volume given by the first reading indicating the free hydrochloric acid, and that of the second the combined hydrochloric acid along with any organic acids which may be present.

The results of this titration are usually recorded in cubic centimetres of decinormal soda per 100 c.c. of stomach contents, and the figures are used to plot a graph against a time basis. On the other hand, they may be converted into the actual weight of hydrochloric acid in grammes per 100 c.c. by multiplying them by 0.0365.

The results with thymol blue tend to be slightly lower than those with Töpfer's reagent and phenolphthalein.

Total and Mineral Chloride.—"Mineral" chloride is that existing as inorganic salts—*e.g.* sodium chloride. Total chloride is the sum of the mineral chloride, the

“combined acid” (*i.e.* the protein chlorides), and the free hydrochloric acid.

Mineral Chloride.—Evaporate 5 c.c. of the filtered sample to dryness in a silica dish, and incinerate the residue. The free acid, and that combined with organic matter, is volatilised. Dissolve the residue in 10 c.c. of dilute nitric acid (equal volumes of concentrated acid and distilled water), add 10 c.c. of $\frac{N}{10}$ AgNO_3 , and 1 c.c. of saturated ferric alum solution.

Titrate with $\frac{N}{10}$ KCNS , stirring vigorously until a red tinge, which persists for a minute, is produced.

The thiocyanate titrates the excess of silver nitrate, so that, if x c.c. were used in the titration, 5 c.c. of sample contained the equivalent of $(10-x)$ c.c. of $\frac{N}{10}$ chloride. Hence 100 c.c. contained $20(10-x)$ c.c.

of $\frac{N}{10}$ chloride, or (expressed as sodium chloride), $0.117(10-x)$ grams, or (expressed as hydrochloric acid) $0.073(10-x)$ grams.

Total Chloride.—The method is the same as for mineral chloride, except that the free and organically combined hydrochloric acid must be converted into sodium chloride before evaporation and incineration. It is thus convenient to use the sample which has been titrated with sodium hydroxide in the determination of free and total acidity.

Microscopic Examination.—Only the fasting sample is worth examining. A drop of centrifuged deposit is examined for erythrocytes, leucocytes, epithelial cells, tumour cells, food residues (note that meat

fibres will invalidate delicate chemical tests for blood), and various organisms (yeasts, sarcinae, etc., when free HCl is absent or in negligible amounts).

EXAMINATION OF BLOOD

Enumeration of Red and White Cells

For this purpose a haemocytometer is used. It consists essentially of two pipettes and a suitable counting chamber.

Each pipette consists of a stem graduated in tenths with a bulb above in which a suitable dilution of the blood can be made, the bulb containing a small glass bead in order to facilitate mixing. Immediately above the bulb will be found a mark, "11" in the case of the white cell pipette, and "101" in the case of the red. Above this each pipette is tapered to connect with a length of rubber tubing which should be about 12 inches long and terminate in a glass mouthpiece.

It is absolutely essential that all pipettes should be thoroughly clean and perfectly dry before use. They should be attached to a filter pump or rubber bulb and rinsed successively with distilled water, alcohol, and ether, and thereafter dried by drawing through them a current of air. When the pipette is clean and dry the glass bead will roll freely in the dilution bulb with no tendency to adhere to the glass. Dirty pipettes are best cleaned by 20 per cent. caustic soda, and a blocked stem can usually be cleared with a piece of thin steel wire.

The counting chamber should be of the Bürker

type, engraved with the Thoma "ruling." It consists of a thick glass slide on which is supported a special cover-glass (an ordinary cover-glass is unsatisfactory) over a raised platform whose surface is exactly one-tenth of a millimetre below the under surface of the cover-glass. The centre of this platform is ruled into a system of squares in which the counting of the blood cells is performed. Both the platform and the cover-glass should be cleaned with acetone and polished with a silk cloth before use so as to remove any trace of moisture or grease.

Blood is obtained from the patient most conveniently from the thumb at the base of the nail, from the pulp of the finger, or from the lobe of the ear. The skin is lightly swabbed with ether and a puncture made either with a vaccinostyle or a straight triangular needle. The first drop of blood should be wiped away and thereafter the pipettes filled, care being taken to avoid any pressure on the surrounding tissues.

With the stem of the pipette held in a horizontal position, blood is drawn into the graduation stem to the mark 0.5 or slightly above it. The tip of the pipette is then removed from the patient's finger, any blood adhering to the outside of the stem being wiped away between the operator's fingers, and the column of blood adjusted exactly to the 0.5 mark by touching the point of the pipette gently against the operator's thumb nail. Should the column of blood have risen above the 0.6 graduation the pipette must be blown empty and discarded, since a too great dilution error is subsequently introduced by reason of the cells adhering to the glass between the 0.5 and 0.6 graduations. The tip of the pipette is finally intro-

duced into the appropriate diluting fluid¹ and filled to the mark "101" in the case of the red cells or "11" in the case of the white cells, the pipette being rotated vigorously throughout the dilution. The upper and lower ends of the pipette are now closed between the finger and thumb and the pipette vigorously shaken in order to secure thorough dilution of the blood in the mixing chamber. Since the column of diluting fluid in the stem of the pipette (measuring one unit) does not mix with the blood and is subsequently discarded, a 1 in 200 dilution of blood is obtained in the red cell pipette (0.5 of blood in a total volume of 100, *i.e.* 107-1), and a 1 in 20 dilution of blood in the case of the white cell pipette (0.5 of blood in 10, *i.e.* 11-1). For all ordinary purposes this dilution will be found satisfactory. In cases of severe anaemia, however, it is sometimes advisable to obtain a 1 in 100 dilution of blood for the red cell count. This is obtained by drawing the blood up to the mark 1.0 and thereafter the diluting fluid to the mark "101." Again, in cases of leukaemia it may be necessary to use a red cell pipette for counting the leucocytes, so that a 1 in 100 or even a 1 in 200 dilution can be obtained.

¹ *Diluting fluid for red cell count.* [Hayom's Solution.]

Mercuric chloride	0.5 grm.
Sodium chloride	1.0 grm.
Sodium sulphate	5.0 grm.
Distilled water	200.0 c.c.

Filter from time to time to remove cells which have fallen from tip of pipettes.

Diluting fluid for white cell count.

Acetic acid (glacial)	2.0 c.c.
Mercuric chloride	0.1 grm.
Distilled water	to 100 c.c.

The solution should be coloured with methyl violet, which stains the nuclei of the leucocytes and facilitates counting. The acetic acid dissolves the envelopes of the red cells and causes the leucocytes to swell. The solution should be filtered each week.

It is desirable to proceed to the actual counting of the cells as soon as possible after the dilution has been made. If this is not convenient then the rubber tubing may be detached from the pipette, which is closed at both ends by a broad rubber band. The contents of the dilution chamber must then be thoroughly remixed before the count is made, which in any case should be within a few hours of the blood having been withdrawn.

The counting chamber is prepared for the diluted blood by placing the cover slip on its supports and pressing it firmly thereon so that a series of coloured concentric rings are seen (Newton's rings). The column of diluting fluid which still fills the stem of the pipette is then expelled (and discarded) and the tip of the pipette touched on the edge of the counting platform, which will be found to project beyond the margins of the cover slip. A quantity of diluted blood then runs into the counting chamber, and this quantity can with practice be adjusted so that it exactly fills the chamber, there being no tendency for it to overflow into the grooves. If overflowing occurs, then the cover-glass must be removed and the counting chamber cleaned, dried, and refilled. The count should be made after two or three minutes, during which time the blood cells settle on to the ruled area.

The counting chamber is transferred to the stage of a microscope and adjusted so that the ruled area is in the centre of the field. The light is cut down and the ruled lines brought into focus under the low-power objective, when it will be observed that the ruled area just fits into the field and that the blood cells are uniformly distributed over the lines. If they

are not, then the chamber must be dried and refilled.

The ruled area is illustrated in Fig. 36. It consists of a single "large" square whose area is one square

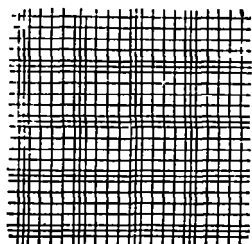


Fig. 36.—Centre Ruling of Thoma.

millimetre, and this is further subdivided into four hundred "small" squares, each of which is $1/400$ sq. mm. Through the centre of every fifth small square is drawn an extra line. Hence there appears to be a series of "medium squares," each containing sixteen "small squares," and divided from its

neighbours by a threefold line. Somewhere in the field there will be observed a block of nine "medium" squares completely bounded by triple ruling, and each consisting of sixteen "small" squares. It is this block which is required for the red-cell count. Bring the left "medium" square of the block into the centre of the field, and, using the high-power objective, count all the red blood cells lying on the sixteen small squares within the triple lines. Repeat this procedure successively with the top right, the bottom right, the bottom left, and the centre "medium" squares. It will be found that in some parts blood corpuscles are lying actually on the lines bordering the block. On the average, every boundary line will cut across the same number of cells. Hence it is customary, in each "medium" square, to count the cells lying across two of the sides and to omit those on the other two sides. The red cells lying on 80 (*i.e.* 5×16) small squares have now been counted, and it is evident that these have settled from a column

of diluted blood 1/10 mm. in height. Let R be the total number of red blood cells counted, then $R/80$ = the number in one small square whose area is 1/400 sq. mm. and which supports a column of diluted blood 1/10 mm. in height.

Thus a volume $\frac{1}{400} \times \frac{1}{10}$ c.mm. diluted blood contains

$$\frac{R}{80} \text{ cells.}$$

200 volumes of diluted blood contains only 1 volume of blood.

Hence a volume $\frac{1}{400} \times \frac{1}{10} \times \frac{1}{200} = \frac{1}{80,000}$ c.mm. blood

contains $\frac{R}{80}$ cells.

$$\begin{aligned} \text{Hence 1 c.mm. blood contains } & \frac{R}{80} \div \frac{1}{80,000} \\ & = \frac{R}{80} \times 80,000 \\ & = 10,000 \times R. \end{aligned}$$

Normally the red cells are between the limits of 4,500,000 and 5,500,000 per c.mm., with an average of 4,800,000 in males. In females the figures are about 10 per cent. less.

The enumeration of the white cells is performed in a similar way. Under the low-power objective count all the leucocytes in the whole square millimetre, *i.e.* in 1/10 c.mm. of diluted blood. Let this number be W. Then $W \times 10 \times 20$ = the number of leucocytes per c.mm. of blood, since on this occasion the dilution was 1 : 20. Three such counts should be made, with

different fillings of the chamber, and the average accepted as the white count. Normally blood contains 5000 to 10,000 white cells per c.mm.

The Neubauer Ruling.—The Neubauer ruling is shown in Fig. 37. The centre part is exactly the same

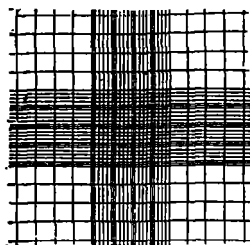


FIG. 37.
The Neubauer Ruling.

as the Thoma ruling and is used for the red cell count in exactly the same way.

Altogether, however, there are nine "large" squares of 1 sq. mm., and for the white cell count the cells are counted in any three of these large squares, or, for still greater accuracy, in the whole nine.

Estimation of Haemoglobin. Sahli Method.

In this method of estimation the oxyhaemoglobin is first converted into acid haematin by means of hydrochloric acid and the colour diluted until it matches a coloured glass standard.

With the teated pipette provided, N/10 hydrochloric acid is placed in the comparator tube up to the mark 15. To this is added 20 c.mm. of blood obtained from the patient by a special clean, dry, graduated capillary pipette. All blood must be wiped from the outside of the pipette, which is then emptied into the comparator tube and washed two or three times with the hydrochloric acid already present in the tube. The comparison, according to the instructions supplied with the instrument, is to be made at the end of two minutes. To the comparator tube distilled water is added drop by drop until its colour

exactly matches the standard, thorough dilution being effected either by gently rolling the tube or by the glass mixing rod provided. It is usually advisable to take one reading when the colour is just too dark and a second when it is just too light, and take the average of these two as being the percentage of haemoglobin.

“100 per cent.” on the Sahli scale represents a concentration of 15·6 g. haemoglobin per 100 c.c. of blood. The normal range may be taken as 85 to 110 for adult males and 80 to 100 for adult females.

The Colour Index

This is an expression of the amount of haemoglobin carried by each red blood corpuscle. It is given by the ratio :—

$$\frac{\text{Hb. as percentage of the normal}}{\text{R.B.c. as percentage of normal}}$$

and the normal colour index ought thus to be unity. The numerator of this ratio is read directly from the haemoglobinometer. The denominator can be obtained conveniently by multiplying the first two figures of the red cell count by 2 (*i.e.* 2,500,000 R.B.c.s per c.mm. is 50 per cent.) if the normal red blood count is taken as 5,000,000.

Actually, since the normal haemoglobin is not 100 per cent. on the Sahli scale, but lower, and the average red cell count is not 5,000,000, but lower, the average colour index in normal persons is not quite unity. It is usually about 0·95, and a colour index of 1·0 should be regarded as high.

Blood Films. The Differential Count.

Although the cover-slip method of producing blood films is probably the best, especially when an accurate differential count is required, an easier and more generally applicable method is as follows. Two thoroughly clean and dry glass slides are selected, on one of which the film is to be made, and the other, which must have an unchipped edge, to serve as the spreader. A small drop of blood obtained by finger prick is touched towards the end of one of the slides, which is placed quickly on some firm surface. The edge of the spreader held at an angle of about 45° to the first slide is then brought into contact with the blood, which is allowed a moment to spread across the edge. A short sharp movement of the spreader then draws a film of blood across the slide, which should be dried immediately by waving it in the air. It can then be examined fresh at once or fixed and stained for more detailed examination.

Although apparently simple, the making of satisfactory blood films requires much practice, and it is no exaggeration to say that probably 50 per cent. of films sent to laboratories for examination are unsatisfactory. It is essential that both slides be absolutely free from grease and dust. If a stock is not kept in alcohol, both slides should be washed thoroughly with "Monkey Brand" or similar soap and thereafter thoroughly cleaned with acetone and polished with a silk cloth. The surfaces of the film slide must not be touched with fingers nor allowed to come into contact with the patient's skin. By far the most common mistake, however, is to take too large a drop of blood, the correct size of which can only be gauged with practice (but say about $1/16$ th inch in

diameter). If an accurate differential count is to be made it is essential that *all four* edges of the smear shall be examined and the drop of blood must accordingly be a small one. The size of the film having been adjusted, its thickness can be varied by altering the angle between the spreader and the slide. Spreaders made of celluloid and other materials are not recommended.

It is now necessary to fix and stain the film. For both purposes the most generally useful reagent will be found to be Leishman's stain. The film to be stained should be supported horizontally above the bench and the undiluted stain dropped on to it from a teated pipette. The blood smear is conveniently demarcated by a grease pencil, in which case 5 or 6 drops of stain are usually sufficient to cover it completely. The undiluted stain is allowed to act for one minute, during which time the alcohol fixes the film. Thereafter the stain is diluted by adding twice as many drops of distilled water and allowing the diluted stain to act for at least 10 minutes. (The longer the diluted stain is allowed to act the better the final result, provided, of course, it is not allowed to become dry.) The stain is poured off and the specimen differentiated with distilled or tap water until pink. It should then be allowed to dry before mounting in Canada balsam.

For the differential count the slide should be placed on a movable stage and focussed under the high-power objective. At least 300 white cells should be counted, and these must include the cells at the edges of the smear as well as those centrally situated, the various types of cell being tabulated into their appropriate categories.

The Reticulocyte Count

A reticulocyte is an immature erythrocyte, and in order to demonstrate its presence in the blood a special staining technique must be used, the cells being stained prior to fixation.

On a clean glass slide a film of stain is made by stroking the slide with a glass rod dipped in a saturated alcoholic solution of cresyl blue. The slide should first be warmed over a small flame so that the alcohol evaporates readily, leaving a thin film of the stain on the slide. No special precautions need be taken to produce an even film. A *small* drop of blood (say 1/16th inch diameter) is now received from the patient's finger (see p. 334) on to the centre of a clean, dry cover-slip ($\frac{3}{4}$ inch square, No. 1 thickness), and, to prevent rouleaux formation, this is immediately placed on the stained slide. The drop of blood spreads itself out as a thin film between the two. During this process some of the stain dissolves in the blood plasma and is taken up by the reticulocytes; the platelets and nuclei of leucocytes also take up the stain to some extent. Since such a preparation will not keep for more than a few hours, it should be examined as soon as possible. The counting of the cells is best performed under the oil immersion lense on a movable stage and by restricting the size of the microscope field by employing an Ehrlich eyepiece, or, more simply, by cutting a small aperture about $\frac{1}{2}$ cm. square in a disc of paper which can be introduced between the glasses of the ordinary eyepiece. The proportion of reticulocytes to mature red blood cells can then be ascertained by examining a number of successive fields and counting therein the numbers of each. At least 1000 red blood cells should be counted.

If the red cell count be also known, the absolute number of reticulocytes per c.mm. of blood can be calculated, although this is not usually necessary.

Normal blood contains less than 1 per cent. of reticulocytes, and an increase above this proportion is indicative of increased activity of the bone marrow. During the liver treatment of pernicious anaemia, for example, up to 30 or 40 per cent. of reticulocytes can often be found.

Blood Grouping

Before blood transfusion can be undertaken with safety, it is essential to be certain that the donor's corpuscles are not agglutinated by the recipient's plasma. Tests for agglutination of the recipient's corpuscles by the donor's plasma are not usually made, since the subsequent dilution of the donor's plasma by that of the recipient obviates the possibility of agglutination occurring to any important extent.

Direct Test.—Undoubtedly the most satisfactory test to perform, and one which in any case should never be omitted, is the direct test in which the compatibility of the donor's blood is examined by testing the donor's blood directly against the recipient's.

Withdraw from the recipient a few drops of finger-prick blood into a capillary tube and centrifuge to obtain the plasma therefrom, which can then be placed on the centre of a microscope slide. (If it be preferred, the blood may be obtained by venepuncture and allowed to clot.) Into a haemoglobinometer pipette withdraw about 20 c.mm. of finger-prick blood from the donor and dilute this with 1–2 c.c. of red cell diluting fluid (p. 328) in a small test-tube. Add a

small drop of this dilute suspension of cells to the recipient's plasma on the slide and mix well by stirring. Place the slide, which should be rocked gently from time to time, on the microscope stage and observe through the lower power objective for fifteen minutes. Incompatibility of the blood is indicated by clumping of the cells within the first ten minutes. Agglutination, if it be produced at all, is usually very marked and can be seen easily with the naked eye. Slight clumping of the cells visible only at the edges of the specimen is usually due to evaporation of the fluid and can be disregarded.

Blood Grouping.—The blood group of the prospective recipient can be determined in order that a suitable donor may be employed. This, however, must be regarded as a convenience and not as a substitute for the direct test.

For this purpose capillary tubes of Group II and Group III sera are required. These can be obtained commercially and must be fresh. A drop of each serum is placed on a glass slide and a drop of the recipient's diluted blood (obtained exactly as described under the direct test for the donor) is thoroughly mixed with each. Both specimens are examined for agglutination. It is evident that four possibilities exist—no agglutination with either serum, agglutination with both sera, agglutination with Group III only, and agglutination with Group II only. The blood group of the recipient can be found, then, from the following scheme (Moss's classification) in which the *plus* sign indicates agglutination:—

	Cells I	Cells II	Cells III	Cells IV
Serum I . . .	0	0	0	0
SERUM II . . .	+	0	+	0
SERUM III . . .	+	+	0	0
Serum IV . . .	+	+	+	0

Group I serum thus produces no agglutination of cells of any group and is hence called Universal Recipient. Group IV cells are, on the other hand, not agglutinated by the serum of any group, and Group IV is, therefore, Universal Donor.

The blood groups of Moss's classification correspond to those of the International classification as follows :—

	<i>Moss</i>	<i>International</i>
Group	I	AB
„	II	A
„	III	B
„	IV	O

The Spectroscopic Examination of Blood

For most purposes a simple direct-vision spectroscope suffices, but the instrument should be sufficiently powerful to show at least the strongest of the Fraunhofer lines. The slit should be so adjusted that

these lines are seen when the spectrum is properly focussed by movement of the eyepiece.

Fairly bright daylight (but not direct sunlight) forms the best light source; artificial light can be used, but in this case the Fraunhofer lines are not present and a useful index of reference is therefore lost. The fluid to be examined is best contained in a vessel with parallel sides about half an inch apart, but an ordinary $\frac{5}{8}$ inch test-tube is reasonably satisfactory. The container is held immediately in front of the spectroscope.

The Fraunhofer lines are due to absorption of light by various elements in the sun's atmosphere. The most prominent are D (in the yellow, due to sodium); b (in the green, due to magnesium); and F (in the blue, due to hydrogen). C (in the red, due to hydrogen) and E (in the green, due to iron and calcium) are less prominent, but should be visible. B (in the red, due to oxygen) and G (in the violet, due to iron and calcium) are shown by really good instruments.

Absorption bands, appearing as more or less dark streaks vertically across the spectrum, are referred both to the colour of the light absorbed and to the nearest of the prominent Fraunhofer lines. Thus oxyhaemoglobin (see Fig. 38) shows two bands in the yellow-green area, one just to the right of the D line, and the other just to the left of the E line.

In examining for certain derivatives of haemoglobin, the concentration of the substance is of importance, and the following instructions as to dilution should be observed. Fig. 38 shows the chief absorption bands of a number of important substances.

Oxyhaemoglobin and Haemoglobin.—The characteristic bands lie between the D and E lines, the one to

the left being narrow and fairly well defined, while the other is broader and more diffuse. With high concentrations of oxyhaemoglobin (*e.g.* a 1 : 50 dilution of blood, or about 0·3 per cent. oxyhaemoglobin) the bands become fused into a single broad band. They are best seen at a dilution of about 1 : 100. If the blood is still further diluted the bands become narrower and fainter, but can still be seen at a dilution of 1 : 500. On addition of a reducing agent (*e.g.* Stoke's reagent, a solution containing 2 per cent. ferrous sulphate and 3 per cent. tartaric acid) oxyhaemoglobin is reduced to haemoglobin, and the two absorption bands are replaced by a single broad band, occupying a similar position in the spectrum, but touching or slightly overlapping the D line. On shaking a solution of reduced haemoglobin with air, oxyhaemoglobin is formed again and the double band reappears.

Carboxyhaemoglobin. — Carboxyhaemoglobin is a lighter red than oxyhaemoglobin, making blood containing it a cherry-red, and in very dilute solutions is a cherry-red or a bluish red, whereas oxyhaemoglobin has a yellowish tinge. With the blood diluted about a hundredfold it shows two absorption bands very similar to those of oxyhaemoglobin, but slightly nearer the violet end of the spectrum. This slight shift to the right cannot be detected with certainty in the small hand spectroscope, especially as the usual sample contains oxyhaemoglobin as well. However, Stoke's reagent is without effect on carboxyhaemoglobin, so that on treatment with this the two bands of carboxyhaemoglobin persist—usually superimposed on the broad haemoglobin band formed by reduction of the oxyhaemoglobin present.

Carbon monoxide in blood is perhaps more easily

detected by the tannic acid test. Dilute about 2 c.c. of blood to four times its volume with water, add 10–20 drops of 10 per cent. potassium ferricyanide solution (to oxidise the oxyhaemoglobin to methaemoglobin), and divide the mixture into two parts. Keep one in a stoppered test-tube, and shake the other vigorously for a few minutes, occasionally removing the stopper to allow fresh air to enter. This removes the carbon monoxide, replacing the carboxyhaemoglobin by oxyhaemoglobin, which is oxidised to methaemoglobin by the ferricyanide still present. To both tubes add 5–10 drops of yellow ammonium sulphide (which reduces the methaemoglobin to haemoglobin) and 10 c.c. of a 10 per cent. solution of tannic acid. The blood which has been freed from carbon monoxide will give a dirty greenish-brown precipitate; the unshaken blood, which still contains carboxyhaemoglobin, will give a precipitate varying in colour from reddish-brown (if only a little carbon monoxide is present) to a definite red (if the blood contains much carbon monoxide). This test will detect carboxyhaemoglobin in the presence of even twenty times as much oxyhaemoglobin.

Methaemoglobin.—Blood containing any considerable amount of methaemoglobin is browner than normal blood. The absorption spectrum in nearly neutral solution has four bands, one in the red on the C line, two in the yellow-green, very like those of oxyhaemoglobin, and one in the green-blue between the b and F lines. The most important of these is the one in the red, but it cannot usually be seen if the blood is diluted sufficiently for the others to be obvious. The blood is, therefore, diluted sufficiently for this part of the spectrum to be plainly visible,

though all the rest has disappeared (1 : 5 dilution is suitable). If methaemoglobin is present the band in the red will be seen, and will disappear on addition of yellow ammonium sulphide or Stoke's reagent (the pigment is reduced to haemoglobin).

Sulphaemoglobin.—As in the case of methaemo-

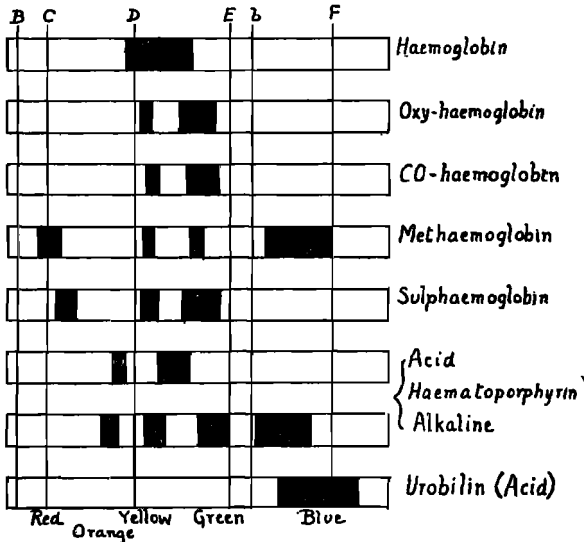


Fig. 38.—Absorption Spectra.

globin, the important absorption band lies in the red (a little above the C line) and is best seen when the blood is not greatly diluted. On addition of ammonium sulphide or Stoke's reagent the band does not disappear.

Estimation of Sugar in Blood

Only one of the many available methods—that of Hagedorn and Jensen—is described here. Protein

and certain interfering substances are removed from the blood by precipitation with zinc hydroxide. The glucose is then allowed to react with an excess of potassium ferricyanide (some of which is reduced to ferrocyanide). The excess of potassium ferricyanide then liberates iodine by oxidation of added potassium iodide, and the iodine is estimated by titration with sodium thiosulphate. Thus the excess of ferricyanide is estimated, and, by difference, the amount required to react with the glucose present. In practice the calculation is abolished by the use of a table.

Reagents.—*Sodium hydroxide*: 0.1 N prepared weekly by dilution of a stock 2 N solution.

Zinc sulphate: 0.45 per cent. solution, prepared weekly by dilution of a stock 45 per cent. solution.

Potassium ferricyanide: 1.65 g. of pure potassium ferricyanide and 10.6 g. of pure anhydrous sodium carbonate in 1 litre. This solution must be accurate. It must be kept in the dark (*e.g.* in a brown glass bottle) and should not be used for longer than a month.

Potassium iodide: 5 per cent. solution, stored in a dark bottle.

Sodium chloride-zinc sulphate: 10 g. of zinc sulphate and 50 g. sodium chloride in 200 c.c.

Acetic acid: 3 per cent. solution by dilution of glacial acetic acid.

Starch: (Indicator): add a thin cold-water starch paste to ten times its volume of boiling water, boil for one minute, and cool.

Sodium thiosulphate: 0.005 N; prepared by dilution from a stock approximately 0.1 N solution. For the latter, dissolve 26 g. of crystalline sodium thio-

sulphate in water and dilute to 1000 c.c. Store in a dark bottle, and use only after 2-3 days. The diluted solution must be standardised each day before use by titration against 0.005 N potassium iodate (which can be made accurately and keeps well). To 2.0 c.c. of the iodate solution add 2 c.c. of the potassium iodide solution, 3 c.c. of the 3 per cent. acetic acid, and a drop of starch solution. Titrate with the thiosulphate until the blue starch-iodine colour just disappears. If the volume used is x c.c., the true normality of the thiosulphate is $\frac{2}{x} \times 0.005$.

Potassium iodate: 0.005 N, containing 0.1784 g. of neutral potassium iodate per litre. This solution must be made accurately.

Procedure.—Measure 0.2 c.c. of blood into a test-tube containing 10 c.c. of 0.45 per cent. zinc sulphate solution and 2 c.c. of 0.1 N sodium hydroxide. The blood is washed out of the pipette by repeatedly sucking up the contents of the tube and allowing them to drain back. Place the tube in a boiling water-bath for three minutes, cool, and filter through a small filter paper.

To exactly 2.0 c.c. of the ferricyanide solution add 6.1 c.c. of the filtrate (which contains 0.1 c.c. of blood), using a large boiling tube, and heat in a boiling water-bath for fifteen minutes. Cool by immersing in cold water. Add 2 c.c. of the sodium chloride-zinc sulphate solution, 2 c.c. of the potassium iodide, 5 c.c. of the acetic acid, and a drop of starch solution. Titrate with the standardised thiosulphate until the blue colour just disappears, using a micro-burette (2 c.c.).

With each batch of estimations make a "blank"

analysis, using 6 c.c. of distilled water instead of the 6.1 c.c. of blood filtrate. (This is to allow for impurities in the reagents which use up a little of the ferricyanide.)

Calculation.—Calculate the volume of exactly 0.005 N thiosulphate equivalent to that actually used in the titrations.

E.g., suppose, in standardising the thiosulphate, 2.10 c.c. were used, so that the thiosulphate was

$$\frac{2}{2.10} \times 0.005 \text{ N.}$$

In the sugar estimation 0.75 c.c. were used, so that the equivalent amount of 0.005 N solution was

$$0.75 \times \frac{2}{2.10} \text{ c.c. (i.e. } 0.714 \text{ c.c.).}$$

In the "blank" 1.80 c.c. were used, so that the equivalent amount of 0.005 N solution was

$$1.80 \times \frac{2}{2.10} \text{ c.c. (i.e. } 1.714 \text{ c.c.).}$$

From Table XV note the amounts of glucose corresponding to these two titration values. In the example chosen, the blood filtrate (containing the glucose of the blood and the glucose-simulating impurities of the reagents) required 0.714 c.c. of 0.005 N thiosulphate, which corresponds to 0.229 mg. glucose; the "blank" (containing only the impurities) required 1.714 c.c., which corresponds to 0.049 mg. Hence the blood filtrate contained $0.229 - 0.049$, or 0.180 mg. of glucose.

But the 6.1 c.c. of blood filtrate used were equivalent to 0.1 c.c. of blood.

Hence 100 c.c. of blood contained 180 mg. of glucose.

TABLE XV
C.C. 0.005 Na₂S₂O₃ USED AND MG. GLUCOSE PRESENT

C.C.	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.0	0.385	0.382	0.379	0.376	0.373	0.370	0.367	0.364	0.361	0.358
0.1	0.355	0.352	0.350	0.348	0.345	0.343	0.341	0.338	0.336	0.333
0.2	0.331	0.329	0.327	0.325	0.323	0.321	0.318	0.316	0.314	0.312
0.3	0.310	0.308	0.306	0.304	0.302	0.300	0.298	0.296	0.294	0.292
0.4	0.290	0.288	0.286	0.284	0.282	0.280	0.278	0.276	0.274	0.272
0.5	0.270	0.268	0.266	0.264	0.262	0.260	0.259	0.257	0.255	0.253
0.6	0.251	0.249	0.247	0.245	0.243	0.241	0.240	0.238	0.236	0.234
0.7	0.232	0.230	0.228	0.226	0.224	0.222	0.221	0.219	0.217	0.215
0.8	0.213	0.211	0.209	0.208	0.206	0.204	0.202	0.200	0.199	0.197
0.9	0.195	0.193	0.191	0.190	0.188	0.186	0.184	0.182	0.181	0.179
1.0	0.177	0.175	0.173	0.172	0.170	0.168	0.166	0.164	0.163	0.161
1.1	0.159	0.157	0.155	0.154	0.152	0.150	0.148	0.146	0.145	0.143
1.2	0.141	0.139	0.138	0.136	0.134	0.132	0.131	0.129	0.127	0.125
1.3	0.124	0.122	0.120	0.119	0.117	0.115	0.113	0.111	0.110	0.108
1.4	0.106	0.104	0.102	0.101	0.099	0.097	0.095	0.093	0.092	0.090
1.5	0.088	0.086	0.084	0.083	0.081	0.079	0.077	0.075	0.074	0.072
1.6	0.070	0.068	0.066	0.065	0.063	0.061	0.059	0.057	0.056	0.054
1.7	0.052	0.050	0.048	0.047	0.045	0.043	0.041	0.039	0.038	0.036
1.8	0.034	0.032	0.031	0.029	0.027	0.025	0.024	0.022	0.020	0.019
1.9	0.017	0.015	0.014	0.012	0.010	0.008	0.007	0.005	0.003	0.002

Estimation of Blood Urea

The method is exactly the same as that used for urine urea (see p. 286), except that 1.0 c.c. of undiluted blood is used, and no correction for ammonia is required, since the amount ordinarily present in blood is negligible.

Estimation of the Total Non-Protein Nitrogen of Blood

Proteins are removed from the blood by precipitation with sodium tungstate and sulphuric acid : 2 c.c. of blood are mixed with about 8 c.c. of water in a 20 c.c. measuring flask ; after addition of 2 c.c. each of 10 per cent. sodium tungstate and $\frac{2}{3}$ N sulphuric acid, the volume is adjusted to 20 c.c. with water ; the mixture is allowed to stand five minutes and then filtered. The filtrate is boiled with a mixture of phosphoric and sulphuric acids with a trace of copper sulphate, whereby the organic nitrogenous compounds are oxidised and the nitrogen is obtained in the form of ammonium sulphate. The mixture is made alkaline with sodium hydroxide, and the free ammonia is distilled into 10 c.c. of N/70 sulphuric acid. The excess of this standard acid is determined by titration exactly as in the estimation of urea.

Estimation of Uric Acid in Blood

Protein-free blood filtrate is used directly, or, preferably, the uric acid is first precipitated from it by silver lactate, and the silver urate is decomposed by a mixture of sodium chloride and hydrochloric acid. In either case the colour produced when the

uric acid reduces a solution of arseno-phosphotungstic acid is compared with the colour similarly produced by a known amount of uric acid.

Estimation of Creatinine in Blood

A portion of the protein-free blood filtrate is treated with alkaline picrate solution, and the orange-yellow colour produced (sodium picramate) is compared with that given by a standard solution of creatinine under similar circumstances.

Estimation of Inorganic Phosphate

Protein is precipitated from the blood by trichloroacetic acid, and a portion of the filtrate is treated with ammonium molybdate. The phosphomolybdic acid so formed is reduced by hydroquinone in presence of sodium sulphite to a blue soluble substance, and is estimated colorimetrically by comparison with the colour similarly produced by a standard solution of sodium phosphate.

Estimation of Chloride

The protein-free blood filtrate, prepared by means of sodium tungstate and sulphuric acid, is treated with an excess of silver nitrate in presence of nitric acid, and the excess of silver nitrate is determined by titration with ammonium thiocyanate. Alternatively, whole blood may be used, the proteins being destroyed, and silver chloride precipitated, by heating for some hours with concentrated nitric acid in presence of a known amount of silver nitrate, the excess of silver nitrate being determined as before.

Estimation of Cholesterol

A measured volume of blood is absorbed by filter paper, dried in a steam-oven, and the paper is then thoroughly extracted with chloroform. The chloroform extract contains the cholesterol, which gives a green colour with acetic anhydride in presence of sulphuric acid. It is estimated by comparing the colour with that given by a standard solution of cholesterol.

Estimation of the Total Serum Calcium

The method generally used for the determination of the total serum calcium (or, after filtration through collodion, of the diffusible calcium) is either that of Kramer and Tisdall, or a slight modification of it. Into a conical centrifuge tube are pipetted 2 c.c. of serum, 1 c.c. of saturated ammonium oxalate, and 2 c.c. of distilled water. The mixture is allowed to stand overnight for precipitation of the calcium oxalate—the half-hour allowed in the original method is not enough to ensure complete precipitation. The tube is then centrifuged for four minutes and the supernatant fluid is poured off. The precipitate is broken up and washed with 4 c.c. of dilute ammonia (2 c.c. of 0.880 ammonia diluted to 100 c.c. with distilled water), the ammonia being used to wash down the sides of the tube. By means of the centrifuge the precipitate is again separated, and the washing is twice repeated. The precipitate is then dissolved in 2 c.c. of approximately normal sulphuric acid, heated for a few minutes on the boiling water-bath, and titrated with 0.01 N potassium permanganate (0.316 g. of KMnO_4 per litre), each cubic centimetre

of which is equivalent to 0.2 mg. of calcium. For this titration a burette holding 2 c.c. and graduated in hundredths of a cubic centimetre is needed. The permanganate solution does not keep well and must be standardised frequently by titration with a 0.01 solution of sodium oxalate (0.67 g. per litre) acidified with sulphuric acid and heated on the water-bath.

Bile Pigment in Blood

The van den Bergh Reaction.—The blood should be drawn with precautions to prevent haemolysis from taking place, since free haemoglobin will mask the colour produced in the test. Thus no ether may be present in needle, syringe, or test-tube, which latter must be dry and chemically clean. Perfect cleanliness of all apparatus is particularly necessary for success in this test. The blood may be allowed to clot, and the serum separated, or plasma may be obtained by centrifuging oxalated blood. The test should be performed within two hours.

For the direct qualitative test, measure 1 c.c. of the serum or plasma into each of two test-tubes. Only one of these is really necessary, but the other serves as a control, rendering it more easy to notice small colour differences. Dilute the control serum with 1 c.c. of water, and add the same volume of freshly prepared reagent to the other. The reagent is prepared by mixing 25 c.c. of stock solution A (sulphanilic acid 1 g., concentrated hydrochloric acid 15 c.c., distilled water to 1000 c.c.) with 0.75 c.c. of stock solution B (0.5 per cent. sodium nitrite in water), both of which keep almost indefinitely. A positive reaction is shown in one of three ways :—

Immediate Direct Reaction.—A bluish violet colour begins to appear at once, and is maximal within a minute.

Delayed Direct Reaction.—After no apparent change for at least a minute, and often as much as fifteen or twenty minutes, a reddish colour begins to appear, gradually deepens, and becomes more violet.

Biphasic Direct Reaction.—A reddish colour appears immediately, and deepens to violet at a rate which may be rapid (prompt biphasic reaction) or slow (delayed biphasic reaction).

The Indirect Reaction is used to confirm a delayed direct reaction, as a further more delicate test when the direct reaction is negative or so faint as to be doubtful, and for quantitative work.

The reagent, 0.5 c.c., is added to 1 c.c. of serum or plasma, just as for the direct reaction, but after a minute 2.5 c.c. of 96 per cent. alcohol and 1 c.c. of saturated ammonium sulphate are added. The mixture is centrifuged and the clear supernatant fluid used for observing the colour or for quantitative estimation. In this procedure azo-bilirubin is formed by the interaction of bilirubin and diazo-benzene sulphonate, and is soluble in alcohol. Hence the precipitation of the proteins by alcohol does not cause loss of pigment.

Quantitative Test.—The serum or plasma is treated as for the indirect reaction, and the coloured solution is composed in a colorimeter with an artificial standard. The standard solution, which keeps well if stored in the dark, consists of 2.161 g. of anhydrous cobaltous nitrate dissolved in 100 c.c. of water, and corresponds to one unit (0.5 mg. per 100 c.c.) of azo-bilirubin. Since the serum has again been diluted to five times

its original volume, the number of units per 100 c.c. of serum is given by multiplying by five the ratio of the two colours.

Icteric Index.—An even simpler quantitative technique, which is sufficiently accurate for clinical purposes, is that introduced by Meulengracht, who, assuming that the yellow colour of the serum is due entirely to bilirubin, compares the serum, suitably diluted (usually twenty times), with a standard solution which keeps indefinitely in the dark and consists of 0.05 g. of potassium bichromate dissolved in water containing 2 drops of concentrated sulphuric acid and diluted with water to 500 c.c. The icteric index is the product of the dilution of the serum and the ratio between the colour of the diluted serum and the colour of the standard. If a colorimeter is used for the comparison, this is given by the formula :—

$$\text{Icteric index} = \text{dilution} \times \frac{\text{reading of standard cup}}{\text{reading of cup containing serum}}$$

If comparison is made by the method of test-tube dilution the formula is :—

$$\text{Icteric index} = \text{dilution} \times \frac{\text{volume in test-tube containing serum}}{\text{volume in test-tube containing standard}}$$

The icteric index divided by ten gives approximately the number of van den Bergh units.

In this method, as in the original van den Bergh, it is essential to avoid haemolysis, and it is advisable to use fasting blood, since the serum is often cloudy during the period of post-ingestive absorption. The

Meulengracht method gives fallacious results in presence of a carotinaemia such as may be found in cases of diabetes, in starvation, and in individuals who have been taking a diet very rich in vegetables. Hence this method should be used, if there is any reason to suspect the presence of a carotinaemia, only after the van den Bergh reaction has shown the presence of a bilirubinaemia, and even then the figures should be accepted with caution.

Fouchet's Test for Bile Pigment in Blood.—This simple test, though it does not differentiate the two types of bilirubin as does the van den Bergh test, is often useful, since it is not prevented by the presence of moderate haemolysis of the sample. It is performed by mixing, in a small porcelain dish, 3 drops of serum with an equal amount of the reagent (2 c.c. of 10 per cent. FeCl_3 , 5 g. of trichloroacetic acid, and 20 c.c. of water) and allowing the mixture to stand for a few minutes. The presence of more than about three van den Bergh units of bilirubin is indicated by the coagulum turning a greenish-blue colour, maximal in about twenty minutes.

The Phenoltetrachlorophthalein Test

After first drawing about 8 c.c. of blood from a superficial vein of the arm, 5 mg. of phenoltetrachlorophthalein per kilogramme of body weight are injected into the same vein. For injection, the required amount of dye, which is obtained in ampoules containing 5 mg. per 0.1 c.c., is drawn into a sterile syringe and diluted to about 25 c.c. with sterile physiological saline. The time of completion of the injection is noted, and the vein wall is then washed free of dye by injecting a

further 20 c.c. of saline through the same needle. At the end of fifteen minutes, counting from the completion of the injection of the dye, 2 c.c. of blood are drawn from a vein of the other arm, and a second sample is obtained at the end of an hour. Later samples may be drawn if the second sample, on analysis, shows the presence of the dye.

The specimens of blood are allowed to clot, their sera are pipetted off, and to each sample a drop of 5 per cent. sodium hydroxide is added for each cubic centimetre of serum. For making the comparison a standard solution is prepared by dissolving 10 mg. of the dye in 100 c.c. of water. This arbitrary standard is chosen as having, on the basis of average blood-volume figures, the same concentration of dye as the serum would have if all the injected dye remained

TABLE XVI

STANDARDS FOR DETERMINATION OF PHENOLTETRACHLORPHTHALEIN IN SERUM

<i>Volume of 100 per cent. Standard</i>	<i>Volume of Control Serum</i>	<i>Volume of Water</i>	<i>Percentage of Injected Dye remaining in Serum of same Colour</i>
	<i>c.c.</i>	<i>c.c.</i>	
0.25	0.60	0.15	25
0.20	0.60	0.20	20
0.15	0.60	0.25	15
0.10	0.60	0.30	10
<i>Volume of 10 per cent. Standard</i> ¹			
0.40	0.60	0.00	4
0.20	0.60	0.20	2

¹ Prepared by diluting 1 c.c. of 100 per cent. standard to 10 c.c. with distilled water.

in it. It is therefore referred to as the 100 per cent. standard, and a serum showing one-fifth the depth of

colour of this standard indicates the retention of one-fifth of the injected dye. The colour of the serum samples is compared with that of a series of standards prepared, as shown in Table XVI, from the 100 per cent. standard diluted with the serum obtained before the injection of the dye. The comparison is made by direct observation, using small test-tubes of uniform diameter. It is obvious, of course, that the comparison can be successful only if the serum samples are clear, and if haemolysis is avoided.

THE TEST FOR OCCULT BLOOD IN FAECES

To demonstrate occult blood in faeces, a technique may be employed similar to that described on p. 320 for the detection of blood in the stomach contents, using a piece of stool about the size of a pea ground up in about 5 c.c. of distilled water. Otherwise, a few drops of a thin emulsion made in this way, and, as usual, thoroughly boiled to inactivate oxidising enzymes, may be added, after cooling, to a mixture of 2 c.c. of a saturated solution of benzidine in glacial acetic acid and 3 c.c. of 3 per cent. hydrogen peroxide. In the presence of blood a blue colour develops in a minute or two. This test is exceedingly delicate.

EXAMINATION OF THE CEREBRO- SPINAL FLUID

Microscopic examination of the fluid or of a centrifuged deposit from it is made by the usual methods.

Total protein is most easily estimated by addition, to a measured volume of fluid, of trichloroacetic acid and by comparison of the turbidity produced with that

of a series of standards. Such a series is obtainable commercially.

Sugar, urea, etc., may be determined by methods used for blood.

Qualitative Test for Globulin.—To 1 c.c. of cerebro-spinal fluid add 1 c.c. of a saturated solution of ammonium sulphate and set aside for a few minutes. A turbidity or strong opalescence indicates the presence of globulin in excessive amounts, with a total protein content of, usually, over 100 mg. per 100 c.c. of fluid. A normal fluid remains clear or, at most, shows a very slight opalescence.

Qualitative Test for Sugar

Fehling's Test.—Reduction of Fehling's solution affords a useful rough clinical test of the sugar content of the cerebro-spinal fluid. Boil 2 c.c. of the fluid with 6 drops of Fehling's solution. In health a heavy reddish precipitate is formed and rapidly settles to the foot of the test-tube, leaving a clear, light-blue supernatant fluid. With a low sugar content there may be no precipitate at all, since the protein prevents the very small amount of sugar present from reacting. This, however, occurs only when the sugar content is very greatly reduced, and with moderate reduction the mixture becomes opalescent, or shows a slight yellowish precipitate.

Estimation of Chloride

The method we advocate is very similar to that described for the estimation of chloride in urine, and the same reagents are used (see p. 303). To 0.5 c.c. of the cerebro-spinal fluid add 1 c.c. of the

silver nitrate solution and 2 drops of the acidified indicator. Mix, and run in the ammonium thiocyanate solution from a micro-burette until the first trace of yellow persists throughout the mixture. A suitable burette may be extemporised without much loss of accuracy by simply using a 2 c.c. pipette graduated in hundredths or fiftieths of a cubic centimetre. The calculation, except for the alteration in the quantities involved, is that used for urine.

APPENDIX II

TABLE SHOWING NORMAL CHEMICAL COMPOSITION OF HUMAN URINE AND VARIATIONS IN DISEASE

It must be noted that the analytical figures for normal urine may vary greatly since they depend so much on the diet. Those given are for an average mixed diet, but figures beyond these ranges do not necessarily indicate disease.

	<i>Amount in gm. in 24 hrs.</i>	<i>Amount in gm. per 100 c.c.</i>	
Volume	1500 c.c.	—	High in azotaemic Bright's disease. Low in nephrotic syndrome, intestinal obstruction, fevers, etc.
Specific gravity	1012-1020	—	Low in azotaemic Bright's disease. High in nephrotic syndrome, intestinal obstruction, fevers, etc.
Urea Urea nitrogen	25-35 12-18	1.6-2.5 0.8-1.2	{ Total high in fevers. Concentration low in azotaemic Bright's disease; high in nephrotic syndrome, intestinal obstruction, fevers, etc.
Ammonia Ammonia nitrogen	0.5-0.9 0.4-0.7	0.03-0.06 0.03-0.05	{ High in uraemia, liver diseases, eclampsia and in acidosis generally.
Chlorides (as NaCl)	.15-20	1.0-1.5	Concentration low in azotaemic Bright's disease, but total normal. Concentration and total low in nephrotic syndrome, intestinal obstruction and pneumonia.
Ammonia coefficient	2-5		Abnormalities as for ammonia.

APPENDIX III

TABLE SHOWING NORMAL CHEMICAL COMPOSITION OF
HUMAN BLOOD AND VARIATIONS IN DISEASE

It must be noted that the abnormalities shown as occurring in disease are not invariably present and that the list of diseases is not exhaustive.

<i>Constituent</i>	<i>Normal Amount in mg. per 100 c.c.</i>	<i>Variations in Disease</i>
Non-protein nitrogen Urea nitrogen	25-35 12-18	High in azotaemic Bright's disease, cardiac failure, intestinal and prostatic obstruction. Low in pregnancy.
Uric acid	2-3.5	
Creatinine	1-3	High in very advanced azotaemic Bright's disease.
Inorganic phosphorus	3-4	High in azotaemic Bright's disease and renal rickets. Low in rickets.
Cholesterol	140-180	High in nephrotic syndrome, gall-stones and diabetes. Low in pernicious anaemia.
Glucose	80-90	High in diabetes, hyperthyroidism, excitement, etc.
Acetone bodies (as acetone)	1.3-2.6	High in diabetes, starvation, severe vomiting, acute yellow atrophy.
Chlorides (as NaCl)	450-500	Slightly raised in nephrotic syndrome. Low in severe vomiting.
Calcium	9.5-10.5	Low in certain tetanies, alkalosis, and acidosis.
Co ₂ combining power	53-78 vols. %	Low in acidosis. High in alkalosis.
Bilirubin (van den Bergh units)	0.2-0.5	High in jaundice, haemolytic anaemias. Low in secondary anaemia.
Diastase (index)	6-25	High in acute pancreatitis. Low in azotaemic Bright's disease.
Plasma globulin " albumen	1.5-3.0 % 3.5-6.5 %	Low in nephrotic syndrome, etc. Increased in kala-azar, and myelomata.
Fibrinogen	200-400	Low in severe liver disease.

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