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THE
PRACTITIONER'S GUIDE
TO
CLINICAL RESEARCH

PUBLISHED BY
THE CLINICAL RESEARCH
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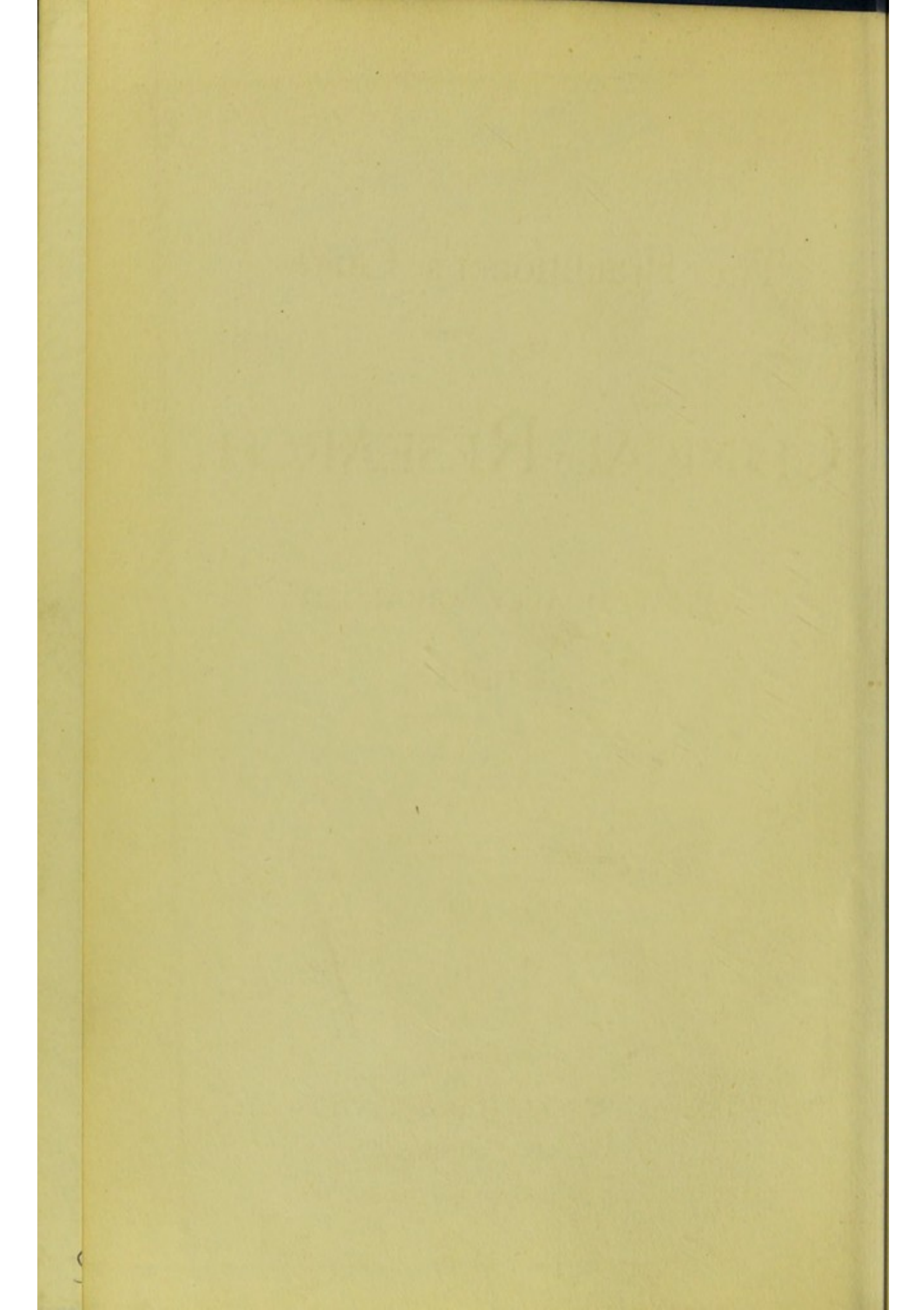
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The Practitioner's Guide
TO
CLINICAL RESEARCH

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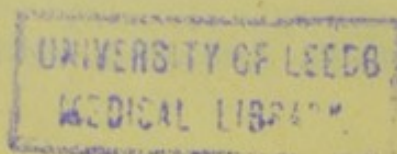
IN SECTIONS.

PUBLISHED BY
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PREFACE.

It is just twenty years ago since The Clinical Research Association, the first institution of its kind in this country, was founded, with the object of providing for every practitioner who desires it the assistance which can be rendered by a fully equipped laboratory in the investigation and treatment of obscure cases of disease. The extent to which such assistance can be afforded has greatly increased in recent years, and is constantly increasing both in volume and complexity. In order to keep the members of the Association, and others, in touch with new developments in connection with their work in the Laboratories, the Directors of the Clinical Research Association started five years ago The Journal of Clinical Research, a periodical appearing thrice each year, in which the possibility of solving new bedside problems by laboratory research is raised, and new methods of performing laboratory tests are described and discussed. Members of the Clinical Research Association receive copies of this Journal gratis.

From experience gained by the Editors of this Journal in dealing with correspondence addressed to them by their colleagues in practice there appears to be a demand for a concise, up-to-date, work dealing with the present position of laboratory investigations in relation to clinical medicine and surgery, setting out in detail the classes of cases in which laboratory assistance is likely to be of value, the exact way in which the necessary material should be collected and forwarded for investigation, and the deductions that may justifiably be drawn from the results obtained.

The Practitioner's Guide to Clinical Research is designed to meet this need. Every fluid and tissue from the body, whether normal or morbid, which may profitably be submitted to examination, is dealt with in turn in a separate section, the sections being alphabetically arranged. The various investigations to which these materials may be submitted are detailed. The apparatus required for, and the method of procedure in, securing and forwarding the

material for examination are described. The relative importance of the different investigations is set out, and the conclusions that may be drawn from the results obtained are indicated. It is recognized, however, that many problems present themselves to the practitioner from the standpoint of obscure symptoms, in face of which there is uncertainty as to the tissue, secretion, or morbid material, the investigation of which might assist in arriving at a diagnosis. In others, some definite disease or morbid state may be suspected, and the question the practitioner asks himself is "Have modern Laboratory methods any help to offer in diagnosing such conditions?" To afford assistance in such cases the fullest possible alphabetical index has been prepared, indicating the section in the Guide in which the value of laboratory investigation in clearing up the particular problem needing solution is discussed.

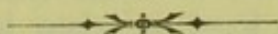
An Appendix to the Guide is published (see pages 123 to 150), giving full particulars of the charges made by the Clinical Research Association for each of the investigations detailed, the prices being set out in parallel columns for members and non-members. The price list follows the order of the sections in the Guide for ease of reference.

A form of application for membership of the Clinical Research Association is enclosed with the Guide and a current copy of the Appendix, and any further information desired will be forwarded at any time on application to the Secretary. The Editors would welcome any suggestions which would enable them to increase the usefulness of the Guide to the medical profession when publishing further editions.

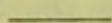
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The Practitioner's Guide to Clinical Research.



SECTION I.



The Blood.



A. BLOOD COUNTS.

A complete ordinary blood count consists in:—

1. Enumeration of the red corpuscles per cubic millimeter of blood.
2. Determination of the percentage of hæmoglobin present in the blood.
3. Calculation of the colour index of the red corpuscles from the figures afforded by 1. and 2.
4. Enumeration of the white corpuscles per cubic millimeter of blood.
5. A differential count of the leucocytes in stained blood-films.
6. A report as to the appearances of the red corpuscles in stained blood-films.

Examination of blood films for filaria parasites, hæmatozoa of malaria, and so forth, requires special staining (page 26), and therefore is not included in an "ordinary blood count." It happens frequently that only part of the above is required in a particular case, a differential leucocyte count, for instance, or a determination of the colour index.

Apparatus and Technique.

The apparatus sent out by the Clinical Research Association for the use of medical practitioners in sending material to the laboratories for the purpose of making a complete blood count is as follows:—

I. For (a) Enumeration of Red and White Corpuscles ; (b) Estimation of Hæmoglobin.

Apparatus supplied.—Two boxwood cases, one containing a bottle of solution, the other of water (both accurately measured quantities), two pipettes fixed in glass holders, fitted case, and an addressed box for its return.

METHOD OF PROCEDURE.

Note.—As the methods are identical, except in respect to the fluid used, which is water in the case of hæmoglobin, and a special solution in the enumeration of corpuscles, only one description is given.

Unscrew the top of the boxwood case, leaving the bottle standing in the lower part of the case. Remove the stopper carefully, touching its pointed end against the side of the bottle so as to drain back any fluid adhering to it. Lay the stopper down on its flat top.

Clean the lobule of the ear (or the back of the thumb) with ether or with soap and water, rub dry, and prick. Do not use any antiseptic; it is unnecessary, and is very likely to interfere with the investigation.

Dip the pipette into the blood-drop that wells up, so as to let it fill by capillary attraction. It will fill more readily if it be held in such a manner as to allow the blood to flow downwards into it. Carefully wipe away with a handkerchief any excess of blood adhering to the point of the pipette. When this is accomplished, eject the blood as soon as possible into the fluid in the bottle by gently blowing. Any undue delay will permit the blood to coagulate in the pipette. Rinse the pipette several times by dipping the point into the bottle and gently blowing back the fluid which rises into it by capillary attraction. Neither the blood nor the fluid should on any account be sucked into the pipette.

Carefully replace the stopper, gently shake the bottle, and screw on the boxwood cap.

If more than one specimen is to be taken, the partially-dried blood should be rubbed away from the ear lobule with a towel or handkerchief, and a fresh drop allowed to exude for each preparation. A separate pipette should be used in each case.

Should the blood flow insufficiently, it is not advisable to obtain a further supply by squeezing, but preferably by making a deeper puncture.

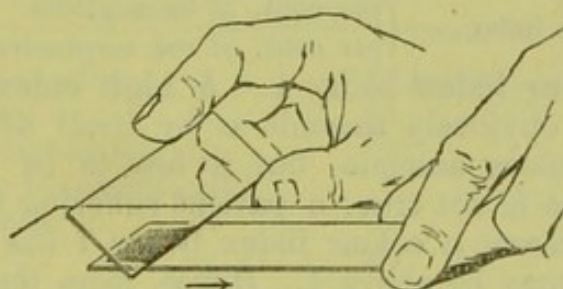
II. For (c) the Differential Count of Leucocytes and Determination of the Characters of the Red Cells.

Apparatus supplied.—Grease-free slides, a needle, rubber bands, box, and addressed envelope. On one of the slides a specimen film has been spread in order to indicate the general appearance and extreme thinness of a "satisfactory" film.

METHOD OF PROCEDURE.

Clean the lobe of the patient's ear with ether or soap and water, rub dry, and prick sharply with the needle.

Bring the surface of one slide in contact with the resultant blood drop so as to get some of it near one end. Lay the edge of a second slide transversely across the first in the way indicated in the following diagram:—



and push the transverse slide steadily along the surface of the horizontal one to the other end of the latter. The blood drop should be on the distal side of the transverse slide, so that it is *drawn* along by the latter and not *pushed* along by it.

The film should be as thin as possible, drying in a few seconds.

Prepare three such slides, allow them to dry completely, wrap them *separately* in tissue paper, fasten together with elastic bands to prevent rubbing, and replace them in the box.

It is of the utmost importance that the blood films be allowed to dry completely before they are repacked.

Printed directions are issued with each set of apparatus sent out by the laboratory.

The Clinical Information afforded by Blood Counts.

Apart from the discovery of protozoal parasites in the blood in filaria (page 27), malaria (page 26), relapsing fever, trypanosomiasis and sleeping sickness (page 27), and so forth, subjects which are not included under the heading of an

ordinary blood count, there are three well-known diseases in which the diagnosis depends upon pathognomonic blood changes, namely :—

- i. Pernicious anæmia.
- ii. Splenomedullary leuchæmia.
- iii. Lymphatic leuchæmia.

(i.) Pernicious Anæmia.

In *pernicious anæmia* there must always be doubt as to the correctness of the diagnosis until it has been demonstrated that there is a great diminution in the percentage of hæmoglobin, and at the same time a still greater diminution in the number of the red corpuscles; so that in association with a marked degree of positive anæmia there is a relatively high colour index. In a typical case, for instance, the figures may be found to be as follows:—

Hæmoglobin	30 per cent. of normal.
Numbers of red corpuscles		1,200,000 per cub. mm.
Equivalent to		24 per cent. of normal.
Colour index =	$\frac{\left\{ \begin{array}{l} \text{per cent. of hæmoglobin} = 30 \\ \text{per cent. of red corpuscles} = 24 \end{array} \right\}}{1} = 1.25$	

the normal colour index being 1. A high index like this in a patient who is obviously anæmic is by itself almost pathognomonic of pernicious anæmia, but it has to be borne in mind that the converse is not true; a patient suffering from pernicious anæmia may present a colour index below 1 one week, and the next the index may be above 1. Hence, more than one negative finding is required before the diagnosis of pernicious anæmia can be excluded, although a single positive finding may clinch the diagnosis. It is also important to remember that as a patient suffering from pernicious anæmia rallies and improves under treatment, the red corpuscles rise faster than does the hæmoglobin; so that the colour index of a convalescing case is nearly always less than 1, even though the index in the same case when the anæmia was more pronounced was well over 1. The following are some counts from an actual case to illustrate this point:—

Figures illustrating the fall in the Colour Index as a Patient suffering from Pernicious Anæmia improves under treatment.

Date.		Red corpuscles, per cub. mm.	Red corpuscles, % of normal.	Hæmoglobin, % of normal.	Colour index.
December 6th	...	1,000,000	20	35	1.75
" 14th	...	1,462,500	29	48	1.65
January 4th	...	4,300,000	86	68	0.79
" 11th	...	3,600,000	72	60	0.83
" 19th	...	5,600,000	112	98	0.8

This being so it is important that the other blood characters that are to be expected in pernicious anæmia should be looked for in each case. These are, briefly, as follows:—

The total number of leucocytes per cubic millimeter of blood is seldom increased above the normal unless there is some complicating inflammation to cause a leucocytosis; rather there is a decided tendency to leucopenia, the readings often being only 4,000 per cubic millimeter, 3,000 per cubic millimeter, or even less.

The differential leucocyte count shows no constant departure from the normal, but in the great majority of cases there is a relative increase in the small lymphocytes, a corresponding diminution in the polymorphonuclear cells, and, in addition to the normal white corpuscles, an occasional basophile leucocyte and one or two myelocytes may appear. The following are examples of differential leucocyte counts in a normal person and in a case of pernicious anæmia respectively:—

Differential Leucocyte Counts.

				Normal.		Pernicious anæmia.
Polymorphonuclear cells	65 per cent.	...	51 per cent.
Small lymphocytes	25 "	...	41 "
Large hyaline lymphocytes	8 "	...	6 "
Coarsely granular eosinophile cells	2 "	...	1 "
Basophile corpuscles	— "	...	0.5 "
Myelocytes	— "	...	0.5 "

The red corpuscles in stained films generally take the eosin well, but they present in a marked degree all those departures from the normal that are common to all forms of very pronounced anæmia of standing, namely:—

Poikilocytosis, that is to say, the red corpuscles, instead of being all the same shape—circular, biconcave bodies—vary in shape, some being oval, others pear-shaped, others hour-glass-shaped, and so on. These corpuscles of abnormal outline are termed poikilocytes. They are not artefacts—from which they are to be distinguished, for they are to be seen in the fresh unstained and unfixed blood.

Microcytosis and megalocytosis.—Many of those red corpuscles which are still circular in outline, depart considerably from the normal diameter of 7 μ , many being much smaller than normal, *microcytes*; many much larger than normal, *megalocytes*. Probably the preponderance of megalocytes in stained films is, after the high colour index, the most characteristic feature of the blood in pernicious anæmia.

Polychromasia.—The red corpuscles tend to take up more or less of the methylene blue or other basic dye in which they have been immersed, instead of only the pink eosin. This may give some of the corpuscles a more or less generally diffused purplish hue, or the blue may appear in an otherwise red corpuscle in the form of minute blue specks or dots, *punctate basophilia*. The latter is seen best in cases of anæmia from lead poisoning, but it also occurs in pernicious anæmia.

Nucleated red corpuscles appear in varying numbers; generally speaking, the worse the stage of the anæmia the more likely are nucleated red cells to be found in films. They may be normal sized red corpuscles with nuclei, *normoblasts*; or large red corpuscles with nuclei, *megaloblasts*; or nucleated red cells smaller than normal, *microblasts*; or occasionally one sees very big red corpuscles containing two nuclei or more, *gigantoblasts*.

The question of *blood platelets* and their significance is one of difficulty. Although these bodies may be present in numbers in different conditions, including pernicious anæmia, there are no definite clinical deductions that can be drawn from them.

Whereas nearly all the above changes in the red corpuscles may be produced by any disease that is associated with severe and prolonged anæmia, they are seen most pronouncedly in pernicious anæmia, especially the preponderance of megalocytes and of poikilocytes.

The essential laboratory investigations required in the diagnosis of pernicious anæmia are:—

Estimation of the hæmoglobin.

Enumeration of the red corpuscles.

Calculation of the colour index.

The diagnostic indications obtained from these may be materially confirmed by:—

Enumerating the white corpuscles to find that there is no leucocytosis.

Making a differential leucocyte count to find that there is a relative lymphocytosis.

Examining stained films to find megalocytosis and poikilocytosis.

(ii.) **Splenomedullary Leuchæmia**; and

(iii.) **Lymphatic Leuchæmia**.

In leuchæmia, whether of the splenomedullary, of the lymphatic, or of the mixed type, the diagnosis cannot be established with accuracy without enumeration of the white corpuscles and

a differential leucocyte count. Only in very exceptional cases is there not a very considerable increase in the total leucocytes per cubic millimeter. In an ordinary case of splenomedullary leuchæmia they generally number some hundreds of thousands, say 400,000 per cubic millimeter, instead of the normal 5,000 to 10,000. The limits of variation are very wide—from 50,000 to 1,000,000. In an ordinary case of lymphatic leuchæmia the total is generally less than in the splenomedullary form of the disease, an average figure being about 100,000 per cubic millimeter; but here, again, the variations are extreme—from 25,000 to 1,500,000. Even, however, when the total number of leucocytes per cubic millimeter of blood is not so extreme that the diagnosis of leuchæmia is obvious from that alone, the differential leucocyte count is generally characteristic. Myelocytes are more numerous in splenomedullary leuchæmia than in any other disease; they often amount to 40 per cent. of all the leucocytes present. In lymphatic leuchæmia the lymphocytes are generally increased to a degree that is almost unknown in any other malady—sometimes to 95 per cent. of all the leucocytes present. In the commoner type of the malady it is the small lymphocytes that are increased in this way. There is, however, another type of the disease in which the size of the lymphocytes is larger—lymphatic leuchæmia of the large lymphocyte type; whilst there is yet another type of the disease in which the differential leucocyte count contains the characters of splenomedullary and of lymphatic leuchæmia, myelocytes being numerous, but small lymphocytes being also very considerably increased both relatively and in total numbers. The following are some characteristic differential leucocyte counts in leuchæmia:—

	Normal blood. Per cent.	Splenomedullary leuchæmia. Per cent.	Lymphatic leuchæmia. Per cent.	Mixed leuchæmia. Per cent.
Polymorphonuclear cells ...	65	33	4	29
Small lymphocytes ...	25	20	91	45
Hyaline lymphocytes ...	8	3	1	3
Coarsely granular eosinophile cells ...	2	3	0.5	1
Basophile cells ...	—	2	2.5	2
Myelocytes ...	—	39	1	20

The occurrence of great leucocytosis, plus these changes in the differential count, gives the diagnosis of leuchæmia forthwith. The changes in the red corpuscles and hæmoglobin do not assist the diagnosis. In the early stages there may be no change in either of these; later, there is a progressive anæmia, usually of the chlorotic type, though occasionally in lymphatic leuchæmia the red corpuscles fall faster than the hæmoglobin, so

that the colour index is temporarily greater than 1. in the late stages of any type of the disease the anæmia becomes extreme, and all the changes in the red corpuscles characteristic of severe anæmia may then be detected (pages 9 and 10). These are not of diagnostic value, but they are important in weighing the prognosis.

Although the above are the only three blood diseases in which the diagnosis is actually made upon positive changes in the blood count, there are a great many other maladies in which the diagnosis remains in doubt until the blood has been examined, and leuchæmia or pernicious anæmia have thereby been excluded. Thus, when one meets with a patient suffering from a *very enlarged spleen*, a complete blood count is required, more especially a total leucocyte count and a differential leucocyte count. If the result is negative, or indeterminate, leuchæmia is excluded, and one of the various other causes of much enlarged spleen is indicated—cirrhosis of the liver, malaria, Kala-Azar, Hodgkin's disease, splenomegalic polycythæmia, infective endocarditis, or splenic anæmia. If there is a pronounced tendency to cyanosis without dyspnoea, and if without pathognomonic changes in the white cells the red corpuscles are materially increased—perhaps to 7,000,000 per cubic millimeter, for instance—the diagnosis of splenomegalic polycythæmia will be confirmed. The other conditions mentioned are not diagnosable by blood count alone. Malaria (see page 26) may be suspected if the patient has resided in the tropics, has had the characteristic pyrexial attacks, has chlorotic anæmia, leucopenia rather than leucocytosis, and exhibits a relative increase in the large lymphocytes in the differential leucocyte count, as in the following example:—

					Normal.		Malaria.
Polymorphonuclear cells	65	...	58
Small lymphocytes	25	...	21
Large lymphocytes	8	...	16
Eosinophile cells	2	...	4
Basophile cells	—	...	0·5
Myelocytes	—	...	0·5

Additional evidence in favour of malaria is afforded by the occurrence of pigment granules of a golden brown or blackish colour both free in the blood stream and within some of the leucocytes. Similar pigment granules may also be found in the urine (page 111). The importance of these minor changes in the blood in malaria is greatest when the patient has had quinine before the blood had been examined, for the administration of quinine rapidly depletes the peripheral blood of the characteristic malaria parasites (page 26) even when the doses

given have been insufficient to check the pyrexia entirely. Under these circumstances the pathognomonic proof of the malarial nature of the disease, namely, the discovery of the hæmatozoa in blood films, may be impossible; and then considerable stress may be laid upon the existence of leucopenia, together with a relative increase in the large lymphocytes in confirming the diagnosis.

As regards cirrhosis of the liver, in which sometimes, owing to the great size of the spleen, leuchæmia may be simulated, the results of blood examination are almost entirely negative. The leucocytes may be moderately increased up to perhaps 20,000 per cubic millimeter, but there is nothing like the enormous increase that is characteristic of leuchæmia. The differential leucocyte count may show minor departures from the normal, especially in regard to a relative increase of the small lymphocytes and the occurrence of an occasional basophile corpuscle or myelocyte, but no definite conclusions can be based upon this small change. It is said that direct microscopical examination of freshly drawn blood smeared sufficiently thickly to make it dry slowly may assist in confirming a suspicion of cirrhosis of the liver, in that in this disease the red corpuscles tend to run together into rouleaux much faster than is the case in most other maladies, but it is seldom possible to convince oneself so emphatically of this departure from the normal as to be able to base the diagnosis on it.

Kala-Azar suggests itself as the diagnosis when the patient has been resident in parts, such as Assam, where this disease is prevalent. Malaria is apt to be the original diagnosis, but this is excluded by the persistent absence of the malaria parasites from the peripheral blood. The ultimate diagnosis depends upon discovering Leishman-Donovan bodies in films prepared from fluid obtained by puncturing the spleen (page 28).

Hodgkin's Disease cannot be diagnosed except in a negative way by an examination of the blood. The nature of the malady has generally been suggested by the occurrence of generalised enlargement of the lymphatic glands of the well-known clinical type, with or without simultaneous enlargement of the spleen. The request is sometimes sent to the laboratory to examine the blood, and to report as to whether it indicates Hodgkin's disease or not. This is more than a blood count can do. Nevertheless, blood examination is essential in order to exclude leuchæmia, either form of which may simulate Hodgkin's disease. When, however, there is no leucocytosis, or at most but a slight one, and when in a differential leucocyte count myelocytes are at most only a small percentage of the total and the lymphocytes are not increased to the very great extent that is the rule in

lymphatic leuchæmia, both forms of leuchæmia can be excluded, and then Hodgkin's disease remains as a possibility. It is by a process of exclusion that the blood count serves to help the diagnosis of Hodgkin's disease. In the early stages there need be no anæmia, but later there is progressive diminution both in the red corpuscles and in the hæmoglobin, until ultimately in a severe case all the changes in the red cells characteristic of a severe anæmia (page 10) may be found. In many cases nowadays the diagnosis of Hodgkin's disease is best arrived at by excising one of the affected glands under local anæsthesia and having it examined histologically. The question of relationship of Hodgkin's disease to lymphadenoma, and of either or both of these to lymphosarcoma, is one which is as yet far from settled. The remarks already made in regard to the value of the negative blood count in the diagnosis of Hodgkin's disease apply also in the diagnosis of lymphosarcoma and lymphadenoma. Some believe that these three are all different degrees of severity of the same malady, whilst others believe them to be different affections. In not a few instances it is difficult to distinguish between lymphadenoma, Hodgkin's disease, and tuberculous glands; ordinary blood examination alone will not serve to distinguish between them; if the clinical characters of the glands and their surroundings do not suffice, it is very often necessary to excise a gland and to have it examined microscopically to settle the diagnosis.

Chloroma is a malady that seems to be allied both to lymphatic leuchæmia and to Hodgkin's disease; the blood changes in it are of a negative character, as in Hodgkin's disease; the diagnosis is made upon the greenish tinge of the subcutaneous tumours when they are incised.

Infective endocarditis is a disease which presents so many different types that a large book could be written about this one disease alone. In the present connection, however, the two types that are of most importance from a point of view of an ordinary blood examination are, first, that in which there is considerable enlargement of the spleen, so that leuchæmia may be simulated unless a blood count is done to exclude this, and, secondly, that type of the disease in which there is progressive and profound anæmia which may simulate pernicious anæmia in many of its features unless a careful examination of the red corpuscles and of the hæmoglobin is made. Whereas in pernicious anæmia the colour index would be found to be high, at any rate at some examination or another (page 8), in infective endocarditis the anæmia is practically always of the chlorotic type, the hæmoglobin being much more diminished than are the red corpuscles. In either case the blood examination is made for the purpose of

the exclusion of some other disease, and the findings do not directly indicate that the patient has got infective endocarditis. In such an instance it would probably be wise to have a small quantity of blood withdrawn from a vein for culture purposes (page 46), the discovery of the infective organism in the blood stream serving not only to assist the diagnosis, but at the same time indicating the possible curative line of action, either by means of an immunising serum, or by means of vaccines prepared from the organism itself.

It might be thought that as infective endocarditis is a microbial disease there would be a leucocytosis similar to that found in connection with abscesses, empyemata, and so forth. In most instances, however, this is not so, perhaps because the purulent foci are not confined in enclosed spaces as is the case with abscesses in general. When pus is confined under pressure, leucocytosis is to be expected, but when once the tension of the pus is relieved, for example, by incising the abscess, the leucocytosis rapidly disappears. The products of the infecting micro-organisms in infective endocarditis are generally not confined under pressure, and this may explain why there is so seldom a leucocytosis in this malady. Sometimes, however, the leucocytes instead of numbering from 5,000 to 10,000 per cubic millimeter may rise to 15,000 or 20,000 per cubic millimeter, and at the same time the differential leucocyte count may exhibit that relative increase in the polymorphonuclear cells which generally accompanies pyogenic processes (page 23).

When this change is found it often serves to distinguish a case in which the cardiac symptoms are the result merely of mechanical failure without infection from an otherwise similar case in which, in addition to mechanical failure, there is an infective process taking place upon the valves. Both a total and differential leucocyte count, therefore, may often be of some value in detecting infective endocarditis, even though the leucocytosis is generally not pronounced. The most important proof of the nature of the disease, however, is obtained by blood culture; particularly when the result is positive, for a negative blood culture does not exclude infective endocarditis.

Splenic Anæmia is not diagnosable by blood count any more than Hodgkin's disease is. The blood examination is required to exclude splenomedullary leuchæmia, which may otherwise closely simulate splenic anæmia. There are no pathognomonic changes in the blood in the latter disease; there is a progressive anæmia of the chlorotic type with ultimately all the changes in the red corpuscles described on page 10 as occurring in any form of severe anæmia; there is generally leucopenia; and in the differential leucocyte count there is apt to be a more or

less pronounced relative increase in the small lymphocytes, together with the appearance of an occasional myelocyte or basophile corpuscle.

A number of other maladies besides infective endocarditis may, from the profoundness of the anæmia associated with them, simulate *pernicious anæmia*. Indeed, in all cases of severe anæmia it is necessary that a blood count should be made in order to exclude *pernicious anæmia*. When the latter has been excluded owing to there being a persistently low colour index instead of a high one (page 8), the diagnosis has generally to be made upon other grounds than those afforded by the blood count itself. *Chronic plumbism* with profound anæmia may be suggested if the red corpuscles exhibit a marked degree of punctate basophilia. This, however, will not be so marked, as a rule, as to clinch the diagnosis, unless it is possible to trace lead into the system from its external source, or unless by evaporating down a bulk of urine it is possible to demonstrate the presence in it of lead that is being excreted (page 112).

Chronic parasitic infection may simulate *pernicious anæmia* rather closely, especially infection by *ankylostomum duodenale*, or by one of the tape worms, notably *Bothriocephalus latus*. Such a parasitic infection might be further indicated by the occurrence of a pronounced eosinophilia, of which the following are instances:—

Differential Leucocyte Count showing Eosinophilia in Parasitic Infections.

		Normal blood.		In a case of Ankylostomiasis.		In a case of Bothriocephalus infection.
Polymorphonuclear cells	...	65	...	44	...	53
Small lymphocytes	...	25	...	31	...	29
Large lymphocytes	...	8	...	6	...	5
Coarsely granular eosinophile cells	...	2	...	19	...	13

The ultimate diagnosis, however, depends upon the discovery in the fæces of either the ova or of portions or the whole of the parasites themselves (pages 67 and 68).

Carcinoma of the stomach sometimes presents a very great similarity to *pernicious anæmia* as regards the colouration of the patient and the profoundness of his anæmia; the colour index in these cases, however, is persistently less than 1. The diagnosis may remain obscure until an abdominal lump becomes palpable, but if vomiting or other gastric symptoms have attracted attention, evidence of the nature of the case may be obtained by analysis of the gastric juice (page 76).

There remain a number of other cases in which the profound anæmia simulates pernicious anæmia, and yet the colour index is persistently less than 1, so that ordinary pernicious anæmia is put out of court. Some observers regard some of these cases as of the same nature as pernicious anæmia, but without any bone marrow reaction, and they have designated this type *aplastic anæmia*. As regards the blood count, it imitates pernicious anæmia except in the colour index, being persistently less than 1. The diagnosis is arrived at, however, by a process of exclusion, though it cannot be made with certainty until a series of blood counts has shown a persistently low colour index.

Chronic sepsis, especially that which results from persistent poisoning by the toxins absorbed from septic tooth sockets, may produce a similar cachexia. The blood changes are like those of aplastic anæmia, but when there is an obviously septic cause the disease is named from this cause, and is so differentiated, as a rule, from actual aplastic anæmia for which no known cause has been found.

Tertiary syphilis sometimes produces a cachectic state which may simulate pernicious anæmia until a persistently low colour index is found. Aplastic anæmia may then be diagnosed instead of tertiary syphilis, unless a Wassermann serum reaction (page 42) is tested.

Eosinophilia, that is to say, the relative increase in the coarsely granular eosinophile cells in the blood, the normal figures for which are about 2 per cent., though sometimes even in the absence of disease they have obtained so unusual a figure as 5 per cent., is prone to occur in certain parasitic affections, especially in patients infected by *Ankylostomum duodenale*, *Bothriocephalus latus*, *Tænia solium*, *Tænia mediocanellata*, *Trichina spiralis*, *Bilharzia hæmatobia*, *Filaria sanguinis hominis*, and hydatid disease, when the hydatid infection is active. It does not occur in all parasitic diseases, however, for it is generally absent in cases of infection by *Ascaris lumbricoides*, *Oxyuris vermicularis*, *Trichocephalus dispar*, *Pediculosis*, *Scabies*, *Ringworm*, and the vegetable parasitic affections of the skin. In those who have returned from the tropics it is of not infrequent occurrence without any direct evidence of parasitic infection being demonstrable. In certain cases, however, the occurrence of eosinophilia in the differential leucocyte count affords valuable clinical evidence of a parasitic infection which may then be verified by investigation of the fæces (pages 67, 68).

It is not, however, only in cases of parasitic infection that eosinophilia may be of diagnostic value. It may attain a pronounced degree during paroxysms of spasmodic or true asthma, and thus serve as a means of differentiating this disease from

those other varieties of spasmodic dyspnœa with or without cough which are so often spoken of as asthma, cardiac "asthma," uræmic "asthma," thymic "asthma," paroxysmal dyspnœa due to thoracic aneurysm or mediastinal new growth, recurrent bronchitis with or without emphysema. The well-known difficulty of deciding whether a given patient who is now obviously suffering from bronchitis and emphysema became bronchitic and emphysematous on account of suffering primarily from asthma, or became emphysematous and bronchitic without having suffered from true asthma at all, is of daily occurrence in practice; so much so that a certain type of bronchitis and emphysema is spoken of as "bronchitic asthma" as though it were in some way related to true or spasmodic asthma, which is probably in the great majority of cases not so. The test of eosinophilia in the blood is less often resorted to than it might be in differentiating true asthma—in which during the paroxysms there is nearly always eosinophilia—from chronic bronchitis and emphysema in which there is no obvious eosinophilia, as a rule. The following are some examples of differential counts; in the fourth the degree of eosinophilia is far more pronounced than the average, but it serves to show how very striking the phenomenon may sometimes be:—

**Differential Leucocyte Counts showing the value of
Eosinophilia in distinguishing true Asthma from
Bronchitis and Emphysema simulating Asthma.**

	Normal blood.	Chronic bron- chitis and emphysema.	True asthma with slight eosinophilia.	True asthma with extreme eosinophilia.
Polymorphonuclear cells...	65	67	61	44
Small lymphocytes ...	25	23	22	18
Large lymphocytes ...	8	7	9	3
Eosinophile cells ...	2	3	8	35

It is worthy of note that in true asthma similar coarsely granular eosinophile corpuscles may be found in abundance in the sputum (page 99) as well as in the blood stream, and this test may also be worth applying. The excess of coarsely granular eosinophile corpuscles disappears from both blood and sputum between the asthmatic paroxysms.

Another group of conditions in which eosinophilia may occur is in association with certain lesions of the skin. It is often stated that most skin diseases may lead to eosinophilia, the statement leaving upon one's mind the impression that one might quite expect to find well-marked eosinophilia, for instance, in eczema or psoriasis. This, however, is, upon the whole, not the case, and there is one type of skin affection which so far outstrips all the others put together in the degree and commonness

with which it produces eosinophilia that it would be almost true to say that it is of these skin lesions in particular that the eosinophilia is characteristic. These are the *bullous dermatoses*, different types of which have been labelled pemphigus, erythema bullosum, hydroa gestations, dermatitis herpetiformis, erythema iris. Sometimes there may be doubt as to whether the blisters which are found upon the skin in a given patient are natural or due to artificial means. The presence of eosinophilia would indicate that the disease was really pathological, and in this connection the differential leucocyte count may sometimes be of extreme importance. It is noteworthy that it is not only in the blood corpuscles, but also in the contents of the fluid of the natural bullæ themselves, that the eosinophile cells abound, whilst if an artificial blister is produced in a patient already suffering from pemphigus with eosinophilia, the contents of the artificial blister, as a rule, do not present eosinophilia.

Polycythæmia and enlargement of the spleen constitute the two main symptoms of a rather rare disease known as splenomegalic polycythæmia (page 12). There are certain other conditions in which polycythæmia may be a pronounced feature of the case, so that a blood count is likely to be of some clinical importance. The best known of these is probably congenital heart disease with cyanosis—so-called *morbis cæruleus*. The leucocytes may not be altered materially, but the red corpuscles may quite often number as many as 7,000,000 per cubic millimeter, whilst figures as high as 12,000,000 or even 14,000,000 per cubic millimeter have been recorded. It is not, of course, every case of congenital heart disease that has polycythæmia of this kind. With patent ductus arteriosus, for example, there is generally neither lividity nor polycythæmia, and if there is a patent septum interventriculorum without any other deformity, there need not be either cyanosis or polycythæmia. In most cases of pulmonary stenosis, however, and in many other cases in which the nature of the congenital heart lesion is difficult to determine during life, cyanosis and considerable polycythæmia may be prominent. Under such circumstances if the red corpuscles, when the patient's heart lesion is best compensated, number 12,000,000 per cubic millimeter, a drop to 10,000,000 per cubic millimeter would indicate a considerable relative anæmia, so that it is important to know not only what is the number of corpuscles which such a *morbis cæruleus* case shows at ordinary times, but also what changes from the normal they may present from time to time, the "normal" applying here not to that which is true of ordinary persons, but that which is the average in the patient himself. In a precisely similar way there is a tendency to considerable polycythæmia in many other

diseases associated with liability to cyanosis, especially, perhaps, in chronic mitral stenosis on the verge of failure of compensation; and also in people suffering from persistent bronchitis and emphysema and plethora. It seems not improbable that this increase in the number of red corpuscles per cubic millimeter of blood in these patients is an additional factor in maintaining compensation, the hæmoglobin being distributed over the larger surface area in a given volume, and therefore it may be of considerable importance to determine the degree of polycythæmia—which is often up to 6,000,000 or even 7,000,000 red corpuscles per cubic millimeter in mitral stenosis—and to adopt therapeutic measures which may assist in maintaining it. In patients suffering from chronic mitral stenosis it may very well be that they might be able to get along with their work if their red corpuscles numbered 7,500,000 per cubic millimeter, but that when their number falls to so low a figure for these patients as 6,000,000 per cubic millimeter there is a relative anæmia which throws additional strain upon the heart. The value of counting the red corpuscles periodically, therefore, in heart cases may be of considerable assistance in guiding therapeutics.

It should, perhaps, be mentioned that the altitude at which a person lives has a bearing upon the number of red corpuscles that he ought to have normally in his blood; roughly speaking, the higher the altitude the greater the number of red corpuscles per cubic millimeter.

Besides being of value in diagnosis, periodic blood counts may often be of considerable help in measuring the rate of recovery from curable anæmia. *Chlorosis* is a good example of this. In a very severe case of chlorosis the leucocytes may be normal both in total numbers per cubic millimeter and in the differential leucocyte count. The red corpuscles may be diminished from 4,500,000 per cubic millimeter down, perhaps, to 3,000,000, but the main reduction is in the hæmoglobin. This may be down, perhaps, to 40 per cent. of normal, so that if the red corpuscles at the same time are diminished to only 70 per cent. of normal, the colour index will be $40/70$, or 0.571. As the patient recovers, the red corpuscles generally mount up to the normal mark faster than the hæmoglobin does, so that if one relied upon the red cell count only one would get quite an erroneous idea as to the degree of the anæmia of the patient. The hæmoglobin should be estimated as well, and if it is a question of determining either rather than both, the percentage of hæmoglobin gives a better measure of the severity of the chlorosis than do the number of the red corpuscles per cubic millimeter of blood. When, under treatment, the hæmoglobin is found to be steadily rising week by week the patient is known to be doing well, but

if on successive counts the hæmoglobin remains stationary, it is probably necessary to alter something in the treatment which has been advised. Similar remarks hold good of the anæmia, sometimes severe, which may result from extreme loss of blood, as for instance, from gastric or duodenal ulcer, from hæmatemesis in association with cirrhosis of the liver, or the like, or from long-continued though less extreme blood loss such as one meets with in association with fibromyomata of the uterus or bleeding polypi of the colon or hæmorrhoids. Estimations of the hæmoglobin and of the red corpuscles in these cases afford the best measure of the effectiveness of the treatment adopted towards cure.

Leucopenia.—The various ways in which a total count of the number of leucocytes per cubic millimeter may be of clinical value include numerous others besides the great increase there is in leuchæmia (page 11); the relative absence of change there is in other conditions which may simulate leuchæmia (page 12), and the leucopenia, that is to say, the relative diminution in the total number per cubic millimeter that may be met with in pernicious anæmia (page 9), splenic anæmia (page 15), and malaria (page 12). *Typhoid fever* is another instance in which leucopenia or at any rate the absence of any marked increase in the total leucocytes per cubic millimeter may be of immediate diagnostic value, especially if the differential leucocyte count at the same time shows a relative diminution in the polymorphonuclear cells and a relative increase in the small lymphocytes. The Widal reaction (page 33) is seldom positive so early that from it alone a diagnosis can be made before the tenth day. It is often of great moment to arrive as nearly as possible at the diagnosis of typhoid fever long before this. The total leucocyte count and the differential leucocyte count together afford a means of such early diagnosis, for, granted that there is obscure fever obviously of considerable seriousness, there are hardly any other affections besides typhoid fever that will at the same time present no leucocytosis or an actual leucopenia together with a relative increase in the lymphocytes such as is exemplified by the following count:—

Total and Differential Leucocyte Counts in Typhoid Fever.

	Normal blood.		Typhoid fever.
Total leucocytes per cub. mm. of blood ...	6,500	...	4,500
Differential leucocyte count:—			
Polymorphonuclear cells ...	65	...	57
Small lymphocytes... ..	25	...	36
Large lymphocytes... ..	8	...	6
Eosinophile cells	2	...	1

These leucocyte changes may not be absolutely pathognomonic, but so constant are they that they have a very considerable diagnostic value applicable before the stage at which Widal's reaction can be applied to good advantage. The fever, leucopenia, and relative increase in the small lymphocytes have proved of great diagnostic value in distinguishing typhoid fever from the other fevers in India. Acute hepatitis or tropical abscess of the liver will produce considerable leucocytosis together with a considerable increase in the polymorphonuclear cells. Malaria, whilst producing leucopenia like typhoid fever, gives a relative increase of the large lymphocytes (page 12) and not of the small lymphocytes.

Leucocytosis.—The presence or absence of leucocytosis may sometimes be of very considerable value in detecting the existence of deep-seated pus in what may otherwise be an obscure case. Unfortunately, the absence of leucocytosis does not mean the same thing as the absence of a suppurative focus, neither can one say definitely that there is a suppurative lesion because there is a leucocytosis. Other conditions will increase the leucocytes, for instance, lobar pneumonia, though this cannot be called a suppurative lesion in the ordinary sense. The number of ways in which the leucocyte count may be of assistance in connection with suspected pus is greater than can be indicated in detail here, but one may, perhaps, give some examples. For instance, one may have a patient suffering from lobar pneumonia in whom during the attack the leucocytes may have risen to 25,000 per cubic millimeter, and in whom they fall after the crisis to 8,000 per cubic millimeter. The patient may seem to be doing fairly well, but pyrexia recurs and there is a doubt as to whether an empyema is developing or not. The leucocyte count is made at intervals of a day or two, and the leucocytes do not show any signs of increase; one begins to wonder what else may be the matter, seeing that the absence of leucocytosis would be against the accumulation of pus in the pleural cavity. Additional attention is given to other aspects of the case, the sputum, perhaps, may be analysed and tubercle bacilli found to account for the persistence of untoward symptoms after the lobar pneumonia had seemed to be successfully past. A precisely similar patient, having reached the crisis in the same way, may exhibit a progressive leucocytosis although the abnormal physical signs may not change very much. A rise in the leucocytes from 8,000 to 15,000 per cubic millimeter, 20,000 per cubic millimeter, 26,000 per cubic millimeter, 30,000 per cubic millimeter, on successive days suggests an empyema so strongly that the chest is needled, when if the physical signs alone had been relied upon, empyema might not have been suspected. Pus may

be found and the condition relieved sooner than might otherwise have been the case. Again, one knows of the difficulties of distinguishing such febrile conditions as typhoid fever from similar pyrexia due to deep-seated pus. Not infrequently the presence of a leucocytosis up to 20,000 or more per cubic millimeter of blood has led to the discovery of a pyosalpinx, or appendicular abscess or other deep-seated purulent focus in a patient who had hitherto been regarded as suffering from typhoid fever. The leucocytosis which may accompany empyema of the gall bladder, tropical abscess of the liver, an abscess in the brain, appendicitis, may in one patient or another prove of value clinically, though, especially in connection with a protean malady like appendicitis with its different degrees and forms, no hard and fast line or rule can be laid down as to what conclusions can be drawn from this or that particular alteration in the leucocytes at a single examination. Experience is essential in interpreting the results found in the laboratory, but this does not imply that the results are not of value. It should be added that it is not only the increase in the total leucocytes per cubic millimeter in the blood which affords evidence of the existence of pus, but that such a suspicion is strongly confirmed if at the same time there is a relative increase in the polymorphonuclear cells in the differential leucocyte count, as in the following examples:—

**Differential Leucocyte Count showing the Relative
Increase in the Polymorphonuclear Cells
in Abscess Cases.**

	Normal blood.		Abscess case (Empyema of gall bladder).
Total leucocytes per cub. mm. of blood ...	6,000	...	27,000
Differential leucocyte count:			
Polymorphonuclear cells...	65	...	79
Small lymphocytes ...	25	...	14
Large lymphocytes ...	8	...	5
Eosinophile cells...	2	...	

It would be necessary to write a very large book if one wished to include all the possible ways in which a blood count may be of clinical importance, but the above notes indicate some of the chief individual diseases in which blood examinations are almost essential. To recapitulate these briefly, they are as follows:—

Spleno-medullary leuchæmia, suggested by great enlargement of the spleen, confirmed by finding a very large number of white corpuscles per cubic millimeter, *e.g.*, 400,000, whilst in the differential leucocyte count there is a considerable percentage of myelocytes which are not present in normal blood.

Lymphatic leuchæmia, suggested by generalised enlargement of the lymphatic glands and possibly enlargement of the spleen; confirmed by the presence of a very large number of leucocytes per cubic millimeter, *e.g.*, 200,000, and a great increase in the relative numbers of lymphocytes in the differential leucocyte count.

Mixed leuchæmia, diagnosed by reason of the blood count exhibiting characters common to both the spleno-medullary and the lymphatic types.

Pernicious anæmia, suggested by progressive asthenia and the primrose-yellow colour of the skin. Confirmed by finding the red corpuscles very greatly diminished, the hæmoglobin greatly diminished, but less so than the red corpuscles, so that the colour index is greater than 1; blood films presenting all those changes characteristic of a severe anæmia (page 10), but especially a preponderance of megalocytes, whilst the leucocytes are generally not increased, and the differential leucocyte count, if it shows any changes at all, presents relative increase in the small lymphocytes.

Other conditions in which there is pronounced enlargement of the spleen are: cirrhosis of the liver, malaria, Kala-Azar, Hodgkin's disease, splenomegalic polycythæmia, infective endocarditis, or splenic anæmia; the blood count, especially the total leucocyte, is required in order to exclude spleno-medullary leuchæmia, but the blood picture does not itself give anything pathognomonic of the disease except in the case of the polycythæmia associated with splenomegaly.

In conditions in which there is widespread enlargement of the lymphatic glands other than lymphatic leuchæmia—Hodgkin's disease, lympho-sarcoma, lymphadenoma, chloroma, tuberculous glands, syphilitic glands—blood examination, especially the total leucocyte count, is necessary to exclude lymphatic leuchæmia, but does not by itself give figures that are pathognomonic of the disease.

In conditions in which there is pronounced anæmia simulating pernicious anæmia blood counts, particularly estimations of the red corpuscles and of the hæmoglobin, are necessary, probably on more than one or two occasions, to determine that the colour index is persistently not higher than 1 in order to exclude pernicious anæmia. Instances of such anæmias are infective endocarditis, some cases of malignant cachexia, especially carcinoma of the stomach, syphilitic cachexia, malarial cachexia, lardaceous disease, chronic parenchymatous nephritis, aplastic anæmia, chronic sepsis, and septic anæmias, notably those secondary to bad teeth.

In chlorosis the severity of the case and the progress towards recovery are best determined by the degree to which the hæmoglobin is diminished or increased.

In anæmia after severe or recurrent hæmorrhage, for instance, from gastric or duodenal ulcer, or hæmatemesis in association with cirrhosis of the liver, or the like, or from long-continued though less extreme blood loss such as one meets with in association with fibromyomata of the uterus or bleeding polypi of the colon, the hæmoglobin should be determined in order to measure the effects of treatment upon the rate of recovery.

Eosinophilia may suggest one or other of certain parasitic affections; may be a useful guide in distinguishing true asthma from other forms of spasmodic dyspnœa; or in distinguishing the true bullous dermatoses from other forms of blisters.

Leucopenia with relative lymphocytosis is confirmatory of typhoid fever.

Leucopenia with relative increase in the large hyaline lymphocytes occurs in malaria, a point of particular value in cases in which the parasites cannot be found in the blood owing to previous treatment by quinine.

Leucocytosis, especially if associated with relative increase in the polymorphonuclear cells often assists in detecting internal suppuration.

Polycythæmia is of value in the diagnosis of splenomegalic polycythæmia or in connection with the diagnosis of congenital heart disease; and it may mask a comparative degree of anæmia, in cases that should have more red cells per cubic millimeter of blood than the normal 5,000,000, for instance, in those who dwell in high altitudes or those who have a tendency to lividity from chronic heart disease or from chronic bronchitis and emphysema.

Punctate basophilia is often well marked in chronic plumbism, but is not confined to this malady.

It is also to be borne in mind that in many cases a particular disease presents itself to one's notice in so unwonted a guise that it may not even be suspected of being what it really is until blood counts give the clue. To mention only one group of such cases one would draw particular attention to acute and chronic purpura in this connection. This often turns out to be incipient leuchæmia, and sometimes the leuchæmia may be of the purpuric type without any great enlargement either of the spleen or of the lymphatic glands. The disease is apt to be missed if blood examination is not resorted to.

B. PARASITES IN THE BLOOD.

The Apparatus sent out.

The apparatus sent out for purposes of detecting parasites in the circulating blood consists simply of microscope slides specially cleaned to make them free from grease. Films of the patient's blood should be spread upon them in the way described upon page 7.

The films will be stained at the laboratory by any method that the sender may desire, but if no special instructions to the contrary are sent they are generally stained with a special modification of Leishman's method.

The Importance of the Examination.

1. *In malaria.*—In a case of suspected malaria the only conclusive proof of the nature of the disease is the discovery in the patient's blood of the causal organism which may be either of the tertian, the quatern, or the malignant crescentic variety. These are illustrated in most text-books. The best time for taking the blood for the purpose of detecting the parasites in it is either just before an expected malarial attack or as immediately as possible after the initial rigor.

It should be borne in mind that a patient may be suffering from malaria associated with pyrexia, and yet if quinine has been given, the parasites may not be detected in the blood films, so rapidly does the drug cause them to disappear from the peripheral circulation. It is important, therefore, that the films should be taken before quinine has been given whenever possible. If, however, the illness of the patient does not warrant waiting until films can be made, and if quinine has been given, it should be remembered that from the total leucocyte count per cubic millimeter of blood, and from the differential count of the leucocytes for the detection of a relative increase in the large lymphocytes (page 12), especially if at the same time there are extensive pigment granular deposits in the serum, within the leucocytes, and perhaps also in the urine, a suspicion of malaria may be converted into a tolerable certainty, even though no malaria parasites can be detected.

2. *Trypanosomiasis.*—Trypanosomes are to be found in the circulating blood in people who, having been resident in sleeping-sickness zones, have been infected with the parasite of sleeping-sickness; and they may sometimes be detected in the blood long before they find their way into the cerebro-spinal fluid (page 57) to produce active symptoms of sleeping-sickness itself. The absence of trypanosomes from a given blood film by no

means excludes the possibility of a trypanosome infection of the individual, but the finding of the trypanosomes as early as possible is very important if cure is to be effected so that in a suspicious case repeated examinations are generally made.

It is worthy of note that in addition to the trypanosomes of sleeping-sickness infecting man there are quite a number of trypanosomes that affect various animals and birds in the tropics, and the diagnosis of this depends upon laboratory examination of stained blood films. The list of conditions in which the circulating blood of animals may be found to contain the protozoa of disease is too long to give in detail here. As a rule, in persons infected by trypanosomiasis a differential count of the leucocytes in the blood will show a relative increase in the coarsely granular eosinophile cells.

3. *Filaria sanguinis hominis*.—Filariasis is suspected at once if a patient who has been resident in the tropics develops elephantiasis either of the legs or of the external genitals, and the diagnosis is confirmed by having blood films appropriately taken and stained for the filaria embryos derived from the mature worm, which itself occupies the pelvic lymphatic vessels. It is by no means only in those who have elephantiasis, however, that the filaria embryos are to be found in the blood stream. Individuals who are living, or who have lived in regions in which the parasite is indigenous, may need to have their blood examined for them, even though there is no elephantiasis. It is important to remember the peculiar diurnal distribution of the embryos, for in the majority of cases they are confined to the deep organs and blood vessels in the day time, migrating to the peripheral circulation only during the night. Films, therefore, need to be made at night and not during the day when the parasite has to be discovered in that which is the commonest type of the malady, namely, *filaria nocturna*. If the disease is suspected, and the parasites cannot be found in the night blood, it may then be necessary to examine the day blood as well, for there is a type of the disease the converse of the above, in which the parasites are only present in the peripheral blood during the day. This is spoken of as *filaria diurna*. Whether it is a distinct variety or only a different type of the same disease is not apparently of great moment. In a rarer form still, known as *filaria perstans*, the parasites are present in the peripheral blood both day and night. In association with filariasis there is generally eosinophilia, which may help to indicate the parasitic nature of any symptoms the patient may have (page 16).

4. *Leishman-Donovan bodies in Kala-Azar*.—Kala-Azar, a parasitic disease best known in Assam, and generally associated with pyrexia and considerable enlargement of the spleen, was

formerly included under the general heading of malaria, but it is a much more serious and fatal malady than ordinary malaria, and is now known to be quite different from the latter. Blood films are examined, in the first instance, under the impression that the patient is suffering from malaria, but when after repeated examinations no malaria parasites can be found, and Kala-Azar suggests itself as the diagnosis, the most certain way of confirming this suspicion is to puncture the spleen with a sharp hollow needle attached to a syringe, and to make a film of the fluid thus obtained by splenic puncture. These films are stained and examined microscopically in the laboratory, and in cases of Kala-Azar the typical Leishman-Donovan bodies are found upon the discovery of which the diagnosis depends.

C. PIGMENTS IN THE BLOOD.

It happens now and again that it is important to determine whether a patient's blood contains an abnormal pigment, the three commonest of these needing laboratory investigation being carboxyhæmoglobin, sulph-hæmoglobin, and met-hæmoglobin.

The Apparatus required.

The apparatus sent out for the collection of blood for investigation for any one of these abnormal pigments consists of a sterile glass bottle in a convenient tin; a small syringe of blood should be obtained from a vein by the method described on page 41; it should be transferred to the bottle, securely corked, and sent direct to the laboratory with as little delay as possible.

The Importance of the Examination.

The clinical significance of carboxyhæmoglobin in the circulating blood lies particularly in connection with death by coal-gas poisoning or by fumes produced by incomplete combustion, such as may be associated with fires in closed spaces, imperfectly ventilated stoves, brasiers, or in association with deaths produced by the fumes from lime kilns, fires in the holds of ships where there has been imperfect access of air, and so forth. Very often the investigation is of medico-legal importance, especially in regard to verifying the cause of death. In case the services of the medical man in the laboratory who carried out the investigations may be required in connection with giving evidence at the courts, attention is drawn to the note on page 87.

As a rule the occurrence of death from the effects of carbon monoxide is suggested by the bright cherry-red colour of the blood, which does not become reduced even in the veins. Indeed, the dead person may have so rosy a hue as to appear still alive. The examinations made in the laboratory are partly chemical and partly spectroscopical.

It is, of course, not only in the case of dead persons that carboxyhaemoglobinæmia may occur. A person may be suffering from the effects of sleeping in a room in which there is an escape of gas. There may be doubt as to the facts. If it can be shown in the laboratory that the patient's blood contains a perceptible quantity of carboxyhaemoglobin, the clinical significance of this may be considerable. Such proof might be very important, for instance, in connection with actions at law for compensation, and the like.

Sulph-haemoglobinæmia is a rare condition, but probably if it were sought for more often it would be found to be less uncommon, at any rate in minor degrees, than is at present thought to be the case. As a rule it is not sought for until the patient already presents symptoms, of which the chief is a peculiar colouration of the face suggesting lividity or cyanosis. The cyanosis strikes one as being different from that due to ordinary heart failure, but it may not be for some time that one thinks that it may be due to autogenous poisoning by sulphuretted hydrogen, resulting from intestinal toxæmia. Cases of the kind are recorded in the literature from time to time. Probably these are late and obvious stages of that which, if discovered earlier, might be found to be a relatively common cause of otherwise obscure symptoms. The diagnosis depends entirely upon the verification of the presence in the circulating blood of the abnormal pigment sulph-haemoglobin, for which special chemical and spectroscopical tests are required.

Met-haemoglobin may be present in the blood along with sulph-haemoglobin, or it may occur without the latter; it has many causes, all more or less rare. It may be due to certain drugs, such as potassium chlorate or to the effects of toxins, especially syphilis; and it occurs in association with paroxysmal hæmoglobinuria. The pigment is detected in the blood in the laboratory by means of chemical and spectroscopical tests. If the patient is thought to be suffering from paroxysmal hæmoglobinuria, it sometimes happens that an attack accompanied by changes in the urine does not occur whilst the patient is under direct observation, and yet met-haemoglobinæmia may be discovered in confirmation of the diagnosis. Another method of confirming the diagnosis under such circumstances is by means of the blood serum reaction devised by Eason, and known as Eason's reaction (page 44).

D. TOTAL VOLUME OF THE BLOOD IN THE LIVE BODY.

When one makes an ordinary blood count, one measures the number of corpuscles and the amount of hæmoglobin in each cubic millimeter of blood, but it does not follow that because the figures obtained happen to be normal the patient necessarily has the normal total quantity of blood in the body. Similarly it may be found that the hæmoglobin is only 50 per cent. of the normal in a given quantity of blood, so that the patient may appear to be absolutely anæmic, and yet if one has an opportunity of determining the total volume of blood in that patient's body, it may be shown that the blood is merely diluted and that the total quantity of hæmoglobin present is normal, though, owing to its dilution, its percentage in a given volume is reduced. In order to determine whether in a given case there is an absolute anæmia or not, and also the degree of that absolute anæmia, it is necessary to measure the total volume of blood present in the patient's body. This has not yet become an everyday clinical investigation, for the process is complicated and not absolutely devoid of risk. At present, therefore, it is mainly confined to the investigation of diseases in which research work is being carried out, especially in relationship to the severe anæmias which at present are still obscure as to their nature. It is probable that as time goes on it will be found that there are certain conditions in which it is material for the patient's good that over and above an ordinary examination of his blood a determination of the total blood in his body should be made. Meanwhile, although these investigations are being carried out for research purposes, they are not in widespread clinical use. The following is an outline of the technique: A patient is given a known mixture of carbon-monoxide and of oxygen to breathe. Curves have been worked out experimentally from which it is known what percentage of the total hæmoglobin present in a given volume of blood will be converted into carboxyhæmoglobin when exposed to various degrees of carbon-monoxide and of oxygen. When the patient has breathed abundantly of the mixture, a little of his blood is taken to the laboratory and analysed in order to determine the percentage of hæmoglobin that has been converted into carboxyhæmoglobin. From experimental data already known, one is then able to calculate what must have been the total amount of hæmoglobin that had been submitted to the mixture breathed in order to give this figure. Knowing the total amount of hæmoglobin present per

cubic millimeter of blood, one is then able by a simple calculation to work out what is the total amount of blood present in the patient's body.

E. SPECIFIC GRAVITY OF THE BLOOD.

The estimation of the specific gravity of the blood requires to be done with a fresh drop. Sometimes it is required to determine the specific gravity of the blood in the case of a patient who is not so ill but that he can be brought by his medical attendant to the laboratory. More often, however, it is in connection with acute or serious illness necessitating the patient being in bed that determinations are required, especially, for instance, in connection with severe diarrhoea or vomiting, cholera nostras, dysentery, and so forth, when the best means of determining the extent to which saline infusion is indicated, especially as to the need for its continuance or repetition, is the change produced by it in the specific gravity of the blood. This has been shown very clearly by Major Rogers, I.M.S., in connection with cholera, and he points out how it is a matter of equal importance in connection with the summer diarrhoea and vomiting of infants and other similar maladies. Instead of the saline infusion being carried out in any blind way, both the need for it and the need for its repetition may be gauged accurately by the determination of the specific gravity of the blood. All that is required from the patient is a single drop of blood such as may be obtained by pricking the lobule of the ear. This is received into a mixture of chloroform and benzene, and the specific gravity of the latter is varied at will by adding benzene to diminish it or chloroform to increase it until the blood drop neither sinks nor floats, at which point the specific gravity of the mixed chloroform and benzene may be taken with a suitable indicator such as a corrected urinometer, and this gives the specific gravity of the blood.

The Importance of the Examination.

Broadly speaking, in persons who are not suffering from acute illness such as cholera, the specific gravity of the blood varies with the percentage of hæmoglobin present. The greater the anæmia the lower the specific gravity. In ordinary health, when the hæmoglobin measures 100 per cent., the specific gravity of the blood is, approximately, 1.056.

Determination of the specific gravity of the blood may sometimes be of great importance in distinguishing between shock after injury and the occurrence of internal hæmorrhage; in cases of shock the specific gravity of the blood is not altered, but in association with hæmorrhage the specific gravity falls.

F. SERUM REACTIONS.

1. Agglutination Tests.

This is hardly the place to discuss the theory of the agglutination or clumping reactions that may be exhibited by blood serum in different maladies. The best known example of it is the Widal reaction in typhoid fever, but there are many other infections also in which the blood serum develops the power of clumping, or agglutinating, the causal organism.

The Apparatus required for collecting the Specimen.

The apparatus sent out by the laboratory consists of a small glass tube drawn out to capillary size at each end; each such tube is enclosed in a small metal case to ensure its passage through the post unbroken. It should be sent by letter post. The method of using it is very simple, and is as follows: The lobule of the patient's ear is cleansed with soap and water, or with a little ether, and it is often a good plan to give it a gentle but brisk rubbing in order to redden it by bringing blood to the part by vaso-dilatation. This makes the filling of the tube easier by reason of the increased rate at which the blood will flow when the lobule of the ear is pricked. The pricking is done in exactly the same way as is described on page 6 in connection with obtaining blood for blood counts. One capillary end of the Widal tube is held in the blood drop that exudes from the pricked spot, and it will be found that without any suction, but simply by capillary attraction and the force of gravity, the blood will run into the tube spontaneously. It is often a good plan to give the lobule of the ear a little squeeze to make a fair sized blood drop collect upon it at the pricked spot and then to insert the capillary end of the Widal tube into this drop until the whole of it has run into the tube, then to squeeze the lobule again, collecting a second drop in the tube in a similar way, and so on, until the Widal tube is about half full. Whilst it is always wise to be as cleanly as possible, there is no intrinsic need for extreme aseptic precautions to be taken; there is, for instance, no need to boil the capillary Widal tube before filling it. The next step is to seal off its two ends by inserting them into some such flame as that of a spirit lamp. The end which does not contain the blood should be sealed first; the heat of the flame speedily melts the glass, so that it closes together spontaneously. On allowing it to cool for a moment it will be found that the blood is now drawn further into the tube

from the end which was filled, leaving that capillary extremity clear, so that it in its turn can be sealed readily. If one tries to seal the blood-containing end first the presence of the moist blood within it generally makes the process difficult because of the tendency of the heated liquid to bubble out and of the glass to crack; by sealing the clear end first and then the blood-containing end, this condition is avoided. The sealed tube is put back into its metal case; this is put into an envelope and sent to the laboratory where the further investigations are carried out according to instructions.

This procedure is the same for all the different agglutinating tests, the chief of which are as follows:—

(a) The Widal Test for Typhoid Fever.

The Importance of the Examination.

The value of Widal's agglutinating serum reaction in the diagnosis of typhoid fever is very great; indeed, there is probably no other single test upon which greater reliance can be put in the diagnosis of enterica. There are, however, limitations to its value. In the first place, it takes time for the patient's serum to develop the reaction, and, broadly speaking, it may be said that the reaction is seldom, if ever, positive during the first week; that it is rarely positive before the tenth day, but that from the end of the second week onwards it is positive in the great majority of cases of typhoid fever. Indeed, the occurrence of a definitely positive Widal reaction in the serum of a patient who is suffering from a fever which, from the clinical symptoms, might be typhoid fever is practically proof that that fever is typhoid. The converse is not quite true, for sometimes patients suffering from typhoid fever do not develop the agglutinating reaction until much later, and some appear not to develop it at all. For instance, in one particular case the pyrexia lasted sixteen weeks instead of three in a boy aged 16. The diagnosis seemed at first to be that of typhoid fever, but as the pyrexia went on so long, and as the Widal reactions remained constantly negative, the original diagnosis was changed to infective endocarditis, whilst some who saw the boy thought he had general tuberculosis. In the fifteenth week, however, the Widal reaction which hitherto had been negative, became positive, and in the sixteenth week defervescence occurred, and the patient ultimately became perfectly well. Such cases of long delay in the defervescence of typhoid fever, and

of delay in the development of the Widal reaction, are, however, uncommon. A greater difficulty arises in connection with infections which, though they clinically resemble those of typhoid fever, are due not to the bacillus typhosus itself, but to one or other of the closely allied organisms, *Bacillus paratyphosus* A or the *Bacillus paratyphosus* B, for example. When, therefore, the Widal reaction is persistently negative to the *Bacillus typhosus* when it might be thought from the clinical character of the case that it should be positive, it is wise to have the serum tested, not only against cultures of the *Bacillus typhosus*, but also against cultures of the *Bacillus paratyphosus* A and the *Bacillus paratyphosus* B.

In this connection the occurrence early in typhoid fever of relative lymphocytosis with leucopenia, or at any rate without leucocytosis (page 21)), merits particular attention as an additional means of checking the diagnosis. It is also important to bear in mind that the typhoid bacilli are often recoverable from the blood (page 48) in the early stages of the disease; this means of diagnosis is less often resorted to than it might be.

As regards the meaning of a positive reaction, different observers have used different criteria in respect to the degree of the dilution of the serum at which the adjective "positive" is first applied. Normal serum will agglutinate bacilli in many cases if it is not diluted; in practically no other disease but typhoid fever, on the other hand, does the serum, when it is diluted 200 times, clump active cultures of typhoid bacilli twenty-four hours old within half an hour. There can be no doubt as to the positiveness of a Widal reaction, therefore, if the serum diluted 200 times clumps bacilli in half an hour, but in some instances this is too drastic a dilution, for the patient may have typhoid fever and yet the serum may not be so potent in its clumping powers as this. For practical purposes, therefore, a dilution of 1 in 50 is accepted, and if the bacilli are not clumped in this dilution the reaction is negative, whilst if they are clumped, the reaction is positive. If the patient's medical attendant desires the serum to be tested in greater dilutions, this will be done on request; in the ordinary routine application of the test dilutions up to 1 in 100 are employed.

One other source of fallacy merits some attention, and that is, the question of whether the serum reaction might not be due to a previous attack. It has been shown by French* that this is not likely to be the case. He has shown how remarkably soon after typhoid fever the Widal reaction ceases to be fully positive in the great majority of cases.

* The Guy's Hospital Reports, vol. lxi., p. 227.

(b) Serum Agglutinating Reaction for Paratyphoid Fever.

The Apparatus required.

The apparatus sent out is the same as that for Widal's reaction (page 32).

The Importance of the Examination.

The results of modern bacteriological investigations have shown that a fever which is clinically hardly distinguishable from ordinary typhoid fever may be produced by micro-organisms which, though probably related to the *Bacillus typhosus* closely, are, nevertheless, sufficiently different to make the disease bacteriologically distinct, and the name paratyphoid fever has been applied to this condition. It has been shown, moreover, that there are two types of organisms which may produce paratyphoid fever: one known as the *Bacillus paratyphosus* A, and the other as the *Bacillus paratyphosus* B. So specific is the clumping reaction of the blood serum that if a patient, apparently suffering from typhoid fever, is infected, not by the *Bacillus typhosus*, but by the *Bacillus paratyphosus* A, his serum will not give a Widal's clumping reaction with cultures of *Bacillus typhosus*, but will clump readily with cultures of *Bacillus paratyphosus* A. Similarly, if a person is suffering from paratyphoid fever due to the *Bacillus paratyphosus* B his serum will clump cultures of the *Bacillus paratyphosus* B, but will not clump cultures either of *Bacillus typhosus* itself or of *Bacillus paratyphosus* A. When, therefore, a patient is thought to be suffering from typhoid fever, and yet gives a distinctly negative Widal reaction with *Bacillus typhosus*, it is often wise to have the serum tested again against either the *Bacillus paratyphosus* A or the *Bacillus paratyphosus* B, or against both. The number of recorded epidemics of paratyphoid fever is increasing steadily now that this clinical method of distinguishing the malady is becoming better known.

(c) Serum Agglutinating Reaction for the *Bacillus* Enteritidis of Gaertner.

The Apparatus required.

The apparatus sent out is the same as that for Widal's reaction (page 32).

The Importance of the Examination.

The *Bacillus enteritidis* of Gaertner is closely related, on the one hand, to the *Bacillus coli communis*, and, on the other, to the paratyphoid bacilli and the *Bacillus typhosus*, but apparently it is different from all of these, or at any rate there is a micro-organism which can produce severe diarrhoea and vomiting, often in an epidemic form of so-called ptomaine poisoning, and it is sometimes of very considerable importance to know exactly what is the offending organism. In the summer diarrhoea and vomiting of infants several different micro-organisms probably take part, but amongst these, in addition to Morgan's bacillus, Gaertner's bacillus is prominent in some epidemics. It is extremely difficult to analyse the intestinal flora sufficiently quickly by cultural methods to be of clinical service, but if in a particular case it is found that the child's blood serum clumps cultures of Gaertner's bacillus in ordinary dilutions, there is strong evidence that the infection in the particular instance was due to this particular micro-organism. Some day it is to be hoped that the malady will be combated by specific antitoxic treatment, and to this end accuracy of bacteriological diagnosis is to be advocated strongly.

Another way in which the patient's serum reaction to Gaertner's bacillus may be of considerable value is in connection with so-called ptomaine poisoning. In many instances this is not in the strict sense of the term ptomaine poisoning at all, but is rather an infection by micro-organisms which were present in what seemed to be perfectly good sweet meat; a relatively common organism in this connection is Gaertner's bacillus. It may be that the infection incapacitates the individuals for a considerable time, and convalescence sometimes is very slow even when the acute stage of the malady does not prove fatal. Medico-legal questions of one kind or another involving the question of responsibility on the one side and of exact diagnosis upon the other, may take the case into the law courts, where the patient's medical man concerned in the case may be put to it to bring forward evidence either for or against the diagnosis of infection by food-poisoning micro-organisms of the so-called ptomaine type. It is very difficult indeed to prove that a given malady was due to the food that the patient took, but if it is remembered that the patient's serum, when taken at the time of the illness, may clump cultures of the offending micro-organism, and if this clumping test has been made through the bacteriological laboratory and the serum is found to give a positive reaction, the strength of the evidence will be very much greater

than it otherwise would be, and, therefore, it is nearly always wise in cases of supposed "ptomaine" poisoning that the patient's serum should be tested against Gaertner's bacillus, and, if the reaction with this is negative, against some of the micro-organisms allied to it. The serum reaction passes off when the patient recovers, so that the blood needs to be taken at the time of the illness. It is also clear that it would be most wise to have any of the food that may be suspected of having contained the micro-organisms in question investigated bacteriologically at the same time (page 73).

(d) Serum Agglutinating Reaction in Affections by the Meningococcus.

The Apparatus required.

The apparatus sent out is the same as that for Widal's reaction (page 32).

The Importance of the Examination.

The agglutinating reaction of the serum when tested against specific micro-organisms infecting the patient at the time is by no means confined to bacillary diseases, but is also found in connection with some non-motile micro-organisms amongst which may be mentioned the Meningococcus, formerly known as the *Diplococcus intracellularis meningitidis* of Weichselbaum which is the cause of posterior basal meningitis and possibly also of epidemic cerebro-spinal meningitis. In a suspected case of either of these maladies it is probable that the diagnosis will be tested by examining fluid obtained by lumbar puncture (page 55); but it is sometimes useful to know of an additional clinical method of verifying the diagnosis, and to this end a serum reaction similar to that of the Widal reaction in typhoid fever is useful. If the meningococcus is not clumped by the patient's serum suitably diluted, it does not, of course, follow that the symptoms are not due to the meningococcus, but if there is a definite clumping reaction with the meningococcus, it is an additional point in confirming the diagnosis which may possibly be in doubt from the results of the examination of the lumbar puncture fluid alone.

(e) Serum Agglutinating Reaction in Malta Fever.

The Apparatus required.

The apparatus sent out is the same as that for Widal's reaction (page 32).

The Importance of the Examination.

Malta fever is due to a distinct micro-organism known as the *Micrococcus melitensis*. The diagnosis may be already fairly clear from the nature of the symptoms and the place in which the patient is or has recently been resident—Malta itself, for instance, or somewhere in the Mediterranean, though the disease is now known to be much more wide-spread than this, occurring also in India and in South America, and probably elsewhere. As a means of clinching the diagnosis the finding of a positive serum reaction is often of great importance.

(f) Serum Agglutinating Reaction with the *Bacillus Coli Communis*.

The Apparatus required.

The apparatus sent out is the same as that for Widal's reaction (page 32).

The Importance of the Examination.

Although the *Bacillus coli communis* is a normal inhabitant of the healthy large intestine, it is now becoming recognised with increasing force that not a few acute as well as chronic infective maladies may be due to this bacterium escaping from the alimentary canal and infecting other parts. A very good example of this is afforded by coli bacilluria and by the further development of this into pyelonephritis due to the *bacillus coli*. This malady, very common in children and not uncommon in adults, and especially women, and that whether they are pregnant or not, escaped recognition until comparatively recently. The diagnosis may be entirely obscure unless, on examining the urine, a trace of albumen is found, and upon microscopical examination an excess of white corpuscles, perhaps barely amounting to actual pus; bacteriologically, a pure culture of *Bacillus coli communis* may be obtained from a catheter specimen obtained with all

aseptic precautions. Sometimes, however, it is very difficult to ascertain the site of the infection by the *Bacillus coli*. There may be no obvious coli bacilluria, and yet the *Bacillus coli* may be infecting the blood stream. When there is doubt it may sometimes be of considerable value to find out whether the patient's blood serum gives a clumping reaction with the *Bacillus coli*, similar to that which it does with the *Bacillus typhosus*. If there is no reaction it does not follow that the patient is not suffering from infection with the *Bacillus coli*, but if there is a positive reaction in moderate dilutions of the blood, not only does it indicate, as a rule, that the patient is suffering from a coli infection of some kind, but also, if there has been doubt hitherto, as there may be, as to whether the *Bacillus coli* found in the urine, for instance, is the result and cause of pathological changes, and not merely an accidental contamination, a positive serum reaction will be strong evidence in favour of it being the cause of the symptoms and not an accidental concomitant. There are probably cases of *Bacillus coli* septicæmia which are not even yet recognised for what they really are. In addition to testing the clumping reaction of the patient's serum it might be advisable to obtain blood cultures in cases of this kind.

(g) Serum Agglutinating Reaction in Cases of Dysentery.

The Apparatus required.

The apparatus sent out is the same as that for Widal's reaction (page 2).

The Importance of the Examination.

Dysentery is rather the name of a symptom or group of symptoms than of a particular disease, and it is becoming increasingly evident that the malady may be caused by a considerable number of different micro-organisms and parasites. Of these, the two best known are Shiga's bacillus, on the one hand, and the amoeba of dysentery, upon the other. A large amount of bacteriological work is still required in the investigation of other causal micro-organisms, not only in tropical dysentery, but also in allied affections at home, asylum dysentery, cholera nostras, ulcerative colitis, and so forth. To this end bacteriological examination of the faeces are required, and they should be carried out more consistently than is the case at present (page 71).

One great difficulty arises from the fact that the fæces ordinarily contain not only the bacillus coli communis in abundance, but also many other micro-organisms which may be found in cultures, but which may have no essential relationship to the disease. One method of testing whether a given micro-organism recovered from the fæces is an accidental concomitant or the intrinsic factor in the malady is to test the patient's blood serum as to its agglutinating power upon the suspected organism. If the serum gives a decided agglutinating reaction, this affords evidence in favour of the organism in question being an important factor in the cause of the disease. In the case of tropical dysentery it is important for many reasons to make sure whether it is due to Shiga's bacillus or to the amœba of dysentery the prognosis in the two cases is different, and the liability to sequelæ, such as hepatic abscess, is much greater in the case of the amœbic than in that of the bacillary type. It may be possible to determine that the dysentery in a given case is amœbic by discovering the characteristic amœbæ in the stools. There are, however, other varieties of amœbæ that inhabit the colon, and these may be mistaken for the amœbæ of dysentery when the latter is really bacterial. In such cases the agglutinating power of the serum should be tested against Shiga's bacillus, and if the reaction is definitely positive it points to the dysentery itself being bacterial and not amœbic. This is a subject upon which a considerable amount of work is still required, and for those who have the opportunity of working at it the research is likely to afford valuable results.

(h) Serum Agglutinating Reaction in Whooping Cough.

The Apparatus required.

The apparatus sent out is the same as that for Widal's reaction (page 32).

The Importance of the Examination.

It sometimes happens that there is doubt as to whether a child is suffering from whooping-cough or not, and it may be important to know for certain, both from the point of view of whether other children should be allowed to associate with the patient or not, and also subsequently from the point of view of whether the patient should be allowed to associate with others who have recently had whooping-cough, or whether from the

fact that the child has not had it, the risk of the infection should be continuously avoided. During an illness, of which cough was the prominent symptom, the nurse may have said that she heard one or two suspicious whoops, but the latter may not have been so definite as to convince the parents and the medical attendant that the condition was really whooping-cough. There is evidence to show that the doubt can sometimes be set at rest by testing the patient's serum as to its agglutinating power upon the Bordet-Gengou organism of pertussis. A positive agglutinating reaction would indicate that the child had whooping-cough. The subject is, however, one upon which comparatively little work has been done hitherto. It merits further investigation.

G. COMPLEMENT FIXATION TESTS.

(a) The Wassermann Reaction for Syphilis.

The Apparatus required.

When possible it is best to obtain a fair quantity of blood, for instance, 10 cc., from the patient's median cephalic or median basilic vein by means of an all-glass antitoxic syringe and hollow needle, the vein being first distended by the application round the upper arm of a bandage, tourniquet, or, best of all, the broad rubber bag of a sphygmomanometer into which the air can be pumped up to any desired pressure, the best being about 100 to 110 mm. Hg. The advantage of the all-glass antitoxic syringe over other kinds is that the piston moves practically without friction, so that the blood itself fills the syringe by its own pressure after the needle has been inserted into the vein. Both the syringe and the needle should be sterilised by boiling in plain water. The tourniquet, or sphygmomanometer band, having been applied as high up the arm as possible, the skin at the bend of the elbow is cleansed with ether and the needle attached to the all-glass antitoxic syringe is plunged through the skin directly into the most distended vein. Blood wells up into the syringe at once; when the desired amount has been obtained, the tourniquet is removed, the needle is withdrawn from the vein, and all bleeding stops at once if the patient's arm is now raised for a moment above the head. The blood so obtained should be ejected from the syringe into a suitable sterile glass bottle, the latter corked securely with a cork that has been sterilised by boiling, and the specimen sent to the laboratory. As alternative, one may use the following:—

Outfit for the Collection of Blood for Wassermann's Reaction.

Box contains.—1. One centrifuge tube with indiarubber stopper. 2. A tourniquet. 3. One needle.

Directions.—The hands of the patient should be washed with as warm water as possible before collecting the specimen. The dorsum of the finger (usually the forefinger of the left hand) is well rubbed with cotton wool and with absolute alcohol, and then with ether. The skin should be thoroughly dry before pricking, and no trace of ether must remain on the finger. The tourniquet is wound round the finger from the proximal almost to the distal extremity. The stopper is removed from the centrifuge tube, and the latter, by means of the cotton wool, is made to stand more or less upright inside the box. The skin is then sharply pricked with the needle in the middle line on the dorsum of the finger, about a quarter of an inch behind the nail. The blood is allowed to drop into the centrifuge tube until the flow appears to cease. Then the finger is mopped with dry cotton wool. The tourniquet is removed. The finger is again massaged with cotton wool. The tourniquet is re-applied, and it will not usually be found necessary to prick the finger again, but the blood will flow from the original puncture. If the blood does not flow another puncture should be made. This should be repeated until at least 20 minims are obtained. If there is any difficulty in obtaining blood in this way it should be collected by vene-puncture (page 41).

Note.—The whole contents of the box (including the label with particulars) should be returned.

The specimens, if they are not sent to the laboratory at once, may be kept in an ice chamber for several days without interfering with the subsequent reaction.

In the case of the medical attendant not wishing to take the specimen of blood himself, arrangements can be made for the patient to attend personally at the laboratory and have the blood taken from him there direct without extra charge. The Clinical Research Association insists, however, upon having explicit instructions from a qualified medical man in all such cases; it will not entertain examinations in connection with patients who may come to the laboratory on their own initiative or through unqualified persons.

The Importance of the Examination.

In the diagnosis of active syphilis, whether congenital or acquired, primary, secondary or tertiary, there is no test more valuable than the Wassermann reaction. There are a great

many methods of performing the test, but so far none of the simpler techniques have been found so reliable as the original one or a slight modification of it. There are a few conditions other than syphilis in which the reaction has occasionally been found positive, in certain cases of scarlet fever, for instance; but, broadly speaking, unless there is obvious clinical evidence to the contrary, the fact that a patient's serum gives a positive Wassermann reaction is practically proof of his infection by active syphilis. The number of ways in which this fact may be valuable is so great that it is impossible even to indicate them all here. One may mention as examples the difficulties that are sometimes met with in determining whether a vulval or penile sore or extra-genital chancre is really a chancre or not. Scrapings from such a sore may show the *Spirochæta pallida* (page 95), but further proof of the nature of the affection is afforded by the Wassermann test. Its value in the differential diagnosis of specific from other ulcers, or in connection with the diagnosis of aortic aneurysm or valvular heart disease possibly of syphilitic origin, or again in the diagnosis of various nerve diseases, including not only locomotor ataxy and general paralysis of the insane, but also pachymeningitis, severe headache, deafness, combined sclerosis of the cord, transverse myelitis, and other affections of a similar nature need only be mentioned to indicate the wide applicability of the test. Again, in the treatment of primary and secondary syphilis the Wassermann reaction should be tested at intervals in order to determine whether the infection is being eradicated from the system or whether it still persists. It is important to remember that during the administration of iodide of potassium and mercury, or immediately after the injection of salvarsan, the reaction may be negative though after an interval it may be found positive again, so that when the test is to be performed an interval of a week or two should be allowed to elapse after the last administration of anti-syphilitic remedies before the patient's blood is taken. There are already a very large number both of papers and of books upon the subject, and these should be consulted for further details in regard to the clinical value of the test.

When there is syphilitic disease of the central nervous system it is often wise to have the test carried out upon the cerebro-spinal fluid as well as upon the blood, for, broadly speaking, when there is syphilitic visceral disease, but no affection of the central nervous system, the blood will give a positive Wassermann reaction and the cerebro-spinal fluid a negative one, whereas when there is syphilitic disease of the central nervous system the reaction will be positive in both the cerebro-spinal fluid and in the blood (page 57).

(b) The Hydatid Serum Test.

The Apparatus required.

The apparatus required for the hydatid serum test is precisely similar to that needed for the Wassermann serum test (page 41).

The Importance of the Examination.

It is often exceedingly difficult to be sure whether a patient is suffering from hydatid disease or not unless by operative or other measures the actual hydatid cyst can be seen. The malady is not common in Great Britain, but in some countries it is far from uncommon. A negative reaction, so far as the blood serum is concerned, does not prove that the patient has not got hydatid disease, for it depends upon the activity of the living hydatids whether the patient's blood will contain the corresponding precipitins or not. When the cyst is quiescent, dead, or obsolete, there will be no positive serum reaction, just as there will be no eosinophilia (pages 16, 17). When the hydatid disease is active, however, there is generally eosinophilia, and the patient's serum, sent to the laboratory, will be found to give a positive hydatid test, which is sometimes of very considerable value in differential diagnosis.

(c) Eason's Reaction.

The Apparatus required.

The apparatus required and the method of collecting the specimen is the same as that described for Wassermann's test (page 41).

The Importance of the Examination.

Eason's reaction is given by the blood of patients suffering from paroxysmal hæmoglobinuria, and it is important clinically in that Eason's reaction may be positive at a time when there may happen to be no passage of hæmoglobin in the urine so that the diagnosis can be made from it even when an actual attack is past. Apparently the test also serves to distinguish essential or paroxysmal hæmoglobinuria from other varieties of hæmoglobinuria, for instance, those due to certain drugs. The disease

hæmoglobinuria is apt to be mistaken for hæmaturia unless special tests are carried out. Even when the blood pigment in the urine is mainly extra-corpuseular there are often a considerable number of red blood discs in the centrifugalised deposit from the urine, and the Eason's reaction is then of very considerable value in distinguishing a case of hæmaturia from one of paroxysmal hæmoglobinuria.

(d) The Complement Fixation Test in Glanders.

The Apparatus required.

The apparatus required for obtaining a specimen for the complement fixation test in glanders is similar to that already described for the Wassermann test (page 41).

The Importance of the Examination.

Glanders is very uncommon in man, but when it does occur the correct diagnosis is very apt to be missed, especially in the early and presumably curable stage of the disease. External lesions may be examined both directly and culturally for the *Bacillus mallei*, but every additional means of diagnosis is of value, and one such is the testing of the patient's serum by the complement fixation method against cultures of the glanders bacillus. The same test is also applicable to the diagnosis of glanders in the horse, and there is evidence to show that it will presently be relied upon even more than is the test by the injection of mallein.

(e) Other Complement Fixation Tests.

It is not at all improbable that, as time goes on, more and more maladies due to infecting micro-organisms will be found to be diagnosable by testing the serum of the patient by the complement fixation method. Investigations in this direction are still in their infancy. The Clinical Research Association is willing to carry out any such tests should it be called upon to do so, but it cannot yet be said that any absolute diagnosis can be built upon the results that we have knowledge of so far. Some observers consider that tuberculosis is diagnosable in this way, for instance, but it is as yet too early to say that this is definitely so. The subject is one that might well be utilised for thesis research work in general practice.

H. BLOOD CULTURES IN THE DIAGNOSIS OF SEPTICÆMIA, MALIGNANT ENDOCARDITIS PUERPERAL FEVER, TYPHOID FEVER, etc.

The Apparatus required.

In order to recover from the blood the living organisms which may be circulating in it, it is necessary to obtain a quantity from a patient's vein with aseptic, but not antiseptic, precautions, to inoculate this into a suitable culture medium immediately, if it can be so arranged, and to transmit the latter to the laboratory where it can be cultivated with as little delay as possible. The method of obtaining the blood by means of a tourniquet, a hollow needle, and an all-glass antitoxic syringe has been described on page 41 in connection with Wassermann test. It is most important that the antiseptic applied to the skin should afterwards be thoroughly removed, for, otherwise, enough of the germ-killing material may be carried in by the point of the needle when the vein is punctured to prevent the growth of the small number of organisms that are obtainable in a syringe-ful of blood. The Clinical Research Association sends out for the purpose two tubes of a gelatine culture medium ready for inoculation. If the procedure is carried out rapidly, there is no need to take steps to prevent coagulation of the blood in the syringe, but in order to make more certain of preventing this, a little solution of sodium citrate in distilled water may be drawn into it before the needle is plunged into the vein. This solution of sodium citrate should be of a strength of 1 per cent. It may be prepared by dissolving sodium citrate in water in the proportion of about 100 grains to the pint. The nutrient gelatine tubes when they are cold are solid; by standing them in a basin of hot water for about five minutes the medium liquifies; care should be taken not to raise its temperature over blood-heat, or otherwise the micro-organisms instead of growing in it will be killed. The tubes of liquid culture medium and the vessel containing the syringe and needle being conveniently at hand, the tourniquet, or better still the broad rubber bag of a sphygmomanometer, is applied round the upper arm, the obstructing pressure being such as will allow the pulse to be still felt. The median basilic and the median cephalic veins will stand out prominently and their prominence may be increased if the patient will open and close the fist several times to drive the blood from the deeper veins into

those that are more superficial. The skin in front of the elbow should be cleansed gently with soap and water, washing off the soap with plain boiled water, and drying with a sterile towel, after which a little ether should be poured over and allowed to evaporate. In puncturing the vein the needle should be struck boldly through the skin directly over the most prominent part of the vein and in the direction of the blood stream, the point being directed obliquely upwards, nearly, but not quite parallel to the skin, so that the point passes into the lumen of the vein and not straight through the vessel and out through its deeper wall. The blood now flows into the syringe by its own pressure, and only the gentlest traction on the piston should be employed. When a quantity of 10 cc. or more blood has collected in the syringe, the bandage, tourniquet, or sphygmomanometer valve should be at once relaxed to stop the flow of blood and the needle withdrawn from the vein. A culture tube is unplugged in the ordinary way, and part or all of the blood in the syringe ejected vertically downwards into the liquid culture medium. If desired, half the blood may thus be inoculated into one tube and half into the other. The mixture should then be gently shaken, care being taken not to allow it to run up on to the plug. The gelatine medium is then re-solidified by placing the tube in a glass of cold water for some minutes, and when it has set again it is ready to be packed and forwarded to the laboratories. The puncture in the vein does not require any dressing as a rule. If there is any tendency to oozing of a few drops of blood through it, this will cease if the arm is held above the patient's head for a minute. A little boric acid powder may be applied over the site of the puncture with advantage.

The Importance of the Examination.

There are a very large number of different microbial infections in which blood cultural investigations are of great importance in diagnosis, and in some of these it is only by blood culture that the actual organisms producing the symptoms can be determined. It is not only in diagnosis, however, that blood cultures are important; if treatment, either by vaccine or by serum is to be successful, it is essential to know exactly what micro-organism is responsible for the malady in order that the vaccine or the serum may be specific. One of the best examples of the value of blood culture is in connection with the diagnosis of infective endocarditis, in which malady it is often only by discovering that there are micro-organisms in the circulating blood that the case can be distinguished from non-infective endocarditis with me-

chanical heart failure. The number of the different organisms which have been found to produce infective endocarditis is considerable, and includes streptococci, staphylococci, pneumococci, typhoid bacilli, diphtheria bacilli, and others whose exact identity has as yet been less well established.

The simplicity of this method of investigating cases is very much greater than might appear from the description of the technique required in obtaining a blood specimen. The latter is obtainable with hardly any disturbance to the patient, and it takes but a few minutes to collect it. In the future, diagnosis by blood culture is certain to take a very prominent place amongst clinical methods.

Not only in fungating endocarditis, but in many other infective maladies, blood culture is of diagnostic value. One need but mention, for instance, cases of obscure pyrexia after childbirth, long continued fevers without very definite objective causation, and chronic septicæmias of all kinds. It is not sufficient to diagnose septicæmia; it is necessary to determine the nature of the infecting organism whenever possible.

Further than this, it is being shown by modern researches that many diseases that were at one time thought to be purely local are really, at one stage or another, associated with the presence of the causal organism in the blood stream. An instance of this is typhoid fever, in which the *Bacillus typhosus* can be recovered from the circulating blood by cultural methods especially during the first ten days, so that the diagnosis can sometimes be made by this test just at the time when the Widal reaction has not yet become positive.

Blood cultures afford the most certain way of arriving at the diagnosis of pneumococcal septicæmia in many cases. It is not possible in a short article to indicate all the possible ways in which blood cultures are likely to be of importance to the patient, but the above may serve to indicate some of them. Moreover, the value of obtaining organisms from the patient's own blood is very considerable when it is desired to use autogenous vaccines prepared from the patient's own organisms.

I. CHEMICAL ANALYSES OF THE BLOOD.

The Apparatus required.

For chemical analysis of blood it is necessary to obtain at least 10 to 20 cc. as a rule. This is best done by means of an all-glass syringe and hollow needle as described on page 41.

It may be required to estimate the percentage of various different ingredients under various circumstances. One may mention in particular:—

- i. Urea.
- ii. Uric acid.
- iii. Fat.
- iv. Dextrose.
- v. Calcium.

The Importance of the Examination.

i. *Urea* in the blood normally amounts to between 10 and 25 parts per 100,000; there are many conditions of albuminuria, pyuria, hæmaturia, and the like in which this figure is not exceeded, and it affords a measure of the degree to which the kidneys are getting rid of excretory products in spite of lesions in them. When, however, there is a renal lesion which is interfering with excretion, estimation of the urea in the blood may show that this has risen from 10 to 25 parts per 100,000 to 70, 150, or even 300 parts per 100,000, the higher the figure the worse being the prognosis. The urea in the blood does not seem to be increased in puerperal eclampsia, but it is in other forms of uræmia. The value of this method of investigation in connection with Bright's disease, for instance, or renal tuberculosis is considerable, and in particular is it of value in association with enlargement of the prostate with obstruction to the urine outflow of some duration. It is so difficult in cases of this kind to determine by most clinical methods the extent to which the kidneys are damaged. In some patients there may be great frequency of micturition with pyuria and ammoniacal decomposition of the urine, and yet the kidneys may be relatively sound, so that prostatectomy is likely to lead to complete cure, whilst in other cases there may be a much less abnormal urine, and yet the kidneys may have suffered so much from obstruction to the urine outflow that notwithstanding prostatectomy, the patients may still remain unwell. Ryffel and French have carried out extensive researches upon this point and have come to the above conclusions.

ii. *Uric acid*.—The question of the relationship between uric acid and gout is a very controversial subject, but there are many who believe that gout and allied states are due to the accumulation of uric acid in the system. The degree to which uric acid has accumulated can be determined, to some extent at any rate, by estimation of the uric acid in the blood, and the figures so

obtained are thought by many observers to afford valuable clinical information, both from a diagnostic point of view and from that of the treatment indicated.

iii. *Fat* is always present in the blood to some extent, but its amount is materially increased under certain conditions which, when they reach an exaggerated degree, are termed lipæmia. The two best known causes of lipæmia are diabetes mellitus and fractures of long bones. It is sometimes possible to detect lipæmia in diabetes by examination of the retinæ with the ophthalmoscope long before the acute danger the patient is in is recognised from the clinical symptoms, but the best proof of the existence of lipæmia is analysis of the blood for fat.

iv. The amount of *dextrose in the blood* merits determination in glycosuric cases far more frequently than is generally the rule at present; for whereas many varieties of glycosuria hardly matter at all, there is always the fear that when there is glycosuria the patient has real diabetes mellitus. By determining the amount of sugar in the blood one can better decide whether the patient is suffering from real diabetes with hyperglycæmia, or merely from kidneys that are relatively too pervious to sugar without there necessarily being any diabetic error of metabolism in the body generally. It is in the distinction between the hyperglycæmic and the non-hyperglycæmic cases of glycosuria that estimations of the sugar in the blood are so important.

v. Errors in the amount of *calcium in the blood* are thought by some authorities to play a very important rôle in various maladies. The calcium in the blood may be in excess with consequent tendency to arterio-sclerosis and other degenerative lesions that are apt to be associated with the deposition of calcium salts locally in the tissues, in which cases decalcifying treatment would seem to be indicated; or there may be too little calcium in the blood, this being, according to Blair Bell and others, a very important factor in some cases of dysmenorrhœa, menorrhagia, and other affections of the female genital system. It is true that some investigators hold that it is hardly possible to vary the percentage of calcium in the blood by any therapeutic measures at present known, but this is a matter of opinion, for others believe that much good is done in different cases by adopting steps calculated either to increase or diminish the calcium salts in the blood according to which process may be indicated by the results of chemical estimations of the calcium present in the blood.

J. OPSONIC INDEX DETERMINATIONS.

The Apparatus required.

The apparatus required for obtaining the necessary specimen for an opsonic index determination consists of a glass pipette and a needle in a small metal case, which can be sent back to the laboratory by letter post in an ordinary envelope. The lobule of the patient's ear or his finger near the root of the nail having been cleansed with soap and water or with ether, but without the use of any other antiseptic, a blood drop is obtained by pricking in the way described on page 6. The first drop of blood should be wiped away; into the succeeding drops one end of the pipette is dipped, the other end being allowed to slope slightly downwards. Sufficient blood should be obtained to half fill the small tube. To seal the latter, the empty end is held in a spirit flame until the glass melts and the sides fall together. When this end cools, the blood is drawn in from the other end so that the latter can then be sealed in a similar way without heating the blood within the tube. An alternative plan is to close the blood-containing end with sealing wax.

The Importance of the Examination.

The clinical value of the opsonic index determinations is probably less than was at one time believed, but, as is so frequently the case, there has been a swing of the pendulum almost from the one extreme to the other, so that whereas opsonic index determinations were but recently all the vogue, they have now gone too much out of fashion. Although they may not be necessary in controlling the administration of tuberculin and other vaccines, they have an undoubted value as a measure of the prognosis of cases suffering from chronic infections, and they also have a diagnostic value in many cases when there is doubt as to whether a given organism recovered from some part or other is the cause of the patient's symptoms, or only an accidental associate. In such cases, if the opsonic index of the patient's blood to the organism in question is normal, doubt will remain, but if the opsonic index, on the contrary, is either materially above or materially below the normal, then this fact affords confirmatory evidence as to the micro-organism in question being the causal factor in the patient's illness. It seems probable that in this direction opsonic index determinations will be resorted to again more generally than they are at present.

K. ARNETH'S TEST IN PHTHISIS.

The Apparatus required.

All that is needed is a blood film prepared in the way described on page 7.

The Importance of the Examination.

Arneth's test consists essentially in determining in a successive series of polymorphonuclear cells in a stained blood film what is the average number of lobes of the nucleus. In healthy persons the polymorphonuclear cells, arranged according as they contain one, two, three, four, or five lobes to the nucleus, give some such differential figures as the following:—

With 1 lobe	With 2 lobes	With 3 lobes	With 4 lobes	With 5 lobes
2 per cent.	19 per cent.	56 per cent.	18 per cent.	5 per cent.

In cases of active pulmonary tuberculosis, on the other hand, there is a marked tendency for the polymorphonuclear cells to contain fewer lobes, a characteristic count showing, for instance, figures such as the following:—

With 1 lobe	With 2 lobes	With 3 lobes	With 4 lobes	With 5 lobes
35 per cent.	58 per cent.	7 per cent.	0 per cent.	0 per cent.

The idea underlying this method of investigation is that it is the older and most mature cells which are likely to contain the multi-lobed nuclei, the less mature cells being fewer lobed as to their nuclei, so that when there is an infective process going on, presumably with destruction of the leucocytes and active proliferation of younger members, then there is likely to be a relative increase in the numbers that have fewer lobes. Even if this does not afford any diagnostic evidence, from the point of view of prognosis it is held that it is invaluable. If routine examinations of blood films are carried out in phthisical cases and differential counts made of the number of lobes in the nuclei of the polymorphonuclear cells, then it appears that so long as this differential count shows a relatively large proportion of few-lobed nuclei the prognosis is relatively bad, whilst when the count changes to one in which there is a relative preponderance of nuclei containing relatively large numbers of lobes, then the patient is improving and the prognosis is correspondingly better.

It is by no means easy to gauge the degree of real improvement in cases known to be actually phthisical. The physical signs, the general feeling of the patient, and his weight may

all give a sense of false security, and therefore any additional method of measuring the degree of actual improvement that is taking place is to be welcomed.

It would seem well worth while, therefore, to have Arneth's method of blood counting carried out as a routine measure at intervals of a few weeks in all cases of established pulmonary tuberculosis, and it is to be hoped that the observations already published by others upon the subject will be confirmed.

SECTION II.

Calculi and Concretions.

Transmission to the Laboratory.

Calculi are best sent to the laboratory suitably wrapped up and enclosed in a small but strong box or tin which will not become crushed in the post.

The Importance of the Examination.

The importance of knowing which urinary ingredient constitutes the main percentage of a calculus from the bladder or kidney is obvious, for to prevent the recurrence of a stone, medicinal and dietetic treatment may be required, and this will vary according as the calculus consists mainly of uric acid, of urates, of phosphates, or of rarer substances such as cystine or xanthin bases.

Another way in which an analysis of any kind of concretion is sometimes of great importance is when a patient fancies that the substance submitted for examination was passed from one of the natural apertures of the body when really it may be an adventitious stone derived from a gravel pathway or some other similar source. The possibility of malingering in this connection is obvious; chemical analysis of the stone will at once distinguish the true from the false.

The importance of distinguishing true intestinal sand from the gritty particles that may be passed with the motions after eating various fruits, especially pears, affords another example of the way in which chemical analysis may be of value.

Occasionally sputum contains a calcareous particle which affords strong proof of the breaking down of previously healed tuberculous mischief, but without chemical analysis such a particle may be mistaken for a fragment of mutton bone, or something of that sort.

The following are the chief varieties of concretion that may be met with in practice and may require chemical analysis:—

Gall stones are either obtained direct from the ducts by operation or recovered from the fæces, or found in an abscess in some way related to the liver. The most characteristic ingredient of a gall stone is cholesterin, which is recognisable both by chemical tests and by the characteristic crystals. When the stone is very small the chief ingredient may be a calcium salt of one of the bile pigments, generally either calcium bilirubinate or calcium biliverdinate. The stone generally results from a former bacterial infection of the gall bladder, for instance, by the bacillus coli, and not infrequently the bacillus typhosus has been recovered in cultures prepared from the nucleus of a fresh gall stone. It is, therefore, advisable in many cases to have bacteriological as well as chemical analyses of gall stones carried out.

Urinary stones.—These may be either very small and passed in the urine in the form of gravel or they may be larger and obtained from the penis, the bladder, a ureter, or a kidney. The chief ingredient varies in different cases, sometimes being uric acid, sometimes urate of ammonium, sometimes calcium oxalate, sometimes phosphate of lime; whilst rarer ingredients are cystine and xanthine bases. Almost any of these are characteristic, the least so being calcium phosphate which may be an ingredient of calculi other than urinary ones. It is very unlikely that an adventitious stone would be introduced into the urinary passages in the male, but in the female with the wider, shorter urethra this form of neurosis or of malingering is by no means unknown.

Phleboliths.—These consist mainly of calcium phosphate and calcium carbonate with some organic matrix. They may be found in the course of a peripheral vein which has been previously thrombosed, for instance, in connection with varicose veins; or more frequently in the abdomen, whether in the form of hard masses in the mesentery or omentum, possibly simulating cretaceous tuberculous glands, but generally distinguished from these by their less regular shape.

Enteroliths.—In addition to intestinal sand mentioned above, which generally occurs in association with, or as an alternative to, muco-membranous colitis in unmarried women of nervous temperament, one occasionally meets with larger concretions in the bowel. These are termed enteroliths. They may consist of

masses of undigested food stuffs matted together with mucus (some cases of the kind have been recorded, for instance, after the chewing and swallowing of large quantities of cinnamon), or they may be composed of precipitated salts that have been administered by the mouth, bismuth stones, for instance, or magnesium stones; or they may be undigested pieces of bone passed per rectum; or they may consist of true concretions of calcium phosphate, calcium carbonate, and calcium soaps, resulting from erroneous secretions from the intestinal wall. A special variety of such enterolith is the well-known appendicular concretion that results from inflammation of the vermiform appendix. In the latter case the calculus generally contains salts of various soaps, particularly calcium stearate.

Salivary calculi.—These are not common. They have to be distinguished from a bony sequestrum, but the diagnosis is generally easy owing to the situation of the mass in one of the salivary ducts. They consist, as a rule, of lime salts, especially phosphates and carbonates. *Pancreatic concretions* have a similar composition. Loose sequestra from necrosing bone may require decalcification and examination microscopically before their exact nature can be determined.

Gouty concretions are seldom rigid and hard, being more often of a pultaceous consistence; they consist of inspissated urate of sodium.

Other concretions that may come before one's notice occasionally are calcified particles from hydatid cysts, calcified particles from dermoid cysts, otoliths, or concretions from the ear, rhinoliths or concretions from the nose; these have no characteristic composition, but consist of varying mixtures of calcium phosphate and calcium carbonate.

SECTION III.

Cerebro-spinal Fluid.

The Apparatus required.

Cerebro-spinal fluid may be obtained either from within the cranium, for instance, in infants suffering from meningitis, or from older people who have cerebro-spinal fluid escaping from the ear or nose after a fracture of the skull, or, as is much more

frequently the case, from the spinal canal by lumbar puncture. It is best sent to the laboratory in a small glass bottle suitably corked and enclosed in a packing or tin that will ensure its safe transit through the post. When bacteriological investigations are required the bottle can be sterilised previously by boiling in water.

The Importance of the Examination.

Large books have been written upon the various ways in which investigations carried out upon the cerebro-spinal fluid may assist the physician in connection with diagnosis, prognosis, and treatment. It is not possible to specify all the investigations that may be helpful here, but a few of the commoner may be referred to briefly as follows:—

After a head injury in which it is not certain whether the skull has been broken or not, the identification of clear fluid that may be dripping from nose or ear as cerebro-spinal fluid may be the crucial point in deciding that there is a serious fracture at the base of the skull. The normal characters of this fluid are briefly as follows: It should be quite clear and free from colour so that in a test-tube it may be difficult to distinguish it from water; its specific gravity is low, lying generally between 1004 and 1007; in reaction it is always alkaline whether in health or in disease; its normal freezing point is $0.55^{\circ}\text{C}.$; its reducing power with Fehling's solution is equivalent to about 1.5 parts of sugar per 1,000, and it also contains about 1.5 parts of urea per 1,000; practically no coagulable proteid should be present, and if it is obtained without contamination from blood vessels or lymphatics it contains practically no cell elements.

Almost any departure from the above characters in the fluid obtained by lumbar puncture points to some disease process affecting the brain, the spinal cord, or their meninges. When there is acute meningitis the fluid is generally no longer quite transparent, but exhibits either some degree of opalescence or it may become quite opaque and purulent; at the same time it is apt to develop a yellowish tinge, and, microscopically, numbers of leucocytes are found. In tuberculous meningitis the cells in the cerebro-spinal fluid are mainly lymphocytes, whilst in other forms of acute meningitis the polymorphonuclear cells predominate. It would be unwise, however, to rely solely upon the character of the leucocytes present in deciding between tuberculous and other varieties of meningitis; it is wise to have films from the centrifuged deposit stained direct for possible organisms and also to adopt cultural methods of identifying these.

The chief importance of arriving as soon as possible at a correct bacteriological diagnosis of the nature of any particular

variety of acute meningitis is in order to pick out the meningococcal cases of the disease from the rest. It is probable that almost all cases of tuberculous, streptococcal, staphylococcal, or pneumococcal meningitis prove fatal, but a considerable percentage of the meningococcal cases recover, and this percentage of recoveries is increased by the adoption of serum treatment. This cannot be carried out whole-heartedly unless one is sure of the exact nature of the case. Meningitis due to other micro-organisms, especially typhoid bacilli, influenza bacilli, and the bacillus coli communis, is not necessarily fatal, but no specific treatment is known for it although vaccines might prove useful. Cases due to these micro-organisms give no distinctive clinical signs, and they have only been discovered lately as the result of routine examination of the cerebro-spinal fluid. Much yet remains to be found out in this connection, and research work in this direction might well be undertaken for thesis purposes.

The diagnosis of sleeping sickness in its early stages depends largely upon the discovery of trypanosomes in the cerebro-spinal fluid. They are generally examined for in stained films prepared from the centrifugalised deposit of fluid obtained by lumbar puncture.

Amongst the various subacute and chronic lesions of the central nervous system in which analysis of the cerebro-spinal fluid may be valuable, the following may be mentioned in particular:—

Syphilis and parasymphilitic diseases, including tabes dorsalis and general paralysis of the insane.—The best test to apply to the cerebro-spinal fluid in these cases is Wassermann's. There is much evidence to show that a person who is suffering from systemic syphilis which does not affect the central nervous system will give a positive Wassermann reaction as regards his blood serum, but a negative one as regards his cerebro-spinal fluid. If, on the other hand, a patient has syphilis affecting his nervous system as well as the viscera, then the cerebro-spinal fluid will give a positive Wassermann reaction as well as the blood. It is important in such cases to have the test applied, therefore, both to the blood and to the cerebro-spinal fluid. When general paralysis of the insane is suspected, or tabes dorsalis, the occurrence of decided lymphocytosis in the cerebro-spinal fluid is confirmatory of the diagnosis. It is important to bear in mind, however, that it is only when upon other grounds the diagnosis has been narrowed down to be something of the nature of general paralysis of the insane that the test is a weighty one, for there are many other maladies in which a similar lymphocytosis has also been observed, notably in sleeping sickness, in herpes zoster, in acute anterior poliomyelitis, in

some cases of cerebral tumour, in lymphatic leuchæmia, in chloroma, and in some cases of mumps. None of the latter, however, are at all likely to be confused with general paralysis of the insane upon clinical grounds, so that the lymphocytosis in the cerebro-spinal fluid in the latter disease is a point of real diagnostic value.

Occasionally in addition to, or instead of, leucocytes, large abnormal cells derived from a neoplasm have been found, and upon the discovery of these the diagnosis of malignant metastases affecting the spinal meninges has been made at a time much earlier than would have been possible upon any other grounds.

When there is compression of the cord by a tumour of the vertebræ or meninges it sometimes happens that the cerebro-spinal fluid contains an abundance of both albumen, globulin, and fibrinogen, so that it clots spontaneously and gives an abundant precipitate upon boiling, and yet upon microscopical examination of the centrifugalised deposit hardly any abnormal cells are to be found. The presence of many polymorphonuclear leucocytes in a highly albuminous cerebro-spinal fluid points to an inflammatory affection of the meninges, whilst when there is an abundance of coagulable proteid with hardly any corpuscles the symptoms in the case are more likely to be due to compression of the cord. In some cases of this kind the cerebro-spinal fluid has a remarkable yellow ochre colour, and this has been recorded particularly by French observers in connection with tumours of the spinal meninges. There is evidence, however, to show that this xantho-proteic reaction, that is to say, the occurrence of bright yellow cerebro-spinal fluid with abundant albumen in it, but few cell elements, is not pathognomonic of a neoplasm affecting the cord and its meninges, but that it may be due to other causes of compression from outside the cord, for instance, by spinal caries, or an abscess. The xantho-proteic reaction, however, is very characteristic and always pathological.

Several observers have drawn attention to the possible value of the estimation of the urea in the cerebro-spinal fluid in a case of coma, for it is sometimes very difficult to determine whether the coma is due to uræmia or to some other entirely different cause, such as general paralysis of the insane, narcotic poisoning, or a gross lesion of the central nervous system, such as a tumour or meningitis. If the urea is present to the extent of more than 1.5 parts per 1,000 this fact would point to uræmia.

Choline is examined for in the cerebro-spinal fluid by a rather complex test which involves the preparation of crystals

that are recognised microscopically and by determination of the melting point. It was at one time thought that the existence of more than a mere trace of choline in the cerebro-spinal fluid would afford a definite point of distinction between organic and functional nerve disorders. It is open to some doubt, however, as to whether this is really the case, and the test is now less frequently applied than at one time was the case.

SECTION IV.

Class Specimens.

The Clinical Research Association is in a peculiarly favourable position to supply large numbers of microscopical sections, bacterial cultures, and other similar material that may be required for class purposes at hospitals, medical schools, universities, and the like. Separate slides of histological tissues of all kinds can be supplied at such charges as to make it cheaper to purchase them from the Association than it is to prepare them in a hospital.

SECTION V.

Cyst Fluids.

The Apparatus required.

Fluid obtained from a cystic accumulation is sent to the laboratory most conveniently in one of the bottles and tins used for the transmission of urine specimens. These can be supplied ready sterilised if special bacteriological examinations are required. If a urine bottle, but not a sterilised one, is ready to hand it can easily be sterilised by immersing it and the cork in boiling water, removing it from the latter and holding it upsidown for a few minutes when it will speedily dry by itself.

The Importance of the Examination.

Cystic fluids may be derived from a large number of different sources in man. In many of these the nature of the fluid is at once obvious without either chemical, microscopical, or bacteriological examination, for instance, in the case of a simple hydrocele or a dermoid cyst of the ovary. There are many cases, however, in which the precise nature of the cyst may be in doubt until the fluid from within it has been analysed either chemically, microscopically, or bacteriologically. It is difficult to indicate all the possible ways in which laboratory investigations of the fluid may be useful, but they will be almost obvious if the following list of various kinds of cysts is considered:—

- Congenital cyst of the neck.
- Cyst of the breast.
- Cyst of the lesser sac of the peritoneum.
- Cyst of the meninges—congenital, traumatic, or resulting from hæmorrhage.
- Dermoid cyst of the ovary.
- Dermoid cyst of the skin.
- Dermoid cyst of the thorax.
- Encysted ascites.
- Hæmatocele.
- Hydatid cyst.
- Hydrocele.
- Implantation cyst.
- Morant Baker cyst.
- Ovarian cyst, malignant.
- Ovarian cyst, simple.
- Pancreatic cyst.
- Parovarian cyst.
- Salivary cyst.
- Sebaceous cyst.
- Spermatocele.
- Thyroid cyst.

In regard to hydatid cysts, the diagnosis must remain in doubt until either daughter cysts have been found within the parent cyst or else the typical hydatid hooklets in the centrifuged deposit from the cystic fluid; the hooklets are the most characteristic feature. Suspicion as to the nature of the case may be aroused, however, if the fluid is of very low specific gravity and contains practically no proteid and no cell elements, and if no hooklets can be found in it owing to its being sterile, further confirmation of the diagnosis may be afforded by the discovery of eosinophilia on examination of the blood (page 17), and by the blood serum giving a positive hydatid reaction (page 44).

The great importance of distinguishing simple ovarian and parovarian from malignant ovarian cysts at the earliest possible moment needs no emphasis. In the two former cases the fluid is relatively thin, watery, and non-albuminous, of a specific gravity of about 1006, whereas in the malignant cases it is often thick and glairy, with a high specific gravity possibly up to 1030; albumen is present in variable quantity, and there is generally much pseudo-mucin or metalbumen which is the cause of its viscosity. This differs from ordinary albumen in not being coagulable by heat, and from mucin by not being precipitated by acetic acid. Alcohol produces a dense stringy precipitate, and, after boiling with hydrochloric acid, metalbumen yields a substance which reduces Fehling's solution. Microscopical examination of the centrifugalised deposit in these cases generally shows much detritus, together with red corpuscles and leucocytes, also epithelial cells which may be arranged in little clumps, and have so characteristic an appearance that a diagnosis of new growth may be made from them at once; there may also be cholesterin crystals, sometimes in large quantities.

Hydrocele fluid has characters similar to those of ascitic fluid (page 92), and they are almost as variable. Cholesterin crystals are often present. Spermatozoa are absent from ordinary hydrocele fluid, but they are generally present in that from a spermatocele; this point affords an important means of differentiating between the two. Although it would be difficult to diagnose hydrocele fluid itself, it is frequently important to examine it bacteriologically and microscopically to determine its cause; the fluid may give a positive Wassermann reaction in syphilitic cases (page 42); it may present an excess of lymphocytes with or without tubercle bacilli in cases of tuberculosis of the epididymis or testis; sometimes microscopic particles of new growth may be found in cases of malignant disease of the testis.

The presence of thyreo-iodine may be an important point in distinguishing between cysts derived from the thyroid gland and other cysts that may occur in the neck.

Encysted ascites may simulate hydatid disease, but the presence in the fluid of an abundance of coagulable proteid, together with the absence of hydatid hooklets, may be important points in distinguishing the two. Encysted ascitic fluid presents the same characters as does ordinary ascitic fluid (page 92).

The presence of urea or other urinary constituents in the fluid may afford an important or even conclusive proof that the cyst is a hydronephrosis, or is at any rate in some way connected with the urinary tracts. It has to be remembered, however, that a hydronephrosis of old date may have lost all the urea it originally contained, so that the absence of urea from a given

fluid does not prove that the latter was not derived from a hydronephrosis.

The difficulty of distinguishing simple encysted ascites or a hydatid cyst from a so-called pancreatic cyst may be considerable. The importance of examining the fluid for digestive ferments in such cases is obvious. Steapsin, trypsin, and amylopsin may all be present in comparatively large amounts in those large cysts in the upper part of the abdomen which are generally referred to as pancreatic cysts, although they are more often big cysts of the lesser omental sac with the pancreas incorporated and included in their wall. In a similar way the discovery of ptyalin in the fluid obtained from a cyst in the mouth will serve to distinguish a salivary cyst from one due to retention in a mucous gland or in a congenital cyst.

The presence of an abundance of butter-like fat, together with hair and possibly other body tissues, such as teeth, bones, muscles, and so forth, is characteristic of the ordinary dermoid cyst of the ovary.

Cysts derived from old hæmorrhage, whether in the meninges or elsewhere, may be identified sometimes by the presence within them of crystals of hæmosiderin.

Almost any cyst may become infected with bacteria; the possible presence of tubercle bacilli in a hydrocele has been referred to above. These bacilli may similarly be present in an encysted ascitic cyst, and so on. The *Spirochæta pallida* may be found in hydrocele fluid. Pyogenic and other bacteria may need to be examined for in some cases when the cyst wall has become inflamed, for instance, in connection with cystic disease of the breast.

Needless to say when other examinations can be carried out at the same time they will often be of material importance in association with the investigation of the fluid, and this applies particularly to histological examination of the cyst wall whenever this is possible.

SECTION VI.

Drinking Water.

The Apparatus required.

The various kinds of examination, and the methods of collecting and forwarding samples, are as follows:—

Chemical Analysis.

For this purpose not less than a Winchester quart bottle of the water should be sent for examination. For some analyses two Winchester quarts are necessary.

The bottle should be cleansed thoroughly with a brush, and by rinsing with strong sulphuric acid, and afterwards several times with the water of which a sample is to be examined. It should be securely stoppered. Corked vessels, and especially stoneware jars, are extremely unsatisfactory for the purpose, and it is frequently necessary when such have been used to delay the analysis for a fresh sample. A suitable bottle, properly cleansed and packed in a basket, will be sent free of cost on application to the Secretary of the Clinical Research Association.

It is highly desirable that the form which accompanies each bottle sent out be carefully filled up:—

Sample of Water for Chemical Analysis.

Special apparatus is required for samples for bacteriological examination. It will be forwarded on request.

Sent by..... Analysis required.....
Of..... Date of Collection.....
..... Number of previous report.....

INSTRUCTION AS TO COLLECTION.—The bottle should be thoroughly rinsed with the water which is to be analysed, and then filled up to the shoulder. The stopper should be rinsed before reinsertion and tied on to the neck, covered with linen or wash-leather, and sealed if necessary. Do not seal direct on the glass. Dispatch the sample as soon as possible after collection. It should be taken direct from the well or main, if possible, and not from a cistern.

Source (River, Well, Public Supply, &c.).....

IF A WELL WATER. { Depth.....
Nature of Stratum from which derived.....
Details as to previous rainfall.....
How lined (steel tubes, bricks laid in cement, &c.).....
Proximity to Sea or Tidal River.....
Proximity to Drains, Cesspools, &c.....
Does the water become turbid, and is there a marked rise in the water-level after heavy rain.....

Whether it is filtered, or allowed to sediment before use.....

Whether from cistern or direct from the well or main.....

An addressed Label for the return of the basket will be found inside the lid.

Under the cost of the Examinations (see Appendix, pages 134, 135) there are set out forms of analysis suitable for use in different conditions.

Bacteriological Examination.

Water is examined bacteriologically by quantitative and qualitative methods.

Quantitative.—By this is meant an estimation of the number of organisms capable of producing visible colonies on nutrient gelatine plates after three days' incubation at 20°C. The number is stated as an average of so many per cubic centimetre of the water.

It is usual also to make a similar estimate of the number of colonies on agar plates after forty-eight hours' incubation at 37.5°C. These agar plates are sometimes useful, because, while the more important sewage organisms grow readily at 37.5°C., most of the common water bacteria show no evidence of growth at this temperature. For this reason the development of any considerable number of colonies on the agar plates affords immediate presumptive evidence of pollution. Strictly speaking, however, the quantitative examination concerns itself with the enumeration of organisms, not with their characters.

Qualitative.—This has for its object the identification of organisms, more especially those characteristic of sewage, of which the most important are:—the *Bacillus coli communis*, the *Bacillus enteritidis sporogenes*, and streptococci.

Although the examination is said to be qualitative, it is performed by methods which are, in a sense, also quantitative. For example, in testing water for the detection of *Bacillus coli*, quantities of 0.1 cc., 1 cc., 2 cc., 5 cc., 10 cc., etc., up to a total of 100 cc., are examined, and should the organism be found, some idea of its numbers can be obtained by noting the minimum quantity of water in which its presence is evident.

For the *Bacillus enteritidis sporogenes* two quantities of 35 cc. each, and three quantities of 10 cc. each, are taken, and for the *Streptococcus* three quantities of 10 cc. each and one of 1 cc.

Bacteriological examinations conducted on such lines afford the best means yet discovered of detecting dangerous pollution with sewage. The methods are extremely sensitive, and are capable of indicating traces of pollution imperceptible by chemical analysis.

It is unfortunate that it is hardly practicable to examine water for typhoid and diphtheria bacilli. The difficulties are so great as to render a negative result practically valueless.

Collection of samples.—Samples must be packed in ice and forwarded without delay, otherwise the quantitative results will be vitiated by the rapid multiplication of organisms in the water.

The Association forwards the ice box, carriage paid, but it requires to be charged with ice by the sender.

Although either a chemical analysis or a bacteriological examination may be sufficient in itself for obtaining specific information with regard to a particular water, these two processes should be looked upon as parts of a complete investigation.

It is particularly requested that in all cases the source whence the water has been obtained should be stated; for example, whether from a deep or a surface well, from a spring, etc., so as to enable satisfactory conclusions to be drawn from the results obtained. In their absence, it is frequently impossible to express any opinion at all.

SECTION VII.

Ear Discharges.

The Apparatus required.

A small bottle or test tube may be used for a copious fluid discharge. Films spread evenly on clean microscope slides are suitable for examination for pus or for the direct staining of micro-organisms. The films should be allowed to dry thoroughly before they are packed. For cultural investigations simple swabs may be sent to the laboratory, or culture tubes suitable for direct inoculations will be sent on request with directions as to their use. These can be sent direct to the laboratory for incubation, and they have the advantage that the micro-organisms are less apt to die in transit, especially in the winter time.

The Importance of the Examination.

The only two discharges apt to be met with from the ear are: (1) pus with or without blood; and (2) cerebro-spinal fluid. The latter is not at all likely to occur unless there has been a head injury such as may produce a fracture of the base of the skull, and then the fact of cerebro-spinal fluid escaping in this way from the ear is one of the most important clinical proofs of the nature of the lesion. For the characters of cerebro-spinal fluid see page 55. When the discharge consists of pus, the fact may be proved by microscopical examination of films that have been stained appropriately (page 88). Too much stress, however, cannot be laid upon the fact that beyond the establishment of the diagnosis of a suppurative discharge from the ear, efforts

should always be made to identify the causal organism. This may be one of the pyogenic cocci, especially either staphylococci, streptococci, or pneumococci. On the other hand, the otorrhœa may be due to various other organisms, including pneumo-bacilli, tubercle bacilli, Klebs-Löffler bacilli of diphtheria, the bacillus xerosis, the bacillus pyocyaneus, the bacillus coli communis, the typhoid bacillus, and possibly others. The nature of the causal organism may be ascertained sometimes by simple examination of specially stained films (page 89); more often cultural methods of identification are required (page 89).

It happens more often than is generally recognised that symptoms that have apparently nothing whatever to do with the ear are secondary to the absorption of toxins from a chronic otorrhœa. Amongst these may be mentioned in particular certain types of rheumatoid arthritis. When vaccine treatment is to be adopted in these cases the importance of identifying the causal organism correctly is obvious.

SECTION VIII.

Fæces.

Few medical men devote as much attention to microscopical examination and bacteriological examination of the fæces as the clinical information to be obtained therefrom warrants. The chief investigations required, as a rule, may be discussed under the headings of general and microscopical examination; chemical analysis for food residues; tests for occult blood; tests for ferments; and bacteriological analysis.

The Apparatus required.

The amount required for any but quantitative tests is small. When chemical analysis of the total food residue in the fæces is required, or when an estimation of the total bacteria in the fæces is desired, the whole bulk of the fæces passed in the twenty-four hours should be transmitted to the laboratory in a clean glass vessel of suitable size, thoroughly well corked, and protected by an outer casing which cannot become broken in the post. Special apparatus for the purpose is supplied by the laboratory on request.

The Importance of the Examination.

1. General and Microscopical Examination of the Fæces.

There are many ways in which a general microscopical examination may lead to discoveries of clinical importance. Amongst these may be mentioned in particular the presence of undigested food residues. In addition to the well-defined constituents of ordinary fæces familiar to all who have made routine examinations of this kind, it may be at once obvious that the patient is passing an excess of undigested fat globules, or starch grains may have passed through the alimentary canal without being acted upon by the digestive juices, or muscle fibres may have escaped digestion in a similar way. If all three are present in excess in a case in which there is diarrhoea suggesting that the food is being hurried through the alimentary canal with excessive rapidity, the fact affords considerable evidence in favour of there being deficiency of the pancreatic juice, and in conjunction with this one may find a deficiency in the corresponding ferments themselves in the fæces (page 71), or the urine may give a positive Cammidge's reaction (page 117), and there may be a pathological relationship between the saponified, unsaponified, and total fats in the fæces (page 70). If either starches, fats, or muscle fibres, but not all three, are being passed undigested, the fact may afford valuable information as to the need for modifying the dietary accordingly, or as to the prescription of a suitable dose of that ferment which will assist in the better digestion of the food which is being passed undigested.

The ova of such parasites as affect the alimentary canal of man are so characteristic that their detection in the fæces on microscopical examination affords one of the best proofs of the presence of the parasites themselves within the bowel. Some of them are not, strictly speaking, pathological, *trichocephalus dispar*, for instance. Most parasites, however, produce symptoms sooner or later, though in varying degree. One may mention in particular *oxyuris vermicularis*, *ascaris lumbricoides*, *tænia solium*, *tænia mediocanellata*, *bothriocephalus latus*, and *ankylostomum duodenale*. In the case of the last four, a differential leucocyte count made upon a stained blood film generally shows eosinophilia at the same time (page 17). When there is infection by *bothriocephalus latus* or *ankylostomum duodenale* the toxic symptoms may be so severe that the patient becomes profoundly anæmic, and presents an appearance which

may be mistaken for pernicious anæmia until this is excluded by determination of the colour index (page 8), and by the discovery of eosinophilia, and of the ova of the offending parasite in the fæces. In the case of bilharzia hæmatobia, the mature worm does not itself occupy the alimentary canal, but lies in the pelvic lymphatics or veins, from which the characteristic spikéd ova are discharged generally into the bladder, where they produce hæmaturia. Not infrequently, however, some of the ova find their way into the pelvic colon as well, and they may then be discovered in the fæces.

When a patient is being treated for tapeworm, and the head has not been passed, it may be possible to detect the fact of the continued activity of the worm earlier by the discovery of the ova in the fæces than can be done in any other way.

Portions or the whole of any of the above parasites may be found in the fæces and identified by microscopical examination.

In addition to the ordinary intestinal parasites it happens sometimes that the larvæ of various flies develop in the alimentary canal and are passed per rectum. Amongst those that have been discovered in this way in various cases may be mentioned the larvæ of the following:—

Stratomyidæ	Microchrysa polita
Syrphidæ	Eristalis tenax
	Helophilus pendulus
Cestridæ	Tachina larvarum
	Gastrophilus equi
Sarcophagidæ	Sarcophaga carnaria
	Wohlfahrtia magnifica
Muscidæ	Musca corvnia
	Musca domestica
	Lucilia cæsar
	Calliphora vomitoria
Anthomyidæ	Fannia scalaris
	Allognota agromyzina
Micropezidæ	Calobata cibaria
Piophilidæ	Piophila casei
Geomyzidæ	Tychomyza fusca

A patient sometimes thinks that parasites are present in the fæces when that which simulates them is really derived from various vegetable food stuffs. These pseudo-parasites can be identified, as a rule, by microscopical examination.

Intestinal sand is generally associated with the passage of mucus, or alternates with the passage of the latter, especially in the condition known as mucous colic, or mucous colitis, or muco-membranous colitis. The sand itself may be passed separately from the fæces in the form of a very fine grit associated with a little fluid, or it may be found in the fæces them-

selves microscopically. It has to be distinguished from false intestinal sand which is composed of gritty particles derived from various food stuffs. The identification is possible to some extent microscopically, but, as a rule, chemical identification is required as well.

Mucus is a normal constituent of fæces, but it should not become sufficiently obtrusive to be recognisable as such. As a general rule the passage of obvious mucus per rectum indicates either a catarrhal or an inflammatory or an ulcerative lesion of the intestines or an error in the physiology of its secretion, associated very often with other neurotic symptoms, especially in unmarried women who are approaching middle age. Mucus can be identified by certain staining reactions; also by chemical tests. There is little doubt as to its nature when it is present in the form of jelly-like masses, but when it forms larger fragments or actual casts of the intestine, it may simulate a tapeworm, on the one hand, or a mass of growth or other abnormal tissue, upon the other. Exact identification is important, and sometimes this necessitates fixing, embedding, and making microscopical sections of it, as in the case of other histological specimens (see page 105).

It very rarely happens that a particle of new growth can be detected in the fæces, even when there is a carcinoma so low down as the rectum. Occasionally, however, such a fragment may be found. It may be identified by direct examination, but more often if the diagnosis is to be established with certainty the tissue should be prepared as a histological specimen (page 105).

2. Chemical Examination of the Fæces.

The chief chemical analyses of clinical importance applicable to the fæces are: Estimation of the total nitrogen in the fæces; Estimation of the total fat in the fæces; and Estimation of the relative amounts of saponified and unsaponified fats in the fæces.

The total amount of nitrogen passed daily in the fæces may be more than 1 gram without its indicating anything necessarily pathological. When, however, there is defective digestion of proteid foods, the total amount of nitrogen in the fæces may exceed this considerably. The clinical deductions that can be drawn from the fact must necessarily vary according to the other features of the case. Analyses of this kind are particularly important in connection with metabolism research work, and when a large number of such estimations are to be made in a particular case for research purposes, specially reduced charges will be quoted upon application to the Clinical Research Association.

The importance of knowing both the percentage and total of unabsorbed fat in the fæces may be considerable. When there is chronic pancreatitis or a pancreatic stone, or a carcinoma of the pancreas obstructing its duct, as much as 70 or even 80 per cent. of the total fat may pass per rectum. The proportion is less in the case of growths or other things obstructing the common bile duct, but leaving the pancreatic duct free. It is not possible to learn so much, however, from a mere determination of the total amount of fat in the fæces as it is from determination of the relation of the unsaponified to the saponified fats; whereas the former are in excess in diseases that interfere with the digestive functions of the pancreas, such as carcinoma of it, or advanced chronic pancreatitis, the saponified fats predominate when gall-stones are obstructing the common bile duct, but not the pancreas.

This broad summary is subject to exceptions, of course, and the results of analysis in any particular case must be interpreted in conjunction with the other clinical features of the case. When actual disease of the pancreas is suspected, Cambridge's reaction should be tested at the same time (page 117). One of the larger works that includes a discussion of this subject should be consulted for details.

In children, analysis of the fæces for fat may afford valuable information as to the direction in which the milk or other food that is being given may need modification if the best results are to be obtained.

Chemical analysis of the fæces for drugs may sometimes be of importance; lead, for instance, is excreted by the bowels, and when there is no blue line upon the gums, and yet plumbism due either to an accidental cause or possibly to the deliberate taking of diachylon pills, is suspected, this diagnosis may be confirmed by chemical analysis of the stools.

When fæces contain large quantities of blood, this may be obvious from the colour, and verified either by the application of the ordinary guaiacum test or by extracting the pigment and using the spectroscope. Over and above this, however, much clinical information is sometimes obtainable from detection in the fæces of minute quantities of blood undiscoverable except by special methods of extraction and the use of the spectroscope.

Occult blood of this kind may be derived from an ulcer of the stomach or a gastric carcinoma, a duodenal ulcer, from acute enteritis or colitis, a carcinoma of the intestines, or other similar lesion. By itself, no great stress can be laid upon its presence, perhaps, but when the symptoms point to the possibility of there being merely dyspepsia, on the one hand, or a gross lesion with breach of surface of the mucous membrane,

upon the other, the persistent presence of occult blood in the stools points to the latter alternative. In testing for it, it is important to exclude from the dietary for two or three days such substances as contain blood pigment, especially meat, meat extracts, and soups containing the colouring matter of meat.

Ferments in the stools have not been investigated very extensively up to the present, but there have been workers in this field of research, the results of whose investigations point to the fact that normal fæces should contain residues of those ferments which have been taking part in digestion higher up in the alimentary canal. The proteolytic ferments are of chief importance, and their presence or absence has been used as a criterion of whether the pancreatic juice is reaching the bowel normally or not.

3. Bacteriological Examination of the Fæces.

Bacteriological examinations of the stools are less often made than they should be. One of the difficulties is that the bacillus coli communis so preponderates, as a rule, that it is apt to overgrow and swamp the other micro-organisms that may be present, and considerable time and repeated subcultures may be required before the laboratory worker is in a position to report upon all the different flora, normal and abnormal, that can be isolated by cultural methods in any particular case. Nevertheless, results of great clinical value are obtained in not a few instances. In a recent case, for example, the patient was suffering from an obscure form of intestinal dyspepsia, and the surprising result was found on cultivation of the fæces that pneumococci were constantly present. This suggested that the intestinal trouble was due to pneumococcal inflammation. Pneumococci were recovered also from the mouth and throat, and the adoption of pneumococcal vaccine treatment cured what had up till then been a very resistant lesion. A form of streptococcus is normally present in the fæces, but pathological streptococci also occur sometimes, and it has been stated that in some cases of neurasthenia the patient may be benefited by the use of a vaccine prepared from mixed cultures from the fæces.

In the diagnosis of typhoid fever the initial leucopenia with relative lymphocytosis (page 21), or recovery of typhoid bacilli from the blood (page 48), or in the second week or later, Widal's serum test (page 33) afford the most certain means of confirming the diagnosis, but sometimes the nature of the case remains in doubt in spite of these tests. Recovery of the typhoid bacillus from the stools may then afford a valuable help in proving the nature of the disease. The same applies with even greater force to the diagnosis of the various types of paratyphoid fever.

Zymotic diarrhoea of infants is apparently due to different organisms in different places and in different epidemics. If vaccine treatment is to be successful in curing this scourge, it is first necessary to determine the nature of the causal organism by cultivation of the stools. Morgan's bacillus I., Morgan's bacillus II., Gaertner's bacillus, the bacillus *ærogenes lactis*, and probably other micro-organisms may each individually be responsible for what clinically may appear to be the same disease.

The value of cultivation of the stools in the diagnosis of asylum dysentery, cholera nostras, and other allied intestinal affections is well known. True cholera is fortunately rare in this country, but bacteriological examination of the stools is the most certain way of establishing the diagnosis in suspected cases. Dysentery may be due to Shiga's bacillus or to the *amœba coli* or possibly to other micro-organisms; establishment of the exact nature of the dysentery requires bacteriological investigation of the stools.

There are, indeed, a very large number of ways in which bacteriological and other investigations carried out upon the *fæces* may be of clinical importance, and it seems a pity that they are made use of so seldom as at present.

SECTION IX.

Foods and Drugs.

(For Milk see page 83).

The Clinical Research Association is willing to undertake examination of food stuffs, either for bacterial infections or for poisons or for the presence of adulterants, etc., in the case of samples taken under the Food and Drugs Act.

The Importance of the Examination.

The importance of analyses of food stuffs by chemical, microscopical, or bacteriological methods lies mainly in safeguarding the public from being supplied with things which have been adulterated or things which are for some other reason unfit for human consumption. A general investigation of samples taken under the Food and Drugs Act includes not only a determination of whether a given sample contains the right percentage

of its various ingredients as laid down by Act of Parliament, but also in the detection of adulterants, potato starch grains in flour, the admixture of various foreign substances such as sand, sawdust, and so forth, either with foods or drugs, the addition of undue quantities of preservatives, and so on.

It is a common practice to sell as free from starch, and therefore as being fit for the use of diabetic patients, special foods which contain an abundance of carbohydrate other than of the kinds which give a blue reaction with iodine. Boiling such foods with hydrochloric acid and then applying Fehling's test will detect this fraud at once. This will serve as an instance of the way in which laboratory investigations may be of service clinically in connection with foods.

Sometimes it is desired to compare one food stuff with another as to their relative nutrient values. The Clinical Research Association will analyse a food stuff quantitatively when desired.

The importance of detecting contamination of food stuffs by the heavier metals, especially by lead or copper or tin, is obvious. Arsenic may be ingested unsuspectedly in beer, as in the celebrated Manchester epidemic.

Ptomaine poisoning is far more often a poisoning by actual microbes than it is by ptomaines proper. The diagnosis depends upon bacteriological investigation of the suspected food stuff—especially pork pies and other foods prepared from the meat of the pig, and the discovery in them of the causal organisms of the ptomaine group.

Histological examination of some food materials, particularly meat, may be required sometimes; for instance, in the demonstration of suspected tuberculosis or in the verification of the nature of some parasitic disease in the meat or in the demonstration of the nature of some peculiar appearance in relation to the food.

Drugs frequently require analysis to see whether they are up to the standard required by the British Pharmacopœia.

It sometimes happens that peculiar reactions take place when a particular mixture has been dispensed, and it becomes a matter of importance to determine what is the chemical nature of the changes so produced.

Crude drugs may be adulterated by the admixture of adventitious powders, etc.; this may be detected by microscopical or chemical means.

Sometimes the laboratory is called upon to identify the nature of leaves or other portions of plants; for instance, in respect to poisoning by the ingestion of some wild plant such as belladonna; or to dermatitis that results when rhus toxicodendron

has been allowed to grow upon a house in mistake for the ampelopsis. Sometimes cattle or other animals are taken ill after eating in a particular place, and the nature of the malady is cleared up by the identification of some poisonous plant which grows upon the ground.

The above will serve to indicate some of the ways in which analysis in the laboratory may be of service in connection with chemical, bacteriological, and microscopical examinations of various foods and drugs.

SECTION X.

Gastric Contents.

The Apparatus required.

Owing to the fact that the normal characters of the gastric contents differ considerably at different stages of ordinary digestion, it is important to know not only what food has been taken by the patient previously, but also how long before the specimen is obtained this food was eaten. Broadly speaking, the greater the proportion of proteid the meal contains the later is it before free hydrochloric acid is present. It is on this account that test meals are generally required if a correct interpretation is to be given to the results of chemical analysis of the gastric juice. The various test meals that may be employed and the correct intervals that should elapse before they are recovered are given in most clinical text-books. The following are some examples:—

EWALD'S TEST BREAKFAST—

White bread	70 grams.
Weak tea	300 cc.

The gastric contents should be recovered by lavage one hour later.

KLEMPERER'S TEST BREAKFAST.

White bread	70 grams.
Milk	500 cc.

The gastric contents should be recovered by lavage two hours later.

GERMAIN SEE'S TEST LUNCH.

White bread	150 grams.
Minced meat	80 grams.
Cold water	300 cc.

The gastric contents should be recovered by lavage two hours later.

RIEGEL'S TEST DINNER.

Soup	100 cc.
Beefsteak	60 grams.
White bread	50 grams.

The gastric contents should be recovered by lavage three hours later.

When, however, circumstances prevent the use of test meals and the subsequent recovery of the gastric contents by lavage, and yet it is desired to have analyses made of the gastric contents after they have been brought up by the patient vomiting spontaneously, results of considerable clinical value can still be obtained provided careful notes are made of the previous dietary and of the interval that has elapsed between the last food and the patient's vomiting. The specimen should be sent to the laboratory in a clean glass bottle, preferably one with a wide neck. It should be carefully and securely corked and transmitted in a suitable tin or box in strict accordance with the post-office regulations (page 126). If bacteriological investigations are required it is necessary that both bottle and cork should have been sterilised previously by boiling, but no antiseptic or preservative should be used. When, however, no cultural investigations are required it is often advisable to add a small quantity of thymol to the specimen, especially in hot weather, in order to prevent the occurrence of fermentation or putrefactive changes during the time that must necessarily elapse before it reaches the laboratory.

The Importance of the Examination.

Monographs have been written upon the various ways in which analyses of the gastric juice may be of value in clinical medicine. It is not possible to do more than indicate here some of the chief ways in which the investigations may be of value in practice.

Much stress has been laid upon the importance of the presence or absence of free hydrochloric acid in distinguishing between gastritis or simple ulcer, on the one hand, and carcinoma of the stomach, upon the other. The test is of real value, but it is important that its considerable limitations should be understood clearly, for otherwise wrong deductions may be drawn. There

is no free hydrochloric acid in the normal gastric contents until all the molecules of proteid in the last meal have been combined with this acid and a surplus is left over. Free hydrochloric acid is not to be expected, as a rule, until three-quarters of an hour or more after the last meal. It is impossible to say, therefore, whether the absence of free hydrochloric acid from a given specimen of gastric juice can have any pathological significance at all unless one knows exactly what were the constituents of the last meal, and also how long it was after that meal was eaten before the stomach contents were recovered. If, however, the meal and the interval after it were both such that in health free hydrochloric acid would be present in the juice and none can be found in the specimen being examined, there is something wrong with the patient. Even then, however, it is impossible to deduce that there is a gastric carcinoma, for almost any condition of chronic ill-health may so interfere with the secretions of the body, the gastric juice amongst the rest, that they are abnormal; and there is deficiency or even absence of free hydrochloric acid in the gastric contents at the proper interval after a test meal in a very large number of maladies besides gastric carcinoma—extensive carcinoma elsewhere associated with cachexia, chronic heart disease, the later stages of cirrhosis of the liver, pernicious anæmia, typhoid fever, and a host of others. When, however, the clinical evidence indicates that the lesion is almost certainly a gastric one, and it is a question as to whether there is a carcinoma of the stomach or not, then a pronounced deficiency in the free hydrochloric acid in the gastric juice at the proper interval after a test meal is an argument in favour of carcinoma rather than of some less serious lesion. Nevertheless, even when the trouble is gastric, and it is not malignant, there may be deficiency of hydrochloric acid in the stomach contents, so that although it is a very valuable test which should be applied in all suspected cases, it is one that needs much skill in its interpretation, and the results of the analysis for it in the gastric juice require to be interpreted in association with all the other clinical signs and symptoms in the case.

As regards the lactic acid in the gastric contents, its importance is, roughly speaking, the converse of that of free hydrochloric acid. In the earlier stages of normal digestion, especially if the meal contains milk, lactic acid is present in moderate quantity before sufficient hydrochloric acid has been secreted for a surplus to be left free. Later, when free hydrochloric acid begins to appear, lactic acid diminishes in amount until in the later stages of digestion it would normally be absent, or present in traces at the most. In pathological conditions in which

the hydrochloric acid secretion is deficient, lactic acid may be present throughout digestion, and when there is undoubted fermentation in the stomach it may be present in excess.

Butyric and acetic acids in any but very small quantities generally indicate undue fermentation; hydrochloric acid is apt to be defective at the same time; they may be of particular importance in connection with the gastric contents of infants and young children.

Too little importance is generally paid to the question of the ferments in gastric juice. Hydrochloric acid may be present in normal quantities, and yet there may be deficiency in the peptic activity of the juice. To test it, incubation of the juice with a measured quantity of proteid is required, a control containing a known quantity of pepsin being incubated at the same time. The rennet activity of the gastric juice is tested by similar incubation of prepared milk. In children who cannot digest milk it will frequently be found that there is deficiency of the rennet ferment, and this can seldom be determined without laboratory investigations of the gastric contents. The value of examining the gastric contents both for pepsin and for rennin is obvious when it is remembered that either of these ferments can be supplied artificially if need be.

The possible importance of analysis of the gastric contents in cases of supposed poisoning is obvious. Sometimes a particular poison is suspected, and it is a question merely of determining whether this is or is not present. More often, perhaps, the nature of the poison is sufficiently unknown to necessitate a routine examination both for various inorganic substances such as arsenic, carbolic acid, or the like, and for some alkaloid such as strychnine or morphine.

Sarcinæ ventriculi are never present in the normal gastric contents. Their discovery is practical proof of there having been stagnation of the food in the stomach extending over a long period, the result, for instance, of pyloric stenosis. They do not, however, indicate the actual cause of the delayed emptying of the stomach contents.

Should it happen that a particle of new growth is seen on microscopical examination of the contents of the stomach, the diagnosis of gastric carcinoma is established with certainty.

The word "gastritis" is often used in a loose sense to cover lesions other than those in which there is actual inflammation of the gastric mucous membrane; various kinds of non-inflammatory dyspepsia are ill-distinguished from conditions in which the symptoms are due to actual inflammation; the discovery with the microscope of numbers of leucocytes in the gastric juice serves to indicate that there is actual gastritis.

When a breach of the mucous membrane is suspected, the occurrence of occult blood confirms this, and its detection is sometimes useful in establishing a suspicion that there may be a gastric ulcer or an ulcerating carcinoma.

The presence of the Oppler-Boas bacillus in the gastric contents is regarded by many authorities as virtual proof that there is a gastric carcinoma. Probably this is too dogmatic a view to hold, but the presence of this organism helps to confirm a suspicion of new growth.

The gastric contents should be sterile when they are recovered at the height of digestion by means of a sterilised lavage tube which obviates the possibility of contamination from the mouth or pharynx. In conditions of gastritis the affection of the gastric mucous membrane may be due to streptococci, staphylococci, pneumococci, or other micro-organisms. Bacteriological investigations of the stomach contents either by direct staining, or preferably by cultural methods, will sometimes lead to exact proof of the nature of a very troublesome and long-continued gastritis, and in some cases cure is said to have resulted from the adoption of the proper vaccine after ordinary measures have failed to relieve the patient.

There are various other ways, less common than the above, in which investigations of the gastric juice may prove of clinical value. These will be found mentioned in the larger text-books and in monographs devoted to the subject.

SECTION XI.

Hair.

The Apparatus required.

No special apparatus is required for the collection of hair and its transmission to the laboratory for examination. A small test tube carefully corked and enclosed in a small box or tin is generally convenient. Hairs that are to be examined for ringworm spores are best removed from the suspicious focus one by one with forceps, the short broken stumps at the margins of the ringworm patch being selected. They should be pulled out

with a short sharp jerk, which the patient will hardly feel. It is not satisfactory to remove them with scissors, for the spore-bearing ends may thereby be left behind. No preservative is required when transmitting the hairs, and when cultural identification of the nature of the ringworm fungus is needed, it is particularly important that no antiseptic should be used, and that the hairs should be taken from a part to which no antiseptic has been applied recently. In the case of alopecia areata the hairs which should be selected for examination are the short club-ended or exclamation-mark stumps at the periphery of the bald patch.

Hairs that are to be examined microscopically for errors of form or of pigmentation should be sent entire.

When analysis of hair for arsenic is to be carried out it should be remembered that that portion of the hair which was growing at the time the arsenic was being administered is the part which will contain the most arsenic. Which part this is likely to be can be calculated from the time relations of the case and the rate of growth of the hair. When the arsenic has been administered recently, the part closest the scalp is that which is important. The larger the amount of hair that can be sent for this analysis the more accurate are the results obtained likely to be, but, broadly speaking, the hair growing upon an area of scalp equal to that of a half-crown will suffice.

Parasites associated with the hair, or nits attached to the hair, can be sent to the laboratory for identification either in a small dry bottle or in some preservative fluid, such as spirit.

The Importance of the Examination.

The diagnosis of ringworm is often sufficiently obvious without examination of the hair for spores upon it being necessary. Sometimes, however, the diagnosis of the nature of the bald patch is uncertain, and microscopical identification of the spores upon the hair may then be very important. Similar importance attaches to the examination in cases of ringworm that have been treated and are thought to be cured; before cure can be assumed for certain more than one negative report as to the presence of spores may be required.

Since the extensive researches of Sabouraud and others have been published it has been recognised that there are many different kinds of ringworm, and that whereas some of these are transmitted almost entirely from child to child, others are derived from animals. Cultural examination of the infected hairs may serve to trace the source of a ringworm infection to some domestic pet, and the possible importance of this is obviously

great. Similar examinations of the hair of pet animals or of farm-yard stock are not infrequently required in order to make certain of the nature of some malady affecting the animals' skin or hair.

Alopecia areata may be extensive without any parasite being found. There seem to be two types at least of the malady, one parasitic, the other possibly not. The value of microscopical identification of the parasitic type is obvious.

The nature of nits or of other various parasites affecting the hairy regions is generally possible without microscopical examination, but in cases of doubt it is useful to have the specimen mounted as a microscopical slide, when the exact nature of the malady can be established beyond doubt.

Various peculiarities of hair, deficiencies in its pigment, atrophy of the hair bulbs, splitting of the ends, moniliform shapes, and so forth, are recognised more easily by microscopical examination than in any other way.

Arsenical poisoning from some unknown source may be suspected in a given case. The practitioner may not wish to divulge his suspicion, and yet he may desire confirmation. The fact that arsenic becomes stored in the hairs will prove useful in such instances. Analysis of that part of the hair which was growing during the time of the suspected administration will prove or disprove the arsenical suspicion. In some cases, again, the origin of peripheral neuritis is discovered with difficulty. The possibility of arsenic being the cause can be converted into a certainty by hair analysis. This was proved in the case of the celebrated Manchester epidemic of peripheral neuritis which, originally thought to be due to alcohol, was proved to be really arsenical. Urine analysis for arsenic (page 112) may afford similar evidence.

SECTION XII.

Inoculations in the Diagnosis of Tuberculosis or other Infections.

Whenever necessary the Association will arrange for inoculation of living animals with clinical material for diagnostic purposes and will report the result.

The importance of the procedure is, in certain instances, very great, and may, indeed, be essential to complete and accurate diagnosis.

It is, perhaps, particularly in the diagnosis of otherwise obscure tubercle that the method is most often needed; sometimes, for instance, there is a serous exudate into the chest or the peritoneum, and no tubercle bacilli can be found in it upon direct examination of the centrifugalised deposit, and yet it is most important to make sure whether the exudate is tuberculous or not. Almost the only way in many cases of this kind is to inject a small quantity of the centrifugalised deposit aseptically into a guinea pig, and to observe whether the latter develops tuberculosis or not. One cannot exclude tubercle rapidly in this way, however, and sometimes it is impossible to give a final report for six weeks after the inoculation. Similarly, in the case of urine from a kidney suspected of being tuberculous, if tubercle bacilli can be found in the centrifugalised deposit, the diagnosis is established, but, in a certain proportion of cases, either the tubercle bacilli in the urine are so few that they cannot be found, or for some other reason they are not detected, and then inoculation of a guinea pig with the centrifugalised deposit may be the only means of determining whether tubercle is present in the urinary passages or not.

The same applies to certain cases of pulmonary tuberculosis in which, even when no tubercle bacilli can be found in the sputum, animal inoculation shows that the sputum is tuberculous.

Glanders, rare though it is in man, requires early diagnosis if the patient's life is to be saved, and although suspicious bacilli may be found in the lesion, and although the patient's blood serum may give the complement fixation test to the bacillus mallei (page 45), inoculation into a guinea-pig, and the consequent testicular changes, may be essential to establishing the diagnosis with certainty.

These are some examples of the way in which the method may be required in diagnosis from time to time. Other instances are met with in connection with establishing the causal connection between the presence of a given micro-organism and its pathogenic effects on the patient, and especially in association with research work upon the nature and character of the new micro-organisms that are discovered from time to time.

SECTION XIII.

Joint Fluids.

The Apparatus required.

Joint fluids are generally obtained in such small quantities that they are best sent to the laboratory either in special sterilised test tubes, or sealed capillary pipettes can be supplied for the purpose.

The Importance of the Examination.

Whenever an inflamed joint has to be opened, in any case it is important that the nature of the inflammation should be confirmed, if possible, by both direct and cultural bacteriological examinations of the fluid. If any portion of the synovial membrane is removed at the same time this should be examined histologically and bacteriologically, too, for very often the joint fluid contains nothing but dead micro-organisms, whilst the surrounding tissues contain living ones. The importance of verifying the diagnosis of a tuberculous joint in this way is clear, and similarly it may be of very material benefit to the patient by reason of the subsequent vaccine treatment that may be adopted if the causal organism of an acute infective arthritis can be demonstrated to be a streptococcus, staphylococcus, gonococcus, pneumococcus, or some other similar organism from cultures of which autogenous vaccines may be prepared (page 119).

Besides those cases in which the joint has already been opened for surgical purposes, however, there is increasing evidence to show that various subacute and chronic kinds of synovitis and arthritis which are so confusedly grouped together under the heading of rheumatoid arthritis, will some day be distinguished from one another bacteriologically. Gonococcal arthritis and synovitis has already been split off from the main mass in this way. It is more than likely that there is a streptococcal form of rheumatoid arthritis, a staphylococcal form, a tuberculous form, a form due to the bacillus coli communis, and so on, the joints responding to the effects of different organisms by pathological changes which are clinically indistinguishable at present.

If the treatment of these cases is to be more successful in the future than it is now, it will be necessary to be more accurate in the bacteriological diagnosis. At present this amounts to little more than guess-work in many cases; for instance, a patient may have a chronic vaginal discharge containing strep-

tococci; she may also have typical rheumatoid arthritis with recurrent exacerbations; no other pathological cause than the discharge from the genital passages can be detected; it is therefore assumed often that the absorption from this toxic source is the cause of the joint changes. Similarly, when staphylococci are persistently found in recurrent nasal inflammations in a patient already suffering from arthritis, the latter is assumed, doubtless with considerable probability, to be staphylococcal; and so on in other instances. One cannot help feeling, however, that it would be much more satisfactory if the supposed causal organism could be demonstrated in these cases in the fluid obtained from within or around the inflamed joints, and there is a strong probability that in the near future an increasing number of cases will be diagnosed with bacteriological correctness by having the fluid obtained by needling such joints examined bacteriologically in the laboratory. The therapeutic importance of this is obvious, for, once the causal organism is known, the nature of the vaccine most likely to do good to the joints themselves is also known.

The subject would lend itself admirably to research work in general practice.

SECTION XIV.

Laboratory Instruction.

The Clinical Research Association always welcomes medical men who wish to see the routine work which is being done day and night at the laboratories. It is best that an appointment should be arranged beforehand in order that one of the qualified medical men attached to the laboratories may be disengaged to explain the different parts of the technique in person. No charge is made for visits to the laboratory of this kind.

SECTION XV.

Milk.

The Apparatus required.

Apparatus suitable for the transmission of milk specimens to the laboratory for chemical, microscopical, or bacteriological

analysis will be supplied upon request. For most investigations the specimen can be sent in a sterile bottle and case similar to that used in the transmission of urine specimens, but when bacteriological investigations are required in which it is important that the multiplication of the organisms in the milk should be checked by the use of ice, the apparatus for this purpose will be sent carriage paid from the laboratory upon request, but it needs to be charged with ice by the sender of the specimen. When cultural investigations of milk are to be made it is most important that no boric acid or other similar preservative should be added, for if any such substance is so added the micro-organisms will be apt not to grow when the centrifugalised deposit is planted upon culture media.

The Importance of the Examination.

Milk, when sold as milk, must comply with the minimum requirements of the regulations under the Food and Drugs Acts as regards its composition. Chemical analysis is required in the detection of watering of milk, the abstraction of cream from the milk, the addition of preservatives to the milk, and so forth.

It is unfortunately a fact that much milk sold for human consumption contains tubercle bacilli, and a very great deal of tuberculosis of joints, glands, skin, peritoneum, etc., in children is due to the fact that tuberculous milk is ingested by these children when this could be prevented if samples were examined more frequently for tubercle bacilli and drastic steps taken to suppress all milk that is tuberculous.

Milk always contains numbers of leucocytes, cocci, and epithelial debris from the udder, and it is not easy to draw any hard and fast line between milk which is normal and milk which is dirty in these respects. When, however, a specimen is found to contain very large numbers of leucocytes and a considerable excess of cocci, the probability is that the cow from which the specimen came was suffering from mastitis, rendering the milk unsuitable for food purposes, whilst if the centrifugalised deposit from the milk contains considerable quantities of hair and other extraneous materials, the probability is that the dairy from which the specimen came is a dirty one.

It is not only in connection with Acts of Parliament that chemical analyses of milk are important; in not a few cases an infant that is being brought up artificially is unable to digest milk from one cow when it can digest milk from another. Chemical analysis will often show that this is due to the indigestible sample containing either too much proteid or too much fat, and by modifying the proportions of this accordingly, milk which may have been indigestible hitherto may be made diges-

tible. This applies also to mothers' milk. Samples of human milk are examined chemically less often than they should be in cases in which the child does not seem to thrive. It will some- be found that although theoretically it is better that the infant should be brought up upon its mother's milk rather than arti- ficially, for one reason or another the mother's milk may depart sufficiently from the normal to be really less good than modified cow's milk or an artificial food. The fact can sometimes be determined at once by chemical analysis of a specimen obtained by the breast pump.

SECTION XVI.

Mounting of Museum Specimens.

The Clinical Research Association prepares specimens for museum purposes, mounting them for colour preservation or otherwise according to instructions. When required, crude speci- mens will be carefully dissected and mounted to show any parti- cular points that may be desired.

SECTION XVII.

Nasal Discharges.

The Apparatus required.

Nasal discharges are best examined bacteriologically in the same way as throat swabbings (page 102), and the charges and apparatus required are the same. The only exception to this is that it is possible for a nasal discharge to consist of some unusual substance, for instance, cerebro-spinal fluid when there has been a fracture of the anterior fossa of the skull (see cerebro- spinal fluid, page 56).

The Importance of the Examination.

Purulent nasal discharges are more often due to diphtheria bacilli than is generally recognised. This organism should be suspected if the purulent material is so acrid as to cause a raw red excoriation of the skin round the nostrils and down the upper lip towards the mouth. It is scarcely possible to diagnose nasal diphtheria in its earlier stages clinically; bacteriological examination is required, and cases of diphtheritic rhinitis are constantly being missed because nasal discharges are not sent to the laboratory for bacteriological examination as a routine procedure.

SECTION XVIII.

Poisons.

The Apparatus required.

Clean stoppered jars of suitable size. Viscera, etc., should not be placed in any preservative fluid, and should be sent as soon as possible after death.

The Importance of the Examination.

The importance of determining accurately the nature of a poison in a case of death from suicide or murder is obvious. Probably the commonest material that needs examination in this way is the stomach and its contents (page 77). Usually, however, it is necessary to analyse for poisons other organs, for instance, the liver for arsenic, mercury, lead; the kidneys for inorganic or alkaloidal poisons; the blood for diffusible poisons such as prussic acid; and so on. The importance of analysis of the hair in cases of chronic arsenical poisoning is mentioned on page 80. Some poisons may be found in the urine (page 112), others in the faeces (page 70). Nor is it only in connection with the viscera or other parts of the body that such analysis may be required. They may be needed in relation to the contents of some bottle, or some food stuff or a suspected powder, or some instrument, as in the case of the arrow-head poisons of the tropics. Any analyses of this kind can be carried out at the laboratories of the Clinical Research Association.

Note.—Owing to the trouble that is apt to arise in medico-legal cases when the actual examiner of a specimen has subsequently to attend at the Courts to give evidence, investigations that may possibly lead to the necessity for such attendance in Court can only be undertaken when a written guarantee accompanies the specimen to the effect that in case of attendance in Court being required a special fee will be paid for each day that such attendance is required.

SECTION XIX.

Post-mortem Examinations.

The Apparatus required.

The Clinical Research Association upon request sends out a man skilled in the removal of the viscera from the body for the purposes of examining them post-mortem. He brings with him all the necessary implements, and subsequently restores the organs in the same way as is usual at a hospital. The Association is also in a position to send a skilled morbid anatomist if desired. In medico-legal cases the necessary jars for receiving the gastric contents, and so forth, for analysis are supplied upon request. When required, analyses for poisons can be carried out at the laboratory (page 86). They are frequently required in connection with dogs and other animals as well as in the case of human beings. Histological preparations of organic lesions can be prepared (page 105), or morbid anatomy specimens can be mounted for museum purposes (page 85). Bacteriological investigations will be carried out upon the heart, blood, the spleen, or other parts in cases in which these may seem to be indicated.

The Importance of the Examination.

No stress need be laid upon the value of post-mortem examinations in all cases in which the diagnosis has been in doubt. In cases of suspected foul play, of sudden death, or of death by accident, and so forth, post-mortem examination is ordered by the coroner. The great assistance that can be rendered by a skilled post-mortem room attendant far more than counter-balances the small cost, and when the death has been due to some

septic cause, and the practitioner who is making the post-mortem examination has an extensive surgical or midwifery practice, and therefore desires to contaminate his hands as little as possible, if at all, such assistance is almost essential. It is only in special cases that the attendance of a skilled morbid anatomist is required as well.

SECTION XX.

Pus.

The Apparatus required.

The two chief methods of examining pus are by direct staining and by taking cultures. When direct staining is required, the best way of sending the specimen to the laboratory is to spread out a small quantity of the pus by means of forceps or a glass rod or some other similar instrument upon the surface of an absolutely clean microscope slide until a thin film has been made. This should be allowed to dry spontaneously in the air. When quite dry, each film should be wrapped carefully and separately in tissue paper, enclosed in a suitable small wooden box or tin, and sent in accordance with postal regulations. When cultures are required the pus should be collected upon a small sterile swab of cotton wool, similar to those used in obtaining throat swabbings (page 102). If no swab is available, the pus may be collected upon a small piece of lint or cotton wool, care being taken that nothing is used which has been impregnated with any antiseptic, for in the presence of the latter the micro-organisms will not grow when planted on culture media at the laboratory. The swab or piece of lint, or whatever it is upon which the pus has been collected, may conveniently be put into a sterile test tube, the neck of which is well plugged with sterile but not antiseptic cotton wool. The latter should then be lighted with a match, the flame being blown out when the surface of the cotton wool has been thus sterilised externally. The test tube should be enclosed in a strong box or tin and sent to the laboratory through letter post. When the amount of pus available is considerable, a quantity may be collected in a suitable small bottle, and sent as such to the laboratory, both bottle and

cork having been sterilised by boiling in plain water before use. It is sometimes an advantage to plant the pus directly on to the culture medium as soon after it is obtained from the patient as possible. For this purpose the Clinical Research Association will, upon request, supply the necessary tubes of culture media with cases for their transmission through the post. Printed directions as to their mode of use accompany the culture tubes.

N.B.—When it is subsequently decided to have a vaccine prepared from micro-organisms obtained from pus in this way, the cost to members of such preparation of vaccine (see separate Appendix) includes the above, a separate charge for the cultural investigation of the pus not being made.

NOTE.—In the case of unusual organisms in which special cultural investigations are required—for instance, for research purposes—special charges will be quoted upon application.

The Importance of the Examination.

In the case of any abscess, purulent discharge, or local septic focus, it is always important to know the precise nature of the causal organism. If bacteriological examinations of pus are carried out in all cases as a routine it is surprising how often the infection is found to be due to some unexpected bacterium. As instances of this, one need, perhaps, only mention how typhoid bacilli may be found in pure cultures in apparently simple or subcutaneous or other abscesses years after the original attack of enteric fever; how diphtheria bacilli may be present in pus coming from the ear or from the vagina or from an apparently simple impetigenous affection of the skin; how gonococci may be present in the pus of a conjunctivitis; how actinomyces may be found in pus from an abscess of the chest wall or of the neck; and how chronic or early glanders is only diagnosable by bacteriological examination of the purulent foci. Besides the commoner organisms such as streptococci, staphylococci, pneumococci, gonococci, and tubercle bacilli, less expected varieties may be found sometimes. Especially when vaccine treatment is to be adopted, too much stress cannot be laid upon the need for verifying the bacteriological diagnosis, not only by direct examination of pus films, but also by cultural methods.

One form of purulent infection may be mentioned in particular here, and that is, infective stomatitis, of which one form is better known under the term of pyorrhoea alveolaris. It is being recognised with more and more force that septic infection of the mouth is responsible not only for much general ill-health, but also for maladies which might at first sight appear to have nothing to do with the mouth at all—subacute and chronic arthritis of various types, arterio-sclerosis, and so on. In the treatment

of pyorrhœa alveolaris, local antiseptics may do much, but in many cases local applications by themselves fail to eradicate the disease, and they can then be much assisted by the use of vaccines. Before vaccines can be employed with any accuracy, however, the precise organism responsible for the pyorrhœa has to be determined; sometimes this is due to streptococci, sometimes to pneumococci, sometimes to Goadby's organism, and there are others in other instances. In a similar way recurrent influenza has repeatedly been traced to a persistent local infection of the mouth or nose, and the same probably applies to corresponding persistent local infection with the micrococcus catarrhalis in those who suffer from cold upon cold.

Examination of pus and purulent discharges by cultural methods as a routine procedure has not yet become sufficiently generalised in practice.

SECTION XXI.

Relative Activities of Antiseptics.

The value of the different antiseptic substances that are sold commercially is estimated by ascertaining the degree of dilution at which the substance in question will either actually kill or stop the growth of micro-organisms upon culture media, and, as a rule, the standard by which the strength of other antiseptics is expressed as that of known dilutions of carbolic acid incubated as controls under the same circumstances as the substance that is being tested. A great deal has been written upon the sources of fallacy that arise in making the tests. It is clear that the effectiveness of a given antiseptic will vary with the temperature, the nature of the organism that is used, with the chemical ingredients that are associated with the antiseptic, and the bacilli in the culture tube. Some antiseptics form a coagulum with albumen, so that whereas they may be very efficient in a non-albuminous solution, in a fluid containing albumen they are much less effective than are antiseptics that do not coagulate albumen. An antiseptic A may be more inimical to one micro-organism than is antiseptic B, but, on the other hand, antiseptic B may be more potent than antiseptic A against some other micro-organism. From facts of this nature it follows that

in determining the antiseptic properties of a given substance it is necessary that all the different conditions under which the tests were carried out should be stated. It also follows that in the selection of an antiseptic for a particular clinical, veterinary or public health purpose it is necessary to know exactly what are the effects of different antiseptics under the precise circumstances under which it is desired to make use of them. One or another antiseptic may be most efficient in disinfecting drains; another may be much more effectual in killing the micro-organisms in materials saturated with an albuminous discharge; yet a third may be the best to employ in the disinfection of other materials. The Clinical Research Association is in a position to carry out laboratory tests which will show the relative effects of different antiseptics under whatever particular circumstances it is desired to investigate them, either for clinical, public health, or veterinary purposes, or on behalf of manufacturers of the antiseptics themselves.

SECTION XXII.

Serous Fluids.

The Apparatus required.

Serous fluids, whether obtained from the pleural cavity, the pericardium, the peritoneum, or a hydrocele, are best transmitted to the laboratory in the same kind of bottle and package used for sending urine specimens (page 106). When bacteriological investigations are required it is important that aseptic, but no antiseptic, measures should be employed either in sterilising the skin or in conjunction with the needle and syringe or other instrument employed in obtaining the fluid from the serous cavity. The bottle to which it is transferred should be rendered aseptic by boiling in plain water; it will dry spontaneously and quickly if it is held upside-down for a few minutes after it has been taken out of the boiling water. The cork should also have been boiled thoroughly, and it should not be touched by any but aseptic fingers afterwards. The fluid having been transferred to the sterile bottle, and the latter corked securely with the sterile cork, the specimen should be put into a tin supplied for the purpose and sent to the laboratory by letter post.

The Importance of the Examination.

Chemical, microscopical, and bacteriological examination of serous fluids may sometimes be of extreme importance in diagnosis in distinguishing non-inflammatory transudates from inflammatory exudates and in determining whether a given effusion is probably tuberculous or malignant or due to some other cause. Books have been written upon this subject alone, so that it is scarcely possible to do more than indicate here some of the ways in which the examination may be of value.

The more an effusion, whether into the pleural cavity or into that of the pericardium or the peritoneum, approaches a transudate such as may be found in cases of anasarca from heart failure, or from some other similar cause, the lower will the specific gravity tend to be, the smaller the total percentage of coagulable proteid, the less the tendency to spontaneous clotting, and the fewer the cell elements seen in films prepared from the centrifugalised deposit. On the other hand, in cases of inflammatory or malignant effusion the specific gravity tends to be higher and higher the more active the inflammation, and at the same time the percentage of coagulable proteid generally is high, the liquid separates from itself a spontaneous coagulum, and the centrifugalised deposit shows numbers of leucocytes, peritoneal cells, or, in malignant cases, particles of new growth. There are many intermediate cases in which by analysis of the fluid alone it may not be possible to distinguish with certainty between an exudate and a transudate, but if clinical details are given at the same time it is generally possible to give a pretty definite opinion from the results of analysis made in the laboratory.

The discovery of particles of new growth in the fluid proves conclusively that there is malignant disease affecting the serous membranes in question.

In tuberculous cases the centrifugalised deposit does not contain a very large number of cells, as a rule, but those that are present generally exhibit a preponderance of small lymphocytes.

In inflammatory affections due to other bacteria, such as the streptococci, staphylococci, pneumococci, and other similar micro-organisms instead of the small lymphocytes, the polymorpho-nuclear cells predominate.

The nearer the relative percentage of serum albumen and serum globulin in the fluid approach to the proportions of these in ordinary blood serum the more likely is the effusion to be a transudate and not an exudate, whilst conversely there is evidence to show that in inflammatory conditions serum albumen is present in relatively larger quantities than serum globulin. Sometimes the fluid does not coagulate spontaneously, but can

be made to do so by the addition of small quantities of fibrin ferment showing that the fluid contains fibrinogen. This is particularly apt to be the case in hydrocele fluid.

Occasionally it is possible to find tubercle bacilli on direct examination of the centrifugalised deposit in cases of tuberculous pleurisy or tuberculous peritonitis. More often, however, the bacilli are relatively so few in number that it is necessary to inoculate a guinea-pig with some of the centrifugalised deposit obtained with all aseptic precautions in order to decide beyond any doubt whether the lesion is tuberculous or not (page 80).

When ascites is secondary to malignant ovarian cysts, this fact may be indicated by the presence in the ascitic fluid of paramucin (page 61).

In cases of inflammatory effusions other than tuberculous, the centrifugalised deposit can be cultivated in the laboratory, and growth of the causal micro-organism obtained. The absence of resultant cultures does not signify that no micro-organism was originally present, however, for serous effusions have considerable antiseptic properties, and the infecting organism may have become devitalised before the cultures could be made. In such cases, however, it is not infrequently possible to find the micro-organisms by direct staining of films prepared from the centrifugalised deposit; streptococci may be discovered in this way, for instance, and so may pneumococci, and others. It is noteworthy that effusions may be due to organisms which ordinarily produce pus, and yet the numbers of these present may not have been sufficient to render the fluid more than slightly opalescent, so that the condition, though due to a pus-forming organism simulates a non-purulent effusion. This is particularly the case with the pleural cavity, in which an inflammatory serous exudate can often be demonstrated to contain micro-organisms before actually developing into an empyema.

In some affections there is a considerable preponderance of coarsely granular eosinophile corpuscles in the centrifugalised deposit. These have been recorded in malignant cases, but it would not be possible from them alone to tell that the condition was necessarily malignant. Their discovery, however, would cause one to look with particular care for other evidence of malignant disease in the case.

Any effusion may become contaminated, even at a first tapping, with small quantities of blood, particularly if the last portions removed from the serous cavity are included in the specimen examined. If, however, at a first tapping the fluid contains obvious blood throughout, grave suspicion of malignant disease will be aroused, and the centrifugalised deposit should be examined with special care for particles of new growth.

Stress is laid by some authors upon the value of analyses of serous fluids, both for the total inorganic residue and also for the relative proportions of salts in this residue. This subject has not been worked at very extensively as yet. It would form an admirable subject for research work, but what clinical deductions can be drawn from the results can scarcely be laid down clearly yet.

Hydatid hooklets in the centrifugalised deposit afford conclusive proof of the effusion being due to hydatid disease.

Effusions are sometimes milky from the amount of fat suspended in them; chylous ascites is not uncommon; chylous effusions into the chest or pericardium are rarer. One of the commonest causes of chylous ascites is infection by *filaria sanguinis hominis* in the tropics, and the discovery of fluid of this kind would lead one to examine the blood for the *filaria* embryos (page 27). There are, however, other causes of chylous ascites, such, for instance, as injury to the abdomen with rupture of the lymphatics or of the receptaculum chyli; malignant disease of the thorax obstructing the thoracic duct; and occasionally it is due to chronic parenchymatous nephritis in some way which is not yet understood. The fat present takes different forms, and sometimes only part of the chylous appearance is due actually to fat, the greater part of the milkiness of the fluid being due to altered proteid and not fat. To the latter condition the name of pseudo-chylous ascites is sometimes given; it is distinguished from true chylous ascites only by direct staining of the fat particles with Sudan III., osmic acid, or other fat stain, or, better still, by chemical estimations of the total fat present in the fluid.

The above are some of the ways in which laboratory investigations of serous effusions may be valuable; others will suggest themselves in individual cases.

SECTION XXIII.

Skin.

The Apparatus required.

In the case of purulent infections of the skin the preparation of microscope films will suffice for direct examination for bacteria. If cultural methods are to be employed, swabbings from the

purulent surface should be sent in a suitable small glass bottle. Portions of excised skin obtained by biopsy should be sent to the laboratory in the way described for histological specimens generally (page 105).

The Importance of the Examination.

The importance of identifying the exact nature of any animal or vegetable parasite of the skin needs little emphasis. Similarly it is clearly important to know what is the bacterium that is causing an inflammatory or pustular affection of the skin. The significance of the bacillus acnes in connection with acne vulgaris has been well established, and successful vaccine treatment of this common but troublesome malady seems to require that the products of the bacillus acnes should be used as well as those of staphylococci. The value of staphylococcal vaccines in the treatment of chronic staphylococcal pustules and subcutaneous abscesses has been demonstrated again and again. There have been similar good results in the treatment of streptococcal inflammations of the skin, including erysipelas and in those due to the bacillus pyocyaneus. It is less generally recognised that the diphtheria bacillus may be responsible for very severe dermatitis, and that this having resisted ordinary forms of treatment may be combated by the use of anti-diphtheritic serum which would probably not be employed unless the bacteriological nature of the malady had been demonstrated. The fact that the bacillus coli communis, the gonococcus, the typhoid bacillus, may produce skin lesions has been recognised of recent years; the nature of the lesions cannot be diagnosed without cultural tests. The great importance of recognising anthrax at the earliest possible moment by bacteriological examination of the malignant pustule lies in the fact that early treatment by Sclavo's serum, with or without excision, leads to rapid cure, whilst if the nature of the spot is not recognised until later, anthrax septicæmia may ensue and life be lost. Glanders of the skin in man is only recognisable with certainty by bacteriological methods, though the patient's employment may suggest the nature of the complaint.

The detection of the spirochæta pallida may clinch the diagnosis when a syphilitic sore simulates one which may otherwise be thought for the time being to be only a soft sore. The spirochætes may be demonstrated before the Wassermann reaction (page 42) is positive. When there is suspicion that a resistant eruption upon the finger, a lip, an eyelid, or elsewhere, may be an extra-genital chancre, the discovery of the spirochæta pallida may be invaluable in clinching the diagnosis early.

The non-discovery of tubercle bacilli in a skin lesion does not prove that it is not tuberculous; frequently the local affection seems to be due to the tuberculo-toxins rather than to the bacilli themselves. Even in lupus vulgaris tubercle bacilli may be absent from the superficial parts that are open to examination; the demonstration of the bacilli, however, clinches the nature of the affection in other cases.

Leprosy is fortunately very rare in this country, but there are places where it is common, and the diagnosis may be in doubt unless the bacillus lepræ can be demonstrated either in the discharge or in a small portion of skin excised.

In the bullous dermatoses, the fluid in those blebs which develop spontaneously sometimes abounds in eosinophile corpuscles. The discovery of the latter may be of material assistance in deciding that the lesion is due to pemphigus or one of the dermatoses related to it, and that it is not an artefact. Curiously enough although the spontaneous bullæ in pemphigus may contain fluid rich in eosinophile corpuscles, artificial blisters in a similar case do not contain these eosinophile corpuscles in the same way. As a rule, in the bullous dermatoses the blood exhibits eosinophilia at the same time (page 18).

Biopsy of the skin, that is to say, excision of a small portion under a local anæsthetic, is a clinical method of diagnosis that is being resorted to with increasing frequency especially when it is desired to distinguish between an inflammatory or simple ulcerative condition and one that is due to epithelioma, rodent ulcer, syphilis, tuberculosis, leprosy, or actinomycosis. Skin biopsies are also being employed to an increasing extent in research work in connection with various diseases of the skin.

SECTION XXIV.

Sputum.

Transmission to the Laboratory.

Sputum is best sent to the laboratory in a small glass bottle well corked and enclosed in a tin and wrapper which will not become crushed in the post. Special bottles and tins for the purpose, ready addressed, are supplied from the laboratory, but

in the absence of these any suitable small bottle can be used, but the postal regulations must be strictly followed. If cultural investigations of the bacteria in the sputum are to be made, both the bottle and the cork should be sterilised by boiling before the sputum is introduced into it.

Specimens should be sent by *letter* post.

The Importance of the Examination.

There is little need nowadays to insist upon the great importance of sputum analysis in connection with the verification of the diagnosis of phthisis. It is most important that it should be realised, however, that when a physician gives a sputum bottle to the patient and asks him to send the next day's sputum off in it to the laboratory, the patient himself may sometimes expectorate little but saliva or the discharge from the posterior nares into the bottle, and then it not only may, but it does happen continually, that no tubercle bacilli are found, when if *sputum* had really been sent it would have been found to contain them in abundance. The laboratory is only able to report upon the specimen that it receives. It is no fault of the laboratory that it fails to find tubercle bacilli in a specimen which is not really sputum at all, although there are sometimes complaints that the so-called "sputum" having been analysed, and no tubercle bacilli having been found in it, the patient, nevertheless, was proved subsequently to have been suffering from tuberculosis of the lung. Whenever possible the physician should himself see the sputum which the patient sends to the laboratory, and, better still, should see the way in which it was obtained in order to be sure that it really came from the lung and not from the mouth or the naso-pharynx. It is a very serious source of fallacy to assume that that which the patient himself calls sputum really is sputum.

Even, however, when actual sputum is obtained and sent to the laboratory, tubercle bacilli may not be present in the particles sent up, and the report will then be negative though the patient really has phthisis. It is very important that this should be borne in mind and that no great reliance should be put upon a single negative examination in a patient in whom phthisis may be suspected upon other grounds. On the other hand, a positive result of the examination is not only almost absolute proof of the existence of pulmonary tuberculosis, but it is practically the only absolute proof of the presence of the disease. Tubercle bacilli may be found in sputum at a stage so early that no abnormal physical signs are to be detected at all. On the other hand, a patient may seem to have all the symptoms

and physical signs of phthisis and yet the lesion may not be tuberculous at all; apical phthisis, for instance, may be simulated by obstruction to a bronchus by a thoracic aneurysm. The extreme importance of having any kind of suspicious sputum examined for tubercle bacilli cannot be insisted upon too much, for by so doing it happens repeatedly that phthisis is discovered in an early and curable stage when without sputum analysis the patient might have been allowed to drift into an incurable stage before the real nature of the malady was ascertained.

It is realised far too seldom, however, that one cannot tell by the discovery of tubercle bacilli alone in the sputum whether the disease is active and progressing, stationary, or even retrogressing towards cure; for when tuberculous granulation tissue is present in the wall of the cavity, the discharge from this may continue to contain tubercle bacilli for months or years without the disease having extended at all in the meantime, just in the same way that the discharge from a similar tuberculous ulcer of the skin may continue for months and contain tubercle bacilli all the time, though the ulcer may be even smaller at the end of that time than at the beginning. Real proof that, when a tuberculous lesion is present in the lung, it is actually extending is afforded by the discovery of elastic fibres in the sputum. The presence of elastic fibres means that lung tissue is being destroyed; in other words, that the disease is advancing. Far too seldom is the request for the examination for elastic fibres made at the same time as the request for the detection of tubercle bacilli. It should be almost a routine procedure in phthisical cases to have sputum specimens tested periodically for elastic fibres as well as for tubercle bacilli.

Almost equally important, especially in cases in which there are cavities secreting pus, is the determination of the degree to which the purulent process in the lung is due to tubercle bacilli only or to a super-added infection by streptococci, pneumococci, influenza bacilli, sarcinæ, tetrads, streptothrix organisms, and so forth. Opinions differ as to the relative importance of these various micro-organisms, and no absolute law can be laid down, but there is a general consensus of opinion that when the process in the lung is due to tubercle bacilli without the secondary organism there may be comparatively little pyrexia or even none at all, and the general health need not deteriorate, but that when secondary infection takes place there is super-added to the clinical picture of the purely tuberculous phthisis the much graver symptoms of phthisis with hectic and cachexia. This is exactly comparable to the condition of a psoas abscess when this is purely tuberculous to begin with, but is apt subsequently to become infected by pyogenic organisms.

The importance of this lies largely in connection with prognosis and treatment. The prognosis is very much worse when secondary infection has taken place, and there is a widespread feeling that the treatment should include the use of vaccines prepared from the secondary infecting organisms in addition to or even instead of the use of the tuberculin which might be employed when the process was purely tuberculous. In order to determine what vaccine should be used, the exact nature of the secondary infecting organism needs to be ascertained by cultural examination at the laboratory, and it is comparatively easy to prepare mixed vaccines in suitable doses from the patient's own organisms (see page 121).

The possible value of determining whether a given sputum contains blood or not is obvious. This blood may not be red to the naked eye, in which case other tests for it are required. On the other hand, sputum may be red from other causes than from the presence of blood. Cases have been recorded recently of red sputum due to the presence in it of pigments of micro-organisms. The diagnosis of hæmoptysis had been made until blood was shown spectroscopically not to be present, whilst cultural methods of examining the sputum isolated the pigment-forming organism.

Coarsely granular eosinophile corpuscles often abound in the pellets of sputum that are brought up in true bronchial asthma as distinct from other varieties of dyspnoea that simulate asthma, so-called "cardiac" asthma, "thymic" asthma, "renal" asthma, and so on. Eosinophile corpuscles in the sputum in true asthma are generally associated with eosinophilia in the blood (page 18).

Curschmann's spirals, Charcot-Leyden crystals, and Dietrich's plugs are regarded by some as important evidence of asthma. They are interesting bodies, but whether they are really of diagnostic importance it is difficult to say.

The exact verification of the nature of different yeasts, moulds, and so forth, in the diagnosis of such maladies as actinomycosis of the lung, aspergillosis, and so forth, can hardly be overstated, for although these maladies are not very common, they do occur from time to time, and they are apt to be mistaken for phthisis unless careful bacteriological investigation of the sputum is carried out as a routine practice in lung cases.

One would like to insist upon the possible value of the presence of the Bordet-Gengou organism of whooping cough in the diagnosis of this malady, but as the micro-organism disappears from the sputum after the first few days, or at least cannot be found on account of its being overpowered by the other bacteria present, it seldom occurs to one to have it examined for early enough to be of value. It may be borne in mind, however,

that whooping-cough is due to this organism, and that the serum of a patient suffering from the malady gives a clumping reaction with it. In certain cases in which only a doubtful whoop or two has been heard, and yet in which it is very important to know whether the cough was due to pertussis or not, this serum reaction (page 40) may be of material importance.

Pneumonia is now known to be due to several different organisms, and as there are many cases in which vaccine treatment has been beneficial, it is important that the pneumonia should be verified not merely by physical signs, but also bacteriologically as to its kind. Some authorities prefer to obtain the offending organism by needling the affected part of the lung through the chest wall after the skin has been rendered aseptic. This is probably a better plan than direct sputum analysis, from the bacteriological point of view, but it is not always possible nor even advisable in practice, so that one has to fall back upon direct and cultural investigations of the sputum to determine whether the pneumonia is due, for instance, to the pneumococcus of Fraenckel, the pneumo-bacillus of Friedlander, the influenza bacillus, or in rarer cases to the Klebs-Löffler bacillus of diphtheria, the typhoid bacillus, or even the bacillus coli communis. If typhoidal pneumonia should be suspected, direct blood cultures may be of assistance (page 46) or the Widal test (page 33) may be positive.

The possible value of albumen in the sputum has been pointed out by some observers. If a comparatively non-purulent sputum containing no tubercle bacilli is found to be rich in albumen coagulable by heat, it is more likely that the patient has tuberculosis of the lungs than if that sputum contained little or no coagulable albumen, as in the case of bronchitis.

SECTION XXV.

Stains on Materials.

The Apparatus required.

No special apparatus is required for transmitting stained material to the laboratory. If the stain is thought to be pus

from which cultural investigations are required, the fabric should be enclosed in a sterilised bottle or box, otherwise the material may be sent in an ordinary package by letter post, provided the postal regulations (page 126) are strictly followed.

The Importance of the Examination.

Stains upon instruments or fabrics are not infrequently of great medico-legal importance, particularly in regard to their being due to blood in cases of suspected murder, or semen in connection with alleged rape. There are certain chemical tests for semen, but the only real proof of its nature is the discovery of actual spermatozoa in the stain; these are pathognomonic. Blood may be detected by chemical, spectroscopic, and microscopic tests. Until recently it was only possible to say whether a given stain was blood or not. It has now been made feasible to determine not only whether a given blood stain is human or not, but if not human, the nature of the animal from which it is derived can be ascertained if sufficient time can be given to the investigation. If an animal of one species is inoculated with blood from an animal of another species, the serum of the first develops specific precipitins which will give certain reactions with the blood of an animal of the second species, but not with that of animals that are not of the same species. This affords a means of detecting the species from which a given specimen of blood was derived. A series of sensitised animals is required, and the procedure is both complex and expensive, but the possible importance of the result in medico-legal cases is obvious. The accused person may attribute blood stains upon his clothes to his having been killing a pig. If by the application of the precipitin tests it can be proved that the blood upon his clothes is not pig's blood, but human blood, the significance of this needs little comment.

The investigation of pus upon fabrics is seldom satisfactory. Linen stained with a vaginal or urethral discharge is sometimes sent to the laboratory with a request that it may be examined for gonococci; in a certain proportion of cases these may be found, but when they are not detected it by no means follows that the discharge was not gonorrhœal. Far more satisfactory than a stained fabric for this purpose is a pus film prepared in the ordinary way upon a microscope slide direct from the patient's lesion.

The determination of the nature of stains that are due to paint, fats, grease, and other things of that kind, is generally of negative value, particularly in excluding their being due to blood or semen.

SECTION XXVI.

Throat Swabbings.

The Apparatus required.

The Clinical Research Association supplies each of its members with one of its registered sterile swabs in a metal case, and when a used swab is returned to the laboratory a fresh one is despatched to the sender without extra charge, so that members can rely on always having one in hand ready for use. Any number can be sent upon request at a charge of 1s. each to members, or on loan for a short period without charge.

At the time of their use, the end of the metal case should be unscrewed and the swab withdrawn, particular care being taken that it touches no contaminating surface before it is actually used in the throat. The specimen should be secured, if possible, before any local antiseptic has been used; failing this, an interval of six hours should be allowed to elapse from the last application of the antiseptic before the swab is taken, otherwise the small amount of antiseptic that may be present will be apt to inhibit the growth of the organisms present, and the report may then be negative, even though the case be really one of diphtheria.

In obtaining a specimen, the patient should be placed in a good light, the tongue depressed, and the cotton swab gently but freely rubbed with a rotatory movement upon the affected area, or against and beneath the membrane if any is visible. If a sterile swab is not at hand, a fairly good substitute may be improvised by means of forceps and a pledget of un-medicated cotton wool, or a camel-hair brush and a test-tube may be employed. Sufficient cotton-wool is wrapped round the handle of the brush to form a plug for the test-tube; a little water is placed in the tube, and the brush and plug are introduced. The water is now boiled over a spirit flame to sterilise the interior and the contents of the tube. The plug is removed for a moment and the water poured away, after which the apparatus is ready for use.

In some cases a portion of the exudation may be loose enough to be picked up by means of forceps, after they have been sterilised by boiling or by heating in the flame of a spirit lamp. The exudation thus removed should be placed in a perfectly

clean, dry, wide-mouthed, and well-corked bottle, both cork and bottle having been sterilised by previous boiling; no preservative should be added.

It is particularly in regard to determining whether diphtheria bacilli are present or not that throat swabbings are required; sometimes, however, throat swabbings need investigation for other organisms such as streptococci, staphylococci, or pneumococci.

The Importance of the Examination.

The great importance of the bacteriological examination of throat swabbings lies in connection with the exclusion or diagnosis of diphtheria. Especially in schools and institutions, outbreaks of diphtheria are generally preceded and accompanied by cases of sore throat, which clinically may seem simple or at any rate non-diphtheritic, but which bacteriologically have been shown again and again to be mild cases of diphtheria. Convalescent cases of diphtheria may still harbour virulent diphtheria bacilli in their throats from which other cases may become infected. It is hardly too much to say that every case of sore throat should be investigated bacteriologically by means of throat swabbings. The cost is very slight and the inconvenience to the patient practically nil.

The examination is quite as important in the exclusion of diphtheria as in its diagnosis. Certain cases of pneumococcal or streptococcal sore throat may simulate diphtheria closely, just as many cases of apparently follicular tonsillitis may really be diphtheritic. Vincent's angina in particular may resemble diphtheria so closely as to be indistinguishable from it unless the characteristic spirilla and fusiform bacilli are discovered in films prepared from the swabs.

All cases of diphtheria are considered urgent, and in those examined for the first time a telegraphic report is despatched at latest on the morning after the receipt of the specimen at the laboratories. Those sent for re-examination are reported upon by post only, unless a telegraphic report be specially requested. In first examinations, if the specimen arrive between the hours of 8 a.m. and 6 p.m. a direct investigation of a smear preparation from the swab is always made, and by the Clinical Research Association's modification of Neisser's method of staining it has proved possible to send immediate reports upon 65 per cent. of all positive cases. Cultures from all primary specimens arriving by the first post are examined the same evening, when most positive cases which have not shown Klebs-Löffler bacilli by direct examination can be reported upon. Swabs received later in the day up to the last post in the evening, and all those for

re-examination, as well as such as prove negative on direct examination or after nine hours' cultivation, are reported upon early the next morning, except in a very few instances where the growth is so scanty that nine hours' further incubation is considered advisable. The results of direct examinations are always confirmed by cultivation, but the method of direct staining has been found so reliable that an immediate report is always sent on such cases as are found to be positive.

There is still much confusion as to the supposed relation of Hofmann's bacillus to diphtheria and to the Klebs-Löffler bacillus. Both organisms are often present in cases of diphtheria, and it has frequently been noticed that the former tends to persist in the throat for longer or shorter periods after the Klebs-Löffler bacillus has disappeared entirely. From this peculiar association of Hofmann's organism with the Klebs-Löffler bacillus it is sometimes held that the former is merely a non-virulent modification into which the latter gradually becomes converted under certain conditions, and in view of this opinion some bacteriologists in reporting upon cases of diphtheria draw no distinction between the two organisms. It is, however, a fact that Hofmann's bacillus differs from the Klebs-Löffler bacillus not only in the absence of virulence towards animals, but also in its morphological and cultural characters, and all attempts to convert the one organism into the other have been quite unavailing, or at least have given entirely inconclusive results. In addition to this, Hofmann's bacillus occurs frequently in healthy throats, and is almost constantly present on the nasal mucosa.

From these considerations it appears imperative that a distinction be drawn between the two organisms in practice. Taking all the evidence into account it may be concluded that Hofmann's bacillus is a distinct organism which has no ætiological relation to diphtheria, and that only those cases in which Klebs-Löffler bacilli are present should be considered as actually diphtheritic. This may be regarded as the commonly accepted view at the present day.

Confusion has, in the past, to a great extent arisen from the difficulty experienced in the detection of a few Klebs-Löffler bacilli when large numbers of Hofmann's bacilli happen to be present at the same time. On account of this difficulty it is sometimes necessary to give a guarded report upon such cases.

For the purpose of telegraphic reports the following code has been adopted: When the Klebs-Löffler bacillus is found the telegram is worded "Organism present." When the Klebs-Löffler bacillus is not found it is worded "Organism not found."

It is hardly necessary to state that a negative report does not conclusively disprove a diagnosis of diphtheria.

SECTION XXVII.

Tumours and other Tissues.**The Apparatus required.**

Suitable bottles containing the appropriate preservative fluid are issued by the laboratory for the purposes of transmitting specimens of which histological examination is required. A piece of the affected tissue, not exceeding an inch in length and half an inch in breadth and in thickness should be put into the fixing fluid in the bottle, and the latter sent to the laboratory with as little delay as possible in the tin which is already addressed. In the case of tumours, the part for section should be selected from the edge of the new growth, and whenever possible some of the adjacent healthy structure should be included on one side. In the case of skin growths it is of great importance to excise a portion at its base; a mere scraping from the surface is generally of little use, it is usually desirable to send the whole tumour. When the fixing fluid has to be made up at home, the best to use consists of 70 per cent. of methylated spirit with 30 per cent. of water.

When an immediate report of the nature of a tumour is required, a thin piece of the tissue can be hardened rapidly in the laboratory and examined by the paraffin method in about forty-eight hours. Though this method serves a very useful purpose for determining quickly the nature of a suspected growth, yet it should be remembered that the results are not nearly so satisfactory as when the tissue has been fixed and embedded slowly—a process which involves a delay of from five to seven days. When it is desired that a rapid examination should be made, this should be stated upon the slip accompanying the specimen, otherwise the ordinary routine, which takes about a week, is adopted.

Delicate tissues such as brain or spinal cord should not be placed in spirit. Satisfactory results are best ensured by hardening small fragments of the diseased tissue in Muller's fluid after placing them in a solution of 2 per cent. formalin in normal saline for twenty-four hours. Eyes should be fixed and hardened in 10 per cent. formalin in normal saline, and the eye should be pricked with a needle in several places to allow the hardening fluid to reach the interior quickly.

Unless other instructions are sent with the specimen, the ordinary method of staining with hæmatoxylin and eosin is that

which is employed. Upon request, however, any other staining method that may be desired will be carried out. When spinal cord or brain tissues are to be stained by the Weigert-Pal or Marchi method the time required extends to several weeks. The same applies to specimens containing spicules of bone or calcareous deposits which need to be decalcified before sections can be cut.

In some cases surgeons require rapid and immediate examination of a portion of a tumour actually during an operation in order to settle whether the lesion is malignant or otherwise, and thus to decide exactly to what extent excision should be carried out. If arrangements can be made beforehand, the Clinical Research Association will send a skilled microscopist with all the necessary apparatus for making sections by the freezing method in this way.

The Importance of the Examination.

Little stress need be laid upon the value of determining the exact nature of tumours and other lesions obtained either by operation or after death. It is often very difficult to be sure whether a given mass is tuberculous, inflammatory, or malignant, without histological examination. The great value of immediate examination of portions of a tumour during the course of an operation is being recognised more and more, and sometimes the nature of the operative procedure adopted is materially modified by the microscopist's report, whilst the patient may be saved from the need for a second anaesthetic.

SECTION XXVIII.

Urine.

The Apparatus required.

For ordinary examination of the urine it is sufficient to send a sample, preferably not less than 6 ounces in a clean glass bottle, securely corked and enclosed in a suitable tin or box which will not become crushed in transmission through the letter post. The Clinical Research Association supplies bottles and tins and postal packets for the purpose, which should in all cases be used in order to comply with the postal regulations. If no bacteriological examination is required, the bottle need not be

sterilised, but, particularly in hot weather when the micrococcus ureæ may multiply very rapidly in the urine during the time of its transmission to the laboratory, it is advisable to add a little thymol or boric acid powder to the specimen to prevent putrefaction. Delicate structures such as renal tube casts are apt to disintegrate before the specimen is examined if this precaution is not adopted.

When bacteriological examination of the urine is required, particularly if cultural methods are to be employed, it is essential that a catheter specimen should be obtained, no antiseptic being employed. The first few drops that come through the sterile catheter should be discarded, the rest being collected in a urine bottle which, together with its cork, has been sterilised previously by boiling in plain water. No boric acid, thymol, or other antiseptic should be added to a specimen from which cultures are to be made.

Even when no bacteriological examination is needed, the specimen should be collected as fresh as possible. Urine which has been standing in a receptacle for some hours may have begun to decompose already before it is put into the urine bottle.

When quantitative analyses are desired, it is necessary that the total urine passed per diem should be measured. The total twenty-four hours' urine should be collected in a clean vessel provided with a covering, the whole stirred well, especially if there is any deposit, the bulk measured, and a sample of it, not less than 6 ounces, sent to the laboratory. If more than two ingredients are to be estimated quantitatively an even larger specimen is advisable.

It is a great help in the interpretation of the results to have the sex of the patient stated, together with brief notes of the case, especially if it is of an unusual character.

In the absence of instructions the urine is tested for reaction, specific gravity, albumen, and sugar, and the deposit obtained by centrifugalization is examined microscopically. Other investigations, qualitative, or quantitative, chemical, microscopic, or spectroscopic, are undertaken as desired.

The Importance of the Examination.

A whole book might be devoted to discussion of the various ways in which different urinary examinations may be of clinical importance. The more ordinary circumstances in which testing of the urine is of value are familiar to all. One need, perhaps, say little about them, but it is important to lay particular stress upon some.

Every specimen of urine containing albumen should be examined microscopically for tube casts. This is not done with as

much regularity as it should be. It is important to remember, however, that specimens sent to the Clinical Research Association are centrifugalised by electrically driven plant, which brings down practically everything in the specimen, so that even in a healthy urine an occasional tube cast and an occasional red blood disc may be seen, and, therefore, a note is appended to the report stating whether the numbers found are within the normal limits or whether they are sufficiently numerous to indicate pathological changes in the kidneys. In connection with life insurance examinations, the value of microscopical examination of the centrifugalised deposit in picking out cases of simple adolescent albuminuria from those in which the symptom indicates gross renal changes is great.

A trace of albumen in the urine is sometimes the first clue to a malady which is now being recognised with increasing frequency, namely, coli bacilluria. The urine in these cases seldom gives a definite deposit of pus, but the diagnosis is arrived at by finding microscopically an excess of leucocytes in the centrifugalised deposit, together with the presence of bacilli which can be demonstrated to be the bacillus coli communis when cultures are made from a catheter specimen.

The value of testing for acetone and diacetic acid in cases of glycosuria is great, for glycosuria without this evidence of acidosis is a much less serious affection than glycosuria with acidosis. The degree of the latter may be determined by estimating the total acidosis products in the urine, or some measure of it may be made by estimating the total ammonia in the urine, this increasing with increasing acidosis, and corresponding with the increased danger to the patient.

The value of determining exactly the nature of any peculiar colouration of the urine is almost obvious.

The discovery of urobilin in the urine may afford confirmation of suspected cirrhosis of the liver in a case, for example, where there has been profuse hæmatemesis and it is uncertain whether this is due to gastric ulcer or to cirrhosis. Of all the profound anæmias, pernicious anæmia gives rise to the largest amount of urobilin in the urine, a fact which may assist the diagnosis in doubtful cases.

Hæmatoporphyrinuria may be mistaken for hæmaturia unless the nature of the pigment is determined accurately; when it occurs it may afford proof that a dangerous dose of some drug such as sulphonal has been taken.

Bile pigment, when present in abundance, is detected easily, but in smaller amounts it is less easy to demonstrate by rough and ready tests than it is by the technique of the special laboratory. Moreover, other pigments may simulate bile.

Methæmoglobinuria may simulate hæmaturia unless the spectroscopic tests are positive, and the absence of a corresponding proportion of red corpuscles in the centrifugalised deposit should be confirmed at the same time.

Various drug reactions may simulate bile pigment in the urine or even hæmaturia, and sometimes it is important, by means of urine tests, to determine whether a patient has been taking the drugs which have been ordered, potassium iodide, for example, salicylates, or arsenic, and so forth.

The eye may have been removed for a new growth; there may be a suspicion that there is recurrence in the liver or elsewhere in the body; the discovery of melanin in the urine may serve to confirm the suspicion earlier than might be possible in any other way.

The diazo reaction used to be employed mainly in the early diagnosis of typhoid fever; it has been replaced in this respect by the Widal test (page 33). The diazo reaction still has a clinical value, however, and particularly, perhaps, in relationship to phthisis. A consumptive patient whose urine gives the diazo reaction is doing badly, whereas one in which the diazo reaction has been present, but in whom it subsequently disappears, is doing better.

The significance of indicanuria may be considerable, in that it is to some extent a measure of the putrefactive changes occurring in the bowel. Small quantities of indican are present in the urine of healthy persons, but larger quantities occur only when there is chronic constipation, colitis, or other intestinal lesion productive of auto-intoxication. The measure of the degree of this is afforded by estimations of the ethereal sulphates and of the total sulphates in the urine. In health, the ratio of ethereal sulphates to total sulphates should not exceed 1 to 10; a higher ratio indicates intestinal auto-intoxication.

The reaction of the urine depends largely upon the dietary; the more meat or other proteid in the food the more acid the urine, broadly speaking; vegeterians may pass persistently alkaline urine. There are not many conditions in which it is important to determine exactly the total acidity of the urine unless possibly in connection with original research work upon the effects of dietetic or drug treatment. There are, however, some occasions when it is important to know exactly how acid a urine is, especially when it is desired by medicinal treatment to increase or diminish it as the case may be. In the administration of urotropin, for example, the effects upon the bacilluria or pyuria for which the drug is being given are greatest when the urine is distinctly acid, and to attain this end, sodium dihydrogen phosphate is often given at the same time as the urotropin; to

determine how much of this salt should be given daily, estimations of the resultant acidity may be required. Again, irritability of the bladder or some degree of actual urethritis may be produced by urine which is constantly too acid; the exact degree of this acidity should be determined if scientific accuracy is desired in correcting it by the administration of such a drug as bicarbonate of soda.

Microscopical examination of the centrifugalised deposit probably affords more valuable clinical information than any other single laboratory method. Stress has been laid above on the importance of determining whether pathological numbers of renal tube casts are or are not present in every case of albuminuria. The characters of the casts, whether they are hyaline, granular, or epithelial, or composed of red corpuscles, leucocytes, or bacteria, may indicate the nature of the renal inflammation. Without microscopical examination it is very difficult to detect small quantities of pus. Mucus may be mistaken for pus until microscopical examination has been made.

Crystals of various kinds can seldom be detected except by the use of the microscope. The various kinds that may be present include calcium phosphate, calcium carbonate, uric acid, ammonium magnesium phosphate, magnesium phosphate, calcium oxalate, cystin, tyrosin, leucin, sodium urate, and ammonium urate. In cases of suspected calculus the probability of the diagnosis being correct may be afforded by the discovery in the centrifugalised deposit of small conglomerations of crystals associated with tailed cells denuded from the renal pelvis together with red blood corpuscles in smaller or larger numbers; and the nature of the calculus may be established from the crystals found.

Mucous cylindroids may be mistaken for renal tube casts by those who are not frequently making examinations under the microscope.

Cells other than pus cells or renal cells may or may not be of pathological significance; large squames are almost constant in the urine of women. Occasionally vesical cells may be seen even in a specimen from a healthy person; larger numbers may indicate catarrh or ulceration of the bladder. Renal cells may be found, sometimes in large numbers, in cases where there may be no casts. Particles of new growth may be found in cases of renal or vesical neoplasm, benign or malignant. Bilharzia ova, when present, afford the only certain means of diagnosing this parasitic infection. In rare instances, hydatid hooklets or even the scolices of the echinococcus are detected. The appearances of the centrifugalised deposit may at once suggest the need for making films which can be stained by the Ziehl-Nielsen

method for tubercle bacilli, the discovery of which micro-organism affords the best proof that a renal or vesical lesion is tuberculous.

Nucleo-proteid is generally present in very small amounts in normal urines. Larger quantities of it are generally associated with minute traces of albumin, and the association of the two together generally points to there being some irritation of the urinary passages, a not infrequent concomitant being an excess of calcium oxalate crystals which are the cause of the irritation. Nocturnal enuresis in young persons, irritability of the bladder in older persons may be the chief clinical symptoms produced. In adult males the centrifugalised deposit often contains numbers of spermatozoa at the same time.

Malarial pigment granules sometimes occur in the urine in abundance. Their detection may point to the diagnosis even in those cases in which, owing to the administration of quinine, the malaria parasites are not to be found in the blood. Blood examination may at the same time show that there is no leucocytosis, and there is a relative increase in the large lymphocytes (page 12).

The difficulty of interpreting the result of a slight reduction of Fehling's solution is familiar. The change in colour from blue to green, or to greenish-yellow, without any definite red or orange precipitate may leave one in doubt as to whether the change is due to sugar or to some other reducing substance, such as uric acid, or glycuronic acid. Even when there is a definite reduction of Fehling's solution, it is important to decide with certainty that glucose is present by applying the phenyl hydrazine and the fermentation tests to the specimen. It is even urged by Dr. Garrod and other authorities that if a case is to be accepted as suffering from glycosuria beyond doubt, not only should the phenyl-glucosazone crystals be examined microscopically, but also their exact melting point should be determined. Other sugars which occur from time to time in the urine may simulate glucose closely to the ordinary tests. Exact determinations of the nature of pentose, lævulose, lactose, galactose, and arabinose, when they occur, necessitate laboratory investigations of a special order, which can be carried out upon request.

Albumosuria is a term applied to two distinct conditions. Small quantities of albumose are present in association with many different inflammatory and pyogenic infections, for instance, lobar pneumonia, empyema, pyosalpinx, appendicular abscess, and so forth. There is another form of albumosuria, however, rarer, but of much greater clinical significance; it was first described by Dr. Bence-Jones. Bence-Jones' albumosuria nearly always indicates that there is a serious lesion of the bone

marrow, especially osteo-sarcoma. It is apt to be mistaken for albuminuria, in that a dense precipitate not soluble at once in acetic acid comes down when the urine is heated, but a remarkable feature which attracts attention when the test is performed is that the precipitate appears at a relatively low temperature, and gets less when the urine is heated beyond a certain point. In case of an anomalous reaction of this kind exact determination of the nature of the substance in the urine may afford the earliest proof of the serious character of the patient's complaint.

Verification of the presence of iodides in the urine may be of importance in several ways; two in particular being, first, that unless it is known that iodides are present, the fact that the urine gives a blue reaction with the guaiacum test may be misinterpreted as being a proof that blood is present; and secondly, that the patient may state that he is taking the medicine which is being ordered, and yet, in fact, may be taking none at all. Sometimes a malingerer pretends she is passing no urine. The trick has before now been detected by giving iodides by the mouth and putting starch and some dilute acid into the bath water.

Lead is excreted both in the urine and in the fæces. If fæces can be analysed instead of urine this is preferable, for more lead is eliminated through the bowel than through the kidneys; but if urine only is available, a bulk may be evaporated down and lead examined for in the residue by ordinary chemical tests. Those who have clean mouths and teeth may not exhibit any blue line. The taking of lead for purposes of abortion may be suspected; urine analysis may clinch the diagnosis. Plumbism from some unknown source may be suspected as the cause of the patient's symptoms; analysis of the urine may confirm the suspicion.

Arsenic is excreted in the urine, and this fact may be made use of in different ways. After the administration of salvarsan, for example, one may wish to know how long the drug is still being excreted from the body; urine analysis is one means of determining this. In cases of suspected arsenical poisoning, again, the same analysis may be useful in determining the fact without mentioning the suspicion. Chronic arsenical poisoning is detected better, perhaps, by analysis of the hair (page 79) than by that of the urine.

Chyluria is not a very common condition in this country, though it is frequently met with in those parts of the tropics in which filaria infection abounds. The fact that the milky appearance of the urine is due to chyle is verified either by direct staining with osmic acid, Sudan III., or other fat stain, or by

extraction and estimation of the fat in the urine. It is sometimes the result not of filaria infection, but of abdominal injury, or obstruction to the thoracic duct or receptaculum chyli by new growth or inflammation, and occasionally it is met with in association with chronic nephritis. It may occur by itself, or it may be associated with chylous ascites (page 94), or chylous effusion into the chest.

Fibrinuria is a rare condition which seldom occurs by itself; it is sometimes associated with chylous ascites.

Iron granules may be present in the urine either free or within cells; the best known condition in which they occur in any quantity is pernicious anæmia. Intra-cellular iron granules may be demonstrated by micro-chemical methods; if Perl's potassium ferro-cyanide and hydrochloric acid reaction is applied to the centrifugalised deposit, the granules within the cells assume a distinct Prussian blue colour.

Calcium carbonate in the urine is chiefly of importance as being one of the substances which is apt to come down as a white cloud along with phosphates when a specimen is being boiled in testing for albumen. The calcium carbonate is responsible for the brisk bubbling that sometimes occurs when a drop of acetic acid is added in completing the test.

Quantitative analyses may be valuable in many ways. It is the general custom to determine the total sugar in the urine at intervals in cases of glycosuria in order to assess the effect of dietetic and other treatment. There is, however, a growing feeling that it does not follow that treatment which lessens the sugar in the urine does corresponding good to the patient, and some hold that it is at least as important to measure the degree of acidosis quantitatively either directly by estimating the acetone present in a twenty-four hours' specimen of urine, or indirectly by estimating the total ammonia.

Quantitative estimations of the total urea in the urine afford one of the readiest methods of determining precisely what quantity of proteid food a patient is receiving, and this may be important when there are doubts as to whether he is taking as much food as he says he is. Conversely, in cases in which it is desired to limit the amount of nitrogenous food, estimations of the urea in the urine afford a convenient check upon the statements of the patient. Some authorities believe that determinations of the total urea in the urine are of importance in deciding whether a patient suffering, for example, from tuberculous kidney is safe to operate on or not. The fact is possibly true, for the more ill a patient is, the less, generally speaking, will he eat, and the less, consequently, will the urea in the urine be. But there is experimental evidence to show that even when the

kidneys are extensively diseased, the amount of the urea in the urine still runs closely parallel with the amount of nitrogen in the food, so that when the urea falls, it is not so much that the kidneys fail to excrete as that the general condition of the patient keeps him from eating so much proteid. From the point of view of determining renal efficiency it is probably more important to estimate the urea in the blood (page 49) than the urea in the urine.

In metabolism researches it is frequently desirable to estimate both the total nitrogen in the urine and the relative proportions of it present as urea and as uric acid. In gouty conditions it may be important to know whether too large a proportion of the nitrogen in the urine is present in the form of uric acid. Normally the ratio of uric acid nitrogen to urea nitrogen should not exceed 1 to 50.

The purin bases include not only uric acid itself, but also allied substances such as xanthin, hypoxanthin, guanin, and adenin. Dietists draw important conclusions from variations in the total purin bases in the urine, especially when the exact dietary is known, so that it is possible to determine how much of the purin bases in the urine is derived from exogenous sources and how much is endogenous, that is to say, produced by the patient's own tissues.

Little stress need be laid upon the obvious value of estimations of the total quantity of coagulable proteid present in albuminuric cases.

Estimations of the total chlorides in the urine are particularly valuable in cases of œdema, whether due to nephritis or to other causes. One of the main factors in many cases of œdema is retention of sodium chloride in the body as the result apparently of inability of the diseased kidneys to excrete the full amounts. The degree to which sodium chloride retention is responsible for the œdema can only be determined by estimating the total chlorides in the urine over a period of several days, the total salt in the food being known at the same time, or a definite quantity of additional salt being added to the food, and the consequent amount passed in the urine determined quantitatively.

Lobar pneumonia is generally associated with a remarkable degree of sodium chloride retention, and in atypical cases where there is doubt as to the diagnosis, determination of the fact that the total sodium chloride in the urine is diminished enormously out of proportion to the sodium chloride in the food may afford a means of distinguishing pneumonia from other pulmonary conditions.

Estimations of the total phosphates in the urine afford the chief proof of the correctness of the diagnosis of phosphatic

diabetes or essential phosphaturia. Similar quantitative estimations are important in connection with certain bone diseases such as rickets, osteo-malacia, or caries; and if there is doubt as to whether a child is receiving a proper quantity of the mineral constituents his dietary should contain, estimation of the phosphates in the urine affords a good test of this point.

Much information as to the degree to which the patient's symptoms are due to auto-intoxication from intestinal putrefactive changes may be obtained from estimations of the sulphates in the urine, particularly if in addition to estimating the total sulphates the relative proportion in which these are present as mineral sulphates and as ethereal sulphates respectively is determined at the same time. The co-efficient of Amann is the ratio of ethereal sulphates to total sulphates in the urine. The relative proportion should not exceed 1 to 10. The greater the degree of intestinal putrefaction the larger will be the total quantity of ethereal sulphates in the urine, and the higher consequently the co-efficient of Amann. Indicanuria is generally associated with a high co-efficient of Amann.

Estimations of the total amount of calcium in the urine may afford important clinical evidence of progressive bone disease, though it is probably easier to estimate the phosphates instead for the same purpose. Errors in calcium metabolism are beginning to receive much more attention than they used to, for it is believed that such errors are responsible not only for premature degenerations such as arterio-sclerosis, neurasthenia, and other maladies of modern life, but also for troubles in the pelvic organs in women, particularly those associated with menorrhagia and dysmenorrhoea. Estimations of the calcium in the blood (page 50), together with estimation of the calcium in the urine, are beginning to throw light upon questions of this kind, and the subject is one which would probably well repay further original research upon it.

Bacteriological examinations of the urine often lead to valuable indications for treatment. The detection of tubercle bacilli in the centrifugalised deposit has been referred to on page 110.

It is sometimes possible to discover gonococci in the urine in a similar way, though when these organisms are suspected it is much better to prepare pus films direct from the discharge or from any suspicious purulent focus.

Other micro-organisms in the urine can be detected better by cultural methods than by direct staining, and to this end it is most important that the specimen should be sent to the laboratory without contamination by any antiseptic, and also that it should have been obtained, if possible, by catheter. When for one reason or another a catheter cannot be used, the urethral

orifice should be washed carefully with a sterile swab and plain water that has been sterilised by boiling. The first few drachms of urine that are passed should be discarded and the rest passed either directly into the sterile specimen bottle or into a sterilised receiver from which, with all aseptic precautions, it can be transferred into the sterile specimen bottle. The urine should then be sent to the laboratory with the least possible delay. It is only of recent years that the frequency of bacilluria (page 108) has been recognised. The commonest organism to produce this is the *bacillus coli communis*, and in resistant cases treatment by suitable doses of *bacillus coli* vaccine, prepared either from the patient's own organisms or from stock cultures, is often effectual. The disease is commoner in women than in men, but is by no means confined to the female sex. It is common in children as well as in adults. The symptoms may not be such as to draw immediate attention to the urinary tracts at all; headaches, for instance, or attacks of vomiting may be the only symptom sometimes, and there can be little doubt that cultural investigations of the urine for the *bacillus coli communis* should be carried out in a far greater number of patients than has hitherto been the case.

The possibility of typhoid bacilli continuing to be excreted in the urine after a patient has recovered from the fever is well known. Such a case may be a source of danger to others; nevertheless, it has not yet become the routine practice as it should be to have cultural investigations of the urine made in all patients who are convalescent from enteric fever.

Bright's disease is often, if not always, associated with some degree of microbial infection. In certain cases pneumococci are the causal germ, in others streptococci, and probably if careful investigations were made in a large number of instances different micro-organisms would be found to be responsible for acute nephritis of the Bright's disease type. Just as lobar pneumonia is, from a bacteriological point of view, by no means always the same disease, so are there different kinds of acute Bright's disease, bacteriologically speaking. Research work upon this point is badly needed.

Organisms that have been found in the urine under various circumstances include streptococci, staphylococci, pneumococci, pneumo-bacilli, *bacillus aerogenes lactis*, diphtheria bacilli, diplococci other than pneumococci, but not yet named, and there are doubtless others.

Cryoscopy of the urine, that is to say, determination of the reduction in the freezing point of the liquid below zero, affords an indirect measure of renal efficiency. If the technique required were not so complex it might become a clinical method

more commonly used than at present. The greater the number of molecules in a given volume of urine, the greater is the reduction in the freezing point of the fluid, so that by cryoscopy a measure is obtained, not of any individual constituent of the urine, but of the total constituents together, and from the result deductions can be drawn as to whether the kidneys are excreting efficiently or otherwise. Similar deductions are to be drawn from determinations of the electrical conductivity of the urine, for this also varies with the number of molecules in a given volume of the liquid. Hitherto, however, both cryoscopy and determinations of the electrical conductivity of the urine have been methods used by those engaged in research work rather than in connection with clinical medicine.

Cambridge's pancreatic reaction consists, broadly speaking, of a determination of the presence in the urine, not of sugar itself, but of a substance related to sugar, which by boiling with dilute hydrochloric acid yields a sugar allied to pentose, and yielding osazone crystals to the phenyl-hydrazine test. By itself Cambridge's test may not yield definite clinical evidence of the presence or absence of any particular lesion of the pancreas, but in conjunction with other analyses it may be very valuable. The following quotation from Cambridge's article in French's "Index of Differential Diagnosis of Main Symptoms" summarises the chief factors about it as follows :—

"Other points to be noticed in examining the urine from suspected cases of pancreatic disease are :—

1. The presence of calcium oxalate crystals in the centrifuged deposit. These are met with in 63 per cent. of cases of chronic pancreatitis, or 73 per cent. if jaundice cases are excluded.

2. A pathological excess of urobilin. This is a very constant indication of cholangitis, and a particularly useful sign of gall-stones in the common bile duct, whether accompanied by jaundice or not.

3. A well-marked indican reaction. Pointing to a catarrhal condition of the intestinal mucous membrane, with abnormal putrefactive changes in the contents of the intestine, and possibly a duodenal or gastric ulcer.

4. Bile pigment in the urine. Showing that there is some obstruction to the free flow of bile into the intestine, due to impacted gall-stones, gripping of the common bile duct by the inflamed head of the pancreas which surrounds the duct in 62 per cent. of cases, malignant disease of the head of the pancreas or a growth in the common bile duct."

"For the purposes of a further differential diagnosis, the results of a qualitative and quantitative analysis of the faeces are most

important. In carrying out the analysis, the points to be noticed particularly are :—

1. The presence or absence of stercobilin. In gall stone obstruction, traces, at least, are nearly always met with, whereas in malignant disease of the head of the pancreas, total blocking of the duct is the rule, although the soft growths occurring primarily in the common duct usually allow some bile to filter through, so that traces of stercobilin are met with in the fæces.

2. The percentage of unabsorbed fat. In cancer of the pancreas this is always very high, 70 to 80 per cent. It is usually somewhat less in growths of the common duct, averaging 60 to 70 per cent., and varies from a subnormal percentage in early catarrh of the pancreas to as much as 50 or, rarely, even 80 per cent. in advanced chronic pancreatitis.

3. More important still, however, is the relation of the unsaponified to the saponified fats, for whereas the former are in excess in diseases that interfere with the digestive functions of the pancreas, such as cancer of the gland and advanced chronic pancreatitis, the latter predominate in obstruction of the common duct by gall stones, without pancreatitis, and in malignant growths not involving the pancreas. It must be borne in mind, however, that, owing to the abnormal activity of fat-splitting bacteria in the lower bowel, such as is met with in some cases of intestinal catarrh, an excess of saponified fat may be found in chronic pancreatitis, where the disease is due to an infection spreading from the duodenum along the pancreatic ducts. A similar excess is often met with in early catarrhal pancreatitis, owing probably to an increased flow of pancreatic juice analogous to the salivation met with in parotitis.

4. Microscopical examination of the fæces for fat globules, fatty acid crystals, undigested muscle fibres, connective tissue, etc., should not be omitted. A large excess of fat globules and free fatty acid crystals, with numerous isolated undigested muscle fibres points to cancer of the pancreas or advanced cirrhosis of the gland, whereas muscle associated with connective tissue points to defective gastric digestion.

5. An acid reaction of the fresh stool is in favour of a diagnosis of pancreatic disease. In simple gall stone obstruction the fæces are usually alkaline.

6. Occult blood, when constantly present in the fæces (page 70), is suggestive of malignant disease or, more rarely, advanced pancreatitis, in which it is well known that there is a hæmorrhagic tendency, while the discovery of blood intermittently points to a gastric or duodenal ulcer, which may be invading the pancreas and setting up pancreatitis.

By carefully considering all the facts thus obtained, and interpreting them in the light of the clinical signs and symptoms, it is possible, not only to diagnose correctly the existence of disease of the pancreas, but also to arrive at a satisfactory conclusion as to its probable cause. Affection of the pancreas is much commoner than is generally supposed, and many trying cases of chronic indigestion, recurring or persistent jaundice, and obscure affections of the upper abdomen would be explained, and satisfactorily treated, if investigated as above. Undiagnosed, and consequently untreated, pancreatitis is probably the most common cause of diabetes. If this were more widely recognised much might be done to stay the further increase of that disease."

In addition to ordinary examination of the mixed urine from patients, it is sometimes of considerable importance to carry out separate analyses upon specimens obtained from the right and left ureter respectively. By means of the cystoscope ureteral catheterisation can now be carried out with comparative ease by those accustomed to the use of the instrument, and the urine from each ureter can be collected separately. If both kidneys are normal, the chemical and microscopical features of each specimen should be identical, but when one or other is diseased, all sorts of differences may be found. The urine from a hydro-nephrotic kidney may be more abundant and more dilute than that from the normal side. Tubercle bacilli, if present in one specimen and not in the other, will indicate the unilateral character of the renal tuberculosis and perhaps the advisability of operation, whilst if both specimens contain tubercle bacilli, both kidneys are diseased, and operation will be contra-indicated, as a rule.

Besides the above, other ways in which simultaneous examination of specimens of urine obtained from each ureter separately may lead to clinical evidence of great importance will suggest themselves in practice.

SECTION XXIX.

Vaccines.

The Apparatus required.

When the causal organism is already known it may be decided that a stock vaccine should be employed, and these can be sup-

plied direct from the laboratory in whatever doses may be desired. Generally, however, it is essential in the first place to determine the exact nature of the causal organism by means of bacteriological cultures. To this end three things are of particular importance, namely, first that the specimen containing the micro-organisms should be obtained free from any antiseptic which can inhibit its growth. It should be collected with aseptic, but not with antiseptic precautions, for very small quantities of an antiseptic will inhibit the growth of micro-organisms in culture media. Secondly, the specimen must be transmitted in a suitable sterile receiver, all precautions being taken against contamination from external objects both in transferring it from the patient to the receiver, and in transmitting the latter to the laboratory. Thirdly, it is necessary that the specimen should reach the laboratory with as little delay as possible, for otherwise either the micro-organisms may die out before they are planted upon the culture media or, especially when there has been a mixed infection, the saprophytic germs may outgrow and swamp those which are responsible for the lesion in the patient. The latter event is particularly liable to occur in cases in which the bacillus coli communis is present along with other micro-organisms. Swabbings from cases of acute peritonitis, for example, may yield only bacillus coli communis on cultivation, though the original lesion may have been due to the pneumococcus. Upon request culture tubes will be sent from the laboratory in order that the first inoculation may be made directly, as in the case of blood cultures (page 89).

The Importance of Vaccines.

There can be little doubt that vaccines have been used with ill-effects as well as good, and it requires considerable knowledge before one can say that vaccine treatment should be adopted in any particular case. Special books upon the subject should be consulted, but better than books is personal experience. The benefits to be obtained from vaccines, including tuberculin in suitable cases, are enormous, but it is imperative that the bacteriological diagnosis should be established with certainty before vaccine is employed. It is not sufficient to diagnose pneumonia and then use a pneumococcal vaccine on the assumption that the pneumonia is pneumococcal. The inflammation of the lungs *may* be pneumococcal, but it is also possible that it may be due to the influenza bacillus, the typhoid bacillus, the pneumo-bacillus of Friedländer, or possibly some other micro-organism. Bacteriological as well as clinical diagnosis is required if vaccine treatment is to be successful. Phthisis pulmonalis is due pri-

marily to the tubercle bacillus, but when there are large cavities in the lungs, infected with pneumococci, streptococci, staphylococci, and other micro-organisms, it is not to be expected that treatment by tuberculin will relieve the patient of symptoms that are due to the secondary infection; and so on in other cases. Bacteriological diagnosis is the first essential, and this may be obtained by cultural investigations of such various different things as urethral discharges, nasal discharges (page 85), discharges from the ear (page 56), vaginal discharges, the circulating blood (page 46), cerebro-spinal fluid (page 56), affections of the skin (page 95), catheter specimens of urine (page 115), serous fluids (page 93), pus obtained by needling the chest, or an abscess, or a joint, swabbings from the throat (page 102), or fluid or other material from internal organs at the time of operation upon, for instance, the peritoneum, the gall bladder, or bile ducts, a liver abscess, and so forth.

It is very important to realise, however, that the mere discovery of a germ in some discharge or fluid is not necessarily the same thing as proving that the symptoms in the patient are due to the discovered micro-organism, and a considerable amount of deliberate thought is required before it can be asserted that a particular organism is so probably the cause of the symptoms that vaccine treatment should be adopted. At the same time it is often found that patients suffer from the effects of microbial toxins at a distance from the place at which they are produced; and whereas, for instance, the discovery of the bacillus coli communis in pure cultures in a catheter specimen of urine affords fairly certain proof that pyuria in these cases is due to the bacillus coli communis, or the discovery of streptococci in the circulating blood indicates the bacterial nature of the endocarditis present in the patient, it may often be less obvious that the streptococci or staphylococci or pneumococci present in the nasal mucous membrane are responsible for a patient's chronic rheumatoid arthritis, or that the bacteria in the suppurating gums are producing the patient's arterial or renal degeneration.

These few remarks may serve to indicate the difficulties that arise in connection with vaccine treatment, but that the treatment itself is of value in many conditions due to microbial infection is widely held; and whereas stock vaccines seem to give good results in some conditions such as gonococcal infection, medical and surgical tuberculosis without secondary infection, and in staphylococcal lesions of the skin, many are confident that autogenous vaccines do more good in many other affections, whilst they have the additional advantage that they necessitate the bacteriological as well as the clinical diagnosis being made with as much accuracy as possible before the vaccine is used.

SECTION XXX.

Veterinary Diseases.

The Clinical Research Association laboratories are at the service not only of those who have to do with the maladies of human beings, but also of those who have to deal with the various diseases of animals. The latter are, in some cases, due to the same parasites or micro-organisms that infect man, but besides these there are a large number of other animal diseases due to micro-organisms which are confined to one or more species of animals other than man. It is hardly possible to specify all the ways in which the Clinical Research Association may be of assistance to veterinary surgeons and others in this connection.

APPENDIX
TO THE
PRACTITIONER'S GUIDE
— TO —
CLINICAL RESEARCH

BEING THE
LIST OF CHARGES
— FOR —
LABORATORY EXAMINATIONS

MADE BY THE
Clinical Research Association
Watergate House,
ADELPHI, LONDON, W.C.

INTRODUCTION.

This list of charges is published as an appendix to the Practitioner's Guide to Clinical Research. The various sections are placed in alphabetical order to correspond with the sections in the Guide. Full particulars with regard to the method of collecting and transmitting material for examination, together with statements as to the deductions that may be drawn from the various results obtained, are given in the Guide. The charges quoted are those in force, and will hold good until further notice. The special regulations for the transmission of specimens by post are given on page 126, and the Clinical Research Association of Watergate House, Adelphi, W.C., supplies members with packing cases, tins, and so forth, that may be required for different purposes (see page 148).

In the case of Hospitals and Institutions the Association is prepared to consider reduced charges, in regard to which special terms can be arranged on application. In the case of poor patients treated outside Hospitals or other Institutions, modified terms will also be quoted to our Members.

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IMPORTANT.

The Postmaster-General has drawn the attention of the Association to packets containing pathological specimens where they have been sent by **Parcel Post**. The sending of such specimens by any other post than the **Letter Post** is strictly prohibited by the Post Office regulations, which are printed below, and renders the specimens liable to be destroyed at any stage in transit. It is hoped that members will do their best to see that the regulations are not infringed.

Transmission of Specimens by Rail.

Samples of drinking-water and heavier packages are best sent by rail; and in many cases this will prove the most convenient means of sending smaller packages, for most of the railway companies are now prepared to collect in the country and deliver in London small parcels under two pounds in weight, at a charge of fourpence for each parcel irrespective of distance.

POSTAL REGULATIONS.

DELETERIOUS LIQUIDS OR SUBSTANCES.

ARTICLES SENT FOR MEDICAL EXAMINATION OR ANALYSIS.

Deleterious liquids or substances, though otherwise prohibited from transmission by post, may be sent for medical examination or analysis by a qualified Medical Practitioner, or qualified Veterinary Surgeon to a Laboratory or Institute, public or private, or to a Medical Practitioner or Veterinary Surgeon within the United Kingdom, by ordinary Letter Post, under the following conditions :—

Any such liquid or substance must be enclosed in a receptacle hermetically sealed, which receptacle must itself be placed in a strong wooden, leather or metal case in such a way that it cannot shift about, and with a sufficient quantity of some absorbent material (such as saw-dust or cotton wool) so packed about the receptacle as absolutely to prevent any possible leakage from the package in the event of damage to the receptacle.

The packet so made up must be conspicuously marked "Fragile with care," and bear the words "Pathological Specimen," and also the signature and address of the Medical Practitioner or Veterinary Surgeon who sends it. The packet must on no account be sent by Parcel Post. Any packet found in the post not packed and marked as directed will be at once stopped and destroyed with all its wrappings and enclosures.

Any person who sends by post a deleterious liquid or substance for medical examination or analysis, otherwise than as provided by these regulations, is liable to prosecution.

It is recommended that if receptacles are supplied by a Laboratory or Institute to Medical Practitioners or Veterinary Surgeons, they should be submitted to the Secretary, General Post Office, in order to ascertain whether they are regarded as complying with the regulations.

Cost of the Examinations.

SECTION I.—THE BLOOD.

A.—Blood Counts.

I. THE COST OF A TOTAL BLOOD COUNT, including enumeration of the red corpuscles, enumera- tion of the white corpuscles, estimation of the hæmoglobin, calculation of the colour index, a differential count of the leucocytes, and a report on the characters of the red corpuscles in stained films	Cost for Members. £ s. d.			Cost for Non- Members. £ s. d.		
	1	1	0	1	11	6
II. THE COST OF PARTIAL BLOOD COUNTS.						
1. Enumeration of red corpuscles	0	5	0	0	7	6
2. Enumeration of white corpuscles	0	5	0	0	7	6
3. Estimation of hæmoglobin	0	5	0	0	7	6
4. Differential leucocyte count	0	10	6	0	15	0
5. Report on the character of the red cells in stained blood films	0	5	0	0	7	6
6. Enumeration of red corpuscles and enumeration of white corpuscles	0	7	6	0	10	6
7. Enumeration of red corpuscles and estimation of hæmoglobin; calculation of colour index ...	0	7	6	0	10	6
8. Enumeration of red corpuscles and differential leucocyte count	0	15	0	1	1	0
9. Enumeration of red corpuscles and a report upon the characters of the red cells in stained films	0	10	0	0	15	0
10. Enumeration of the white corpuscles and estima- tion of the hæmoglobin	0	7	6	0	10	6
11. Enumeration of the white corpuscles and differ- ential leucocyte count	0	15	0	1	1	0
12. Enumeration of the white corpuscles and a report on the characters of the red cells in stained films	0	10	0	0	15	0
13. Estimation of the hæmoglobin and a differential leucocyte count	0	15	0	1	1	0
14. Estimation of the hæmoglobin and a report on the characters of the red cells in stained films	0	10	0	0	15	0
15. A differential leucocyte count and a report on the characters of the red cells in stained films	0	10	6	0	15	6
16. Enumeration of the red corpuscles, enumeration of the white corpuscles, estimation of the hæmoglobin, and calculation of the colour index... ..	0	10	6	0	15	0

	Cost for Members. £ s. d.	Cost for Non- members. £ s. d.
17. Enumeration of the red corpuscles, enumeration of the white corpuscles, and a differential leucocyte count	0 15 0	1 1 0
18. Enumeration of the red corpuscles, enumeration of the white corpuscles, and a report on the characters of the red corpuscles in stained films	0 10 0	0 15 0
19. Enumeration of the red corpuscles, estimation of the hæmoglobin, calculation of the colour index, and differential count of the leucocytes	0 15 0	1 1 0
20. Enumeration of the red corpuscles, estimation of the hæmoglobin, calculation of the colour index, and a report on the characters of the red corpuscles in stained films	0 10 0	0 15 0
21. Enumeration of the red corpuscles, a differential count of the leucocytes, and a report on the characters of the red corpuscles in stained films	0 15 0	1 1 0
22. Enumeration of the white corpuscles, estimation of the hæmoglobin, and a differential leucocyte count	0 15 0	1 1 0
23. Enumeration of the white corpuscles, estimation of the hæmoglobin, and a report on the characters of the red corpuscles in stained films	0 10 0	0 15 0
24. Enumeration of the white corpuscles, a differential count of the leucocytes, and a report on the characters of the red corpuscles in stained films	0 15 0	1 1 0
25. Estimation of the hæmoglobin, a differential count of the leucocytes, and a report on the characters of the red corpuscles in stained films	0 15 0	1 1 0
26. Enumeration of the white corpuscles, estimation of the hæmoglobin, a differential count of the leucocytes, and a report on the characters of the red corpuscles in stained films	0 15 0	1 1 0

B.—Parasites in the Blood.

Staining, examining, and reporting upon the films for the presence of a suspected parasite	0 7 6	0 10 0
Staining films and returning them to the sender without report	0 2 6	0 3 6
Cost of report and film	0 8 6	0 11 3
Examination for tubercle bacilli in films	0 10 6	0 15 0
Examination for tetanus bacilli in films	0 10 6	0 15 0
Examination for trypanosomes in films	0 7 6	0 10 0

C.—The Detection of Abnormal Pigments in the Blood.

Spectoscopic examination of blood	1 1 0	1 11 6
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D.—Determination of the Total Volume of Blood in the Patient's Body.

	Cost for Members.			Cost for Non-members.		
	£	s.	d.	£	s.	d.
The cost of the investigation necessarily varies according as the laboratory is asked merely to analyse the blood sent to it in order to determine the percentage of carboxyhæmoglobin present in it,						
Or whether the laboratory is required to send out the mixed carbon monoxide and oxygen that is to be respired, together with an attendant who can supervise the giving of the mixture, take the blood, bring it back to the laboratory and analyse it.						
The cost in either case will be quoted on enquiry.						

E.—Estimation of the Specific Gravity of the Blood.

If, at the desire of the patient's medical attendant, a representative is sent from the laboratory to make the specific gravity estimation, it naturally depends upon the distance that has to be travelled, the number of estimations that have to be made, and the length of time over which the observations extend, what the cost will be; the charge for one, two, or three estimations of the specific gravity at intervals during a period of about two hours at the patient's house, within the London area, would be	3 3 0	5 5 0
The cost of the supply of the necessary chloroform and benzene, and specific gravity indicator sent on loan to the patient's house for the medical attendant himself to use it there, is	0 5 0	0 7 6
The cost of estimation of the specific gravity of a patient's blood when, at the request of the medical attendant, the patient comes to the laboratory for the purpose, is	1 1 0	1 11 6

F.—Serum Reactions.

(a) The Widal Test for Typhoid Fever.

Widal's Test for Typhoid Fever (including reply by telegram).

The report is sent in the first instance by telegram, followed by a typewritten report of the details ...	0 4 0	0 6 0
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(b) Serum Agglutinating Reaction for Paratyphoid Fever.

Testing the serum reaction against <i>Bacillus paratyphosus</i> A	0 5 0	0 7 6
Testing the serum reaction against <i>Bacillus paratyphosus</i> B	0 5 0	0 7 6
Testing the same serum against both <i>Bacillus paratyphosus</i> A and <i>Bacillus paratyphosus</i> B	0 7 6	0 10 6
Testing the same serum against both <i>Bacillus typhosus</i> and <i>Bacillus paratyphosus</i> A	0 7 6	0 10 6

	Cost for Members. £ s. d.	Cost for Non- members. £ s. d.
Testing the same serum against both <i>Bacillus typhosus</i> and <i>Bacillus paratyphosus</i> B	0 7 6	0 10 6
Testing the same serum against both <i>Bacillus typhosus</i> and <i>Bacillus paratyphosus</i> A and <i>Bacillus para-</i> <i>typhosus</i> B	0 10 0	0 15 0

(c) Serum Agglutinating Reaction for the *Bacillus Enteritidis* of Gaertner.

Testing the serum reaction for Gaertner's bacillus ...	0 5 0	0 7 6
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(d) Serum Agglutinating Reaction in Affections by the *Meningococcus*.

Testing serum against cultures of the meningococcus	0 10 6	0 15 0
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(e) The Serum Agglutinating Reaction in Malta Fever.

Testing serum against the <i>micrococcus melitensis</i> ...	0 7 6	0 10 0
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(f) Serum Agglutinating Reaction with the *Bacillus Coli Communis*.

Testing serum against the <i>Bacillus coli communis</i> ...	0 5 0	0 7 6
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(g) Serum Agglutinating Reaction in Cases of Dysentery.

Testing the serum in a case of dysentery	0 15 0	1 1 0
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(h) Serum Agglutinating Reaction in Whooping Cough.

Testing the serum in a case of whooping cough ...	0 7 6	0 10 0
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G.—Complement Fixation Tests.

(1) The Wassermann Reaction for Syphilis.

Wassermann blood serum test and report	1 1 0	1 11 6
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If a series of Wassermann tests are required, either from the same patient at separate times, or from several patients at the same time, special reduced charges will be quoted upon application.

(2) The Hydatid Serum Test.

Examination of the patient's serum for the comple- ment fixation test (Bordet-Gengou) in hydatid disease	4 4 0	5 5 0
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	Cost for Members.			Cost for Non- members.		
	£	s.	d.	£	s.	d.
(3) Eason's Reaction.						
Testing a patient's blood serum for Eason's reaction	4	4	0	5	5	0

(4) The Complement Fixation Test in Glanders.

Testing the serum by the complement fixation test for glanders	4	4	0	5	5	0
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H.—Blood Cultures in the Diagnosis of Septicæmia, Malignant Endocarditis, Puerperal Fever, Typhoid Fever, etc.

Cultural examination of blood	0	10	6	0	15	0
Cultural examination of blood together with the preparation of twelve graduated doses of vaccine						
from	2	2	0	3	3	0
to	3	3	0	4	4	0

I.—Chemical Analyses of the Blood.

Estimation of the urea in the blood... ..	each	1	1	0	1	11	6
Estimation of the uric acid in the blood							
Estimation of the fat in the blood							
Estimation of the dextrose in the blood							
Estimation of the calcium in the blood					0	10	0

J.—Opsonic Index Determinations.

Estimation of the opsonic index to any one of the following organisms:—Tubercle bacilli, strepto- cocci, staphylococci	0	15	0	1	1	0
Estimation of the opsonic index of the same specimen of serum to any two of the above organisms ...	1	5	0	1	11	6
Estimation of the opsonic index to all three of the above organisms from	1	11	6	2	2	0
Two or more opsonic index determinations in the same case, at intervals, within a period of not more than a year each	0	10	0	0	15	0

The cost of carrying out estimations of the opsonic index for micro-organisms other than the above will be quoted upon application to the Clinical Research Association.

K.—Arneth's Test in Phthisis.

Arneth's test	0	10	6	0	15	0
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Note.—An additional fee of Five Shillings for Members and Seven Shillings and Sixpence for Non-members is charged if the patient attends personally at the Laboratories for the specimen of his blood to be taken.

SECTION II.—CALCULI AND CONCRETIONS.

Qualitative examination to determine the nature of the chief ingredients of a calculus... .. from	0	5	0	0	10	0
to	0	10	0	0	15	0

Quantitative analysis for the total percentage of each ingredient present from	Cost for Members.			Cost for Non-members.		
	£	s.	d.	£	s.	d.
	1	1	0	1	11	6

Although the above are the average charges made, the kind of analysis required in different cases varies so much that it is generally advisable to ask for special terms to be quoted if anything other than a routine examination is required.

SECTION III.—CEREBRO-SPINAL FLUID.

The cost of examination of the cerebro-spinal fluid naturally varies with the extent of the analysis required. The following are the charges made for the usual tests:—

General examination, chemical, cytological and direct bacteriological, but not including cultures	0	10	6	0	15	0
Direct examination for <i>spirochaeta pallida</i> in the centrifugalised deposit	0	7	6	0	10	6
Cultural identification of:—						
Meningococci from	0	10	0	0	15	0
Streptococci... .. "	0	10	0	0	15	0
Staphylococci "	0	10	0	0	15	0
Pneumococci "	0	10	0	0	15	0
Influenza bacilli "	0	10	0	0	15	0
Tubercle bacilli "	0	15	0	1	1	0
Typhoid bacilli "	0	10	0	0	15	0
Paratyphoid bacilli "	0	10	0	0	15	0
Bacillus coli communis "	0	10	0	0	15	0
Examination for trypanosomes	0	7	6	0	10	6
Wassermann's test for syphilis applied to the cerebro-spinal fluid	1	1	0	1	11	6
Wassermann's test for syphilis applied to the cerebro-spinal fluid, if it is at the same time applied to a specimen of blood from the same patient; for the two... ..	2	0	0	3	0	0

Other investigations that may be required in any particular instance can be carried out according to directions, and upon request a special charge would be quoted.

SECTION IV.—CLASS SPECIMENS.

For histological sections of any ordinary morbid tissues the following are the charges made:—

Per slide from	£ s. d.		
	£	s.	d.
	0	1	6

The Association is also prepared to supply cabinets containing characteristic sections of the various tissues that are likely to be useful to medical students in preparing for their examinations in pathology. These cabinets can be made up according to the requirements in any particular case, and a price would be quoted by the Association on application.

In addition, almost any histological specimens can be obtained upon request if a little notice is given. Special charges will be quoted upon application; the cost must necessarily vary according to the rarity of specimens required.

Blood films can be supplied in sets at a cost of 2s. 6d. each for each specimen to members and 3s. 6d. each to non-members.

Examples of any particular variety of bacterial film can generally be supplied in a similar way either in sets or in bulk.

							Cost for Members.			Cost for Non-members.		
							£	s.	d.	£	s.	d.
Cost of one set of 12 slides of common pathogenic bacteria							1	1	0	1	7	6
Cost of one set of 25 slides of common pathogenic bacteria							2	2	0	2	10	0

Any of the above films can also be supplied in large quantities for class purposes from :—

					Per 100.			Per Gross.			
					£	s.	d.	£	s.	d.	
For Members	1	1	0	...	1	5	0
For Non-members	1	11	6	...	2	2	0

CULTURES OF THE FOLLOWING MAY BE OBTAINED AT THE UNDERMENTIONED PRICES :—

Ordinary non-pathogenic organisms generally	each	0	2	6	0	3	6
Anthrax, Typhi Abdominalis, Coli Communis, Cholera, Staphylococci, Streptococci, Diphtheria, Pseudo-Diphtheria, Sarcinæ...	...	0	2	6	0	3	6
Pneumococcus, Glanders, Actinomyces, Tubercle, Tetanus	...	0	5	0	0	7	6

The above cultures may be obtained for demonstration purposes permanently "fixed" in formalin, with the tubes sealed, at an extra cost of 6d. per specimen.

SECTION V.—CYST FLUIDS.

The cost of the examination of a cystic fluid will necessarily vary with the investigation that is required. In some cases a microscopical examination of the centrifugalised deposit is the most important; in others, a chemical examination for proteids and other ingredients; in others, a bacteriological examination; in others again, a test for ferments. Special charges will be quoted in special cases; the following are those made in ordinary cases :—

		Cost for Members.			Cost for Non-members.		
		£	s.	d.	£	s.	d.
General examination; chemical, direct bacteriological, and cytological	... from	0	10	6	0	15	0
Quantitative estimation of urea	...	1	1	0	1	11	6
Quantitative estimation of total inorganic residue	...	1	1	0	1	11	6
Wassermann reaction applied to cystic fluid	..	1	1	0	1	11	6
Wassermann reaction applied to cystic fluid, if done against blood at same time; for the two	...	2	0	0	3	0	0

SECTION VI.—DRINKING WATER.

Combined Chemical and Bacteriological Examination of
Drinking Water. £ s. d.

* Sanitary examination of drinking water; being a combined chemical and bacteriological examination to determine its freedom from contamination and suitability for drinking purposes, with opinion 3 3 0

This combined examination will generally be found the most useful in judging of the quality of public and private supplies.

CHEMICAL ANALYSIS.

*Analysis A.

The estimation of ammoniacal nitrogen; albumenoid nitrogen; oxygen absorbed in four hours at 27°C.; chlorine; sodium chloride; nitrates; presence or absence of nitrates, lead, copper; with opinion... 1 1 0

This analysis is a limited one, but will frequently be sufficient to show any contamination of the water, and will be found suitable for private well-water, and other domestic supplies.

*Analysis B.

Total solids; chlorine; sodium chloride; nitrates; nitrites; ammoniacal nitrogen; albumenoid nitrogen; oxygen absorbed in four hours at 27°C.; total hardness; lead or copper; with opinion... 1 11 6

Unless the particular examination required is specified, analysis B will be made.

*Analysis C.

Appearance; colour (through two-foot tube); total solids; mineral solids; organic matter; chlorine; sodium chloride; nitrogen as nitrates; nitrites; ammoniacal nitrogen; albumenoid nitrogen; oxygen absorbed in fifteen minutes at 27°C.; ditto in four hours at 27°C.; total hardness; permanent hardness; temporary hardness; lead; copper; iron; phosphates; with opinion... 2 2 0

*Analysis D.

Total solids; mineral solids; organic matter, etc.; carbonates of calcium, magnesium and sodium; sulphates of calcium, magnesium and sodium; chlorides of calcium, magnesium and sodium; nitrate of calcium; iron; phosphates; total hardness; permanent hardness; temporary hardness; with opinion ... 3 3 0

This will be found desirable in cases where the water is required for boiler and manufacturing purposes.

* For those investigations marked thus, special apparatus is necessary, and will be supplied on request.

	Cost for Members.			Cost for Non- members.		
	£	s.	d.	£	s.	d.
Analysis E.						
Appearance; colour (through two-foot tube); total solids; total mineral solids; organic matter, etc.; carbonates of calcium, magnesium and sodium; sulphates of calcium, magnesium and sodium; nitrate of calcium; iron; phosphates; total hardness; permanent hardness; temporary hardness; ammoniacal nitrogen; albumenoid nitrogen; oxygen absorbed in four hours at 27°C.; nitrites; lead; copper; with opinion...	4	4	0			
This includes Analysis D, with the addition of such determinations as will show the water's fitness for drinking purposes.						
When Spa waters are to be analysed for special ingredients in addition to the above—strontium, radium, iodides, etc.—the cost will be quoted on application.						
The action of the water on lead pipes, with quantitative determination of amount (if any) dissolved	1	1	0			
For all the above chemical examinations a specially cleansed bottle will be sent in a basket.						
Estimation of total chlorides only	0	5	0			
Detection of lead only	0	5	0			
Estimation of absorbed oxygen... ..	0	5	0			
General microscopical examination	0	5	0			
Determination of hardness: temporary, permanent, and total	0	7	6			
Determination of ammoniacal and albuminoid nitrogen	0	10	6			

BACTERIOLOGICAL EXAMINATION OF DRINKING WATER.

Qualitative examination of drinking water to determine its freedom from contamination, and suitability for drinking purposes... ..	1	11	6			
General bacteriological examination of water for evidence of sewage pollution	2	2	0			
Cultural analysis for typhoid bacilli	1	1	0	1	11	6
Cultural analysis for diphtheria bacilli	1	1	0	1	11	6

Sewage or Effluent.

Chemical examination of sewage or effluent	2	2	0			
* Qualitative bacteriological examination of sewage to determine presence and approximate number of sewage organisms... ..						
* Quantitative bacteriological examination of sewage, i.e., the enumeration of organisms	1	1	0			

* For those investigations marked with an asterisk, special apparatus is necessary and will be supplied on request.

SECTION VII.—EAR DISCHARGES.

	Cost for Members.			Cost for Non-members.		
	£	s.	d.	£	s.	d.
Testing for cerebro-spinal fluid	0	10	6	0	15	0
Verification of the presence of pus	0	2	6	0	3	6
Report as to the presence of micro-organisms in stained films, from	0	5	0	0	7	6
Report as to the presence of tubercle bacilli in stained films	0	5	0	0	7	6
Cultural verification of the organisms present, without preparation of vaccine (see p. 141), from ...	0	5	0	0	7	6

SECTION VIII.—FÆCES.

General and microscopical examination of fæces ...	0	10	6	0	15	0
Examination of fæces for ova of parasites in identification of the commoner intestinal parasites or of segments of them	0	7	6	0	10	6
Ditto in identification of larvæ in cases of intestinal myiasis	0	7	6	0	10	6
Identification and general report on intestinal sand from	0	5	0	0	7	6
Detection of fat in	0	2	6	0	3	6
Detection of pus in	0	2	6	0	3	6
Complete investigation (for fats, bile, occult blood, &c., fermentation, tryptic activity, microscopical and bacteriological examination)	3	3	0	4	4	0
Estimation of the total nitrogen in the fæces ...	0	10	6	0	15	0
Estimation of total and of the relative amounts of saponified and unsaponified fats in the fæces ...	1	11	6	2	2	0
Detection of occult blood in the fæces	0	5	0	0	7	6
Detection of stercobilin or urobilin in	0	2	6	0	3	6
Detection of bile in	0	2	6	0	3	6
Detection of carbohydrates in	0	7	6	0	10	0
Investigation of tryptic activity ("pancreatic insufficiency")	0	5	0	0	7	6
Fermentation test for excess of carbohydrates ...	0	5	0	0	7	6
General chemical examination	2	2	0	3	3	0
General bacteriological examination (including tubercle bacilli, &c.)	1	11	6	2	2	0
Cultural examination for typhoid bacilli	1	1	0	1	11	6
Cultural examination for cholera vibrios	1	1	0	1	11	6
Cultural examination for dysentery bacilli	1	1	0	1	11	6
Cultural examination for bacillus bifidis	1	11	6	2	2	0
Microscopical examination for tubercle bacilli ...	0	5	0	0	7	6

	Cost for Members.			Cost for Non- members.		
	£	s.	d.	£	s.	d.
Microscopical examination by antiformin process ...	0	10	6	0	15	0
Examination for actinomycetes	0	5	0	0	7	6
Search for head of tapeworm	0	10	6	0	15	0
Examination of casein digestion by Gross's method	0	5	0	0	7	6

SECTION IX.—FOODS AND DRUGS.

It is very difficult to lay down any hard and fast charges for the various examinations that may be required, but the following may serve to indicate the usual fees:—

Chemical analysis of butter for adulteration ...	0	15	0			
Detection of food preservative, such as boric acid ...	0	10	6			
Examination of diabetic foods for starch and cane sugar	0	5	0			
Chemical, microscopical, or bacteriological examina- tion of foods (other than milk, for which see p. 139), <i>e.g.</i> , cream, bread, cheese, meat, flour, etc., etc., from	0	10	6			
Examination of beer for arsenic	0	15	0			
Examination of cocoa for starch and cane sugar ...	0	5	0			
Qualitative examination of tinned foods for lead or tin, each	0	15	0			
Quantitative examination of tinned foods for lead or tin, each	2	2	0			
Examination of drugs to see that they comply with the requirements of the British Pharmacopœia from 1 1 0 to 5 5 0						
Examination of ice-cream for diphtheria bacilli ...	1	1	0	1	11	6
Examination of foods for ptomaine-organisms ...	1	1	0	1	11	6
Examination of shell fish for evidence of sewage pollution	4	4	0			
Examination of condensed milk, including full chemical analyses and bacteriological tests ...	2	2	0			
Bread or flour, estimation of total carbohydrate in ...	0	10	6			
Bread or flour, estimation of total starch and total sugar in	0	15	0			
Bread or flour, estimation of total carbohydrate and total moisture in	0	15	0			
Bread or flour, estimation of total starch, total sugar, and total moisture in	1	1	0			
Wine, quantitative analysis for glucose	0	5	0			
Wine, quantitative analysis for cane sugar	0	5	0			
Wine, quantitative analysis for glucose and cane sugar	0	7	6			

SECTION X.—GASTRIC CONTENTS.

The cost of the examinations of gastric contents naturally varies according to what is required. When more than one of the following are required upon a single specimen, the charge made would be less than the cost of each examination separately		Cost for Members. £ s. d.	Cost for Non-Members. £ s. d.
General examination of gastric contents, including report upon microscopical examination for blood corpuscles, sarcinæ, food particles, leucocytes, particles of new growth, and Oppler-Boas bacilli, together with estimation of free and combined hydrochloric acid and organic acid	0 10 6	0 15 0	
Direct microscopical examination for blood corpuscles, leucocytes, food particles, sarcinæ, Oppler-Boas bacilli, or particles of new growth	0 3 6	0 5 0	
Qualitative test for free hydrochloric acid	0 2 6	0 3 6	
Qualitative test for lactic acid	0 2 6	0 3 6	
Quantitative test for free and active hydrochloric acid	0 7 6	0 10 0	
Quantitative estimation of the total acidity, the total free hydrochloric acid, the total combined hydrochloric acid, and the total organic acids	0 7 6	0 10 0	
Direct examination for Massol's bacillus	0 3 6	0 5 0	
Isolation of Massol's bacillus, estimation of total acidity and detection of lactic acid	0 10 6	0 15 0	
Estimation of total acidity and of lactic acid, and isolation of Massol's bacillus	1 1 0	1 11 6	
Culture of Massol's bacillus	0 2 6	0 3 6	
Quantitative test for pepsin and rennin	0 5 0	0 7 6	
Quantitative test for occult blood in gastric contents	0 5 0	0 7 6	
Fee for withdrawal of test meal, if within 5 miles of Charing Cross (mileage extra for further distances).	3 3 0	4 4 0	

SECTION XI.—HAIR.

Staining and examination for ringworm spores, with report	0 3 6	0 5 0	
Supply of stained specimen without report	0 2 6	0 3 6	
Staining of hair for ringworm spores with report, together with the stained specimen itself	0 5 0	0 7 6	
Qualitative analysis of hair for arsenic	0 15 0	—	
Identification of the variety of ringworm fungus by culture from the hair from	0 7 6	0 10 6	

SECTION XII.—INOCULATIONS IN THE DIAGNOSIS OF TUBERCULOSIS OR OTHER INFECTIONS.

Inoculations into animals for diagnostic purposes ...	1 1 0	1 11 6	
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SECTION XIII.—JOINT FLUIDS.

General examination of the fluid, including ordinary chemical, microscopical and bacteriological report, without cultures being made	0 10 6	0 15 0	
General examination of the fluid, including ordinary chemical, microscopical and bacteriological report, including the making of cultures and reporting upon them from	0 15 0	1 1 0	

SECTION XV.—MILK.

	£	s.	d.
Ordinary chemical analysis of fresh milk for adulteration	0	10	6
Ordinary chemical analysis of milk samples taken under the Sale of Food and Drugs Act	1	1	0
Complete quantitative analysis of cow's, human, or other milk for the percentage of carbohydrate, of proteid, of fat, water, and salts present ...	0	15	0
Quantitative estimation of total proteid present in milk	0	10	6
Quantitative estimation of total carbohydrate in milk	0	5	0
Quantitative estimation of total fat present in milk	0	5	0
Quantitative estimation of total fat and total carbohydrate in milk	0	7	6
Quantitative estimation of total proteid and total fat in milk	0	12	6
Quantitative estimation of total proteid and total carbohydrate in milk	0	12	6
Microscopical examination of centrifugalised deposit for pus cells, blood corpuscles, dirt, etc. ...	0	5	0
Examination of centrifugalised deposit for tubercle bacilli by direct staining	0	10	6
Bacteriological examination of milk for diphtheria bacilli	1	10	0
Bacteriological examination of milk for typhoid bacilli by cultural methods, from	0	10	6
Bacteriological examination of milk for other organisms by cultural methods, from	0	10	6
Quantitative bacteriological examination of a milk sample, i.e., enumeration of organisms	1	1	0
Animal Inoculation for detection of tubercle bacilli	1	1	0
Detection of sodium bicarbonate in milk	0	5	0
Detection of boric acid, salicylic acid, formalin, } hydrogen peroxide in milk }	0	2	6 each or 0 5 0 for three

SECTION XVI.—MOUNTING OF MUSEUM SPECIMENS.

The cost of making such preparations varies according to the particular requirements. Prices will be quoted on request.

SECTION XVII.—NASAL DISCHARGES.

The charges are the same as for Throat Swabbings (page 142),

SECTION XVIII.—POISONS.

	£	s.	d.
Testing for one specific metallic poison	0	15	0
General examination for metallic poisons, viz., Arsenic, Antimony, Mercury, Lead, Copper and Zinc	1	11	6
Testing for one specified alkaloidal poison	1	1	0
General examination for alkaloids from	2	2	0
Testing for one specified poison in the following list:—Hydrocyanic acid (or alkaline cyanides), Oxalic acid, Mineral acids or Caustic alkalies, Phosphorus, Chloral, Chloroform	0	15	0
Examination of Wall Paper or fabrics for Arsenic ... from	0	15	0
For the examination of the Viscera, 50 per cent. will be added to these fees.			

These fees apply only to cases in which the Directors of the Laboratories are absolved from all legal responsibility. If personal attendance at Court be necessary, the Association's expert must be guaranteed a special fee in addition to the charge for the examination of the specimen.

SECTION XIX.—POST-MORTEM EXAMINATIONS.

	Cost for Members.			Cost for Non-members.		
	£	s.	d.	£	s.	d.
The services of the post-mortem room attendant with the necessary implements	0	10	6	1	1	0

N.B.—The above charges are for attendance within a five-mile radius of Charing Cross. An extra charge of 6d. per mile is made when the distance is greater, or a special inclusive charge will be quoted upon application to the laboratory. Whenever possible at least twenty-four hours' notice should be given.

For the attendance of a qualified Morbid Anatomist to report upon the lesions found, special fees will be quoted on request.

SECTION XX.—PUS.

Examination of a pus film by direct staining for tubercle bacilli	0	5	0	0	7	6
Examination of pus for amœba dysenteriae	0	5	0	0	7	6
Examination of a pus film by direct staining by Gram's method	0	5	0	0	7	6
Examination of a specimen of pus by the ordinary cultural methods, from	0	5	0	0	7	6
Cultural examination for <i>B. pestis</i> (plague)	1	1	0	1	11	6
Cultural examination for typhoid bacilli	0	10	6	0	15	0
Cultural examination for <i>B. coli communis</i>	0	10	6	0	15	0
Cultural examination for <i>B. mallei</i> (glanders)	0	7	6	0	10	6
Cultural examination for gonococci	0	10	6	0	15	0

SECTION XXI.—RELATIVE ACTIVITIES OF ANTISEPTICS.

	£	s.	d.
The cost must necessarily vary with the extent of the work required; broadly speaking, however, the cost of determining the carbolic acid co-efficient of a given antiseptic by the Rideal Walker method with the bacilli typhosus is	2	2	0
Special quotations will be given upon request in any particular case or for a large number of samples.			

SECTION XXII.—SEROUS FLUIDS.

	Cost for Members.			Cost for Non-members.		
	£	s.	d.	£	s.	d.
General, chemical, and microscopical examination of a serous fluid	0	10	6	0	15	0
As above, with the addition of cultural examination	0	15	0	1	1	0
Estimation of the urea in serous fluid	1	1	0	1	11	6
Animal inoculation with centrifugalised deposit, e.g., in the diagnosis of a tuberculous lesion	1	1	0	1	11	6

SECTION XXIII.—SKIN.

1. Identification of Animal Parasites of the Skin, from	0	5	0	0	7	6
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	Cost for Members.			Cost for Non- members.		
	£	s.	d.	£	s.	d.
General microscopical examination of sputum, including presence of blood, pus, mucus, the presence or absence of tubercle bacilli	0	5	0	0	7	6
General microscopical examination of sputum as above, including microscopical examination for pyogenic organisms	0	7	6	0	10	6
Slides of any of the above, each	0	1	0	0	1	3
Bordet-Gengou reaction in sputum	5	5	0	6	6	0
Sulpho-cyanide test for saliva	0	2	6	0	3	6
Testing for mercury in saliva	0	7	6	0	10	6

SECTION XXV.—STAINS ON MATERIALS.

Examination of a given stain for blood	0	10	6	0	15	0
If due to blood, determination of the nature of the blood by the complement-deviation tests	4	4	0	5	5	0
Examination of a stain for spermatozoa	0	10	6	0	15	0

N.B.—The above charges refer to the cost of actual examination and report, but owing to the trouble that is apt to arise in medico-legal cases when the actual examiner of the stain has subsequently to attend at the Courts to give evidence, investigations that may possibly lead to the necessity for such attendance in Court can only be undertaken when a written guarantee accompanies the specimen to the effect that in case of attendance in Court being required a special fee will be paid for each day that such attendance is required.

Re-examinations will be reported by letter only, and charged	0	4	0	0	3	6
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SECTION XXVI.—THROAT SWABBINGS.

Cultural examination and report as to presence or absence of diphtheria bacilli, including telegraphic report	0	5	0	0	6	0
Examination of separate swabbings from right nostril, left nostril, and throat	0	10	6	0	15	0
Isolation of diphtheria bacilli in pure culture from throat swabbings	0	15	0	1	1	0
Re-examinations will be reported by letter only and charged	0	4	0	0	5	0
Direct examination of a swabbing for streptococci, staphylococci, pneumococci, etc.	0	5	0	0	7	6
Direct examination of swabbing for Vincent's angina	0	3	6	0	5	0
Cultural confirmation of micro-organisms other than diphtheria bacilli	0	10	6	0	15	0

When a large number of swabbings is sent to the laboratory all at one time from cases in a school, asylum, barracks, infirmary, or other similar institution, or by a Medical Officer of Health in connection with an epidemic in his district, reduced charges are made.

SECTION XXVII.—TUMOURS AND OTHER TISSUES.

	Cost for Members.			Cost for Non- members.		
	£	s.	d.	£	s.	d.
Macroscopic examination of tissue, and report ...	0	5	0	0	7	6
Preparation of microscopical section from an ordinary tissue and a written report. One slide in addition to the report	0	7	6	0	10	6
Ditto, when two or more specimens are received at the same time from the same case, each	0	5	0	0	7	6
Preparation and supply of one microscope section from an ordinary tissue, without report	0	2	6	0	3	6
Additional slides, each	0	1	0	0	1	3
Examination and report upon slides sent to the laboratory already prepared	0	5	0	0	7	6
Preparation and supply of a block without report or slide	0	2	6	0	3	6
Ditto, with one slide without report	0	4	0	0	6	0
Preparation of a section from a tissue requiring decalcification, with a written report. One slide in addition to the report	0	15	0	1	1	0
Preparation and supply of a section from an ordinary tissue requiring decalcification, without report...	0	10	6	0	15	0
Additional slides, each	0	2	6	0	3	6
Preparation and supply of a section of brain or nerve tissue, special staining, report, and slide ...	0	10	6	0	15	0
Additional slides, each	0	2	6	0	3	6
Celloidin sections, Weigert's method, etc.	0	3	6	0	5	0
Ditto, additional slides, each	0	1	6			
Sections stained for tubercle bacilli by Ziehl's-Nielsen method	0	3	6	0	5	0
Search by means of special stains for micro-organisms of tubercle, leprosy, anthrax, and actinomycosis, each	0	7	6	0	10	6

N.B.—The charges made for the preparation of sections stained by special methods do not generally differ greatly from those given above. Quotations will be made upon application.

SECTION XXVIII.—URINE.

General examination and report upon the reaction, specific gravity, naked eye appearances of the deposit, the result of tests for albumen and sugar, and microscopical examination of the centrifugalised deposit	0	5	0	0	7	6
General qualitative and quantitative examination, including the above general examination, with the addition of estimation of albumen and sugar (if necessary), uric acid, urea, chlorides, and phosphates	0	15	0	1	1	0
"Continental" analysis, as above, with the addition of total solids, total and ethereal sulphates, urobilin, bile, indican and acetone	2	2	0	3	3	0
Examination of urine qualitatively for albumin ...	0	2	6	0	3	6
Examination of urine for blood... ..	0	3	6	0	5	0
Examination of urine for pus	0	3	6	0	5	0

	Cost for Members.			Cost for Non- members.		
	£	s.	d.	£	s.	d.
Examination of urine qualitatively for sugar ...	0	2	6	0	3	6
Report upon chemical examination of urine, including the reaction, specific gravity, albumin, and sugar, with estimation if necessary	0	3	6	0	5	0
Microscopical examination of the deposit	0	3	6	0	5	0
Special examination for urinary pigments:— Hæmoglobin, methæmoglobin, hæmatoporphyrin, bile pigment, urobilin, urochrome, uroerythrin, melanuria, carboluria, alkaptonuria, from ...	0	5	0	0	7	6
The diazo reaction... ..	0	2	6	0	3	6
Complete examination for abnormal carbohydrates (e.g., galactose, pentoses, lævulose, lactose, maltose)	1	1	0	1	11	6
Qualitative examination for globulin	0	5	0	0	7	6
" " " acetone	0	2	6	0	3	6
" " " aceto-acetic acid	0	2	6	0	3	6
" " " oxybutyric acid... ..	0	5	0	0	7	6
" " " glycuronic acid	0	5	0	0	7	6
" " " lead in urine	0	15	0	1	1	0
" " " arsenic in urine... ..	0	15	0	1	1	0
" " " chyluria	0	5	0	0	7	6
" " " leucin and tyrosin	0	5	0	0	7	6
" " " Bence-Jones protein	0	10	6	0	15	0
" " " Bile, chemical tests	0	2	6	0	3	6
" " " Alcohol	0	5	0	0	7	6
" " " Phosphoric acid	0	2	6	0	3	6
" " " Methylene blue	0	5	0	0	7	6
Estimation of acidity	0	2	6	0	3	6
Determination of Joulie's acid and phosphate ratios	0	5	0	0	7	6
Quantitative estimation of urea	0	2	6	0	3	9
" " " uric acid	0	3	6	0	5	0
" " " both urea and uric acid	0	5	0	0	7	6
" " " total nitrogen	0	10	6	0	15	0
" " " urea, ammonia, and total nitrogen for "ammonia-co-efficient"	1	1	0	1	11	6
Quantitative estimation of total purin bases	0	10	6	0	15	0
" " " ammonia	0	5	0	0	7	6
" " " sugar	0	2	6	0	3	6
" " " albumin	0	2	6	0	3	6
" " " sulphates	0	10	6	0	15	0
" " " ammonia co-efficient	0	15	0	1	1	0
" " " of inorganic and ethereal sulphates	0	15	0	1	1	0
" " " of total sulphates	0	10	6	0	15	0
" " " calcium	0	10	6	0	15	0
" " " chloride	0	2	6	0	3	6
" " " phosphates	0	2	6	0	3	6
" " " albumose	0	10	6	0	15	0
" " " acetone	0	10	6	0	15	0
" " " oxalic acid	0	10	6	0	15	0
" " " creatinin	0	10	6	0	15	0
" " " indican	0	5	0	0	7	6

	Cost for Members.			Cost for Non-members.		
	£	s.	d.	£	s.	d.
Quantitative estimation of creatin and creatinin together	0	15	0	1	1	0
" " " " separated by	1	1	0	1	11	6
Determination of exact nature of proteid in ..	0	5	0	0	7	6
Determination of degree of rotation of polarised light	0	5	0	0	7	6
Examination for the detection of atropine, morphine, or apomorphine in urine ...	1	11	6	2	2	0
Examination for the detection of veronal ...	0	15	0	0	17	6

Bacteriological Investigations.

Examination for tubercle bacilli by direct staining ...	0	5	0	0	7	6
" gonococci by direct staining ...	0	5	0	0	7	6
" bacillus coli, cultural ...	0	10	6	0	15	0
" streptococci by direct staining ...	0	5	0	0	7	6
" " cultural methods ...	0	10	6	0	15	0
" staphylococci by direct staining ...	0	5	0	0	7	6
" " cultural methods ...	0	10	6	0	15	0
" pneumococci by direct staining ...	0	5	0	0	7	6
" " cultural methods... ..	0	10	6	0	15	0
" the bacillus typhosus, cultural ...	0	10	6	0	15	0
Cambridge's pancreatic reaction ...	0	10	6	0	15	0
The supply of urine bottles and metal cases, ready addressed for posting, per doz. ...	0	4	0	0	4	0

SECTION XXIX.—VACCINES.

Autogenous Vaccines.

PRIMARY.

Group 1. Acne bacillus. Bordet-Gengou bacillus. Gonococcus. Influenza bacillus. Koch-Weeks bacillus. Meningococcus. Pneumococcus. Septus bacillus. Streptococcus.	Group 2. Coli bacillus. Coliform organisms. Staphylococcus albus. " aureus. " citreus. " mixed.
Group 1. Fee £3 3s. Group 2. Fee £2 2s. for one organism, £3 3s. for two or three organisms.	Including isolation of organisms in the case of Members.

In the case of Non-members the fee for report is additional.

Ordinary autogenous vaccine consists of 12 graduated doses of not more than five different dilutions. For more than five dilutions in the 12 doses—£1 1s. extra to ordinary vaccine fee.

REDILUTION OF DOSES.

Charge, 10/6 for any number up to one dozen doses.

VACCINES PUT UP IN BULK (25 C.C.).

The charge is the same as for ordinary vaccines 12 graduated doses £3 3s. or £2 2s.

SECOND OR THIRD VACCINES (if ordered within 12 months).

Half the original fee is charged for a similar number of doses, prepared from a similar specimen from the same patient at a later date, if the material is of the same origin.

FURTHER DOSES FROM ORIGINAL EMULSION (after one year's interval).

Half dozen doses—one third original fee.

One " " one half " "

Stock Vaccines

FOR PRICE PER DOSE SEE BELOW IN CORRESPONDING DIVISION.

SERIES 1.		SERIES 2.		SERIES 3.	
Gonococcus		Acne Bacillus		Acne Mixed	
Influenza Bacillus		Coli Bacillus		Micrococcus Catarrhalis	
Micrococcus Melitensis		Cystitis Organisms		Coryza Organisms	
Meningococcus		Diphtheria Bacillus		Morax Axenfeld Bacillus	
Streptococcus		Friedländer's Bacillus		Bacillus Neoformans	
Conglomeratus		Para-colon Bacillus		Bacillus Paratetragenus	
or Rheumaticus		Pneumococcus		Pyorrhœa Organisms	
Whooping Cough		Proteus Bacillus		Staphylococcus	
12 graduated doses not		Pyocyaneus Bacillus		Albus, Aureus,	
higher than 750		Septus Bacillus		Citreus, or Mixed	
million, £2 2s.		Streptococcus		Bacillus Typhosus	
		Pyogenes		Prophylactic	
		12 graduated doses		or Therapeutic	
		not higher than		12 graduated doses not	
		750 million: series		higher than 1,000	
		of 12 doses		million: series of	
		£1 11s. 6d.		12 doses, 10/6	

SERIES 1.		SERIES 2.		SERIES 3.	
Million.	Per dose.	Million.	Per dose.	Million.	Per dose.
1	1/6	1	1/-	50	1/-
5	1/6	5	1/-	100	1/6
10	3/-	10	1/6	250	1/6
25	3/-	25	1/6	500	1/6
50	3/-	50	1/6	750	1/6
100	4/6	100	3/-	1,000	3/-
250	4/6	250	3/-	2,000	4/-
500	4/6	500	3/-	3,000	5/-
750	4/6	750	3/-	4,000	6/-
1,000	9/-	1,000	6/-	5,000	7/-
2,000	10/-	2,000	7/-		

ACTINOMYCES.

0·001 mgr.	}	2/6 per dose or 25/- per dozen.
0·002 "		
0·003 "		
0·004 "		
0·005 "		
0·0075 "		
0·01 "		

GENERAL NOTES.

The price per dozen doses of stock vaccine = 10 times the price of one.

The price per half dozen = 5½ times the price of one.

The prices of each special dose of stock vaccine = twice the price of the stock dose next lower in strength.

25 c.c. bottles of stock vaccine:—

When a single dose of stock vaccine costs 1/-, 1/6, or 3/-, a 25 c.c. bottle of stock vaccine is supplied for 21s.

When a single dose costs from 3/- to 10/- per dose, a 25 c.c. bottle of stock vaccine is supplied for 42/-

SECTION XXX.—VETERINARY DISEASES.

The cost is that for carrying out investigations upon the blood, urine, pus and purulent discharges, tumours and so forth in man.

Radiography.

For Radiographs taken at the Radiographers.

	£	s.	d.
A. Hand or Wrist	0	10	6
B. Foot, Arm below Elbow, or Leg below Knee	0	10	6
C. Elbow Joint, Knee Joint, or Ankle Joint	0	15	0
D. Thorax, Shoulder Joint, or Thigh	1	1	0
E. Hip Joint, Abdomen, Pelvis, or Head	1	11	6

The above prices are inclusive of a mounted print.

If more than two positions are required, an additional charge of half the fee for each extra position.

For Radiographs taken at the Patient's House.

Within 5 mile radius of Charing Cross, A and B	4	4	0
" 5 " " C, D and E	4	4	0
" 10 " " A and B	5	5	0
" 10 " " C, D and E	5	5	0
Beyond 10 miles ... " A, B, C, D and E	By arrangement.		

Travelling expenses charged extra at cost.

Miscellaneous.

Sterilisers, bacteriological testing of efficiency of	1	1	0
Examination of air for CO ₂ , including its collection ... from	2	2	0
Examination of air for organisms. Special fee.			
Additional copies of reports each	0	1	0

Reports drawn up for scientific publications and special researches are undertaken at special charges.

Catgut, Silk, etc., Bacteriological examination of ... from	0	10	6
Spermatozoa, microscopical detection of	0	3	6
Semen, verification of nature of	0	5	0
Spirochæta Pallida, direct examination for	0	10	6
	0	15	0

All *urgent* specimens are charged for at the rate of double the ordinary fee with a minimum of 10/6.

Photomicrographs.

Of Sections of Growths—

Magnification 120 diameters	0	5	0
" 320 "	0	7	6

Of Bacteria—

Magnification 1000 diameters	0	10	6
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These charges include the negative and one print.

Standard Culture Media.

Liquid media can be obtained, in tubes, *at the Laboratory*, or can be sent in bottles, with sterilized tubes, into which it can be filled on arrival. Solid media can be supplied in either form.

The following list shows those Media which can generally be supplied immediately. Other varieties can be prepared, when required, at special prices:—

Nutrient Gelatine	per dozen tubes	0	3	0
Nutrient Agar	"	0	3	0
†Nutrient Broth	"	0	3	0
McConkey's Bile Salt Media:—								
†Glucose Litmus Bile Salt Broth, single strength,	per dozen tubes					0	3	0
†" " " double "					"	0	4	6
Lactose Neutral-Red Bile Salt Agar	"	0	3	0
†Peptone Water, Glucose Peptone Water, etc., etc.					"	0	3	0
†Litmus Milk	"	0	3	0
Potato	"	0	3	0
Blood-Serum, as used by the Association	"	0	3	0
Glycerine Agar	"	0	3	0
Culture	(without sterilized tubes)	per	$\frac{1}{4}$ litre	0	3	0
Media	(with 25 ")	"	0	4	6
in bulk	(without ")	$\frac{1}{2}$	0	4	0
	(with 50 ")	"	0	7	6

† These being liquid media, can only be sent by rail or post in bulk, but they can be supplied in tubes at Laboratories when called or sent for.

All the above media can be supplied in tubes provided with rubber caps, to prevent drying of the medium, at an additional cost of 1s. per doz.

N.B.—For quantities of less than three dozen tubes, carriage and packing are extra. Single tubes, or small numbers, are charged for at the rate of 6d. per tube.

Packing Cases, &c.

For Members only.
£ s. d.

A case containing one dozen bottles for sputum, or tissues for histological examination, and half-a-dozen bottles for urine, with labels and metal cases to meet the postal regulations	0	4	0
The Emergency Box containing a <i>large</i> bottle suitable for urine, vomit, milk, and other fluid specimens; a <i>small</i> tin containing a bottle with preservative fluid for growth and other tissues for histological examination; a <i>small</i> tin (grey label) containing a receptacle for sputum and dry specimens; a <i>blue envelope</i> containing a tube with needle and directions for collection of blood for the serum diagnosis (Widal) of typhoid or Malta fever or for opsonic index; a <i>white envelope</i> containing a sterile swab with directions for taking rubbings from suspected throats, for bacteriological exami-					

							For Members only.		
							£	s.	d.
nation; a pink envelope containing a box with slides and directions for the preparation of films for the examination of blood corpuscles, including a differential count of white cells; or for the examination of pus for the gonococcus and other organisms	0	2	6
A sterile swab, in metal case	0	1	0
An apparatus for Widal's reaction or opsonic index...							0	0	6
A box of slides for films of pus or blood					0	0	6
A blood-culture outfit, containing tubes of culture media for inoculation with blood for examination for micro-organisms			0	1	0

N.B.—These cases are supplied at cost price, carriage paid. The contents will be varied to suit individual requirements upon special application. Single bottles in regulation metal cases can be supplied from twopence each, postage extra.

A blood cabinet, containing apparatus for the estimation of hæmoglobin and enumeration of the red and white corpuscles: this is not charged for, but is sent out on loan.

A pus apparatus, for cultivation of organisms in pus, necessary when a vaccine is to be prepared from the organism: on loan, or price 2s. 6d.

Drinking-water apparatus: supplied on loan.

Directions as to the method of use accompany all apparatus sent out.

Modes of Payment.

Members of the Association may either forward the amount of the charge for each examination at the same time as the Specimen by crossed postal order or cheque, or they may send a larger sum, which will be credited to their account.

Members may, if they prefer it, have quarterly accounts furnished of the Association's charge for work done on their behalf.

Non-members are expected to forward a crossed postal order or cheque at the same time that a Specimen is sent for examination.

The Association cannot hold itself responsible for coins enclosed with the specimen.

The fee charged for examination is now stated on each Report.

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