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STUDIES IN PUNCTURE-FLUIDS

STUDIO IN WASHINGTON

STUDIES IN PUNCTURE-FLUIDS

A CONTRIBUTION TO CLINICAL
PATHOLOGY

BEING A THESIS APPROVED FOR THE DEGREE OF DOCTOR
OF MEDICINE IN THE UNIVERSITY OF LONDON

BY

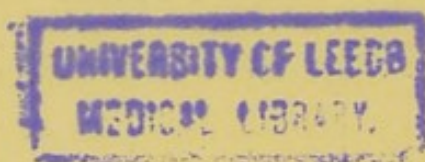
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LONDON

H. K. LEWIS, 136 GOWER STREET, W.C.

1908



P R E F A C E


It has now become generally recognised how great is the assistance which the detailed chemical and physical examination of morbid material affords the physician or surgeon in arriving at or confirming a diagnosis. In carrying out the research recorded in this book, the requirements of the clinical pathologist have therefore been steadily kept in view.

Probably the greatest value of future investigations into the nature and composition of puncture-fluids will be found to lie in the additional light they may be expected to throw upon the subject of metabolic processes in diseases. At present the available data are so scanty that it is impossible to treat this subject in detail, but it is hoped that the special index referring to the puncture-fluids met with in different morbid conditions may prove useful.

My grateful thanks are due to the members of the Honorary Staff of this Infirmary, who have kindly permitted me to publish the results of my work upon their cases ; to Dr. G. W. Watson and Mr. J. A. Coupland for assistance in tracing the clinical records of the cases studied ; and to the various members of the Infirmary Staff who have preserved material for analysis. Lastly I must gratefully acknowledge the debt I owe to the writers of the works I have consulted during the course of my researches.

O. C. GRUNER.

PATHOLOGICAL LABORATORY,
GENERAL INFIRMARY, LEEDS,
July 1908.



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The decomposition products of puncture-fluids

ERRATA

- Page 8, line 17 from top, for "hysico-" read "physico-"
Page 30, Table III, for "single" read "simple"
Page 31, line 5 from top, for "Burians'" read "Burian's"
Page 32, line 2 from bottom, for "togatose" read "tagatose"
Page 44, line 14 from bottom, for "marrows" read "marrow"
Page 51, line 17 from top, omit "in"
Page 55, line 8 from footnote, for "orhistid in" read "or histidin"
Page 64, line 2 from bottom, for "H + " read "H+ "
Page 68, line 10 from bottom, for "oxidations" read "oxidative ones"
Page 104, to formula in middle of page add "atmospheres"
Page 139, line 2, omit "fluid"
Page 173, line 6 from top, for 1'22 read 1'31
Page 180, in the Table, for "hydrops. folliante" read "hydrops. folliculi"
Page 182, line 11 from top for "cetylalcohol" read "cetyl alcohol"
Page 185, last line of Table XX, for "trypisn" read "trypsin"
Page 206, line, 15 from top for "Wideroe" read "Müller"
Page 234, last line, before Fig. 5 insert "Plate II"

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THE investigation of the characters of puncture-fluids may be made with two objects: first, to supply, if possible, the means of diagnosis in those cases where the clinician considers an exploratory puncture indicated; and, in the second place, to supply a gap in the knowledge of the chemistry of these fluids. Each of these aims has been pursued in the present work; and though there is comparatively little to be said about differential diagnosis that has not been said before, there still exists a need, it is thought, for bringing together the various diagnostic points, both for reference and for criticism.

In regard to the first aim, the scope and limitations of diagnosis call for primary consideration. As it has been with cytodiagnosis for the past eight years, so it has been for a much longer period of time with chemical differential tests of puncture-fluids. The clinician has had his hopes of infallible means of distinguishing between, say, exudate and transudate raised, only to find that exceptions to the rules arise and nullify the value of the particular test. Or, again, he finds that the method advocated, though good, is yet too tedious for him to make use of it during routine clinical work.

Both of these adverse views need modification, for, in the

first place, it is unlikely that there will ever be a distinctive test for any given disease that can be furnished by an effusion arising during its course, simply because it may be stated as a fundamental truth that there is nothing fixed and immutable in pathology; and, in the second place, it has to be conceded that though there are many tests which are tedious, yet a rapidly performed test is not likely to be as reliable as a more thorough one, because the very thoroughness will strike out variables in the factors which contribute to the diagnosis. It is true that a complicated procedure is impossible in the course of practical clinical work, but, on the other hand, it is equally true that the careful study that can be made in suitably equipped clinical laboratories is worthy of pursuit.

The endeavour to add to the knowledge of the composition and constitution of puncture-fluids has, however, been the main object of the studies which are recorded in the present work. It is, however, readily seen that it is impossible to carry out every form of analysis in a given fluid simultaneously, so that it becomes necessary to make a selection of the particular investigations desired and to confine oneself rigidly to them in a given series of specimens. Other forms of investigation can be arranged, and carried out in another series of fluids. This has been found the only way to cope with the subject in the absence of co-workers—the ideal method of investigating this subject where autolytic changes or decomposition-phenomena require to be forestalled. It is necessary to mention such difficulty in order to make clear that in no one fluid which one has examined have all the aspects of its chemistry been gone into, and that to this extent an absolutely full and just summing up of the properties of an effusion in a given disease or given patient must fail. On the other hand, it must be remembered that for the purposes of the clinician this line of study is quite impossible, and, moreover, exploratory puncture often only allows a few cubic centimetres of fluid to be submitted for examination.

It will be found from the subsequent pages that the attempt has been made to compel the physico-chemistry to supplement the chemistry of the puncture-fluid. This has been applied, as is well known, by several renowned scientists, to the examination of *blood* and *urine*, but so far as can be made out, there has been

no attempt hitherto to apply the principle to the study of the ordinary puncture-fluids. It is believed, however, that such an application of the newest lines of study—those of ionic constitution—will afford an insight into the metabolic processes of those diseases which are associated with the pouring out of fluid.

Not only this, but the attempt has been made to compel chemistry and physico-chemistry together to afford an explanation of the concordance or discordance which exists between the diagnosis hitherto made by their aid, and the actual nature of the disease; this has, however, frequently led one into the subject of the pathology of metabolism, a subject which must of necessity be left in the background in the following pages. Still, it must be reasonable to suppose that the study of the fluids which are poured out must give great aid not only to the pathology of metabolism of a given disease, but must assist in forming a diagnosis of the disease. To illustrate this idea, the deviations from normal metabolism which obtain in disease of the liver may be referred to. They will depend to a large extent on the nature of the disease with which the liver is affected, and more than this, these changes will be different according to whether the disease is primarily one of the liver, or primarily one of the heart. Each of these is bound to result in a different succession of changes from those due, for instance, to renal disease, and the chemical composition of the fluid poured out will be correspondingly different. The excessive amount of chlorides present in nephritic effusions as compared with back-pressure effusions instances the correctness of this contention. Then, again, the investigation of the various decomposition products of proteid that occur in an effusion will afford a not inconsiderable light on the catabolic changes met with in the disease with which the effusion is associated.

To consider for a moment another example of this principle. The fluid in a case of peritoneal carcinomatosis presents, on cytological examination, desquamated carcinoma cells, some of which are living and some necrotic. These cells have a specialised form of metabolism, though the only known fact about them is that their proteid is essentially different in nature from normal cell-proteid.* This difference in constitution must involve a

* Bergell. See also Hoffmann, *Münch. med. Woch.*, 46, 1907.

difference in the products of breakdown of the proteid, though exactly what the differences in the two series of cases (healthy metabolism, and carcinoma-cell metabolism) may be, and where they are to be located in the configuration of the proteid molecules, is quite unknown at the present time. The presence of both living and dead cells too, not only in the fluid, but all over the surface of the peritoneum, means that these metabolic products occur in the fluid which they have caused to appear, and a chemical study of such a fluid must afford an insight into the metabolic changes of carcinoma cell-life. Not only this, but there is the question of ferment action to be considered. There are ferments in the peritoneal fluid of carcinomatosis cases, which may be specific, and are of importance, because they have actuated this particular form of metabolism. We must not forget meanwhile that the whole of the serosa is not one mass of proliferating carcinoma. There are extensive areas in which there is no carcinoma, and the chemical composition will depend to some extent on the normal processes of life of these cells, and on their permeability-phenomena. That is to say, we shall meet with the resultant of two series of processes—quite a different matter from having only a single factor to deal with. If reference be made to the possibility of a *diseased* condition of the hitherto intact serosa following the changes produced in the organism as a whole (cachexia, etc.), we shall at once see clearly that there are so many factors that it would be remarkable if all diagnostic rules in the case of carcinomatous puncture-fluids were always infallible. Perhaps it is more important to suggest that herein lies the means of justly appreciating the facts on which one can base a diagnosis, for by bearing the variables in mind, one may attempt to secure more accuracy in one's opinion.

These considerations have been gone into at such length, because it is felt that there is an undue tendency to expect such investigations as are recorded in the following pages to enable a positive and certain diagnosis to be made as to the nature and origin of an outpouring of fluid. It is not so. We must say, "such and such a substance (of which the tests are given) is more abundant in a certain percentage of cases of this class of effusion than it is in another class of case." Even these limitations do not deprive the methods of all utility, for clinical symp-

toms often help, and just as pros and cons have to be weighed in ordinary clinical diagnosis, so they frequently have to be weighed in clinical pathological diagnosis.

Attention must be directed also to the errors that may arise from the difference in the structure of various proteids. Many proteids contain the same fundamental substance as an integral part of their molecule. The following table, which has been abridged for the present purpose, from the valuable work of Gustav Mann (*Chemistry of the Proteids*, 1907), will illustrate what is meant. We see, for instance, that glycocoll forms a part of the molecule of serum-albumen, of serum-globulin, of hetero-albumose, and of keratin; that tyrosin occurs in serum-albumen, in protalbumose, in Bence-Jones proteid, and in keratin; that tryptophane is only present in keratin (in this table), and so on. Our chemical analysis may reveal the presence of all these bodies, and of course it would only be possible by a careful separation of each of these bodies to say to which the derivative is to be ascribed. If we find tryptophane or tyrosin in a puncture-fluid, for example, we cannot be sure that it was there beforehand, or whether the analysis has resulted in its being split off from a proteid containing it.

	Serum- albumen.	Serum- globulin.	Hetero- albumose.	Prot- albumose.	Bence- Jones proteid.	Protamin (Sturin)	Horn- keratin.
Glycocoll	+	3.52	+	0	0	..	0.34
Alanin	+	2.22	1.2
Leucin	+	18.7	+	+	+	..	18.3
Phenylalanin ..	+	3.84	+	0	3.0
Prolin	+	2.76	3.6
Glutaminic acid	+	2.20	+	..	14
Aspartic acid ..	+	2.54	2.5
Cystin	1.2	1.51	0	6.8
Serin	5.70
Tyrosin	2.7	..	0	+	+	0	4.58
Lysin	3.5	3.5	+	12	..
Histidin	2.2	2.2	..	12.9	..
Arginin	4.9	4.9	..	58.2	2.25
Tryptophane	0	0	+
Ammonia	1.75	0.8	0.8	1.6	0	+
Amino-valerianic acid	5.7
Glucosamin	0	0	0

The table reveals a further fact, namely, that the *amount*

of each derivative varies according to the different proteid. Thus, if we find seven parts of lysin we shall not know whether this means that it has all been derived from equal parts of hetero-albumose or protalbumose, or whether it is a proportion characteristic of some other proteid not included in this table. We might even say that we cannot be sure that the numbers in this table do really characterise the particular proteid under which it is placed. But assuming the table absolutely correct, and assuming that our analysis is correct, it is evident that by slightly altering the relative proportions between one derivative and another we shall really be having a different proteid before us. Such slight differences, which involve us in endless permutations and combinations, would afford an explanation of the *biological* distinctions that obtain between one proteid and another, between fish-albumen, and mollusc-albumen; not to mention the possibility that stereo-isomeric variations might result in still more refined and none the less absolute distinctions.

To speak of such possibilities as these is but to touch on a fringe of the difficulties that arise in interpretation of results of analysis, and would at first sight deter one from ever attempting an opinion in a case. Fortunately, however, there remain many tests of easy application which will help the clinician to form his diagnosis in a case associated with effusion. Even in spite of all the chances of error which have been indicated, these tests preserve their utility, and the fact that failure may sometimes arise should not be allowed to relegate them into the background; and we may hope that with time, a better knowledge of the details to which reference has been made will explain the cause of the failure of such tests in some cases, and enable a better interpretation, with more frequently successful diagnoses, to be made.

The statement may be made that a transudate with decided characters may be certainly distinguished from an exudate possessing decided characters. The border-line cases, or the cases in which inflammation supervenes on transudation, are those which are responsible for the small utility which chemical diagnosis and cytodiagnosis have possessed. But if we remember that the microscope diagnosis of innocent tumours from malignant tumours also fails when we come upon the

border-line cases, we shall see that there is no more justification for condemning the one method of study than the other.

In pursuit of the study of puncture-fluids it has therefore been borne in mind that we shall learn far more from the failures in diagnosis than from a long succession of successes, and the endeavour to find an explanation for the failures will lead us deeper and deeper towards the solution of the ultimate problems of metabolism.

Turning now to the actual methods of study which have been employed in the course of this work, it will be seen that an ordinary chemical examination has been supplemented by a physico-chemical examination of the puncture-fluids, with the object of obtaining an analysis of the grouping of the ions present in any given case.

A consideration of the details of the ionic theory on which so much of biochemical research is based comes more under the domain of theoretical chemistry.* A brief account of this ionic theory, as well as of the theories on which cryoscopy is based, have, however, been included for the sake of completeness ; but it has been felt desirable that the details of the method of performing both cryoscopy and electro-conductivity be omitted. The question of the significance of the concentration of the hydrogen ions as affording an understanding of the meaning of the term acid and base has been entered into, in order to give an explanation of much special work that has been performed, especially in Italy, and to indicate where such conceptions may be erroneous. The fact that hydrogen ions possess practically the same concentration as the hydroxyl ions in puncture-fluids does not really alter the fact that they have more basic properties than acid, that, indeed, they are not really functionally neutral fluids.

The mention of the term cryoscopy inevitably calls up visions of a vigorous controversy between exponents of the theory that renal disease is diagnosable by its aid and the opponents of the theory. The fact must be admitted that cryoscopy was unduly advocated for a purpose for which it is unsuitable, since the estimation of the working power of the kidney cannot be based solely on the molecular concentration of the urine, because the osmotic work performed by the kidney con-

* See Svante Arrhenius, *Theories of Chemistry*, 1907.

sists of the osmotic work of water-secretion *plus* that of water absorption *plus* that of selective action on the constituents of the blood,* a datum which cryoscopy fails to supply. However, the method is useful as affording a value for the osmotic concentration of a puncture-fluid which, by association with a determination of the electro-conductivity, or a determination of the substances impermeable to red cells (Hamburger's method), enables us to classify the varieties and amounts of the different kinds of ions present. The determination of the relations between the amount of chlorine ions and of the achloride ions to the substances for which red cells are impermeable,† is valuable for deciding on the molecular constitution of various fluids. Such a study demands, of course, considerable apparatus,‡ unnecessary for the clinician, it is true, but valuable to the biochemical student to exactly the same extent as is the microscope to the histologist, with the difference that much more care and practice are necessary before the physico-chemical apparatus can be effectively used.§

The scheme of chemical analysis which is submitted is the result not only of practical experiments but also of study of the various methods which have been advocated by eminent chemists. It was, however, felt that a combination of the different methods of separation of the proteids might be advantageous, and an abstraction of the methods described in many numbers of the *Zeitschrift für physiologische Chemie* should be found convenient, especially to clinicians who have not the time to turn up the volumes needed. The confirmatory tests which are given are

* Pauli, *Physical Chemistry in Medicine*, 1907.

† The difference between the molecular concentration as given by the cryoscopic method and that given by Hamburger's blood-corpuscle method gives the concentration of the bodies for which red cells are permeable, and those very bodies are all the most important *products of proteid catabolism*.

‡ All this special apparatus barely costs as much as one of the better microscopes.

§ The physico-chemical methods of examination may be grouped under the following headings :

1. The determination of the total osmotic concentration of a fluid.
2. The determination of the concentration of the chloride electrolytes in the fluid.
3. The determination of the achloride electrolytes.
4. The determination of the concentration of the non-electrolytes.
5. The determination of the concentration of the hydrogen ions.

also a compilation from the literature, but it has been felt that the many confirmatory tests desirable before stating definitely that a given substance is present should be collected together.

The fluids which have come under consideration have been classified as follows, and are dealt with in the same order :

1. The fluids which occur in the large serous cavities :
 - (a) Pleural. Exudations, transudations, pus.
 - (b) Peritoneal. The same varieties.
2. The fluids which occur in the small serous cavities ; such as the joint-effusions.
3. The cerebro-spinal fluid.
4. Fluids derived from cysts : (a) intra-abdominal ; ovarian ; pancreatic ; rare cysts ; (b) in other regions of the body.

Each of these classes of fluids will be studied from their chemical and their physico-chemical aspects.

Finally, a reference will be made to the cytological characters of various puncture-fluids, as the cellular elements which are present in an effusion have some bearing on its chemical composition, and are probably largely, if not entirely, responsible for the ferments which it may contain.

SECTION I

THE CHEMICAL EXAMINATION OF PUNCTURE-FLUIDS

CONTENTS: Preliminary remarks—Difficulties attached to the analysis of fluids—Precipitation of albumen by mastic, etc.—Adsorption—Scheme for analysis of puncture-fluids. (A) Preliminary processes. (B) Separation of the globulins—Albumen—Globulin—Albumoses and peptones—Monaminoacids—Ammonia—Hydrolysis—Sugars—Purins and urea. (C) Diaminoacids—Residual nitrogen. (D) The glycoproteids; pseudo-mucin, its properties and reactions; paramucin; synovin—Lecithin, its importance, constitution, allies, and symbiotic substances, and methods of analysis—The diazo-reaction—The α -naphthol reaction—Ehrlich's glucosamine reaction—Tryptophane—Pigment—The inorganic constituents of puncture-fluids—Ferments, their detection, their occurrence, and their importance.

THE great strides which physiological chemistry has made during recent years enable us to obtain a far deeper insight into the processes of pathological metabolism than was before possible. The flood of light which has been thrown on the constitution of proteid matter by such an eminent organic chemist as Emil Fischer has perhaps furnished the most striking increase in our knowledge not only of pathological chemistry but of pathology as a whole. The application of this knowledge to a study of the pathological effusions in various parts of the body seemed a promising field of research, especially as these fluids can be made to afford interesting evidence of the processes of breakdown of proteid in disease. As Umber * has said, an effusion into the peritoneum is an accumulation in a sterile reservoir of the intermediate retrograde products of proteid metabolism which are preserved intact for a considerable length of time owing to the processes of absorption being exceedingly slow.

* The references to the original articles whose authors are quoted are collected into a separate bibliography for each section and placed at the end of this book.

The first problem which presents itself is that of devising a suitable *method* of analysis which shall enable the various bodies likely to be present in a given puncture-fluid to be detected, and, if possible, estimated quantitatively. There is, however, the difficulty that in some cases there is only a very small amount of fluid available for analysis, so that methods have to be found which will enable as many of the important substances as possible to be searched for in the successive fractions obtained. There is also the difficulty that the results obtained are awaited by the clinician, who does not wish to delay with his final diagnosis any longer than necessary. Moreover, the sooner the analysis can be completed the less chance there is for autolytic decomposition of the fluid to take place.

Of all the constituents of puncture-fluids the most abundant is *albumen*, and the interest attached to this substance is more than that as to its mere quantity, since, in the first place, it may become involved in autolytic or in fermentative changes which lead to the production of dissociation products, while, in the second place, it is responsible for certain important physical properties that such fluids manifest. It is perhaps too much an exaggeration to speak of the peritoneal fluid, for instance in tuberculous peritonitis, as really an *albuminous* solution of various substances, but, on the other hand, one cannot fail to be struck by the *volume* of the coagulum when such a fluid is boiled in the presence of acetic acid. On this ground it is not altogether incorrect to speak of most puncture-fluids as proteid solutions, an aspect which will emphasise the essentially *colloidal* properties of these fluids, that are so important to realise when forming a conception of the part which they play in pathological metabolism.

Not only this, but the albuminous nature of the fluid (90 per cent. by volume in many exudates) justifies the statement that exact quantitative analysis of a puncture-fluid is impossible, since the removal of the albumen destroys its essential nature, apart from the fact that its removal disturbs the osmotic and ionic relations of the other constituents.*

To remark "remove the albumen by boiling," especially if there be much albumen, is merely to ignore all these difficulties.

* This aspect of the subject will call for further comment on a subsequent page.

We have then to study the properties of our fluids, not from the standpoint of a water-basis, but from the standpoint that the properties are those possessed by colloids towards electrolytes in (a) colloidal and (b) in watery solution. We have also to bear in mind the fact that the proteids present in these fluids are the bearers of electrical charges, in virtue of the fact that electrolytes *are* present,* and that the process of boiling, or, indeed, any method of removal of proteid from the solution, destroys or alters the electrical charges, and thus interferes with physico-chemical properties, besides allowing free play for new associations to take place.

METHODS FOR REMOVING ALBUMEN

Mere mention of the *familiar* processes of removal of albumen from a fluid is sufficient, and those which are advocated for the study of puncture-fluids are given in Tables A to D. But an important method of removal of albumen, devised by Michaelis and Rona, calls for more careful consideration, since it makes use of the phenomena of adsorption for separating off the albumen. These authors point out the fact, already laid stress on, that the ordinary methods of de-albuminisation of a fluid are unsatisfactory for many purposes, since boiling damages the fluid, alcohol is undesirable in subsequent processes, and salting out introduces a new element altogether. Starting with the fact that colloids of opposite electrical sign will cause one another to separate out, provided the two bodies are present in just sufficient quantity, the two authors mentioned hit upon the use of a watery solution of mastic which should exert this electrical effect. They found (1) that if a *small* quantity of mastic be added to a large proportion of albuminous solution, the mastic would not be separable, owing to a protective action exerted on it by the albumen; (2) that if the proportions of the mixture were reversed the opposite held good. The explanation of the phenomenon is afforded by the ultra-microscope, which shows the particles of albumen in combination with particles of mastic, in such manner that each particle of mixture consists of mastic, plus albumen. If there be excess of the latter, it becomes possible

* Albumen, pseudo-globulin, and euglobulin show no electrical charge in the absence of electrolytes (Pauli).

for each particle of mastic to be completely enveloped by albumen. The surface tension will thus only have effect on the albumen and not on the mastic. This will hold good until so much mastic has been added that it cannot all be "masked," and at that point the whole of the albumen will separate out.

The methods which they advocate are :

Method A.—A 10-per-cent. solution of mastic in alcohol is suddenly diluted with twice its bulk of water, and the resulting mixture is added to the fluid to be de-albuminised in the proportion of 10 parts mastic mixture to 1 of fluid ; 2 parts of 10-per-cent. acetic acid are then added, in order to acidify the mixture. In half an hour a similar quantity of mastic is added gradually, and acidified by a further similar quantity of the acetic acid ; 2-3 parts of 10-per-cent. magnesium sulphate are now added gradually until a definite precipitation takes place, and after a short period of digestion in a tepid water-bath, the fluid will be found to filter easily and leave a completely albumen-free filtrate. In the event of the removal being incomplete, a third addition of mastic is indicated.

Method B.—50 cc. of fluid are diluted with 12-15 parts of water, and as much acetic acid is added as will clear up the turbidity. To every 100 cc. of fluid 20 gm. of kaolin are added in four or five instalments, vigorous shaking being carried out during this process. The filtrate will now be quite free of albumen. This method, published in July 1907, obviates the necessity in method A of first getting rid of excess of albumen by a preliminary alcohol precipitation, which renders method A applied to fluids rich in albumen of no advantage over former methods of de-albuminisation.

The filtrate from these methods contains monaminoacids, diaminoacids, and polypeptids ; and it is necessary to remember that there is present in this filtrate a nitrogen-free substance of unknown nature, soluble in water, which will come down with phosphotungstic acid unless an excess of tannin be added to prevent its precipitation by phosphotungstic acid.

The precipitate will contain albumen, globulin, and albumose, but no sugar.

Pickardt's Method of Removing Albumen.—200 cc. of fluid are boiled for three-quarters to an hour ; then add dilute acetic acid, drop by drop, till just acid. Heat three-quarters of an hour in a

current of steam. Boil the residue with water, and squeeze it out in a hand-press. The mixed filtrates are filtered while hot and the residue washed with hot water. The filtrate will be absolutely albumen-free.

A *clinical method* of estimating albumen has been devised by Deycke and Ibrahim. 2 cc. of fluid are mixed with 3 cc. of $n/5$ soda (forms a soluble alkali-albuminate), and is then made up with soda to 100 cc. in a flask. 25 cc. of this diluted solution are placed in a flask of 200 cc. capacity, and 2.3 cc. of glacial acetic acid and 20 cc. of potassio-mercuric iodide solution are added. The volume is made up with distilled water. The precipitated mercury albuminate is filtered off and 100 cc. of filtrate treated with 10 cc. potassium cyanide and 10 cc. ammonia. The amount of unaltered cyanide is estimated by titration with $n/20$ silver nitrate.

ADSORPTION.—Before proceeding with the actual methods of analysis there remains one more theoretical consideration, namely, the effect of removal of proteid *on the electrolyte contents*. It will be seen that considerable importance is being attached to the amount of electrolytes present in exudates as compared with transudates (Section IV), and the question arose not only as to the most simple method of analysis but as to whether there was any source of error likely to arise in any of the methods adopted for removing the albumen. It is well known that if albumen be treated with hydrochloric acid a certain amount of the latter will enter into combination with the albumen, and cannot be detected in the filtrate after boiling to remove proteid. The albumen is considered to have *adsorbed* some of the Cl.

What is to be exactly understood by the term "adsorption" it is difficult to say, and this word has been invoked perhaps to give an apparent explanation for phenomena which were not correctly understood. Thus it is admitted, even by Ostwald, that there is no sharp difference between chemical affinity and adsorption. In fact, when one comes to consider it, it is often really very difficult to be sure that the union between two bodies is physical and not chemical.

The question at issue which renders it desirable to discuss adsorption at all is as to whether the chlorine met with in a puncture-fluid is "free" or "combined" with the proteid.

We wish to know how much of the chlorine present in the fluid is really unattached to the proteid, consequently it is not enough to know the total amount of chlorides as estimated, say, by incineration. Moreover, it is specially convenient to use a titration method of analysis. If proteid does hold sodium chloride in its meshes during the process of coagulation by heat, then the chloride estimated by analysis will only represent that portion of the NaCl which is unattached to the proteid, the rest having passed irrevocably into the coagulum. We should say, with Schulz, that some of the Cl is "essentially" bound up with the proteid, and the rest is "accidental."

In a series of studies on the physiology of glands (1907) Asher discusses the very same question, though with a different object. He is concerned with the relation of secretion to the physical condition of the blood-serum, and endeavours to make out, on the one hand, whether the substances in serum are held in solution or not, and, on the other hand, whether chlorine in tissues or body-fluids is free or bound. To solve the problem he uses cryoscopy and electro-conductivity determinations. Some reference to this work will, however, be made in the next section.

While Bayliss goes at length into the effect of Congo red on filter-paper as an "undoubted" example of adsorption, we receive a rebuff from the writings of others,* who insist that the reaction is really a *chemical* one. The same author describes the adsorption of salts by gelatine; but in the experiments narrated it cannot be taken as complete proof in this case either that adsorption, and not chemical affinity, is the mechanism. If gelatine be washed with water, the first washing will remove so many salts. The second washing will remove much less if there be no chemical union, because the percentage of salts on commencing the second wash is so much less than those present at the first wash. Again, at the third wash there is still less salt, and there will be very much less loss of salt at the fourth wash.

On this ground there is not, at first sight, any difference between the process described by Bayliss in washing out the electrolytes of gelatine and ordinary washing out by diffusion. In each case the rate of loss of salts will be slow.

According to v. Bemmelen, the factors on which adsorption depends are: the adsorbing substance, the solvent, the

* Above all, of Gustav Mann.

substance to be adsorbed, the state of its molecules, and the temperature.

These conditions, however, are not sufficient, since there must be a *time* element to consider.

Fortunately, the subject becomes more intelligible from Höbers' clear and distinct diction. This author points out three properties which are characteristic of adsorption.

1. The equilibrium between the adsorbed substance and the nonadsorbed substance varies according to whether the second is added *en masse* or by instalments. It is a surface tension phenomenon, and if the surface be altered by a preliminary addition a further addition to that surface will not meet with the same conditions as did the first portion.

2. The reaction between adsorbent and solvent is reversible only for a short time after its inception.

3. The equilibrium-constant alters with time ("old-age").

To use a mathematical expression, we may say that if an albuminous fluid adsorbs chlorides from a solution of chlorides, the amount adsorbed will depend on the concentration of the fluid and of the solution. Expressed graphically, a curve representing the ratio of the adsorbed to the unadsorbed substance (abscissa) will always be concave towards the abscissa, since the power of adsorption of the adsorbing substance is greater at the beginning than it is at a later stage in the process. When this is put into a mathematical formula, we have:

$$\frac{C_{\text{adsorbed subst.}}^n}{C_{\text{unadsorbed subst.}}} = k.$$

where k is called the adsorption coefficient, and n is a constant greater than 1.

This formula, however, does not necessarily hold good *only* for processes of adsorption, *i.e.* of depression of the surface tension between fluid and colloid, by the dissolved substance. It would equally well express that a *solid solution* is formed in which the chloride is divided over the fluid and the solid solvent. There may be a question of electrical charges in this aspect of the conditions.

Without pursuing the theoretical side of the question further we may come to the conclusion that if the effect of adding sodium

chloride to an albuminous solution can be expressed by the formula given above or by this formula :

$$C_{\text{Cl in alb.}} = k. \sqrt[n]{C_{\text{Cl in water}}}$$

we may assume that adsorption exists between these two substances.

Several tests were applied in order to endeavour to arrive at a conclusion as to the accuracy of the chloride analyses which have been made in puncture-fluids. The variations in conductivity shown by different strengths of saline solution in the presence of different percentages of albumen were determined, and in another series of tests, different solutions of egg-white and sodium chloride were made up, and the amount of NaCl estimated in each volumetrically. In a third series of tests a known strength of natural albuminous fluid was treated with a known quantity of NaCl, and different dilutions made, the conductivity of each of these being tested, and in a final series of experiments a known strength of albuminous solution (human) was treated with a known weight of albumen, and the coagulum was washed with distilled water on successive days till no more chloride could be detected in the filtrate. The strength of NaCl and the conductivity of each washing was determined. The washing was carried out by taking up the coagulum out of the filtrate, transferring to a small flask, and adding about 80 cc. of water. The contents were then well shaken up and allowed to stand for a time. The mixture was then filtered, and additional water poured on to the precipitate until 100 cc. of filtrate was obtained.

Without entering all the results, we may illustrate the method and the results obtained by the following observations.

1. By titration, adopting the usual method of de-albuminisation, 100 cc. fluid, containing—

8 % albumen (egg) +			1 % NaCl yielded		0.85 % NaCl on titration,		
4	"	"	+ 0.5	"	"	0.45	"
4	"	"	+ 0.25	"	"	0.25	"
2	"	"	+ 1	"	"	0.92	"
2	"	"	+ 0.5	"	"	0.5	"
2	"	"	+ 0.25	"	"	0.25	"
2	"	"	+ 0.1	"	"	0.1	"

2. By successive washing,

First filtrate:	$k_{26^\circ} = 1800$
Second "	" = 103.5
Third "	" = 23.2
Fourth "	" = 4.04

From these experiments we see that when the percentage of albumen is low there is no adsorption of chlorides by proteid,

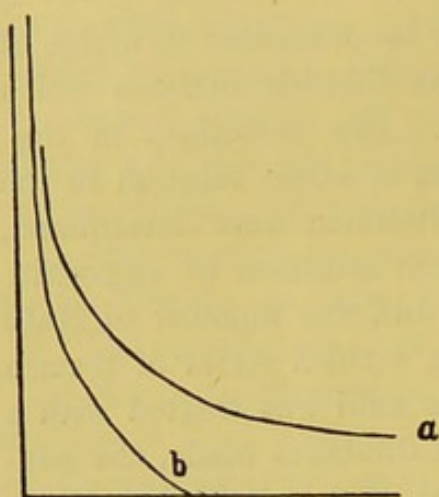


FIG. 1.

a represents the varying adsorption occurring in different concentrations of Congo red and cellulose (Bayliss), while *b* shows the conditions which obtain in the case of serum albumen and NaCl (Author).

while with higher concentrations of albumen, however, there is a decided loss, which is greater the more chlorides there are. Putting the results of the last table in the form of a curve, and comparing it with that from an ordinary case of adsorption (fig. 1, *a*), it is at once evident that there is a difference between the two. From our point of view, then, there is no important error likely to arise in the ordinary method of analysis, *provided the percentage of proteid be not large*.

Bugarsky and Liebermann have settled the question of adsorption of NaCl by albumen by determining the freezing-point depression of

an $\frac{n}{20}$ solution, containing varying weights of albumen. Thus:

Amount of Albumen dissolved in 100 cc. $\frac{n}{20}$ NaCl	Freezing-point Depression.	Freezing-point Depression if no Adsorption.
0	0.183°	0.183°
0.4	0.186	$0.183 + 0.004 = 0.187$
0.8	0.191	$0.183 + 0.006 = 0.189$
1.6	0.194	$0.183 + 0.009 = 0.192$
3.2	0.199	$0.183 + 0.015 = 0.198$
6.4	0.203	$0.183 + 0.022 = 0.205$

They conclude that the similarity of the figures in the second

and third columns indicates that no union has taken place between the salt and the albumen. However, the fact that the observations are made at ordinary temperature and at about 0° C. shows that such an experiment does not decide whether adsorption might not occur at 100° C., a temperature which all the puncture-fluids have to attain in the course of the analysis recommended in this work.

SCHEME FOR ANALYSIS OF PUNCTURE-FLUIDS

After these preliminary observations on the difficulties with which one has to cope, we may proceed to formulate the method of systematic analysis which has been adopted for the chemical examination of puncture-fluids.

The following scheme is based on a very considerable literature, and as by its aid more than 200 puncture-fluids of all kinds have been examined, its practical advantages have been carefully tested. It may, however, be noted that there is not always a need for following out each table from start to finish; a judicious selection can be made of those portions of the scheme which are likely to give information in the particular class of fluid under examination. Where any modification of the methods has been found convenient a mention of it will be made in the appropriate places.

In order to make this work more useful to those who wish to apply these suggested methods for purposes of diagnosis, the confirmatory tests, especially for the less known substances, are entered along with the description of the particular substance. Inasmuch as there are constantly being devised for many of these bodies, new tests, which the ordinary reader cannot ascertain without a very extensive search through literature, it has been thought of value to gather together these innovations, especially those new tests which have been found of use in the Leeds General Infirmary.

TABLE A. PRELIMI

The fluid is heated in the presence of *just enough* acetic acid in a water-bath placed on a sand-bath, in order to prevent to boil, a few drops of dilute acetic acid are added until a coagulum this can be controlled by litmus).

Coagulum.—This consists of albumen, globulins, and nucleomeshes (such as mucoids).†

The **Filtrate** should still be acid, and if an estimation of by adding distilled water to the coagulum in the filter-paper. the remainder is tested in successive portions, thus :

Add an equal part of methylated spirit.			Add basic lead acetate.		
Precipitate is hetero-albumose. (coag. by 65° C.) s. p. 26	Solution. Test with potass, ferrocyanide, and acetic acid.		Precipitate Proteids not required.	Solution. Remove the lead by H ₂ S.	
	Precipitate not required.	Solution Protalbumose. s. p. 26		Precipitate not required.	Solution
				Evap. in a drop of pure nitric acid. Dissolve residue in a few drops of soda. Warm in a platinum capsule. If oily drop forms = <i>leucin</i> . s. p. 27	Add Ammoniacal Silver Nitrate: Precipitate = <i>purins</i> . Or evap. to dryness with HNO ₃ ; add one drop Ammonia. If violet forms = <i>purins</i> or <i>uric acid</i> . (See p. 31 for confirmatory tests; also footnote p. 76.)

* Devoto.

† To detect *nucleo-proteid* (Jolles). Boil the original fluid with excess After 24 hours, the asbestos filter which has been used, and the precipitate, the filtrate with acetic acid, redigest, and finally purify the precipitate by for phosphorus as described on p. 49. The filtrate from ammonium molybcondenser. The fluid, after clarification, polarises light to the right, and with phenylhydrazin (therefore mucin).

To exclude mucin and nucleo-albumen from nucleoproteid, boil the filter while hot. Ammoniacal silver nitrate precipitates nucleoproteid

‡ Willanen.

§ This is a modification of Seliwanoff's test (p. 32), which I believe to be

NARY SEPARATION

until coagulation results.* It is convenient to perform this pro-
excessive heating in the initial stages. When the water is about
ceases to appear (only some half dozen drops will be needed, but

proteid †, apart from substances that may be entangled in its

chlorides be desired, it is made up to 100 cc. (the original bulk)
10 cc. of the filtrate are then set aside for the determination, and

Saturate two-thirds with Ammonium Sulphate.		Saturate the neutral solution with Am- monium Sulphate.			
Precipi- tate	Solution not re- quired.	Precipi- tate	Solution not re- quired.	Concentrate another portion of the filtrate in vacuo, at not more than 40° C., or leave to concentrate spontaneously. Place the fluid in a porcelain basin, and add resorcin till a fairly strong solution results. Heat the basin over a Bunsen flame, after the manner of the Gunzberg test for free HCl, and if a red colour appear, soluble in alcohol, then <i>levulose</i> is present. § The confirmatory tests and other details are to be found on p. 32.	Neutralise a portion of the filtrate with potash, and then concen- trate on the water-bath. If a precipi- tate forms on adding alco- hol, suspect the presence of <i>succinic</i> <i>acid</i> (con- firmatory tests, p. 194).
Deutero- albu- mose <i>a</i>		Deutero- albu- mose <i>β</i>			

of water, and acidulate with acetic acid. The precipitate is nucleoproteid.
are digested in an Erlenmayer flask with 4 per cent. soda. Reprecipitate
washing with alcohol and ether. The dried substance is incinerated and tested
date is hydrolysed for 2 hours with 30 cc. of 4-per-cent. HCl, with a reflux
on removing lead will give the orcein reaction, as well as give a glucosazone

fluid with 5 per cent. H₂SO₄, and neutralise with baryta while hot, and
and purins. Mucin and nucleo-albumen remain in solution.

convenient.

TABLE B. SEPARATION OF GLOBULINS AND THEIR ASSOCIATES

1. Add an equal part of thoroughly saturated pure ammonium sulphate, and leave to stand overnight (or longer).

Residue.	Solution.
Dialyse, dry, and weigh.	Contains chiefly albumen and secondary albumoses.*
Contains globulins, nucleo-albumen, primary albumose, peptone, histones, and lecithin.	Not required.
(1) Fuse some of the residue with KOH + KNO ₃ and test for phosphorus (nucleo-albumen, lecithin).	
(2) Dissolve a portion in water and add acetic acid (up to 1 %). After prolonged standing, Precipitate = serosamucin.	

2. To another portion add double the bulk of saturated ammonium sulphate to precipitate the euglobulin.†

* As the filtrate contains albumen, it may be boiled as in Table A and the weight of albumen ultimately obtained. The ratio between albumen and globulin will then have been obtained by the one process.

† A simple experiment which was made will illustrate the importance of delay before collecting the precipitates obtained by salting out proteids. 100 cc. of a pleural fluid were treated with 200 cc. of saturated ammonium sulphate (thus forming a 66-per-cent. solution). As usual, there was no effect for some time, but next morning abundant flocculi had separated. They were, however, left for two more days, and then filtered off. The fluid, which, it may be incidentally mentioned, was turbid, now yielded a clear filtrate, and pending further study of this filtrate the specimen was left in a plugged Erlenmeyer flask for ten days, at the end of which time it was noticed that there was a not at all inconsiderable further flocculation. This of course shows that salting out may take several days to complete (there could be no concentration of the fluid during this time, otherwise one might argue that with increasing concentration of ammonium sulphate a different fraction was separating).

In studying the different globulins, by weighing, and so on, or in collecting globulin in order to estimate associated lecithin, one consequently needs to allow a considerable interval to elapse before regarding the salting out as complete.

On the other hand, in a note by Cecil Bosanquet (*Lancet*, 1907) he states that the globulins alter on standing, so that the relations between the eu- and pseudo-globulin fractions to albumen will be different in the same fluid according as it may be examined immediately or after a time. He considered that an enzymatic change occurred, by which albumen may be converted into globulin. The view is probably too pessimistic, because if one adds the values given for "ascitic fluid A" one finds that the immediate estimation gives total globulin : albumen = 2.3 : 1.1, and the later estimation still gives 2.3 : 1.1. The other case certainly shows a change from 2.5 : 1.5 to 2.8 : 1.3, the pseudo-globulin having decidedly increased. The duration of time allowed for separation of the precipitate before weighing might easily account for this, in the same way as mentioned above in illustrating the need for delay before collecting and weighing one's precipitates.

Before proceeding farther it will be advantageous to pause to consider the substances separated by Tables A and B, and discuss those properties which are of interest in connection with the study of puncture-fluids. In this way we can take the opportunity to indicate in which fluids the various substances occur, and their significance.

The method of classification adopted consists in dealing with the proteids first, then their derivatives, and finally the substances detected in the latter part of Table A. The diaminoacids, which demand a special method of separation, will therefore come apparently out of turn, but owing to their infrequency in puncture-fluids, and to the complicated procedure necessary to their detection, this deflection will be found convenient, as the more important substances will be taken first.

Albumen.—The chief interest in this substance as regards its presence in puncture-fluids lies in the evidence which it affords in differential diagnosis of exudates from transudates. This side of the subject will be found discussed fully in Section IV. The estimation of the amount of albumen is also of great importance in the detection of diseases of the nervous system in the case of *cerebrospinal fluid*, and this will be discussed later (Section III.). In passing, it is merely sufficient to say that there are four types of effusion, each of which has a different albumen-content.

a. Due to disease of the serous membrane (tubercle, cancer, etc.). Albumen 4–6 per cent.

b. Due to venous stasis (general or local). Albumen 1–3 per cent.

c. Extreme hydræmia (nephritis; amyloid disease). Albumen less than 0.5 per cent.

d. Mixed types. Albumen varies.

The amount of albumen is of interest in other kinds of fluid, as, for instance, hydatid-cyst fluid, which contains very little as compared with, say, pancreatic cysts. In ovarian cysts there is often comparatively little coagulable material.

These points will be referred to under the appropriate headings.

Reale has endeavoured to show that there are two varieties of serum-albumen, a "euserumalbumen," precipitable by weak acid after saturation with NaCl or magnesium sulphate, and coagulating at 71 to 72° C., the other

a "pseudo-serumalbumen," which is not precipitable in the same manner, and coagulates at 84° C.

Globulin.—The scanty attention which was paid to the existence of this proteid, both in blood-serum and in (pathological) urine, in former years is gradually giving place to a much more widespread interest than it has enjoyed in the hands of the few who concerned themselves with pathological chemistry. It is now known that globulin is the bearer of many important functions of the blood-serum, of which the relation to antitoxin is perhaps the most conspicuous. It is first necessary to describe the varieties of globulin which are met with, because it is important to distinguish between them in practice.

Freund and Joachim have classified globulins as follows :

Name.	Precipitant.	Solubility in Water.	Solubility in 0.6% NaCl.	Solubility in 0.25% Na ₂ CO ₃ .	Precipitation limits.	Coagulation Temperature.
1. Paraglobulin or Ovi-mucin * ...	$\frac{1}{3}$ rd sat. Am ₂ SO ₄	Insoluble	70-77°C
2. Euglobulin	"	Soluble	Soluble	Insoluble	2.8-3.6	64-70°C
3. Parapseudo-globulin or dysglobulin * ...	$\frac{1}{2}$ sat. Am ₂ SO ₄	Insoluble	Insoluble	Soluble	...	74-76°C
4. Pseudo-globulin	"	Soluble	"	Insoluble	3.6-4.4	76°C
5. Nucleoglobulin (contains P.)...

These authors lay stress on the physiological importance of these varieties of globulin by stating that pseudo-globulin contains the antitoxin for tetanus, paraglobulin has the property of precipitating egg-white, euglobulin favours the precipitation of myosin, while pseudo-globulin inhibits the action of euglobulin.†

The same authors point out that a globulin may be (1) soluble in water (pseudo-globulin), (2) soluble in 0.6 per cent. NaCl, but insoluble in water (euglobulin), (3) insoluble in either 0.6 per cent. NaCl or in water, but soluble in 0.25 per cent. Na₂CO₃. This will explain the characters of certain kinds of peritoneal exuda-

* Of Ohrmeyer and Pick.

† Pseudo-globulin thus plays the part of an "antiferment," as it were.

tions, since, if the carbonates be present in excess, the third variety of globulin will be in solution, while if there are deficient salts, or if the chloride concentration of the fluid be less than that of the blood, one may expect euglobulin not to remain in solution, and so cause a turbid peritoneal fluid. We shall have to refer to this matter again when we come to deal with opalescent and milky peritoneal fluids.

As regards the *amount* of globulin which is present in a fluid, it cannot be said that this shows any constant relation to the nature of the fluid, though, as a rule, there is less globulin present in pleural than in peritoneal fluids. The abundance of globulin in peritoneal fluids is probably an explanation of the strikingly more frequently occurring opalescence in them than in pleural fluids.

The varying globulin-content of fluids is shown by the following table of some of the analyses from cases in the Leeds General Infirmary.

TABLE I
GLOBULIN-CONTENT OF PUNCTURE-FLUIDS

Peritoneal	Monolobular Cirrhosis	4.585 %
	Tuberculous Peritonitis	0.568 %
	Cardiac	0.25 %
	Cardiac and Renal...	0.4 %
	Monolobular Cirrhosis	0.06 %
Pleural	Exudation (simple)	0.58 %
	Another case	0.84 %
	Tuberculous Exudate	1.8 %
	" Idiopathic "	0.87 %
	"	1.36 %
	"	0.77 %
Ovarian	Cardiac Failure	0.04 %
	" " "	1.84 %
	Simple Unilocular Cyst	2.079 %

THE SUBSTANCES DETECTED BY THE FILTRATE OF TABLE A

Albumoses and Peptones.—To confirm the presence of albumoses, a portion of the filtrate from Table A may be (1) saturated with NaCl, (2) saturated with MgSO₄, (3) dialysed. The protalbumose will pass through, while hetero-albumose will remain behind.

Protalbumose made alkaline with potash, and treated with 2 per cent. CuSO₄ will give no precipitate. Hetero-albumose is precipitated.

The differences between these two substances may be tabulated thus :

Test.	Protalbumose.	Secondary Albumose.
NaCl to saturation + acetic acid saturated with salt	Precipitated	No
Biuret (2 : 100) in neutral solution ...	+	No
Potass, ferrocyanide and acetic acid	+	No
Nitric acid	precipitated in salt-free solution	only precipitated in presence of salt
Half saturation with ammonium sulphate	complete precipitation	No precipitation

The investigations which have been made in order to ascertain in which fluids albumoses and peptones were or were not present are shown in Table II. It will be at once noticed that peptone is uniformly absent from both pleural and peritoneal fluids, and that purulent fluids contain both forms of albumose. This is as might be expected, for, as we shall see, proteolytic ferments are a conspicuous component of the polynuclear leucocytes.

Umber states that primary albumose is always present in a fresh exudate, and that deutero-albumose is often met with, while true peptone is absent. It will be seen from Table II that while protalbumose is absent in a few cases, the results are otherwise in accordance with Umber's statement.

TABLE II

Nature of Case.				Prot-albumose.	Hetero-albumose.	Peptone.
Pleural ...	Bilateral (single)	Absent	Trace	Uniformly absent.
	Tuberculous	"	Absent	
	"	Present	Present	
	Cardiac Failure	Absent	...	
	Idiopathic	Present	Absent	
	Chronic Tubercular Nephritis	"	...	
Peritoneal	Empyema	"	Present	Uniformly absent.
	"	"	"	
	Cardiac (back-pressure)	Absent	Absent	
	Monolobular Cirrhosis	Present	"	
	Peritoneal Cancer	"	...	
	Adt. Pericardium (back-pressure)	"	Trace	
Chronic Peritonitis (non-tuberculous)	"	...	Uniformly absent.
	"	...	

The Monaminoacids (glycocoll, leucin, aspartic acid, serin).

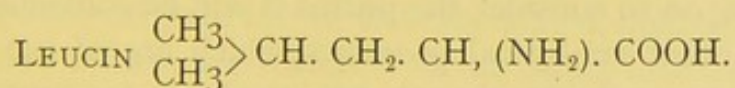
Traces of these substances have been described by various authors as occurring in exudates, especially in peritoneal fluids. Umber states that they are always present, but from the experience of the cases in the Leeds General Infirmary they are not found at all frequently. It must be admitted, however, that possibly too small a quantity of fluid was utilised for their detection: 200 cc. or more might furnish better prospect of detecting them, but hitherto it has been necessary to study other constituents more particularly, by which time there was less material left than would serve to reveal the presence of a trace of monaminoacid.

The *method of separation* of these bodies is as follows. The filtrate (portion 2, Table A) is carefully neutralised after the treatment with H_2S , and 2 cc. * of $nKOH$ added. It is now shaken for eight hours with 2-4 gm. of β -naphthalene-sulphochloride (Merck) by the aid of a mechanical shaker.

A separating funnel is now used and the watery fluid is shaken with ether, and rendered acid. The ether takes up the β -naphthalene-sulphaminoacids out of this acid fluid, especially if a little powdered ammonium sulphate be added first. The aminoacids crystallise out and can be recognised from their physical properties and their microscopic appearance.

This method is employed by Erben as a quantitative method, but since he admits that sometimes only 57.7, and never more than 80 per cent.—varying according to the particular aminoacid—is recoverable, it can hardly be recommended for quantitative purposes.

Confirmatory Tests.



1. Very dilute copper sulphate gives a blue colour.
2. Very dilute ferric chloride gives a red colour.
3. Mercuric salts, in the presence of soda, give a white precipitate.

4. *Urea Test*.—Add urea in slight excess, and baryta (not to excess), and boil till ammonia ceases to come off. Filter, wash

* More if necessary. The fluid must be quite alkaline.

with water, and evaporate down filtrate on a water-bath. If necessary, filter again, and carefully acidify with acetic acid. A crystalline precipitate will appear, insoluble in ether, but soluble in alcohol and alkalies. Melting-point, 205° C. It crystallises out from alcohol in long needles of isobutylhydantoic acid. Detects 0.01 gm. leucin.

5. *Scherer's Test*.—Evaporate the substance in a drop of pure nitric acid. Dissolve the residue in a few drops of soda. Warm in a platinum capsule. If leucin be present, an oily drop will appear, rolling about without wetting the platinum.

6. The aqueous solution is lævorotatory $a_D = -6.65^{\circ}$.

7. It sublimes on heating, and gives off an odour of ethylamine.

GLYCOCOLL $\text{CH}_2(\text{NH}_2) \cdot \text{COOH}$.

1. Add four times the weight of the substance to be tested of alcoholic solution of picric acid. Glycocoll picrate will separate out on cooling. Melting-point, 190° C.

2. The crystals from β -naphthalene-sulphochloride melt at 159° .

3. Copper sulphate gives a blue colour. $(\text{C}_2\text{H}_4\text{NO}_2)_2 \text{Cu} + \text{H}_2\text{O}$.

4. On oxidation with H_2O_2 , glyoxylic acid and formaldehyde are formed.

5. Ferric chloride gives a deep red colour.

ASPARTIC ACID $\text{COOH} \cdot \text{CH}(\text{NH}_2) \cdot \text{CH}_2 \cdot \text{COOH}$.

1. Copper acetate gives a blue crystalline precipitate.

2. Strongly acid solutions are dextrorotatory ($a_D = 25.7^{\circ}$).

3. Melting-point, 270° .

The chief interest of this particular substance lies in its association with the albumen of carcinoma tissue. It may constitute as much as 5 to 10 per cent. of the albumen molecule in these cases, and should therefore possibly be met with in the fluids of carcinomatous serositis. In its detection it is necessary to remove the other monaminoacids first. Aspartic acid is an essential constituent of the globulin molecule.

Before passing on to consider the purins it will be convenient to describe two other analytical processes which are of use in the study of puncture-fluids, though not available for routine work, owing to the time which is involved, and the absence (so far) of any definite diagnostic or prognostic deductions to be made from the results.

The first and most important is Hausmann's method of *hydrolysis of puncture-fluids*. 10 cc. are boiled with 29 cc. of concentrated HCl for five hours on a sand-bath, using a reflux condenser. Saturate the residue with calcined magnesia and

distil over the ammonia into decinormal sulphuric acid. It is more safe to place sufficient magnesia into another flask and add the result of hydrolysis through a funnel. In this way no gas will be lost. This first step gives the *amid-N*. As soon as distillation is complete the residue is dissolved in HCl, brought down to a small bulk, and phosphotungstic acid added.

After 24 hours the precipitate is washed with dilute phosphotungstic acid, acidulated with HCl till the fluid ceases to have a yellowish tinge.

a. Filtrate. Make up to 500 cc. and Kjeldahlise 100 cc., to get the *monaminoacid-N*.

b. Residue. Dissolve in as little alkali as possible, and make up to a definite volume, and filter. Kjeldahlise, to get the *diaminoacid-N*.

Schlösing's Method of Estimating Ammonia.*—10 cc. of normal sulphuric acid is placed into a sporulating dish in an exsiccator, and 10 cc. of the fluid to be tested is placed in the exsiccator. 50 cc. of milk of lime is added to the fluid and the cover rapidly placed in position (vaseline joint).

After three days the sulphuric acid is titrated with $\frac{n}{4}$ soda, using methyl orange as indicator (turns yellow). Every centimetre of the soda which is used in titrating less than 32.6 signifies .106 gm. ammonia.

The Purin Bodies, and Urea.—The purin bodies have been the subject of careful study in many quarters, and it is unnecessary to enter into any description of them other than the facts about their occurrence in puncture-fluids.

As regards *urea*, this may be tested for in the filtrate from Table A by pouring the filtrate into excess of 95 per cent. alcohol. After several hours, the extract is evaporated over a low flame, re-extracted several times, and the final extract dried, when cold a few drops of nitrate acid are added, and the typical crystals of urea nitrate are searched for 24 hours later. †

The addition of hypobromite to a portion of the original fluid will also reveal the presence of urea owing to the effervescence which results. As a rule, however, there is so little gas evolved that an estimation of the amount of urea is not possible.

* Durig gives numerous useful little practical details in *Biochem. Zeit.* iv.

† Salkowski.

TABLE III
UREA-CONTENT OF PUNCTURE-FLUIDS

Absent in.		Faint Trace in.	Trace.	Decided Amount in.
Peritoneal.	Thrombosis of Portal Vein	Carcinomatous Ascites (2 cases)	Pleural Effusion (3 cases)	Single Pleural Effusion.
	Cirrhosis of Liver			" " " " Cardiac Back-pressure (2 cases). ...
Peritoneal.	Monolobular Cirrhosis	Carcinomatous Ascites (2 cases)	Pleural Effusion (3 cases)	Empyema. Hydronephrosis.
	Toxic Nephritis			Peritoneal Fluids: Cardiac Back-pressure. Chronic Peritonitis (single). " " due to Cirrhosis of Liver. Peritoneal Cancer.
Peritoneal.	Sarcoma of Omentum	Carcinomatous Ascites (2 cases)	Pleural Effusion (3 cases)	Syphilitic Cirrhosis of Liver, 1.42% (Poljakoff).
	Tuberculous Peritonitis			

From this table it will be seen that urea is much more frequently present in cases of pleural than in cases of peritoneal effusion. The presence of urea in purulent fluid such as empyema may perhaps be expected from the fact that pus contains so many extractives, in virtue of the cellular elements in it (see Section III.).

The practical deductions which can be made from the presence of urea are, however, scanty. Its occurrence in various pleural and peritoneal fluids suggests that one cannot be sure that a fluid from the abdomen is from a hydronephrosis just because urea is present in it. Probably we need to know the *amount* of urea present, in order to be able to say that there is more than ordinary autolysis (rather than renal secretion) could account for. This subject is again referred to under "Renal Cysts" in Section III.

The *purin bodies* are stated by Umber to occur in minimal traces in exudates.

They are best separated by precipitating the phosphates with ammoniacal Ludwig's magnesia mixture, and then adding 0.5 per cent. silver nitrate in 50 per cent. ammonia to the filtrate. The precipitate is placed on an ash-free filter-paper, and washed till the filtrate is no more alkaline. The residue is boiled in a Kjeldahl flask with water and some magnesia, and is then Kjeldahlised. In this way the nitrogen of the purins is estimated. The uric acid-nitrogen is also determined in another portion of

fluid, and the difference between the two values gives the nitrogen of the purin bases.

The time involved in separating out the individual purins is not profitably spent in connection with puncture-fluids.*

Confirmatory Tests.—1. Burians' Test.—Make the solution alkaline with soda, and add a solution of diazobenzenesulphonic acid. An intense red colour results. (Cf. Test 6 for tyrosin, p. 35.)

2. Weidel's Test.—A small portion is evaporated in a porcelain basin with freshly prepared chlorine water, and when dry the basin is inverted over an open ammonia bottle. If purins are present, a red or purplish red colour will appear, turning violet when warmed with a little potash. In place of chlorine water, hydrochloric acid and a small quantity of potassium chlorate may be added.

Reaction: xanthin = alloxan + NH_3 = murexid reaction.

3. Nitric Acid Test.—If the fluid be heated with strong nitric acid and evaporated to dryness, the residue treated with soda gives a violet or purple red (turning indigo-blue when dry).

TABLE IV
PURINS IN PUNCTURE-FLUIDS

Present in	Absent in
Chronic Pleural Effusion (non-tuberculous)	Pleural effusion { Tuberculous Transudatory Empyema Peritoneal fluid { Thrombosis of Portal Vein Cirrhosis of Liver Sarcoma of Omentum Peritoneal Carcinomatosis Cardiac Failure.
Simple Chronic Peritonitis	

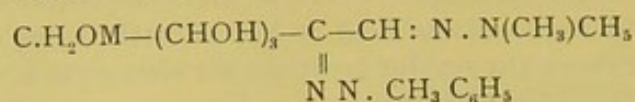
From this it will be seen that purin bodies are really only rarely met with, at any rate in appreciable amount. If they only occur in infinitesimal quantities it is certain that the amount of fluid available in the cases detailed was not great enough to allow of the detection of these bodies.

The **Sugars**.—The presence of *levulose* was discovered by Pickardt, who found it present in very many ascitic and peritoneal fluids, as will be seen in the table on page 150. The method advocated for its detection has nevertheless failed to show its presence in several of the fluids examined by me.

The method to be adopted, where an estimation of the amount

* The same applies to Krüger and Schittenhelm's method, though in the case of *fæces* its value is different.

of reducing body is desired, is as follows: After concentrating the filtrate from albumen, the fluid is boiled five minutes with half as much 98-per-cent. alcohol as there was of original fluid. Cool and filter.* The filtrate (or mixture of alcoholic filtrates) is decolorised with animal charcoal. The amount of reducing substance in one portion may be estimated, and the amount of methylphenylhydrazin necessary can be calculated from this, adding three molecules of hydrazin compound to each molecule of levulose found. This substance is added to the alcoholic solution and allowed to stand some hours, and the filtrate is kept at 40° for twenty-four hours to allow the osazone to crystallise out † fructose methylphenylglucosazone



Confirmatory Tests.—1. When boiled with resorcin and HCl, a red colour soluble in alcohol results (Seliwanoff's reaction.) ‡

2. It is lævorotatory. ($\alpha_D = -113.96^\circ$).

3. In presence of ethereal salt of HBr a deep purple colour appears (brommethyلفurfurol).

4. With phenylhydrazine it gives an osazone exactly like glucosazone or mannosazone or chitosaminosazone.

5. It ferments.

6. The proof is the finding of the methylosazone, which melts at 158–160°C. If the crystals have a reddish colour (being impure) they may melt at 153°. Optically, 0.2 gm. in a mixture of 4 cc. pyridin and 6 cc. absolute alcohol will rotate 1° 40'.

Pentoses.—These are rarely met with.

Confirmatory Tests.—Use the filtrate from Table A after concentration.

1. To 0.5 gm. orcin dissolved in 10 cc. of 25-per-cent. HCl add 1 cc. of 10-per-cent. ferric chloride, and add the fluid to be tested. Shake thoroughly while warming. A green colour will appear if pentose be present.

* The precipitate consists of salts and a little fructose sometimes. If fructose does come down the alcohol extraction must be repeated.

† If only an oil forms (sorbose), it is separated and washed with distilled water by decantation, and then dried completely over H₂SO₄ in vacuo. The resulting resin is dissolved in absolute alcohol, filtered, and frozen crystals will be found to separate at once and may be purified by recrystallisation and final extraction with pyridin.

‡ This test is also given by all polysaccharids which will yield fructose on hydrolysis; it means that a ketose of the 6-CHO-series is present, e.g. togatose, galactose, pseudo-fructose, α-oxyglycuroic acid, cellulose (filter-paper!)

2. It does not ferment.
3. Spectrum analysis. Heat with fuming HCl and phloroglucin = cherry-red colour, giving a dark band between D and E.
4. To the fluid add 1 part *p*-bromphenylhydrazin, $3\frac{1}{2}$ parts 50-per-cent. acetic acid, and 12 parts water = bromphenylhydrazone.
5. Heat with 1 vol. xydidin, 1 vol. glacial acetic acid and $\frac{1}{2}$ vol. alcohol. An intense red of furoxyldin is produced.

The **Diaminoacids**.—*Lysin and Arginin*.—Umber has only found faint traces of diaminoacids in exudates, but probably they are only occasionally present in puncture-fluids. Their separation is, however, a matter of some importance, because with this is bound up the question of the residual nitrogen, to which interest is attached in reference to pathological metabolism.

TABLE C. SEPARATION OF DIAMINOACIDS

Add phosphotungstic acid till the fluid is only just alkaline. An excess of acid must be avoided in order to prevent albumen, albumoses, nucleo-albumens, etc., from coming down.

The **precipitate** * is made up of only diaminoacids, uric acid, purins, and phosphates. Shake with boiling baryta water.

Precipitate = phos- phates.	The Solution is divided into two parts :					
	A Neutralise with H ₂ SO ₄ .			B Pass in CO ₂ and boil.		
	Precipitate = BaSO ₄ .	Solution.			Precipitate = BaCO ₃ .	Solution. Add AgNO ₃ and filter. Concen- trate the filtrate and add alcohol. <i>Lysatinin</i> will crystallise out.
		Neutralise with HCl and add alcohol.	Add AgNO ₃ and then baryta water. Pass in CO ₂ .			
	Precipitate = <i>lysin</i> <i>chloride</i> .	Precipi- tate = BaCO ₃ .	Solution concen- trated by boiling. <i>Arginin</i> will crystal- lise out.			

Solution not required.

Solution not required.

Alternative Method.—Acidify the solution with dilute HCl, dry on a water-bath, dissolve the residue in alcohol, and add

* That portion of the nitrogen of the precipitate which remains after subtracting the nitrogen of the ammonia and the nitrogen of the purins is called *residual nitrogen*, and it is determined by the use of Kjeldahl's method.

concentrated alcoholic picrolonic acid till no more precipitate falls (cholin, neurin, lysin). The crystals may be studied and identified (melting-point, *e.g.*).

Lysin gives brilliant yellowish orange prisms with platinum tetrachloride (a platinum double-salt).

The Residual Nitrogen.—Attention to the amount of residual nitrogen, not only in blood but in various effusions, was especially drawn by Neuberg and Strauss. After removal of albumen, the amount of nitrogen which is precipitable by phosphotungstic acid, as also the amount which is not precipitable by that reagent, are determined. These authors make the definite assertion that by the use of α -naphthylisocyanate* ($\text{CO. N. C}_{10}\text{H}_7$) there is no risk of finding an aminoacid which was preformed in the albumen molecule. The cases may be divided into the following three groups:

1. Where the nitrogen of the amino compound is less than 0.5 per cent. This group includes cases of subcutaneous œdema associated with tubal renal disease, as well as similar cases associated with cardiac incompetence.

2. Where the amino-nitrogen lies between 0.5 and 1 per cent.—as occurs in pleural exudates (0.56 per cent.), cardiac and renal cases (0.65, 0.81 per cent.), and in a case of Banti's disease (0.62 per cent.).

3. Where the amino-nitrogen lies above 1 per cent. This has been found in a case of ascites associated with cirrhosis of the liver.

Glycocoll is never found in subcutaneous œdema fluid, or in cases of cardiac back-pressure.

The Oxyaminoacids.—The only member of this group which calls for attention is *Tyrosin*, which is not rarely met with in association with leucin. Its presence has been recorded in the peritoneal fluids in cases of alcoholic cirrhosis of the liver.

* Aminoacids in alkaline solution react rapidly with α -naphthylisocyanate. The mixture is shaken two to three minutes and then left to stand for a half to three quarters of an hour. The dinaphthylurea is filtered off, and the filtrate acidified with HCl. The corresponding naphthylantoic acids [$\text{R.CH. (NH. CO. NH. C}_{10}\text{H}_7\text{CH)}\text{-COOH}$] which are insoluble, are formed. The precipitate is dissolved in dilute ammonia, then in a little alcohol. Baryta water produces a precipitate of α -naphthylisocyanate of glycocoll—needle-like crystals [$(\text{C}_{10}\text{H}_7\text{. NH. CO. NH}_2\text{CH}_2\text{. COO})_2\text{-Ba.}$] melting at 191° (mean). The leucin derivative melts at 163.5° , the tyrosin derivative at $205\text{--}206^\circ\text{C}$. The mixture of the isocyanates can be subjected to fractional distillation.

This substance is generally identified with leucin. The following tests may be used:

Confirmatory Tests.—1. Millon's reagent gives the same reaction as when used for proteids, the reaction in their case being due to the presence of tyrosin in the molecule.*

2. Quinone Test.—Add dry quinone to the solution. A deep ruby colour forms, turning to violet red on adding sodium carbonate.

3. Piria's Test.—Add sodic sulphate, pour this into water and neutralise with barium carbonate. Filter, add a drop of neutral ferric chloride, when a beautiful violet colour appears.

4. Aldehyde Test.—Acidify with H_2SO_4 (2 cc.) and add aldehyde (four drops of 30-per-cent. alcoholic solution). A carmine colour forms (condensation product), giving an absorption band covering green and nearly all the yellow.

5. Formol in the presence of H_2SO_4 gives a brownish yellow colour on heating, and on boiling with twice its bulk of glacial acetic acid, it turns green (Denigés' test).

(Mörner's Test.—Solid substance added to 1 vol. formalin, 45 vols. water, and 55 vols. concentrated sulphuric acid, gives a permanent green colour on boiling.)

6. Diazobenzenesulphonic acid, in the presence of potash, gives a red colour. (The only fallacy to this test is the presence of histidin.)

GLYCOPROTEIDS

The diagnosis of a puncture-fluid from an ovarian cyst is occasionally necessary, so that it is important to discuss the properties of those substances which lend a distinctive character to the contents of ovarian cysts from a chemical point of view.

The most important of these *glycoproteids* is pseudo-mucin, which was first carefully studied under the name of metalbumen by Hammarsten in 1882. It is so called because it is not precipitated by acetic acid, and yet has a mucinous consistence. The presence of allied substances in the peritoneal fluid and in the synovial fluid renders the subject not only one of academic interest, but also of interest to the diagnostician.

Glycoproteids are substances which contain a carbohydrate radicle (mostly in the form of glucosamin) attached to the proteid molecule, and it is the glucosamin which is responsible for the similarity of the reactions between the various substances above named.†

* The use of this reagent for distinguishing between tubercular and non-tubercular exudates is referred to in Section IV.

† The glycoproteids are fully described in Mann's "Chemistry of the Proteids," but many points of interest in the present connection find only scanty notice there, so that one feels justified in entering thus fully into it.

Glucosamin is dextrose with the H atoms of one of the two CH_2OH groups replaced by an amino group (NH_2), so that it forms, as E. Fisher pointed out, a link between carbohydrate and proteid. The reactions of this substance are :

1. The Molisch reaction is positive (alcoholic α -naphthol followed by strong sulphuric acid, gives a violet colour which is turned yellow by alcohol, ether, or potash).
2. It does not ferment with yeast.
3. It gives Trommer's test.
4. It gives a glucosazon melting at 202°C .
5. It gives Ehrlich's glucosamin test after adding an alkali such as baryta, and then warming. (This test consists in a red colour obtained on adding a 2- to 5-per-cent. solution of *p*-dimethylaminobenzaldehyde dissolved in normal hydrochloric acid * till acid.)

TABLE D. SEPARATION OF GLYCOPROTEIDS

To the fluid add three times its bulk of absolute alcohol. Shake occasionally.

Portion 1 Allow to stand 24 hours.		Portion 2 Allow to stand 2 to 3 months, then evaporate the alcohol at 40°C .		Portion 3 Filter at once.	
Precipitate. Shake with slightly alkaline distilled water. Filter.		Precipitate. Shake with slightly alkaline distilled water. Filter.		Precipitate. Shake with distilled water. Filter.	
Resi- due. not re- quired	Fil- trate. Test for Pseudo- mucin	Resi- due. not re- quired	Fil- trate. Test for Cholin, Lecithin, Mucin	Resi- due. not re- quired	Fil- trate. Add to solution. or test separate- ly for albu- moses
					Solu- tion. Test for Albu- moses, Mucin.

Serosamucin (Umber).—This has been found in peritoneal fluids due to inflammatory processes or to new-growth dis-

* Normal HCl is made by adding 5 cc. water to 1 cc. pure hydrochloric acid.

semination. The substance will come out of solution if to the de-albuminised fluid a small quantity of very dilute acetic acid is added. The precipitate will have the following reactions and properties :

1. Let one drop of solution drop from a glass rod into a 100-cc. measure of distilled water to which a couple of drops of glacial acetic acid have been added. A cloud will appear if serosamucin be present. The precipitate is soluble in moderate excess of acid, and is reprecipitated by further dilution, while it may be redissolved by rendering the fluid neutral or slightly alkaline (Rivalta's test).
2. It contains a minimal amount of reducing substance.
3. It is precipitated by alkaloidal reagents, such as potassium ferrocyanide, nitric acid, copper sulphate, ferric chloride, lead acetate.
4. It is precipitated by an equal volume of saturated ammonium sulphate.
5. It gives the biuret reaction.
6. It gives Millon's reaction.
7. It gives the xanthoproteic reaction.
8. It gives a strong furfural reaction (Molisch reaction).
9. It gives Adamkiewitz' reaction.
10. It gives Liebermann's reaction.
11. It is not coagulated when boiled in neutral solution.
12. It is not separated by dialysis.
13. It forms a precipitate when digested with pepsin.
14. It gives an absorption band in orange, and slight darkening to the left of red when treated with orcein hydrochloric acid, and extracted with amyl alcohol, possibly indicating pentoses or glycuronic acid.

The percentage of nitrogen and sulphur has been remarked on by Umber as showing the body to belong to the mucins.

The question as to the nature of this substance is still unsettled. Whereas Umber considered it to be a mucin, though not a true mucin, because it contains too much nitrogen, Langstein objected to this view, on the ground that a reducing substance can be obtained from all proteids. Stähelin considers the body to be related to globulin, since it possesses similar solubilities, and is precipitated by half saturation with magnesium sulphate, and is not separated out by dialysis.

A similar discussion has been raised about the mucin of the urine, which was once regarded as a mucin, then as a body related to Bence Jones' proteid, then as a globulin, then as a nuclealbumen, then as nucleohiston. Stähelin believes the urinary substance to be identical with that of the peritoneum, and with that found in blister fluid. Rivalta and Primavara, more recently, regard it as a mixture of euglobulin and pseudo-globulin. That

it is not a histone is established by the fact of its yielding no histone even after treatment with 0.8 per cent. HCl for days.

Pseudo-mucin.—The reactions of this substance are :

1. On boiling with a small quantity of dilute sulphuric acid the fluid acquires the power of reducing Fehling.
2. The opalescent filtrate gives a turbidity but no precipitate on boiling.
3. Acetic acid gives no precipitate.
4. Acetic acid and potassium ferrocyanide render the fluid viscid and impart a yellow coloration to it.
5. Millon's reagent gives a bluish red colour on boiling.
6. Glyoxylic acid * followed by sulphuric acid gives a violet coloration.

Pseudo-mucin has been fully studied by Otori in order to determine what decomposition products could be obtained from it. The substance was boiled with hydrochloric acid and tin chloride, and the filtrate extracted with ether, the fatty acids being thus removed and estimated ultimately as silver salts. Silver sulphate was used to extract histidin, arginin, and lysin, and picrolonic acid was used to obtain arginin and guanidin.

The following result of analysis shows the various derivatives which have been found in pseudo-mucin, and which are therefore presumed to occur preformed in the pseudo-mucin molecule :

100 parts pseudo-mucin yield.	On splitting up by H_2SO_4 (grammes).	On splitting up by $\text{HCl} + \text{SnCl}_2$ (grammes).
Ammonia	0.7517	3.239
Guanidin	0.0393	0.0250
Arginin	0.2875	0.7773
Lysin	2.6389	2.582
Tyrosin	1.089	0.4422
Leucin	4.677	4.431
Glycocoll	—	0.146
Glutaminic acid	—	0.5945
Asparagic acid	—	Trace
Oxalic acid	0.1275	—
Levulinic acid	1.971	—
Valerianic acid (?)	—	0.765
Formic acid	Present	Present
Acetic acid, Propionic acid, reckoned as acetic acid	Not estimated	0.161
Reducing body, reckoned as glucose	0.7333	0.429
Insoluble humin substances	6.056	7.005

A means of identification of pseudo-mucin which is absolutely reliable was worked out in 1900 by Zängerle, who prepared a glucosamin compound after benzoylising the substance. The method is not of clinical value, as it

* Glyoxylic acid is prepared by putting sodium amalgam into saturated aqueous oxalic acid. Filter.

THE CHEMICAL EXAMINATION OF PUNCTURE-FLUIDS 39

is far too tedious, but it is important when there is a need to be certain that a given mucinous substance is or is not identical with some other mucinous substance already known. Briefly, the method consists in separating out the substance by the aid of alcohol, after which it is treated with hydrochloric acid, and freed of albumoses, and the fluid benzoated by the use of benzoyl chloride and soda till the filtrate ceases to reduce Fehling. The benzoyl compound is dried and dissolved in hot alcohol, from which, after standing, crystals of tetrabenzoylglucosamine separate, mixed with a certain amount of pentabenzoylglucosamine. The crystalline compound is dissolved in alcohol, precipitated (cold) and redissolved (heat), and then poured into excess of distilled water to get rid of inorganic salts. The purified substance is heated to 100°C . for nearly two days in sealed tubes, which are three quarters full of strong HCl. Crystals will then be found in the tube, and can be purified by the use of ether, and dried in vacuo over lime to get rid of water and HCl. A brown syrup is ultimately obtained, which deposits glittering rhomboidal crystals insoluble in alcohol, soluble in water (a fact made use of for purifying them). The following table shows that the crystals are practically identical with other glucosamins, and, indeed, one may say that the mucinous secretions of goblet cells in any part of the body, be it in the air passages, or in the intestine, or in cysts, or in ovarian tumours, all contain the same form of glucosamin, the same form of reducing substance.

The crystals when dissolved in water are dextrorotatory and readily reduce Fehling.

Table showing the crystallographic characters of glucosamin as obtained from pseudo-mucin, as compared with that obtained from other sources (Schwanke's analysis) :

		p : p 110 : 110	c : r 001 : 101	c : q 001 : 011	c : p 001 : 110	e : p 101 : 110	e : q 101 : 011	p : q 110 : 011	p : q 110 : 011
Glucosamin hydrochloride from	lobster shells	$67^{\circ}48'$	$67^{\circ}01'$	$35^{\circ}33'$	$59^{\circ}55'$	$65^{\circ}20'$	—	$84^{\circ}38'$	$43^{\circ}09'$
	sputum	$67^{\circ}46'$	$67^{\circ}04'$	$35^{\circ}18'$	$59^{\circ}53'$	$65^{\circ}49'$	$71^{\circ}40'$	$84^{\circ}58'$	$42^{\circ}39'$
	mucin	$67^{\circ}57'$	$66^{\circ}56'$	$35^{\circ}29'$	$59^{\circ}44'$	—	—	—	$42^{\circ}58'$
Pseudo-mucin ...		$67^{\circ}53'$	$67^{\circ}08'$	$35^{\circ}25'$	$59^{\circ}56'$	$65^{\circ}31'$	$71^{\circ}35'$	$84^{\circ}52'$	$42^{\circ}49'$

Paralbumen.—The following reactions may be used to identify it :

1. Salkowski recommends the following test: A few drops of alcoholic rosolic acid are added to 25 cc. of fluid and, after boiling, drops of decinormal H_2SO_4 are added till the colour becomes yellow. If the filtrate after boiling is cloudy, there is paralbumen present.

2. Shake the residue resulting from the addition of alcohol in excess, with hydrochloric acid (1 in 3), and boil. On cooling,

Trommer's test is applied and the test tube left in water. A red precipitate means paralbumen or mucin.

3. Acetic acid does not precipitate the paralbumen.

Mucin.—The reactions of mucin demand a brief consideration in order that it may be distinguished from pseudo-mucin, for this is important. Its distinction from nucleo-albumen lies in the fact that the latter contains phosphorus and does not reduce Fehling after hydrolysis * while mucin contains no phosphorus, and does yield a reducing body.

Other properties.—1. Swells up in water into a slimy mass.

2. Is precipitated by acetic acid, insoluble in excess.

3. The solution is clarified by very dilute soda. If neutral, boiling will not precipitate it.

4. Hydrolysis with an alkali converts it into animal gum.

5. In tissues it is recognised by staining with thionin and toluidine blue.

The *significance of the presence of mucin* in a fluid is that it indicates an origin either from a mucous surface or from some tissue which possesses goblet cells. Therefore if present in peritoneal fluid it means that there must be secondary growths from a papilliferous or colloid tumour, or that the fluid obtained by puncture has come from within a papillomatous cyst of the ovary.

In the diagnosis of this substance one has to bear in mind the following errors or sources of error :

In the first place, serosamucin, which has some similar reactions, may occur in an inflammatory peritoneal fluid.

In the second place, both sputum and saliva may contain mucin, and if present as impurity, would lead to an erroneous diagnosis unless the possibility of such contamination be excluded. Whereas submaxillary mucin contains 23.5 per cent. glucosamine, the mucin from a mucous membrane contains 35 per cent. of glucosamine ; moreover, submaxillary mucin contains chondroitinsulphuric acid, just as does the mucoid obtained from cancers. Of course such details cannot be used clinically for differentiating the mucins.

* Other bodies may yield a reducing substance after hydrolysis, especially with sulphuric acid. Thus, paranucleins do so, and also yield an osazone : the same is true of nucleohistone. Paranucleins are nucleins which form no xanthin bases in the presence of acids, while true nucleins do so.

A substance allied to mucin has been described as occurring in the blood by Zanetti, Eichholz, and others.

The chief difficulty, however, lies in differentiating mucin from mucoids, which include colloid substance, pseudo-mucin, and paralbumen. However, pseudo-mucin is not precipitated by acetic acid, though it comes down readily in the presence of excess of absolute alcohol.

The reactions of this body have been given above.

The paramucin of Mitjukoff is a jelly-like mass, and consequently hardly calls for consideration, though it is often found in ovarian cyst. It differs from pseudo-mucin in reducing Fehling without previous treatment with acid.

Paralbumen is only imperfectly precipitated by acetic acid, and is supposed to be really pseudo-mucin with albumen as an impurity.

The mucin of bile is regarded by Landwehr as a globulin mixed with bile salts, while Paijkull regards it as a nucleo-albumen.

TABLE V
MUCINS IN PUNCTURE-FLUIDS*

Absent in		Present in
Pleural	2 Tuberculous Pleurisies	3 Tuberculous Pleurisies
	Effusion secondary to Abdominal Cancer	
	Renal	
	"Idiopathic" effusion	
	Cardiac Failure (4 cases)	Empyema (2 cases)
Pericardial, Cardiac Failure		—
Peritoneal	Cardiac Failure (3 cases)	Carcinomatosis
	Renal Disease (3 cases)	Cardiac Failure (1 case)
	Sarcomatosis	" + Renal Disease (1 case)
	Gastric Cancer	
	Pancreatic Cancer (not invading peritoneum)	Renal Disease
	Cirrhosis of Liver (2 cases)	Simple Chronic Peritonitis
	Tubercular Peritonitis	
Broad-ligament Cyst		Ovarian Cysts (4 cases)

* Not necessarily "serosamucin."

LECITHIN

The chemistry of lecithin is gradually becoming more defined, and the fact that there are many substances present in the body which can be grouped under the name of "lipoids" or of "phosphatids," taking lecithin as a basis, is being realised as a result of several recent important researches. There is, in addition, an increasing knowledge of the physiological and pathological properties of this substance. Since this literature is largely inaccessible to the general reader, it may be usefully discussed, with especial reference to the influence which the properties of lecithin have on the nature and constitution of puncture-fluids.

The importance of lecithin is shown by the following discoveries: It plays a somewhat obscure part in the processes of immunity; thus it is essential to cobra-venom in the hæmolytic process characteristic of poisoning by snake-bite.* It plays an important part in the process of production of anæsthesia, since it is assumed that lecithin renders the anæsthetic readily permeable into nerve-cells, and calculations have been made by which laws governing the distribution of anæsthetic over nerve-cells could be formulated.† Thirdly, lecithin is presumably the source of cholin (see p. 170), whose presence in cerebrospinal fluid has been found by Halliburton to be pathognomonic of organic nervous disease. Fourthly, lecithin is an essential constituent of all growing tissues ‡ and in bone-marrow. § Fifthly, it is frequently associated with cholesterin, which leads one to suspect some subtle relation to exist between the functions of these two substances. Their association recalls the phenomena of symbiosis in living organisms, and there are several observations in the literature which show that the two substances may act in combination better than when kept separate. Thus, both bodies may take a part in the hæmolytic action of bile.|| Sixthly, the suggestion that pseudo-globulin retards the action of certain lysins may be brought into line with Joachim's discovery that lecithin is mainly attached to the pseudo-globulin fraction in ascitic fluids (and therefore presumably also in the blood-serum).

* Morgenroth; Keys.

† Researches on the distribution-coefficient by Overton.

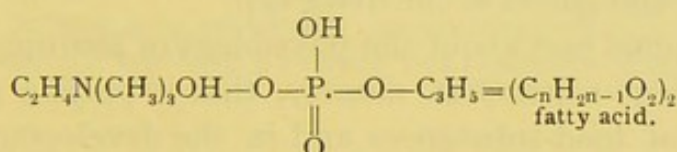
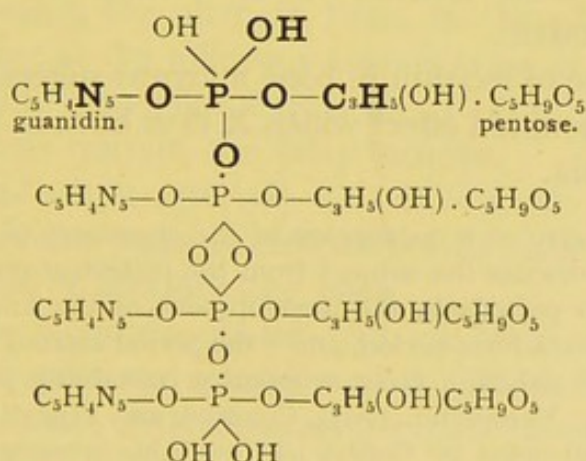
‡ Franchini. § Otol ski; Glikin. || Gustav Bayer. |

Lastly, the important discovery has recently been made by Porges that if a 0.2 per cent. aqueous suspension of lecithin be added in equal volume to the blood-serum of a syphilitic subject, there will appear a flocculent precipitate after five hours' incubation at 37°C. The reaction has, so far, never been obtained in the case of a non-syphilitic subject.

Some of these points demand further consideration. Thus, we may refer to the presence of lecithin in growing tissues,* among which a developing hen's egg forms a suitable example, for its yolk contains a large quantity of lecithin, and may be said to be a repository for that substance.

The growing embryo within this egg contains, of course, a large quantity of nucleic acids in the cells which are so rapidly being formed, and it becomes of interest to study the formulæ of nucleic acid and lecithin in order to ascertain if there be any sort of relation between them. If we take α -guanylic acid as an example of a nucleic acid since a graphic formula of this substance has been made out by Bang and Raaschon, we have—

LECITHIN.

 α -GUANYLIC ACID.

The symbols printed in thick letters will show at once

* It is most likely that much of the confusion which has arisen as to the precise constitution of lecithin is due to its having been mainly studied in this material rather than in the various adult tissues.

how the formula of lecithin can be fitted into that of the nucleic acid, or, more correctly, that the two substances are built up in a similar manner, so that the nucleic acid might readily give rise to lecithin in the course of metabolic breakdown. It will thus be seen that we have here only another example of what must be going on all over the body during life, producing the redundancies of physiological chemistry, since a few substances of very complex constitution will suffice to allow of the appearances of an endless number of breakdown products (vital as well as laboratory) which might be looked upon either as post- or pre-formed in the molecule. It is the determination of whether we are dealing with the one or the other which is the most difficult of the problems of physiological chemistry. But enough has been said on this subject in the first few pages.

The interest which the decision of the structure of the lecithin molecule has to us just at present lies in the fact that those two formulæ allow us to understand (vaguely, it is true) the vital part which lecithin plays. It is remarkable to find that lecithin has a considerable tenacity of its complex structure as long as it remains part and parcel of the *living* cell.

An additional fact about the physiology of lecithin is supplied by other recent researches, namely, that it plays a part in the assimilation of food-substances and in the development of cell-ferments. There has been found a marked similarity in the interaction between ferments and lecithin to that between ferments and mastic.

The existence of lecithin in bone marrows suggests an explanation for the undoubted effect which X rays have in the treatment of leucocythæmia.

In order to arrive at a conception of the chemistry of lecithin it will be convenient to discuss the subject from the historical point of view.

There are two periods in the development of the knowledge of this subject,* a pre-Thudichum period, and † the period started by Thudichum. We may say that although fatty substances containing phosphorus were known to Fourery, Vauquelin (1793), Couerbe, and Freny (1840), the name lecithin was not invented till Gobley had made his investigations (1846) on egg-yolk, whence he obtained glycerophosphoric acid. Four years later Liebreich had come to consider that a body which he called "protagon" must be the mother-substance of lecithin, a conception which finished in a complete proof as a result of modern research of the fact that there is no

* Danilewsky.

† Michaelis and Rona.

entity "lecithin," but that there are a number of different fatty phosphorus-containing bodies present in, say, nerve-substance, but yet able to be isolated by suitable procedures.

In and about the year 1846 we find several names prominent in the story of the research into lecithin—Hoppe-Seyler, his pupils, Diakonow and Stecker. These observers showed in the first place that protagon was not the only phosphorus-containing substance in the body, and, in the second place, they not only obtained pure lecithin, but also showed how cholin and glycerophosphoric acid combine to form lecithin when in association with oleic acid.

In 1876 we come to the second period of study, when Thudichum began to publish his researches, ultimately, in 1901, culminating in a most thorough account of all the phosphorus-containing fats which occur in the brain. He grouped them all under the heading of "phosphatids," and subdivided these into cerebrosides, cerebrinazids, and amidolipoids. Although this investigator planned out methods of quantitative analysis and means of distinguishing all these bodies, and although he adversely criticised Bergell's suggestion that phosphatids could be estimated as cadmium compounds, it may be safely said that neither Thudichum nor any one else has yet succeeded in devising a scheme for quantitative analysis which is above criticism. The methods which can be adopted are only endowed with approximate accuracy.

Perhaps the presence of *jecorin* * in the mixtures in which lecithin has been sought will explain to some the confusion which has arisen in the quantitative analyses. We must, however, regard all opinions about these bodies as at present only temporary, merely remembering that the word "lecithin" does not represent an entity, but represents many closely allied substances.

The difference between one lecithin and another which has been utilised by Erlandsen as a basis for classification consists in the ratio which the nitrogen bears to the phosphorus. We may then arrive at the following classification of lecithins:

1. Monamido-monophosphatids ($N : P = 1 : 1$), *e.g.* lecithin, cephalin. These contain two fatty radicles.
2. Monamido-diphosphatids ($N : P = 1 : 2$), *e.g.* cruorin—three fatty radicles (unsaturated and easily oxidised) + glycerophosphoric acid + alkaloid-like base.
3. Diamido-monophosphatids ($N : P = 2 : 1$), *e.g.* Thudichum's amidomyelin and sphingomyelin. These only occur in the body *combined*, say, with albumen (lecithin-albumen).
4. Diamido-diphosphatids ($N : P = 2 : 2$).

It will be seen from these varying ratios that the classification does not provide us with a means of determining *how much of each variety* may be present in a mixture of phosphatids. A

* Drechsel-Baldi. This substance is briefly discussed below.

complicated series of separations by fractionation with acetone, ether, and alcohol has been utilised for this purpose, and the only means of identifying the body so isolated consists in the ordinary ultimate organic analysis. Such a procedure is only of interest to the student, and for this reason the method published by Stern and Thierfelder last year is given below.

A procedure such as this gives a fairly correct estimate of the amount of each phosphatid present; but in the study of, say, ascitic fluid the presence of so many nitrogenous bodies would involve very careful preliminary separations in order to arrive at anything like a pure enough substance for analysis. Before discussing the methods of detection and analysis the following account of the properties of lecithin will be advantageous:

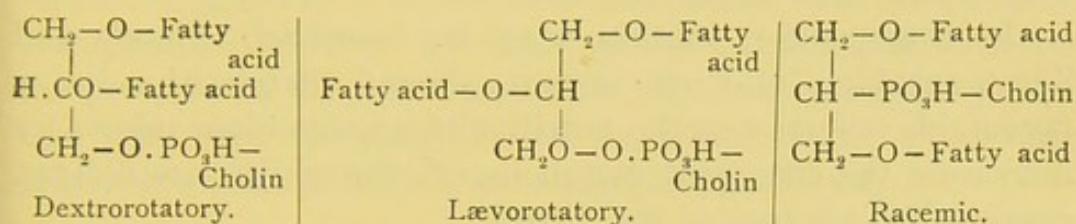
Properties and Confirmatory Tests.—Lecithin is a monophosphatid which can be obtained in the form of a very hygroscopic mass which is insoluble in acetone, but soluble in absolute alcohol. It can be thrown out of its alcoholic solution by both cadmium and platinum, in the form of addition-compounds.

It gives Pettenkofer's reaction with concentrated H_2SO_4 .

It melts at about 68°C ., and begins to decompose at 70°C ., the decomposition products depending to a certain extent on the exact composition of the lecithin. While on the one hand lecithin is made out to consist of cholin, glycerophosphoric acid and a fatty acid, the latter may be sometimes distearic acid, sometimes palmitic acid, and sometimes dioleic acid. As a rule, each of these occurs in one given sample of lecithin, the exact proportions varying, and giving rise to variations in ultimate analysis, though differences in solubility in absolute alcohol enable them to be separated by fractionation. The differences in content and variety of fatty acid explain some of the differences in physical properties. As a rule, lecithin is a waxy mass which is softer the more oleic acid there is, and, on the other hand, special methods of extraction and purification may yield it in the form of a powder. It is only crystalline when salts have been formed with it, the crystals being generally in the form of thin microscopic hexagonal plates.

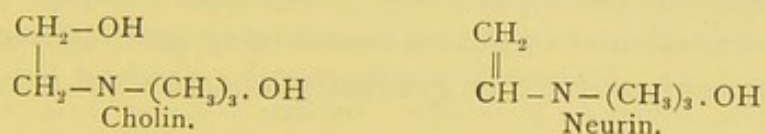
The substance may also have allotropic forms, for Thudichum distinguished between a water-soluble lecithin and a lecithin insoluble in water, the latter form being produced if it be extracted with alcohol and dried.

Then, again, there are optical differences between different forms of lecithin, for while the compound as usually obtained is dextrorotatory ($\alpha_D 24 = +11.3-11.4^\circ$), the action of steapsin on it results in a lævorotatory form. But besides this there is a racemic form, produced by heating the dextrorotatory form with ten times its bulk of absolute alcohol for 5 to 6 hours. This variation in optical activity has been explained by Ulpiani in 1901 by the following formulæ:



The racemic form is seen to be quite symmetrical.

Yet another consideration. If we compare the formulæ of cholin and neurin we see that in each case there are three methyl groups attached to the nitrogen of ammonia, while in cholin the



remaining H atom is replaced by the group $\text{C}_2\text{H}_4 \cdot \text{OH}$ instead of by C_2H_5 , which completes the formula of neurin. It is reasonable to suppose that if a lipid (phosphatid) can be formed of cholin, another may exist which is formed of the allied neurin. The association of the three substances in the phosphorus-containing material of the brain suggests that they may all be associated and possibly may be all in some kind of chemical association. Not only this, but the discovery of a number of bodies allied to cholin by Kutscher of Marburg (1907) in the urine, opens up a large field of possibilities. These new bases are:

1, methylguanidin; 2, novain; 3, reductonovaine (is to novain as neurin is to cholin); 4, methylpyridin chloride; 5, gynesin; 6, mingin; 7, vidiatin; 8, oblitin; 9, neosin.

All these bodies yield trimethylamine on decomposition, and form platinum salts, which enable their identification. Up to the present they have only been met with in meat extract, but their excretion by the urine suggests that they may play an important

part in purin metabolism, and, inasmuch as two bases are associated with lecithin, these other bases may at some period of their formation come into relation with the same lipoid.

The importance of these considerations lies in the light which they throw on certain physiological questions. Thus, the enzymes of the animal body have been found by Paul Mayer to act entirely differently on dextro- from lævo-rotatory lecithin. Possibly its action in activating cobra-venom will depend to some extent on its optical character.

In therapeutics, lecithin may be beneficial. Claude and Zaxy, speaking at Paris in 1901, showed that lecithin has a favourable influence on the nutrition of a tuberculous subject by increasing the energy of metabolism,* though its use did not prevent the advance of the disease.

The association of cholesterin with lecithin is a fact of very great interest, and has engaged the attention of Dr. Craven Moore, who considers that cholesterin exists in the cell in a *colloidal* state, through the agency of lecithin. When the latter undergoes dissolution, the more stable cholesterin becomes unable to maintain its colloidal condition unless fatty acids or their derivatives be present, and it then gradually separates out in crystalline form.

The observation of Pascucci shows up what I term the symbiotic relations between cholesterin and lecithin. The addition of cholesterin to a hæmolysin neutralises the activity of the latter, while lecithin has no action, the varying proportion of lecithin and cholesterin thus affording a means of regulating the effect of hæmolytic substances on the cell. If the OH groups of cholesterin be rendered inert, this neutralising property is lost.

Lecithin and cholesterin together form means by which substances otherwise unable to penetrate the cell-envelope become enabled to do so.

Methods of Detection and of Extraction of Lecithin.

—Otoliski made use of the separating power of an excess of 96 per cent. alcohol; but if we search through his figures, they do not seem to show very constant N : P ratios.

Manasse used warm absolute alcohol for extracting the

* Glycerophosphoric acid appears in the urine when lecithin is taken.

lecithin, and employed Salkowski's method of detecting phosphorus in the evaporated extract. This method is simple and apparently sufficient for clinical purposes.

In several of my own specimens I have used the precipitate with ammonium sulphate (Table B) and applied Salkowski's method of incineration for phosphorus. If known weights are used, this method can be made a quantitative one, although it must be remembered that all the lecithin present is not necessarily estimated, as not all of it may be in association with the globulin. If this method be adopted we shall be able to endeavour to detect the presence of this substance with the same quantity of fluid that was used for globulin—an important point when only moderate amounts of fluid are available for analysis.

The absolute proof that a substance is lecithin will involve an estimation of the N : P. ratio, an estimation of the rotatory power, a preparation of the distinctive cadmium salts, and finally the extraction of cholin (means of detection, see p. 171), glycerophosphoric acid, and of fatty acids.

Incineration Method.—1. A weighed quantity of substance is dried at 120°C . If there be a negligible residue there is no inorganic phosphorus. Otherwise the residue is fused with soda and saltpetre in a platinum crucible by the aid of a blowpipe. The residue should be white, and is dissolved in dilute nitric acid (minimal amount), filtered if necessary, and then one portion made strongly alkaline with ammonia, while the other is rendered still more acid with pure nitric acid. If a precipitate results from the addition of ammonia, then mineral phosphates are present $[\text{Ca PO}_3]_2$, MgPO_3 , FePO_3 .

The other portion is treated with ammonium molybdate, and the resulting precipitate may be weighed in order to estimate the quantity of phosphorus.

0.0024 gm. of precipitate corresponds to 0.0175 gm. lecithin.*

The objection to this simple method is that one cannot well distinguish between the organic and the inorganic phosphorus.

2. The simplest procedure then would be to add three times the bulk of fluid used of absolute alcohol, and wash the residue well before evaporating it to dryness. The residue is then taken up with water, and shaken repeatedly with ether. The ethereal extract is allowed to dry and the residue fused with 3 parts KNO_3 and 1 part sodium carbonate. The white residue is dissolved in a trace of water and nitric acid added. Ammonium molybdate is added (avoiding excess) and the precipitate weighed as in method 1.

3. A still more accurate method is that of Neumann. 500 cc. fluid are treated with pure nitric acid and gradually poured into a round-bottomed flask containing 30 cc. of boiling nitric acid. There should never be more

* It is assumed that 1 gm. lecithin contains 0.0384 gm. phosphorus.

than 100 cc. of fluid in the flask at once. The fluid can then be rapidly reduced to a small bulk. 5-10 cc. of a mixture of equal parts of pure nitric and sulphuric acids are added, moderate heat in a draught-chamber being used. Additional quantities of the acid mixture are added till a litre has been used, and the fluid can then be allowed to cool. The fluid in the flask should now be as clear as water. (Rapid method of oxidising organic matter in any fluid, perhaps especially useful for urine.)

For each 40 cc. of residual fluid add water to 150 cc. and 50 cc. of ammonium nitrate. Heat till bubbles rise, and then add 40 cc. of ammonium molybdate. The precipitate of ammonium phosphomolybdate is thoroughly shaken till granular and left a quarter of an hour, and then washed by decantation till the washings cease to be acid. The residue on the filter is returned to the flask, 150 cc. water are added, and seminormal soda added from a burette till solution occurs. 5 or 6 cc. over this point are added, noting the number of centimetres used. Boil till ammonia ceases to come off, and then titrate with seminormal acid against phenolphthalein. (The number of centimetres of soda used—number of centimetres of acid—+ 1.268 gives P_2O_5 in milligrammes.)

4. The complicated but more accurate methods.—(a) *Erlandsen* first dried the material in a current of air for several hours, and then in vacuo. The powdery mass was extracted with ether, and the final extraction treated with alcohol. The alcohol fraction was made to yield the phosphatids by successive extraction with ether and acetone, while the ether fraction was treated with acetone to extract lecithin, cruorin, etc.

(b) The details of this method are lengthy and cannot be dealt with in this place. A table (pp. 52-53) showing *Stern and Thierfelder's* scheme is given, however, in order to give some idea of the lengthy procedure necessary in order to make accurate researches on this subject. The special feature of their method was the carrying out of the methods in the dark in an atmosphere of CO_2 .

(c) *Manasse's* method is similar to that of *Erlandsen* (1906).

It must again be emphasised that even these quantitative methods do not give absolutely correct figures, owing to the loss that necessarily takes place during the extraction processes. Besides, it is not possible to absolutely separate the monamidophosphatids from the diamidophosphatids.

The accompanying table shows that the process is essentially one of fractionation with different solvents, some of the phosphatids being more insoluble than others.

There is an additional source of error in the fact that the fluids which we are concerned with may contain nucleic acids or their derivatives, i.e. other phosphorus-containing substances, and these will work out as lecithin in the estimation as phosphorus. In the methods of series 4, however, the substances are identified by their *percentage composition*. In spite of the errors, the last series (4) are as accurate perhaps as one can hope for.

In respect to the puncture-fluids it was thought possible to identify certain of these complex substances by applying the tests which are used as micro-chemical reactions. The difficulties so far have been insuperable. The fact that lecithin crystals do not stain with osmic acid after previous fixation with potassium bichromate has not been found available as a

quantitative test. There can be no doubt that if such reactions could be utilised it would simplify the work of examining the chemical constituents of puncture-fluids for clinical purposes very considerably.

In referring to the work of Otolski reference was made to the byefinding of jecorin. It is purposed to discuss this substance more in detail. Its formula has been given by Drechsel as $C_{105}H_{185}N_3P_3SO_{16}Na_7$. It is a body which is extremely hygroscopic and forms a slimy mass in the presence of water. The chief interest lies in its ability to reduce alkaline copper sulphate, though it is precipitated by strong salt solutions and by silver nitrate. In the latter case the precipitate is soluble in excess of jecorin, and when heated with ammonia turns a port-wine red.

To judge by Manasse's analysis, jecorin may be regarded as a compound of lecithin with glucose, for he obtained glucosazone from it, but Meinerz regards it as a mixture of many organic and inorganic bodies.

Just as activity of research on lecithin led to its detection in very many tissues, so with jecorin. The chief sites of jecorin in the body are the spleen, the brain, the blood, and in the suprarenals. It is worthy of note that the suprarenals contain also a considerable amount of lecithin. Whether cholesterin is associated or not has not been made out. However, it is important to note that according to Jacobsen, Henriques and Kölsch, the glucose of the blood may possibly circulate as jecorin, although it has to be admitted that in their process of extraction of the blood with ether some free glucose might be extracted with the ether, and thus give rise to an error. It is another instance of the extreme care which is necessary before one formulates any opinions as to what substances are and what are not present in any given fluid. It bears out the view that many of the simpler proteid derivatives which may be detected in a body fluid may be merely the result of decomposition of the proteids which are present. Paul Mayer endeavoured to definitely settle this problem of the jecorin and sugar combination, and he comes to the conclusion that the glucose is retained by lecithin as a solid solution or possibly by adsorption, although he admits the possibility that it is a molecular additive compound. However, he attaches great importance to the presence of S and Na in jecorin, and says they are essential constituents of it.

He thinks that if a ferment exists in the blood which can split off sugar from its combinations, it might split off the sugar of jecorin and cause the sugar to appear free in the blood. But special experiments led Paul Mayer to the conclusion that there was no such ferment in the blood, so that the sugar which is present in the blood cannot exist in combination with jecorin, but must be free at the outset.

The discovery that jecorin taken from different animals possesses a varying percentage of attached glucose shows that probably there is no constant relation between the two.

Jecorin acts on cobra-venom in exactly the same way as does lecithin, the only difference being that if glucose is present, the hæmolysis takes place very much more rapidly.

THE ISOLATION OF PHOSPHATIDS (STERN AND THIERFELDER).

Use ACETONE for Primary Extraction.

Shake with ether, and centrifuge the turbid fluid.

Weigh the Phos- PHATIDS Iodine No. 693. (Not further in- vestigated.)	Concen- trate these equal parts of fat + Phos- PHATIDS.	Turbid solution.	Solution. Concentrate and add acetone.	Residue.	Dry, dissolve in ether and add acetone.
Evap. Rub residue with acetone.	Solution.	Turbid solution.	Residue.	Dry, treat with ether, centrifugalise.	(White residue.)
Evap. Rub residue with acetone.	Solution.	Turbid solution.	Residue.	Dry, treat with ether and centrifugalise.	(White residue.)
Evap. Rub residue with acetone.	Solution.	Turbid solution.	Residue.	Dry (Iodine No. 684), treat with ether and centrifugalise.	(Very scanty white residue.)
Evap. Rub residue with acetone.	Solution.	Turbid solution.	Residue.	Dry, dissolve in ether and add acetone.	(White residue.)
Evap. Rub residue with acetone.	Solution.	Turbid solution.	Residue.	Dry, dissolve in ether, add acetone as long as oily deposit forms.	(White residue.)
Evap. Rub residue with acetone.	Solution.	Turbid solution.	Residue.	Dry, dissolve in ether, and precipitate with acetone.	(White residue.)
Evap. Rub residue with acetone.	Solution.	Turbid solution.	Residue.	Dry, dissolve in ether, and precipitate with acetone.	(White residue.)

	with acetone.	rub with alcohol and acetone.
Mix; concentrate equal soft mass. Dry; dissolve in ether and alcohol. Iodine No. 573. (Not further investi- gated)	Turbid solution.	Turbid solution. Deposits insoluble; precipitate on concentration.
	Soft mass. Dry, add alcohol, centrifugalise.	Powdery mass. Dry, add ether, centrifugalise.
	Solution. concentrate, precipitate with acetone.	Solution. Concentrate; add alcohol and test precipitate with acetone.
	Residue. Soft mass. Dry, add alcohol, centrifugalise.	Trace of greyish residue.
	Turbid solution. Concentrate, dry, add acetone. Solution. (Residue.) Concentrate, precipitate with acetone.	Turbid solution. Deposits in soluble; precipitate on concentration. LECITHIN. Powdery residue, soluble in ether. CEPHALIN. Iodine, No. 70'4, P: N = 1: 0'77 + Ca.
Mix; only slight residue on con- centration.	Turbid solution.	Soft mass. Dry, add alcohol at 0°; centrifugalise.
	Solution. Concentrate, precipitate with acetone.	(Residue.)
	Turbid solution.	Soft mass (iodine No. 52'96), soluble in alcohol, turbid at 0°; centrifugalise.
	Solution. Concentrate, precipitate with acetone.	(Residue.)
	Turbid solution.	Soft mass. Dry, dissolve in alcohol (turbid at 0°), add ether, centrifugalise.
	Solution. Concentrate, dry, add ether; centrifugalise.	White residue.
	Solution. Concentrate, dry; residue is soluble in alcohol and ether.	White residue.
		SPHINGO- AMIDO- APO- MYELIN-LIKE BODIES.

TABLE VI
LECITHIN IN BODY FLUIDS

Fluid.	Disease.	Lecithin.
Pleural	Inflammatory, (Abdominal Cancer)	Trace.
	Chronic Effusion (nature ?)	Conspicuous
Peritoneal	Renal Disease	Present.
	Peritoneal Carcinomatosis	"
	Unilobular Cirrhosis	"
	Renal Disease	Absent.
	Cardiac Failure	"
	Tubercular Peritonitis	"
Ovarian Cyst	Unilocular Cyst	Absent.
	Broad Ligament Cyst	Present.

The study of the presence of lecithin in body fluids may be said to date from Joachim's investigations in 1903, when he was endeavouring to find the cause of the turbidity of certain ascitic fluids. In one special case studied by him the patient was suffering from cirrhosis of the liver, and the fluid which had been poured out into the abdomen was turbid, though no fat was present. The essence of this study lay in the fact that pseudo-globulin was found to be present in abundance, and that with this pseudo-globulin there was associated lecithin. That this was the cause of the milkiness was proved by the fact that dialysis gave a clear fluid, and that the residue contained lecithin, while the clear fluid did not. The lecithin was extracted by the use of ether, and the extract tested for phosphorus.

It was found that only the pseudo-globulin fraction contains phosphorus, namely, 2.45 gm. in every litre of ascitic fluid, which corresponds to 0.35 gm. lecithin.*

Jolles records a case in which the peritoneal fluid (disease not stated) contained abundance of lecithin.

These observations proved beyond doubt that lecithin may occur in ascitic fluids, and would be the cause of the turbidity. Moreover, it will be noted that this occurred in a case of effusion due to cirrhosis of the liver. It became a matter of no little interest to know if lecithin occurred in other fluids besides

* The precipitate resulting on adding $\frac{1}{2}$ vol. of sat. Am_2SO_4 consisted of euglobulin, and if the filtrate be half saturated again with Am_2SO_4 a precipitate of pseudo-globulin results which is soluble in sodium chloride and sodium hydrate solutions.

ascitic fluid, and if it occurred in ascitic fluid under any other conditions.

The actual diagnostic importance which is to be attached to the occurrence of lecithin in a body fluid can hardly be made out from so few examples, although one hopes to have shown that the question may be of use to the clinician.

The Diazoreaction.—This reaction, which has been applied to the study of urine under certain febrile conditions, was carefully studied by Clemens in 1904, who found that the bodies which are responsible for the reaction are precipitated by basic lead acetate and are insoluble in alcohol. Tyrosin and histidin both give the reaction, and as the former may be present in a puncture-fluid, it was thought that this test might give useful results in puncture-fluids, especially when the test, as applied for the detection of histidin, is extremely delicate.

The results of the experiments showed, however, that it was very rarely present. It was obtained distinctly in a case of pleural effusion associated with abdominal sarcoma, and also in the fluid from a tuberculous pleurisy. In only two peritoneal fluids was it found—namely, one from a case of polyorrhomenitis and another from a case of chronic peritonitis associated with an old gastric ulcer. If the investigations of Clemens represent complete knowledge on the subject we should be able to assume that in these four cases either tyrosin or histidin or both was present,* and the fact of one being a case of carcinoma their presence would be explicable because the products of carcinoma metabolism might well appear in this as in other fluids of the body. However, the reaction was not obtained in any other case of the same kind nor in a definitely carcinomatous effusion. The reaction was uniformly absent in subcutaneous oedema fluids.

* Other views as to the nature of the reaction are :

- (1) That it is due to phenols and amines (Ehrlich).
- (2) That it is due to paired sulphur acids (Dolgow).
- (3) That it is derived from breaking-down leucocytes (Geissler, Saliev).
- (4) That it will appear in urine after taking opium, chrysarobin, naphthalin, morphine, while it is prevented by internal administration of tannin.
- (5) That it is due to the chromogen of urochrome (Weiss).

For literature and full details see v. Noorden, *Handbuch der Path. des Stoffw. I.*, 660. Berlin, 1906.

The Molisch Reaction; the α -Naphthol Reaction.—

The Molisch reaction consists in obtaining a violet colour with an alcoholic solution of α -naphthol in the presence of pure sulphuric acid. This test was applied to the original fluid in many puncture-fluids, mainly as a matter of curiosity in the first place (since the reaction depends on the presence of carbohydrate radicles), and with the hope that it might form a ready test for the presence of such in a fluid.

It was soon found, however, that there were striking differences in the effects in different cases, so that it has since that time been applied as a routine procedure. Up to the present it is hard to formulate any rules as to diagnosis which might be made from its presence or absence. The difference in the degree of reaction is perhaps the most striking, but at the same time it must be admitted that a violet colour is never obtained. The reaction which one does obtain is the formation of a red ring or a red staining of the acid.

A 5-per-cent. solution of α -naphthol in 95 per cent. alcohol is prepared, and an equal volume is added to the fluid examined. Strong sulphuric acid is allowed to run down the side of the test-tube, and the contents are now oscillated gently. A red line will appear at the junction in a well-marked case, almost instantly; and, with oscillation of the heavy acid, the latter will become uniformly red (carmine). The results which have been obtained are shown in Table VII.

From this table it is evident that as a rule pleural fluids give a much more marked reaction than do peritoneal fluids, and that in cases of "idiopathic" effusion the colour-reaction is very decided. On the other hand, peritoneal fluids, especially those dependent on back-pressure (mechanical effusion), show a very scanty reaction.

The difference in the degree of reaction depends, presumably, on difference in constitution of the proteid or on difference of substitution products. The presence of reducing substance in the proteid of many puncture-fluids (some form of carbohydrate radicle) has been specially remarked on by Landolf of Buenos Ayres.

The absence of the reaction in such a peritoneal fluid as is derived from a case of polyorrhomenitis is suggestive, for the fluid from such a case has very marked differences from any

other puncture-fluid; it is clear, or slightly opalescent, quite watery, and contains only a trace of globulin and comparatively few cellular elements.

TABLE VII
THE α -NAPHTHOL REACTION OF PUNCTURE-FLUIDS

Absent.	Very Slight.	Slight.	Distinct.	Decided.	Very Decided.
Pericardial Fluid Subcutaneous Edema (Cardiac) Peritoneal Fluid: Polyorrhomenitis. Cardiac Disease Tubercular Peritonitis Hydatid Cyst	Peritoneal Fluid in Cardiac case in Tuberculous Peritonitis Cirrhosis of Liver Pancreatic Cyst	Peritoneal Fluid in Cardiac case in Cirrhosis Multi-lobular Cirrhosis of Liver	<i>Pleural Fluid</i> (Simple Inflammation)	<i>Pleural Fluid</i> (Tuberculous) Hydatid Cyst	<i>Pleural Effusion</i> (3 Tuberculous cases). Peritoneal Effusion in multi-lobular cirrhosis, Chronic Peritonitis (simple).

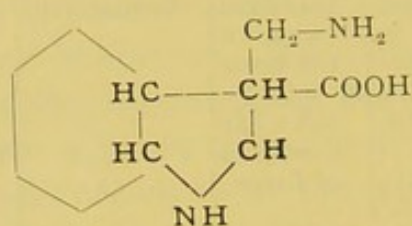
Ehrlich's Glucosamine Test.—As in the case of the Molisch reaction, this test has been applied to a considerable number of fluids, and similar marked variations in degree of reaction obtained. The most striking point seen on comparing Table VII with Table VIII is that the latter shows mainly peritoneal fluids in the marked positive cases, while in the former the pleural fluids gave the more decided reaction.

The test is performed thus. To a small portion of fluid an equal quantity of a 5-per-cent. solution of *p*-dimethylamino-benzaldehyde in 10 per cent. sulphuric acid is added, and strong sulphuric acid is run down the sloping test-tube. A deep violet colour, varying in intensity, will appear at the line of junction, and gentle oscillation of the acid will induce a very intense coloration in a positive case.

The results of these experiments are shown in Table VIII, and the differences in the reaction must find some explanation.

The explanation of the test itself has been the subject of much controversy, and the present opinion seems to be that it is the tryptophane radicle which is the cause of the colour re-

action. The test is called by its name because glucosamine will give the reaction, as well as other osamines. Neubauer considered that urobilinogen would give the reaction, but it is not generally accepted that this is the explanation of the test when applied to proteids in general. Pappenheim regards it as settled that this reaction is a pyrrhol reaction, though no confirmation of this can be found in the literature available, although if the reaction be looked on as a *tryptophane* reaction, the two views are readily reconciled, for both tryptophane and pyrrhol contain the imido-group in the same position; indeed, Holland's formula for tryptophane contains pyrrhol:



Tryptophane (Holland).

The thickened letters represent the pyrrhol group.

The question of pyrrhol will again come up in discussing the ferments in leucocytes.

If we look upon the reaction as indicating the presence of tryptophane in the fluids under question, we shall be able to obtain a more tangible idea of the significance of the test as applied to puncture-fluids, and the intensity of colour will presumably depend on the amount of tryptophane * present.

* Tryptophane (indolaminopropionic acid) is to some extent an index of the amount of destruction of proteids that is going on. The substance bears some relation to melanins. It exists in three optical forms—a lævrotatory, a dextrorotatory and a racemic. The following tests may be employed for its detection:

- (1) The glyoxylic test (see below).
 - (2) Bromine water in acetic acid gives a rose-red or violet colour (mixed mono- and di-bromide of tryptophane according to Levene and Rouiller), which can be taken up by amyl alcohol.
 - (3) Pyrrhol reaction. A match-chip put in strong HCl, and then into the solution, turns cherry red.
 - (4) *p*-dimethylaminobenzaldehyde test.
- It is soluble in cold, readily in hot, water, but only slightly soluble in absolute alcohol.

To separate it from leucin and lysin, Levene precipitates the filtrate of Table A with phosphotungstic acid (four parts to one of water), and the filtrate therefrom contains the substance in question.

TABLE VIII
TRYPTOPHANE IN PUNCTURE-FLUIDS

	Absent.	Faint.	Distinct.	Very Distinct.	Extremely Decided.
Pleural {	Simple Inflammation Simple Inflammation Empyema	Simple Effusion Adherent Pericardium, etc.	Simple Effusion Empyema	Non - tuberculous Effusion (2 cases) Tuberculous Pleurisy Simple Effusion	Simple Inflammatory Effusion (3 cases) "
			Pericardial Fluid		
Peritoneal {	Cardiac Back-pressure Renal Cardiac Back-pressure	Cardiac Back-pressure (2 cases) Tuberculosis (2 cases) Atrophic Cirrhosis of Liver (2) Monolobular Cirrhosis Carcinomatosis Renal Adherent Pericardium, etc.	Cirrhosis of Liver Polyorrhomenitis	Cirrhosis of Liver Tuberculous Peritonitis Tuberculous Peritonitis (deep crimson colour)	Back-pressure (cardiac) (2 cases) Monolobular Cirrhosis Chronic Inflammation- (Non-tuberculous) Tuberculous Peritonitis
Cysts {	Pancreatic Ovarian Hydatid	Ovarian Cyst Pancreatic	Hydatid (crimson colour)		Unilocular Ovarian Cyst

The fact that the Adamkiewitz reaction * depends on the presence of tryptophane will also serve as an indication of the presence of this body in puncture-fluids. In the cases which I have examined, the test has, however, been seldom applied, and only in the Table D section of analysis (p. 36). The results are, however, incorporated in the above table (VIII.).

Pigments.—The colour of puncture-fluids is not of very much interest. The presence of blood will be revealed in the ordinary way, and the presence of bile (which can enter in cases of jaundice, or from perforation of the bile passages) is easily made out.†

* Adamkiewitz reaction. Glyoxylic acid (see p. 38) is added to the solution to be tested, and strong sulphuric acid is added. A blue-violet colour results.

† Nakayama's modification of Huppert's bile pigment test is a useful one, and but little known and used. The fluid to be tested is mixed with

The usual colour of effusions is due to a lipochrome which can be extracted with amyl alcohol and has a characteristic spectrum. In cases of hydræmia, the subcutaneous fluid will be remarkably pale, owing to the dilution of the fluid.

Salkowski's method of extracting the colouring matter consists in collecting the deposit after acidification of the fluid with acetic acid, and purifying it by successive solution and reprecipitation, and dissolving in absolute alcohol. The residue from this is dissolved in chloroform, and the solution examined by the spectroscope. The addition of nitric acid to the chloroform solution results in a transient blue coloration.

The spectrum shows partial absorption of the right half of the spectrum, and a dark band at F and a paler one between F and G.

Gases.—Free gases may occur in puncture-fluids from the rupture of hollow viscera or from the perforation of a pulmonary cavity. In rare cases the gas is derived from bacterial growth.

The intrinsic gases of the various fluids are mainly carbon dioxide with traces of nitrogen. The details which are available are mentioned in Section III under the appropriate headings.

The chief interest in this subject lies in the influence which carbon dioxide has on the proportion of electrolytes.

The Inorganic Constituents of Puncture-Fluids.—

The most important inorganic constituent which we have to deal with is of course sodium chloride. The presence of carbonates, sulphates, phosphates, is of less importance, and is conveniently considered under the one heading of "Achlorides" (see Section II.).

The subject is more fully discussed in connection with the theoretical considerations on electro-conductivity and later, again, in Section IV. We have already discussed the risk of error likely to arise from adsorption (p. 17) and shown that, at any rate in fluids containing only a small amount of albumen, the volumetric method of analysis is accurate enough. In the case of an equal quantity of 10-per-cent. *barium chloride*. After a short centrifugalisation the clear fluid is decanted, and to the residue $\frac{1}{2}$ -inch in a test-tube of the following mixture is added: 1 per cent. fuming HCl in 95 per cent. alcohol, with ferric chloride added to the acid in the proportion of 4 per cent. After mixing, the fluid is boiled and the supernatant fluid will turn green or blue-green, turning violet or red on adding nitric acid.

TABLE IX.—CHLORIDES IN PUNCTURE-FLUIDS
PLEURAL

2%	3%	4%	5%	6%	7%	8, 10%
	Cancer of Ovary, 2.6 0.85 Tuberculous 2.5 Idiopathic... 2.1	Tuberculous 3.75 " 3.6	Cardiac failure ... 4.75 Chronic Nephritis ... 5.1 Cancer of Ovary ... 5.7	Pleural ... 6.5	Tuberculous 7.25	Cardiac failure
PERITONEAL.						
	Back-pressure (Cardiac) ... 2.5 Monolobular Cirrhosis ... 2.5 Disseminated Cancer ... 2.7	Tuberculous 3.5 Chronic Nephritis ... 3.5 Chronic Peritonitis with arterial disease ... 4.6 Peritoneal Cancer ... 3.0 Disseminated Peritoneal Cancer (stomach)... 4.0	Monolobular Cirrhosis ... 5.2 Cirrhosis of Liver ... 5.7	Sarcoma of Omentum... 6.3 Tuberculous 6.4 Disseminated Cancer ... 6.6 Cardiac Back-pressure ... 6.3 Thrombosis of Portal Vein 6.3	Chronic Nephritis ... 7.5 Polyorrhoenitis ... 7.5 Toxic Nephritis ... 7.7 Cirrhosis of Liver ... 7.8	Toxic Nephritis ... 9.1
MISCELLANEOUS.						
Fluid from Leg 0.85 (Atrophic Cirrhosis of Liver)	Empyema Pus 2.4		Fluid from Leg. 5.5 Cardiac failure	Ovarian ... 6	Fluid from Leg 7.5 (Interstitial Nephritis. Portal Obstruction)	Pericardial ... 8

exudates we have to regard the quantity of chlorides estimated as those which are "unattached." A parallel estimation by incineration would be of interest in deciding how much combined chloride there was present.

The accompanying table of the amount of chlorides present in various fluids is of considerable interest, inasmuch as it shows striking differences in chloride-content in different classes of fluid. Thus, in the pleural series there is a high percentage of chlorides in back-pressure cases, and the same is the case with renal effusions into the peritoneal cavity. The subcutaneous fluids in cases of renal disease also often show a relatively high chloride-content.

The methods of analysing chlorides.

1. Volumetric.

The volumetric method which has been adopted in the studies recorded in this work is that of Salkowski's modification of Volhard's method.

A solution of 29.075 gms. per litre of silver nitrate is needed, and 10 cc. of this are placed in a flask graduated to 100 cc.; 4 cc. pure concentrated nitric acid are added, and distilled water is used to fill up to the mark. The mixture is poured into an 8-oz. flask, and 5 cc. of a saturated watery solution of double sulphate of iron and ammonia added. A solution containing about 8 gms. of ammonium sulphocyanide is now run in from a burette until the red colour produced by interaction with the double salt has become permanent. From this reading it is easy to find out how much water is to be added to the sulphocyanide solution to make 25 cc. of it correspond to 10 cc. of silver nitrate. It is well to test that this is so before using it for actual analyses.

The de-albumenised solution is now used, 10 cc. being put into the graduated flask, 30 cc. (about) of distilled water, 4 cc. of nitric acid and 15 cc. of the silver nitrate. The contents are made up to 100 cc. with water. After thorough shaking, the fluid is filtered through a thick filter-paper (Scheicher and Schüll, No. 598) until the 80-cc. mark in a perfectly clean and dry 100-cc. measure has been reached. This quantity is then poured into the 8-oz. flask and 5 cc. of the saturated solution of double salt is added.

Titration is then carried out with the sulphocyanide as with the control, and the reading on the burette is noted.

The 15 cc. of silver nitrate will be found more than sufficient

to precipitate all the chlorides present in these fluids, and the ammonium sulphocyanide measures the excess of silver solution. The formula—

$$\text{Parts NaCl pro mille} = (37.5 - 0.8 R) 0.4$$

gives the number of grammes of chloride per litre. R represents number of cubic centimetres of sulphocyanide used.

Since 10 cc. silver correspond to 25 cc. of sulphocyanide, 15 cc. correspond to 37.5. But only 80 cc. (0.8 per cent. of original fluid) have been titrated, hence only .8 of the reading is really required in order to represent the original filtrate. The last figure in the formula is due to each centimetre of AgNO_3 representing 0.01 gm. NaCl, so that each centimetre of sulphocyanide represents 0.4 gm. NaCl.

To save mathematics, a "ready reckoner" is given in the appendix.

A practical hint may be used in these volumetric procedures. The difficulty of reading accurately to a tenth of a cubic centimetre on the ordinary burette is got over by the following simple procedure which occurred to me: A strip of white paper is cut sufficiently narrow to prevent it wrapping right round the burette. Down the centre of this strip is ruled a clear thick black line. The strip is now attached with elastic bands to the burette, so that the Figures are foremost (Fig. 2).

The upper limit of the fluid causes a break in the line as seen through it, which enables a very exact reading to be taken at any level in the burette. With suitable care the slips will last a very long time.

2. The other methods depend on removal of proteid, thus:

(1) 10 cc. fluid are treated with 20 cc. saturated pure ammonium sulphate, and the mixture heated in a closed flask on the water-bath.

(2) 10 cc. of fluid are diluted with 90 cc. of distilled water and placed in a flask closed with a slitted cork. Boil, add a few drops acetic acid till the albumen separates out in big flakes.

(3) Dry the fluid, and heat to dryness. Extract the ash with

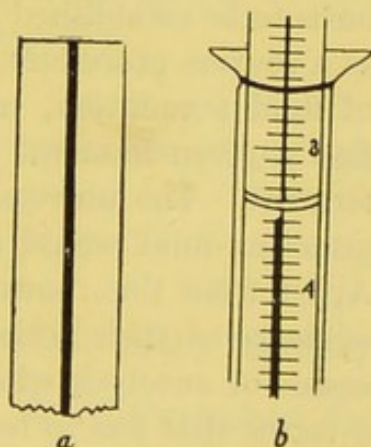


FIG. 2.

boiling small portions of water, and boil each extraction. Then dilute to 70 cc. with water, and filter. The objections are that phosphates, sulphates, and carbonates interfere, and if the heat applied be too great the chlorides may volatilise.

(4) 10 cc. fluid are treated with excess of alcoholic tannin, moderately heated and filtered.

In each of these cases the chlorine is ultimately estimated titrimetrically with silver nitrate and ammonium sulphocyanide in the way described.

THE FERMENTS

In the case of certain puncture-fluids the presence or absence of characteristic ferments serves as a valuable aid to diagnosis. The fluid contained in pancreatic cysts affords the best example of this truth, since the presence of trypsin or of lipolytic ferment, or of both, is almost constant. A type of case, again, in which the detection of ferments would be of great value is illustrated by the following: a marked swelling appeared in the left hypogastric region after an operation for colotomy, and was thought to be a fluid collection—possibly purulent, for the temperature was high. An exploratory puncture produced a fluid which contained a considerable quantity of pepsin, and the swelling proved to be formed by an enormously dilated stomach.

The presence of ferments in a fluid not only enables a diagnosis to be established in certain cases, but requires to be studied as a routine procedure, with a view to determining in which class of fluid (exudation, transudation, or cyst) one may expect to find a given ferment, and in which one may expect to find no ferment. The universal absence of ferments of any kind in a puncture-fluid would enable one to exclude certain diagnoses. Apart from this, however, the presence of these bodies, or the evidence of their action, has an important bearing on the processes of autolysis which occur in body-fluids; and autolysis is a factor that has to be borne in mind in interpreting the results obtained by this section of the chemical analysis.

It may be said that the two main peculiarities of ferments are that they are very specific in their action, and that they are intimately dependent for their action upon the nature of the substrate. The specificity is shown by the fact that while H^+ will produce a great variety of chemical changes analogous to

ferment action, a proteolytic ferment, for instance, will not act on starch or fat, in spite of each of these bodies undergoing a hydrolytic change when fermented. The second point is exemplified by the discovery made by E. Fischer, that certain ferments depend for their action on the stereo-isomerism of the substrate in which the ferment acts.

Speaking broadly, then, we may say that ferment action consists in a preliminary *anchoring of molecules of the ferment* to specific groups in the substrate (phase 1), followed by a *catalytic decomposition* of the same (phase 2). This catalytic decomposition continues up to a certain point and no further; for eventually a state of equilibrium is reached which does not allow any further change except a reverse change (reversible reaction).^{*} The catalytic agent, the "ferment," simply alters the rate at which the catalytic decomposition occurs, so that in order to understand the practical application of the theories of ferment action it is necessary to refer to the laws bearing on velocity of reaction.

If the velocity of reaction v between two substances of concentration C_a and C_b be represented by

$$v = k C_a C_b,$$

where k is the velocity when the concentration of a and of b is unity, the catalysis or ferment action would simply consist in altering k . But as the process involves the production of two new bodies, c and d , in increasing concentration,

$$v_1 \text{ will } = k_1 C_c C_d$$

and $v - v_1 = k C_a C_b - k_1 C_c C_d$ until the diminution of $C_a C_b$ is counterbalanced by the increase $C_c C_d$

$$\frac{k}{k_1} \text{ then comes to } = \frac{C_c C_d}{C_a C_b}.$$

The increase or decrease of $C_c C_d$ over $C_a C_b$ must therefore vary with $\frac{k}{k_1}$, and $\frac{k}{k_1}$ becomes a constant K , characteristic for the particular ferment.

It is well known that such a metal as "colloidal platinum" [†] exerts a catalytic action which bears great resemblance to

^{*} This would in actual fact be a simultaneous process.

[†] Prepared from the metal by establishing an electric arc between platinum electrodes under water.

ferment action. At first sight there is a marked difference between the action of colloidal platinum and an organised ferment,* because a substance acted on by the former becomes decomposed till no more remains, and when acted on by the ferment the process only continues up to a certain point, beyond which it is not possible to proceed. It is, however, now established that there is no essential distinction between the two series of processes; for if the medium in which the organised ferment is acting be diluted, the reaction will again commence, because the concentration of the products of action is diminished by the dilution. The state of equilibrium which was reached was only a false equilibrium.

The law of mass action states that the chemical action is proportional to the active mass of the bodies which enter into the reaction, i.e. is proportional to their concentration. This law holds good in the case of ferment action also.

Whether this statement is absolutely correct or not has been disputed by Lovatt Evans, who states that the velocity of catalysis shows three periods: (1) A *rectilinear* period, where the masses of the substrate converted in equal intervals of time are approximately equal. The values of the constant k in the formula given below steadily increase during the earlier phases of the reaction. (2) A *logarithmic* period. At this stage the law of mass action is followed. The values for k are constant. This period is best seen when the substrate used is in very dilute solution, and the amount of enzyme is very small. (3) An

* The chief points of comparison are:

Colloidal Platinum.	Ferments.
1. The reaction takes place according to the formula $\frac{dx}{dt} = k(A - x).$	The formula is $\frac{dx}{dt} = k_1 \left(1 + \frac{x}{A} \right)$
2. The velocity of reaction increases to a maximum on adding more OH^- ions.	Ditto.
3. Cl^- ion diminishes the velocity.	Ditto.
4. K_2SO_4 accelerates the velocity.	Ditto.
5. There is a temperature optimum.	Ditto.
6. H_2S and HCN act as anti-ferments.	Ditto.
7. There is no limit to the reaction.	The action ceases when the products of catalysis reach a certain concentration.

infra-logarithmic period, due to modification of the logarithmic course of the reaction with the progressive destruction of the catalase. The k values now fall. We see, then, that though the *net* result conforms to the law of mass action, it may be that the details show definite fluctuations and deviations therefrom.

We have to deal with two cases (1) in which there is only one molecule involved in the decomposition—monomolecular reaction, (2) in which there are two molecules interacting.

The velocity of a monomolecular reaction becomes progressively less as the reaction proceeds. This is indicated in Fig. 3, where the curve drops rapidly during the first few seconds, and then falls more gradually until at last it comes to lie almost parallel to the base line. Expressed mathematically we have :

$$-\frac{dx}{dt} = k(A - x),$$

which means that the velocity of reaction with which as small an amount, x , as you please is

transformed during as small an interval of time, t , as you please is proportional to the original concentration, A , of reacting molecules, minus the amount of substance transformed during that time ($A - x$), k being a constant depending on the nature of the reacting system. When integrated, the formula becomes :

$$k = \frac{1}{t} \ln \frac{A}{A - x},$$

where t is the duration of the reaction in seconds. Ostwald gives a table which enables $\log \frac{A}{A - x}$ to be read off without further calculation.*

The bimolecular reaction, where two molecules are supposed to be interacting, is frequently met with in ferment processes. This type of reaction has really been described on page 65, where it is stated that $K = \frac{C_c C_d}{C_a C_b}$. If a be the concentration of the first substance, and b that of the substance acted on,

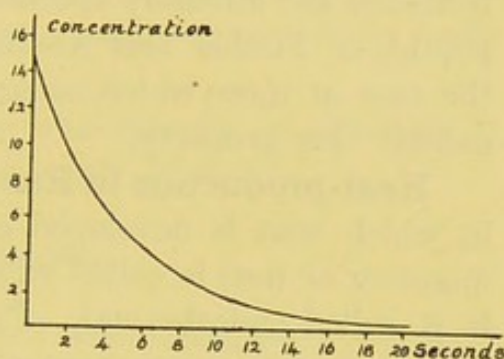


FIG. 3.

* See also Section II., Concentration of Hydrogen Ions.

then, as a becomes transformed b will disappear. When the system contains $a - x$ gram molecules of the first substance, it will also contain $b - x$ gram molecules of the second. When integrated, we have:

$$k = \frac{1}{t} \left(\frac{1}{A - x^1} - \frac{1}{A - x} \right).$$

Specificity of Ferment Action.—It has been stated above that one of the most striking characters possessed by ferments is that their action is specific. The investigations which Jacoby has made on ferment action have led him to discuss whether all ferments do possess this property. While some proteolytic ferments are intensely specific, inasmuch as the synthetic polypeptids of Fischer and Aberhalden are not acted on except in the case of those which occur in nature, other ferments do not exhibit this property.

Heat-production in Enzymatic Reactions.—Any reaction in which heat is developed externally (whether measurable in quantity or not) is called exothermal, while if heat is absorbed it is called endothermal. Passing up from the absolute zero, we come upon a succession of reactions where the development of heat becomes less and less exothermal until we reach a point at which all the reactions are endothermal. At this stage of the series we come upon a condition in which the substances ordinarily split up by the ferment are regenerated.

The more nearly endothermal the reaction the more it approaches a reversible reaction. The relation between ferment action and heat production affords an explanation of the processes of absorption in the intestine, for instance, where reversible reactions are constantly going on. In a table given by Höber, it is shown that the hydrolytic fermentations have a low production of heat, while the oxidations have a high one. Thus, maltose, cane-sugar, and lactose give a value of 3 to 7, fermentation of dextrose into lactic acid gives a value 14.7, while for the conversion of salicylaldehyde into salicylic acid the value is 72.6, so much greater is the heat of reaction.

The occurrence of endothermal reactions has some bearing on the question of autolytic decomposition.

The *production of heat by ferment action* may provide a means of detecting and of estimating the amount of ferment present in a fluid. Such a method has been elaborated by Tangl and his

pupils. The chemical energy of the mixture to be fermented was determined both before and after the action of the ferment by determining the dry substance, the ash, the nitrogen, and the calorimetric energy. It was thus established that when the trypsin acts on albumen the chemical energy is not turned into any other form. On the other hand, in hydrolytic ferment action the energy progressively diminishes as digestion proceeds. Herzog found that oxidising ferments are associated in their action with the production of thermal energy, whereas reductases show no such change, and the ferments, acting on polysaccharoses, glucosides, fats, and proteids, only show a small production of heat. When heat is produced by the action of a ferment within the body this will be a factor in the maintenance of the body heat.

The effect of temperature on the velocity of reaction is given by the following formula of van't Hoff :

$$\ln k = \frac{q}{RT} + \text{constant},$$

where k is the equilibrium constant, and q represents the heat developed during the reaction, and T is the absolute temperature. R is a constant.

The bearing which all these considerations have on the study of puncture-fluids is that the progress of an enzymatic reaction in a puncture-fluid can be observed by their means. For instance, the conversion of cane-sugar into invert-sugar can be watched by means of polarimetry, and calculated by the use of the formulæ given, the degree of rotation being proportional to the velocity of reaction. For instance, if A be the initial concentration of the cane-sugar, A must be expressed in terms of degree of rotation. Let t be 60 (duration of reaction, one hour). The change of rotation, x , divided by A , can be calculated by means of Ostwald's table, which gives the value for $\log \frac{A}{A-x}$. If, however, the velocity be compared with that of a standard reaction, the calculation becomes more simple :

$$k = \frac{1}{t} \log. \frac{\text{total rotation possible.}}{\text{total rotation} - \text{rotation at the end of time } t.}$$

Further remarks about this polarimetric method will be found in Section II., under "Ionic Concentration."

The Ferments which may be met with.—The following is a list of ferments according to the classification by Jolles :

- I. *Hydrolytic*.—Insoluble bodies are compelled to take up water and become converted into soluble bodies.
Diastase, invertase, lactase, trehalase turn bioses (cane-sugar, malt-sugar, milk-sugar) into monoses by taking up water (= glucose, levulose).
Lipase or *steapsin* saponifies neutral fats (including lecithin and oils).
Emulsin breaks up glucosides, *e.g.* amygdalin.
Urease.
Proteolytic ferments, including erepsin.*
- II. *Autolytic*.—Break up bodies without loss of water, *e.g.* zymase. These enzymes are intracellular and play an important part in the metabolic processes.
- III. *Coagulating*.—Form jellies (rennet, thrombose).
- IV. *Oxydases* accelerate oxydative processes (guaiacum test) and play an important part in the life of the tissues, *e.g.* xanthinoxydase.†
- V. *Catalases*, which decompose H_2O_2 .
- VI. The *Antiferments*.

METHODS OF DETECTING AND ESTIMATING FERMENTS ON PUNCTURE-FLUIDS

The fact that these ferments do not all occur in the different puncture-fluids with which we are concerned renders it unnecessary to discuss the means by which *each* is identified. We are only concerned with diastase, invertase, lipase, proteolytic ferments, oxydase, catalase, precipitins, and antiferments.

1. **Diastase**.—Ascoli and Bonfanti's method.—Add 2 cc. of the fluid to be tested to 100 cc. rice starch (1 per cent.), and 1 cc. toluol. Shake, and incubate twenty-four hours at 37° C. The fluid should remain sterile, and 1 per cent. sodium chloride is then added, and the fluid boiled in Soxhlet's apparatus. A few drops of dilute acetic acid are added, and the fluid again raised to the boil. Rapidly filter, and estimate the sugar. Potato starch may be used.

* Erepsin acts on peptone, deuterio-albumose, and clusein, converting them into leucin, tyrosin, ammonia, arginin, lysin, etc., but cannot act on albumens of blood and ascites, globulins, or Bence-Jones proteid (Sieber and Schumoff-Simonowski).

† Xanthinoxydase is one of the four ferments which play the chief part in nuclein metabolism ; (1) *uricolytic ferment*, met with in kidney, liver, and muscle ; (2) *nuclease*, breaking up nucleic acid and liberating purin bases, met with in the liver and muscles ; (3) a *desamidising ferment*, turning aminopurins into oxypurins ; (4) *xanthinoxydase*, which turns hypoxanthin into xanthin, and this into uric acid (Schittenhelm and Schmid).

Walther devised a method similar to that of Mett for estimation of pepsin. Narrow tubes are filled with starch paste, and the length of column after digestion is read off with a lens.

Wohlgemuth quite recently (1908) advocates a method in which the degree of reaction is estimated colorimetrically by the use of decinormal iodine.

2. **Invertase.**—This is detected by the formation of reducing substance after incubation with a 5-per-cent. solution of cane-sugar, toluol having been added. The glucose may be estimated.

3. **Lipase.**—This ferment is more abundant in exudates than in transudates.

(a) The neutral fluid is treated with a few drops of ethyl butyrate in a test-tube and litmus solution added. After twenty-four hours' incubation the litmus is found red if lipase is present, while a control tube is unaltered.

Titration with baryta would enable the amount of ferment to be calculated, supposing that the same length of incubation were given in every experiment.

Robson and Cammidge point out that a trace of calcium salt must be added to the fluid to be tested in the case of pancreatic cyst fluid, as pancreatic ferment alone will not react.

(b) Alternative. Ethyl butyrate may be replaced by an ethereal extract of olive oil containing sodium carbonate, the extract being allowed to evaporate. The advantage of this is that one has a ready means of preparing the requisite neutral fat, instead of having to prepare or purchase ethyl butyrate.

4. **Pepsin.**—The number of methods which have been advocated for estimating the amount of pepsin in a fluid is remarkable, and may be taken to indicate that no one of them is quite satisfactory.

The chief methods may be classified as follows :

1. Methods where the amount of albumen left untouched is noted, *e.g.* measurement of the column of albumen left in Mett's tubes.

2. Methods where the products of digestion are examined (estimation of alteration in acidity; Volhard's method).

3. Methods based on the determination of nitrogen in the residue and in the solution after digestion.

4. *Special methods :*

Liebmann observes the time occupied in clarifying an emulsion of coagulated egg-albumen in which hydrochloric acid is present. He gives a table which he has prepared by taking known strengths of pepsin solu-

tions (Armour's pepsin) and observing the time occupied in dissolving the egg-albumen.

The method has the great objection that one is *helpless without his table*.

Ricin Methods.—Solms places 2 cc. of a 0.25-per-cent. solution of ricin in 50 per cent. NaCl in each of a series of test-tubes, and also places 0.5 cc. decinormal hydrochloric acid into each tube. Successive strengths of the pepsin solution are added to each tube (1 cc. boiled fluid; 0.9 cc. of boiled fluid + 0.1 cc. of unboiled fluid diluted 100 times; 8 cc. boiled + .2 cc. unboiled diluted fluid; .5 cc. boiled + .5 cc. unboiled diluted; 10 cc. unboiled diluted fluid). The corked tubes are incubated for three hours, and it is then noted in which tube the fluid has become clarified.

A fluid of which 1 cc. in a hundred-fold dilution clears up the turbidity in three hours is a fluid containing 100 pepsin units.

This method is most useful, of course, in the analysis of ordinary gastric contents. It may be doubted whether there is any gain in estimating the amount of pepsin in a puncture-fluid from a cavity which might contain pepsin.

A discussion on this method occurred at the Berliner Med. Gesellschaft in July of 1907, Klemperer there advocating the use of *edestin* in place of ricin (1-per-cent. solution), but Jacoby and Fuld failed to see any advantage in such a modification. Reicher recommends Jacoby's method.

Fuld's Method.—This was devised in order that the presence of pepsin could be detected within a minute.

To 2 cc. of a 1-per-cent. solution of *edestin* in $\frac{3}{100}$ normal HCl is added enough of the hundred-fold dilution of fluid to be tested as shall cause a precipitate. After incubation, ammonia is poured on till a distinct white ring appears. If the ring does not appear it means that more than four-fifths of the albumen has been digested. The temperature of incubation and the time of digestion may be arranged as desired by adjusting the strengths of the solutions. The test is described as exceedingly delicate.

Blum and Fuld describe a method of estimating pepsin by *estimating the rennet (chymosin)*, which they assert runs parallel with pepsin. For this purpose a special preparation of dried milk is used. It is not, however, necessary to refer more closely to this method, as it is applicable only to gastric contents.*

An ingenious method of estimating the progress of proteolysis by the use of the *viscosimeter* has been utilised by Spriggs. Starting with a given proteid solution, the more the proteid becomes resolved into its decomposition products the less viscous will the fluid become. The diminution of the viscosity may be used as a measure of the progress of digestion, since the addition of acid to a proteid alone does not alter the viscosity for a very considerable time.

* In any case, the method is cumbersome, as, to estimate the rennet, a succession of tubes with different dilutions is used. Twenty or thirty tubes being necessary, the method cannot be described as convenient, nor can it be of ready application (an argument which applies with equal force to Brandberg's method of estimating albumen in urine). A half-gross of test-tubes used for any one estimation is to be avoided unless there are plenty of assistants!

The velocity of reaction was deduced by this formula, based on a number of observations :

$$y = -k (pt^2)^n,$$

where y is the degree of viscosity, which is still capable of further diminution; p is the relative acidity of the pepsin; t is the time of reaction in seconds, and k and n are constants.

The only objection to the method is the elaborate apparatus that is advocated. Of course one could at intervals abstract a portion of fluid and test it with the Hess instrument (see Section II.), keeping the room at a constant temperature within, at any rate, a degree. However, this would be apt to become tedious, as the instrument would have to be cleaned out so often during a short time.

5. Estimation of Trypsin.—The best method is that published by F. Volhard. The principle of this method is that if a special preparation of casein, to which hydrochloric acid has been added, is treated with pepsin, the acid caseoses formed cease to be precipitable by sodium sulphate, so that the fluid is more acid than before. When the casein is exposed to trypsin instead of pepsin, in the presence of alkali, and after this is acidified with a certain amount of hydrochloric acid, and sodium sulphate added, the increase in acidity is again a measure of the amount of ferment action. In the case of pepsin the increase in acidity is equal to—

$$k\sqrt{f.t},$$

where f = quantity of pepsin, and t = time of digestion.

In the case of trypsin, increase in acidity = $k^*.f$.

The method is a valuable one, and the procedure is as follows :

Preparation of the Casein Solution.—100 grammes of finely granular casein (Chem. Fabrik, Rhenania, Aachen) is dissolved in $1\frac{1}{2}$ litres chloroform water, and 80 cc. of n NaOH added. As soon as the casein has dissolved in the water-bath the fluid is heated rapidly to 90°C . to kill ferments and bacteria, and, on cooling, the bulk is made up to 2 litres, and toluol added. The reagents must be kept in Woulff bottles.

Solutions required :

Casein solution

$n/4$ NaOH

n HCl

20% Na_2SO_4

Chloroform water

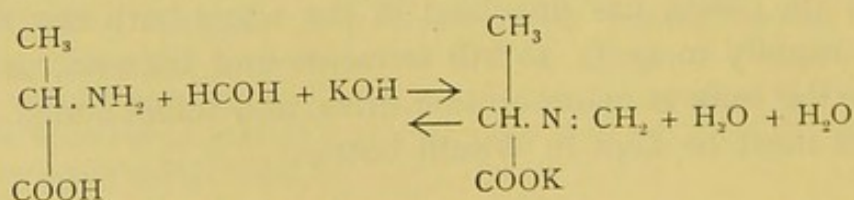
* In each case k is a constant.

PROCEDURE.—Three flasks are used, each having a 300-cc. mark at the lower, and a 400-cc. mark at the upper end of the neck. Each receives 100 cc. of casein solution, and chloroform water is added up to the lower mark. To No. 1 flask add 5 cc. neutralised fluid and 11 cc. $n\text{HCl}$, to another (No. 2) add 5 cc. neutralised fluid alone. Flask 3 is the control. Incubate at 40°C . for about eighteen hours, and then add 11 cc. $n\text{HCl}$ to flasks 2 and 3, shaking till the precipitate is dissolved. 20 per cent. sodium sulphate is added up to the 400-cc. mark, shaking well. This precipitates the undigested casein. Filter through a dry filter, and titrate 200 cc. with $n/4$ soda, against phenolphthalein.

The increase of acidity is a measure of the amount of ferment. The number of cubic centimetres of soda needed to neutralise 200 cc. of flask 1, minus the number required for flask 3, gives the amount of pepsin, the number required for flask 2, minus that required for flask 3, gives the value for trypsin, so that the ratio pepsin/trypsin is readily obtained.

In spite of its apparent complexity, this process is not at all troublesome to carry out, and can be highly recommended.

A series of most important researches dealing with the estimation of proteolytic ferment action has been made by Sørensen. He started with the assumption that *in the main* proteolysis is a hydrolysis with the formation of carboxyl and amino groups. The other groups taking part in the decomposition are assumed to be negligible. The amino groups can be "fixed," as it were, by formol, and the carboxyl groups can then be estimated by titration with $n/5$ barium hydroxide against *thymolphthalein*. The number of centimetres used multiplied by 2.8 gives the number of milligrammes of nitrogen, since the formation of each carboxyl corresponds to the formation of an amino group. The reaction is exemplified thus:



The conditions for the reaction are (1) a suitable strength of formol; (2) a suitable concentration of hydrogen ions; (3) a suitable indicator, which shall be sensitive for the particular degree of concentration of hydrogen ions; (4) a suitable volume of fluid. If these conditions be not fulfilled the reaction will reverse.

The objections to the method are that it gives inexact results with prolin and tyrosin and guanidin salts. Thymolphthalein cannot be used if the

alcohol in it separates out in the fluid titrated where the proteids are only soluble in alcohol with difficulty. A great objection to the method lies in the third condition, since one has to modify one's procedure according to the products to be estimated and according to the ferments employed. The difference in the value for enzyme action as measured by formol titration from that as measured by precipitation with tannic acid * and Kjeldahlising, is very great indeed. However, important improvements in the method, with more concise instructions as to its use, may be expected to follow.

6. Guanase and Nuclease.—The ferments may be isolated † by suspending the precipitate resulting from addition of ammonium sulphate to the extent of 66 per cent. The pseudo-globulin so precipitated carries the ferment, just as the same body carries lecithin. If this suspension be shaken with chloroform for an hour and then dialysed against running water till free of ammonia—a procedure which involves 6 to 8 days—the filtrate will be found to be slightly coloured and to be devoid of purins.

Since the ferment is characterised by converting free purin bases into uric acid the presence of this ferment can be detected by ascertaining if guanin be converted into uric acid or no.

The guanin is dissolved in normal soda, and the chloroformed mixture kept at body heat, and air at 43° is passed through for three days. The oxidising action is necessary in order that uric acid may be produced.

If the fermentation is allowed to take place in the absence of an air current (six days' incubation at 37° C.) xanthin ‡ may be searched for in order to prove the presence of the guanase.

This ferment action is a desamidisation, whilst the other action is a pure oxidation.

Nuclease may be detected, according to Abderhalden and Schittenhelm, by the action of (10 cc.) the fluid on *α -thymonucleate of soda* (10 gm. dissolved in 250 cc. water) at 37° C. for several days, toluol having been added to prevent decomposition. The resulting medium will have become fluid and clear, and will not set on cooling if nuclease be present.

* Tannin method. 20 cc. fluid are neutralised with HCl or soda, and 2 cc. normal sodium acetate, neutral to litmus, are added. Then add 10 cc. of 10-per-cent. aqueous tannin and fill up to 50 cc. with water. Shake well. Stand overnight. Filter next day. Estimate the N in 25 cc. Filtrate by Kjeldahl's method. Multiply the result by 2 to obtain the N-content of 20 cc. in milligrammes.

† Schittenhelm.

‡ To detect and estimate xanthin.—The value of this lies in the adoption of the method for estimating the amount of guanase present in a fluid. The albumen is first removed by boiling in faintly acid (acetic) reaction and silver nitrate is added to the filtrate. The precipitate is washed in water, and then warm hydrochloric acid is added. The filtrate is dried, and boiled with dilute ammonia to remove the hydrochloric acid, and left on ice overnight. The filtrate contains xanthin.

Purin bodies can also be searched for as evidence of the action of this ferment.*

The nature of nuclease has been the subject of much discussion. Some authors consider it to be the same as trypsin, but if trypsin be added to nuclease the action of trypsin will cease. This ferment occurs normally in the thymus, pancreas, and kidneys in cows.

7. **Oxydases.**—Sieber gives the characters possessed by three varieties of oxydases.

	I	II	III
Solubility { in water	+	—	—
in neutral salts	—	+	—
in alcohol and water	—	—	+
Salting out	↓	↓	↓
Effect of heat	destroyed	easily destroyed	destroyed
Amount of sugar acted on in two days	69%	...	80%
Rate of inversion of disaccharids	slow	immediate	slow
Rate of inversion of polysaccharids	rapid	less rapid	less rapid

Detection and Estimation of Oxydase (Jolles' Method).—0.5 cc. of the fluid to be tested is mixed with about 30 cc. of 0.9 per cent. NaCl in a 50 cc. flask and filled up to the mark with the saline. 10 cc. of this mixture are treated with 30 cc. of neutral H_2O_2 and incubated in the cold incubator for twenty-four hours, the time of commencement being noted. After this, sulphuric acid is added to stop the action, and the time is again noted. Potassium iodide is added drop by drop. Iodine separates out, and after an hour the iodine is titrated with decinormal thiosulphate. The original strength of the hydrogen dioxide, less the strength at the conclusion of the experiment, shows the amount decomposed by 0.01 cc. of blood. The amount decomposed by 1 cc. is the "catalysis-figure," which is normally 18 to 30 for blood.

A similar method has been used by v. Dalmady and v. Torday, but their work is confined to the blood itself.

Van Itallie has used the method, with slight modifications, in order to distinguish between the blood of different animals by the varying catalytic power.

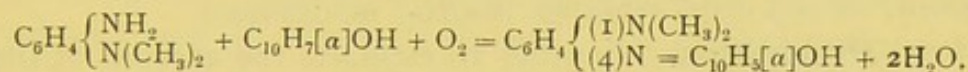
Xanthin oxydase was estimated by Burian by ascertaining how much uric acid was produced, and the velocity-constant of this action was made out to be as of a reaction of the first order.

* To test for purins.—Add sulphuric acid to the filtrate to remove free nucleic acid, and to the filtrate add mercury sulphate, which will throw down the purins. The purin precipitate is soaked in water, and HCl and H_2S added to remove the mercury, the H_2S being removed by an air-current. Ammoniacal silver nitrate may now be used to precipitate the purins. Filter and wash. Heat the precipitate with HCl, filter off the silver chloride, and add H_2S and again filter. The filtrate is evaporated and the purins separate out, are purified, dried, and weighed.

The following reaction may be used to detect the presence of oxydase in *leucocytes* :

A recently prepared solution of *a*-naphthol in water (1 per cent.), containing 1 per cent. of sodium carbonate, is poured over the film and left for one or two minutes. After a momentary wash in distilled water, a few drops of 1 per cent. aqueous dimethyl-paraphenylenediamine (Marke Schuchardt) is poured over the film. If blue granules appear, oxydase is present. The film should be fixed either with alcohol or with formol vapour. The preparation has to be examined in water and will not keep. The solutions should be fresh.

The explanation of the reaction is that the ferment in the granules converts the two reagents used into naphthol blue according to the formula :



Eosinophile granules come out blue also. According to Pappenheim, it is a pyrrhol reaction (see p. 58).

8. Peroxydase.—O. v. Fürth has described a method of estimating this ferment spectrophotometrically with the aid of malachite green. He found this ferment within leucocytes, so that one might expect to find its presence in inflammatory fluids or purulent exudates.

Whether oxydase and peroxydase are special enzymes is uncertain, as the action is a property common to very many enzymes.

9. Catalase.—Jolles' and Oppenheim's method of detecting catalase involves the following procedure :

(a) Preparation of a 1-per-cent. solution of hydrogen dioxide. The solution is neutralised with decinormal soda, and titrated in presence of sulphuric acid with decinormal potassium permanganate (standardised against decinormal oxalic acid).

$$1 \text{ cc. } \frac{n}{10} \text{ oxalic acid} = 1.701 \text{ mgm. H}_2\text{O}_2; 1 \text{ cc. } \frac{n}{10} \text{ oxalic acid} = 1 \text{ cc. } \frac{n}{10} \text{ KMnO}_4.$$

The number of cubic centimetres of permanganate will therefore enable the number of milligrammes of hydrogen peroxide present in 100 cc. of the solution to be calculated. It is then diluted till it reaches 1 per cent. The authors recommend permanganate titration rather than the iodometric method.

(b) Preparation of a solution of sodium hyposulphite to estimate the H_2O_2 which remains unaltered by the catalase.—(A) 25 gm. are dissolved in a litre of water ; (B) 3.874 gm. purest potassium bichromate are also dissolved in a litre of water (20 cc. of this = 0.201 gm. iodine). 20 cc. of this second solution are placed in a stoppered flask, and 10 cc. of 10-per-cent. KI

added. In five minutes add 100 cc. water and titrate the iodine with the hyposulphite solution against starch paste as indicator. Each cubic centimetre of hyposulphite corresponds to $\frac{.201}{\text{No. of cc. of A used}}$ ("x") gm. H_2O_2 .

(c) Dilute the fluid to be tested up to 500 cc.

Now ascertain (i) number of cubic centimetres solution A needed to react with 20 cc. of the standard solution of H_2O_2 , (ii) number of cubic centimetres solution A needed to react with a mixture of 2 cc. of fluid to be tested, plus 20 cc. solution of H_2O_2 .

$$(i-ii) \times x \text{ gm. H}_2\text{O}_2 = \text{No. of gm. H}_2\text{O}_2 \text{ decomposed.}$$

The time of exposure to the H_2O_2 should always be the same.

This ferment is most likely to be present in effusions containing blood, since it has been found by Silbergleet and Mosse that the activity of the catalytic power depends on the varying content of red cells, whether healthy or diseased.

10. **Precipitins.**—In order to estimate the amount of these, Hamburger employs the special tubes which he has devised (Fig. 5). The serum and the antiserum are measured, mixed, and then centrifuged till the precipitate has a constant volume. The exact reading is made with the help of a lens. This procedure has not, so far, been applied to puncture-fluids.

EXPERIMENTS ON THE FERMENT-CONTENT OF VARIOUS SEROUS EFFUSIONS

The accompanying table will show the results which have been obtained by testing peritoneal and pleural fluids for their ferment-content. The most convenient method of applying such tests has been found to consist in using short test-tubes (4 by $\frac{1}{2}$ inches) duly sterilised and plugged. A series of four-ounce flasks were also sterilised, and the following media were prepared in them: a thin emulsion of boiled starch, 1-per-cent. solutions of glucose, lactose, mannite, dulcite, cane-sugar, and ordinary centrifuged milk coloured with litmus. 100 cc. was found sufficient in each case. A number of the sterile tubes were then filled with these media and, as a precaution, further sterilised in the usual way. In the sugar media Durham's tubes were inserted (prepared from ordinary glass-tubing), the air being expelled during the process of sterilisation. The cotton-wool plugs were dyed with methylene blue, methylene green, aurantia, saffranin,

and gentian violet, so as to distinguish those media which were colourless.

In testing a given fluid, one of each of these tubes was charged with a small quantity (not measured) * of the fluid, and enough toluol added to form a covering. The tubes were shaken to distribute fluid over medium, and were incubated at 37° for either twelve or twenty-four hours in different cases. At the end of that time fermentation was noted, if present, and the reaction ascertained by drops of litmus solution.† In the case of starch, the medium was tested first with liquor iodi, and, secondly, with Fehling's solution. In the case of the cane-sugar Fehling's test was alone applied.

In addition to these media, plain test-tubes of the same size were used to test the lipolytic ferment by the ethyl butyrate method, and for proteolytic ferments by the use of Mett's tubes in (a) acid and (b) alkaline medium. The tubes were not used for quantitative tests, but were found convenient, and the egg-albumen was more easily and more economically stored in this way.

The Reducing Power of Puncture-Fluids.—This is met with in inflammatory exudates, and also in the cerebrospinal fluid. It has been best studied in the blood, urine, and bile (Melon, Migliarini).

The reducing power of 1 cc. of blood is equivalent to 65 cc. of decinormal KMnO_4 , that of serum to 51, and is found to be increased in pneumonia and diabetes. The chief value of the observations lies in the fact that the reducing power is constant at all times, but it varies according to the amount of KMnO_4 used, according to the action of the light and according to the time occupied by boiling the blood with the KMnO_4 .

Ferments in Leucocytes.—It is to Leber in 1891 that we owe the discovery that aseptic pus has the power of digesting proteid matter. He found that such pus will liquefy gelatine at 25°C . Not much notice of these observations seems, however, to have been made till Erben in 1903 published an account of ferment action in the blood of leucæmics, shown by the presence of albumoses, a fact which Schumm pointed out in specimens

* Quantitative analysis was deemed unnecessary in this series of experiments.

† More rapid than the use of litmus paper.

To Müller and Jochmann we owe the further discovery that if purulent sputum be placed on Löffler's serum, digestion of the latter will take place, and, moreover, that the blood of leucæmic patients produces the same solvent effect. The solvent action did not occur if the blood had been heated, a fact which proved that the leucocytes of leucæmic blood contain a ferment capable of acting on dried blood-serum. This ferment is a proteolytic, and of a tryptic nature. Besides this discovery, there is that of the fact that the serum of blood contains a body which will inhibit the liquefying action of pus-cells, a property which is also thermolabile, being destroyed at a temperature above 55° C.

Many other observers have corroborated these observations, though they were not absolutely new. Thus, Opie found that in inflammatory exudates due to bacterial causes a proteolytic enzyme was present, and he tested it by the effect on aleuronat, and a N-estimation by Kjeldahlising after coagulation. Opie also noted the interfering action of the serum on this leucocyte ferment, and that it was thermolabile. Pfeiffer found the proteolytic ferment in the polymorphonuclear neutrophile leucocytes, which explained the autolytic processes noticed in leucæmia. The autolysis was estimated by the amount of non-coagulable nitrogen.

Stern and Eppenstein showed that the ferment can be detected just as easily by gelatin as by blood-serum.

The interesting fact was found that this proteolytic ferment will withstand admixture with formalin perfectly well, so that its occurrence can be demonstrated in specimens that have been subjected to the Kaiserling process even years ago.

Objection has been made to these facts by Baer, who pointed out that the digestion was tested for by the use of a denaturalised albumen, and that the process could not be compared with autolysis. The ferment is acting on a foreign albumen, and the process is therefore heterolytic. The distinction becomes important from the fact that the spleen of cows and horses can show autolytic effects, but cannot be made to act heterolytically, and this absence of heterolytic power might be looked on as an antiferment action when there is really no antiferment action in the process.

We may say, then, that the serum of pus contains an antiferment for autolysis, but not for heterolysis, while the pus-cells contain a ferment for autolysis *and* a ferment for heterolysis.

The preponderance or otherwise of the polynuclear leucocyte in an effusion will account for the diminution or increase in the amount of antiferment present. Thus, in septic diseases* the antiferment is almost abolished, while in tuberculosis antiferment is present in increased amount. In tuberculosis the effusion usually contains an excess of lymphocytes (see Section V.), which are not characterised by ferment granules. In anæmia, whether primary or due to hæmorrhage or to malignant tissue, the antiferment-content is normal or even diminished.

Autolysis.—The fact that autolytic processes may occur in exudates and transudates seems to have been first established by Salkowski in 1890. The problem which is most discussed is as to whether autolysis is in any sense a vital process. Wiener, Poll, Langstein, and Neubauer considered that autolytic processes could not take place in a living cell; but in order to establish this it would be necessary to ascertain whether autolysis can occur in an alkaline medium. That this is actually the case has been made out by Drjewzki (1906).

The practical value of the subject of autolysis lies in the fact that in certain fluids no autolytic changes are to be made out. The existence of certain ferment reactions might be accounted for, on the other hand, by assuming autolytic changes to have taken place.

Drjewzki's method of study was as follows: The substance to be tested is diluted with water and 75 per cent. chloroform is added. Another mixture is alkalised with sodium carbonate. A third mixture is boiled. The three mixtures are incubated for a given time, then brought to the boiling-point, made up to a litre, and the fluids filtered. Next day the filtrates are concentrated to 400 cc., and in the filtrate the following processes are carried out: (a) From 20 cc. estimate the N. (Kjeldahl) = *total N.* (b) To 50 cc. add 5 cc. dilute HCl and 10 per cent. phosphotungstic acid till a precipitate ceases to fall. The filtrate is Kjeldahlised, to get *monamid-N.* (c) 50 cc. receive 1 cc. dilute sulphuric acid. Saturate with zinc sulphate, and leave to stand twenty-four to forty-eight hours. The precipitate (albumose) is Kjeldahlised = *albumose-N.*† (d) 100 cc. are alkalised with a few drops of

* Wiens.

† The precipitate must be thoroughly dry before Kjeldahlising, else the flask will burst.

ammonia, and the phosphates are filtered off. Add 3 per cent. ammoniacal silver nitrate to precipitate the purins, and after twelve hours (darkness) the precipitate is collected, washed, and Kjeldahlised = *purin-N*.

a.—(*b* + *c* + *d*) = nitrogen of *diamino-acid*, *peptone*, and *ammonia*.

The following results were obtained in one of his cases :

Analysis after 72 hours' autolysis.	A without Na ₂ CO ₃		B with Na ₂ CO ₃ and ferments.		C with Na ₂ CO ₃ , no ferments.		D coagulated at once.	
	g	% total N	g	% total N	g	% total N	g	% total N
Total nitrogen ...	6.545	...	4.1125	...	3.570	...	3.15	...
Monamino acids ...	4.022	61.60	2.184	53.16	1.722	48.23
Albumoses ...	0.490	7.48	0.532	19.93	0.601	16.83	0.756	24.00
Purin bases ...	0.826	12.62	0.490	11.91	0.049	1.37
Diamino acids and peptones ...	1.197	18.3	0.9065	15.06	1.198	33.57

This shows that autolysis still occurs in an alkaline medium, although it is true that the velocity is much slower than in an acid medium. Column D is a control. Another answer to the question as to ante-mortem existence of an autolytic ferment lies in the following consideration : Why should a ferment exist in a cell during its whole life, and only come into operation after the death of the cell ?

The Importance of Autolysis.—The following are examples of autolytic decomposition : (1) breaking down of malignant tumours ; (2) acute yellow atrophy of the liver ; (3) effusions in process of absorption ; (4) absorption of pneumonic exudate—the proof of this lies in the occurrence of amino acids in the urine ; (5) relaxation of rigor mortis.

Besides these, the puerperal changes taking place in the uterus and the changes of phosphorus poisoning are examples of autolysis. It is natural to suppose that if autolysis occurs in diseases associated with effusions we should be able to find evidence of it in the effusion itself. Consequently, the detection of albumose in such fluids acquires a considerable importance, and a list of the cases in which albumose has been found is given in Table II. It is there seen that albumoses frequently occur in tubercular

pleural cases, and also in empyema. In a purulent effusion one would expect autolytic processes to take place in any case, as, from experience of empyemas which have been opened, one has frequently seen a digestive action being exerted on the exposed surfaces.

In the case of peritoneal fluids albumoses are frequently present, just as peritoneal fluids in general have been found much more complex in composition than are pleural fluids.

That the presence of albumoses does not necessarily mean preceding autolytic processes has been pointed out by Magnus-Levy and by Chvostek, the latter having found albumoses in the urine as a result of ulceration of the intestine.*

Again, there is the possibility that the appearance of such bodies as deutero-albumose in a fluid may be the result of heterolysis (Jacoby), some ferment, say, in the liver having something to do with the process.

The substances which demand search in order to study this point are :

- (a) Leucin, tyrosin, glycocoll, xanthin bases.
- (b) Ammonia, cystin, pentamethylenediamine, lysin, arginin, tryptophane, asparagic acid, glutaminic acid, histidin.
- (c) Thymin, uracil, H₂S, succinic acid, pentoses.
- (d) Peptones and albumoses are merely intermediate products.

From this formidable list we may refer especially to leucin, tyrosin, lysin, arginin, tryptophane, as these have been most searched for in peritoneal fluids.† The results, which will be found tabulated under the various headings, go far to show that either some autolytic changes are going on in exudates and transudates, or that such bodies appear as the result merely of accidental transmission.

The only way of distinguishing between autolysis and extraneous causes of decomposition is to watch the changes which the fluid undergoes on being kept under aseptic conditions. Such observations may be made without going into lengthy analytical

* Such authorities as Abderhalden have denied that albumoses occur in normal blood, while Freund asserts that the methods employed for their detection in blood have not been sufficiently sensitive. (See *Biochem. Zeit.* vii., p. 343.)

† The special tests for each of these bodies will be found indicated under their respective headings.

processes by determining the variations in the electro-conductivity of the fluid from hour to hour (thymol added), since decomposition of proteid would result in diminished inhibition of the conductivity of the electrolytes of the fluid (cf. p. 99).

The following results were obtained :

TABLE XI
AUTOLYSIS IN PUNCTURE-FLUIDS

Duration in hours.	Fluid from Ovarian Cyst.	Pleural Fluid Ruptured Thoracic Aneurysm.
At outset.	888.8 at 19.3° C.	843 at 23° C.
22	818	889
46	893	...
70	954	900
92	...	960
117	981	...
142	...	1062
170	...	1073
189	985	...
381	1059	...

The figures represent the changes in conductivity (expressed in terms of 10^{-5}) and it will be seen that in each case there was a steady increase in the ionisation of the fluid. This seems to point to the existence of an autolytic decomposition, though only of slow development and moderate in amount. The initial fall of conductivity in the case of the ovarian cyst is peculiar.

According to Müller and Magnus-Levy, the autolytic decomposition of albumen is accompanied by breaking down of lecithin and of CHO groups into volatile acids, lactic acid, and succinic acid. There is also a remarkable amount of ammonia liberated.

Ascoli and Izar discovered that autolytic processes are *very greatly* accelerated by the presence of inorganic colloids such as colloidal silver, platinum, or gold. Arsenic, on the other hand, may inhibit oxidation (C. Foà).

As regards the location of the enzyme there are two possibilities to consider. In the first place, the autolytic ferment may occur as an endo-enzyme, within the cells of the puncture-fluid, just as it occurs in the liver-cell, the spleen-cell, the lung-cell, and the thymus-cell. In this case one would expect that on filtering the fluid the ferment would be removed. The other possibility, however, prevents this from affording any definite evidence. That is to say, the ferment in the fluid, just as

is the case with any of the other ferments met with in puncture-fluids, may have become liberated, owing to the breaking up of cells; remnants of such cells are easily seen in any film preparation. In whichever situation the ferment may be, the final result is the same, and it is a matter of no very great importance in which way exactly the ferment came to be in the fluid at all.

But when we consider how many ferments there may occur in any one cell it comes to be a question by what means they can all be accommodated in so small a compass. Pfeffer and Hofmeister considered that the colloidal structure of protoplasm allows the ferments to be within the cell, distributed not only in space, but in time within the cell; and Jacoby, who has contributed many papers on the subject of ferments and anti-ferments, has pointed out that ferments may remain in a state of zymogen for a long time, the active period being relatively only a momentary one. This author compares a ferment to a toxin, and supposes it possible that a ferment may consist of a haptophore and a zymophore group. The haptophore group would become attached to part of the cell substance and lead to the formation of new compounds, which, being liberated into the serum, act the part of an anti-ferment.

It is interesting to note that the solubility of ferments is altered by the presence of lecithin. The bearing which this observation has on the study of puncture-fluids is that if lecithin be absent from a puncture-fluid its absence may account for the presence or absence of any given ferment.

Antiferments.—The nature of anti-ferment reaction is difficult of study, and indeed it is a very great matter for debate as to whether the phenomena assigned to the presence of anti-ferments are really the result of specific entities at all, and not rather the effect of adverse conditions of a general nature. It is easy to see that if a given ferment does not receive activation to exactly the degree requisite for its action, an apparent inhibition of action might occur. Under such circumstances the observer might assume that there was an anti-substance present when, in point of truth, the effect is due to the absence of a particular activator.

There are many arguments in favour of the idea that there is no such thing as an anti-ferment *per se*. The term "anti-ferment" may be used in two senses: it may either mean a

ferment exhibiting properties antagonistic to those of an ordinary ferment, or it may mean a substance which interferes with the action of a ferment.

The following facts may tend to show that antiferments do not exist: The catalytic decomposition of hydrogen peroxide can be inhibited by the addition of NaCl (Lockemann); blue light inhibits catalysis; peroxydase cannot convert pyrogallol into purpurogallin unless hydrogen dioxide be present, or unless an iron compound such as hæmoglobin be present, or unless NaCl * be present; peptic digestion can be inhibited by amino-bodies (Jastrowitz); tryptic digestion can be inhibited by the presence of excess of NaCl and other neutral salts, and especially by sulphates. Fuld's experiments show that the appearance of antiferment can also be mimicked by those reactions in which the *order* of addition of the reagents is of importance. This is exemplified by the need for adding potassium ferrocyanide to acetic acid in the test for proteid, and not *vice versa*. Possibly something similar will prevent or allow a ferment action.

The experiments which Hedin made with charcoal and trypsin might be interpreted in favour of the idea that antiferment action does not depend on any *specific* substance.

We see, then, that certain ferment actions can be brought to a standstill or prevented from occurring altogether by the occurrence of certain antagonistic conditions. It is as reasonable to speak of blue light as anticatalase or of chloroform as antitrypsin as it is to give a specific name to any "antiferment."

If antiferments really exist one must rely upon biological experiments to establish the fact. The most important evidence is certainly furnished by such hæmolytic phenomena as have been carried out by Morgenroth and many others. Thus, the injection into a rabbit of a ferment will result in the production of a serum which has the power of rendering the particular ferment inactive. On the other hand, Liebermann found that active rabbit immune serum only prevents hæmolysis if calcium chloride be present. In this connection the observation by

* This fact imparts an inestimable importance to the presence of sodium chloride in the body, with respect to the dependence of every metabolic process upon it.

Wohlgemuth that lecithin activates the hæmolytic action of pancreatic juice also derives special importance as showing that not only salts but lipoids may have some power of functioning as antiferments. Even the existence of antiferments in the serum of specially treated rabbits does not prove that antiferments exist in the body naturally.

It is specially significant that Beitzke and Neuberg describe an anti-emulsin as connected with the globulin fraction, when we have seen that lecithin is also associated with the globulin fraction. Jacoby, in his summary of his opinions on the nature of ferment action, says, "The fact that rennet and pepsin can be separated from fibrin by alkalies, whilst rennet is separated from antirennin by acid, is in favour of the view that the union of ferment with substrate is not the same as the union of ferment with antiferment." The association of antiferments with globulins reminds us that the action of an antiferment may merely be one of alteration of electrical charges in the colloids concerned in the reaction.

One more consideration. In the case of trypsin it is now well known that without enterokinase this ferment will not act on proteid. Suppose now that this fact were not known, one would have found that certain solutions would not digest proteid, and have assumed that these solutions contained antiferment (antitrypsin). The truth is, of course, that the activator of trypsin is merely absent. But suppose, further, that the enterokinase could be interfered with by some other body, then we shall be introducing a third factor into the case, and it would be reasonable to describe this *third* body as antiferment, though its name would be anti-enterokinase, and not antitrypsin.

It is, however, out of place to discuss this subject further, although the amount of information which is being gathered together by observers in all countries is increasing by geometrical progression. So much is this the case that it becomes almost impossible to fix the state of knowledge on the subject.*

* The views above expressed were written down before the work "Recent Advances in Physiology and Biochemistry" came to notice. The whole subject of ferment action is so fully dealt with there (by Prof. B. Moore) as hardly to need supplement. On the other hand, many valuable contributions have appeared since its publication, in the *Biochemical Journal*, in the *Biochemische Zeitschrift*, etc., as also a work by Schade, "Diabetes und Katalyse." Many of the references to literature for 1907

METHOD OF DETECTION.—The fact that pus-cells contain a proteolytic ferment which will produce a small dimple in Löffler's blood-serum* at the seat of inoculation after incubation at 50° C. for twenty-four hours furnishes the means of detecting anti-proteolytic ferment in a puncture-fluid.

One part of the fluid to be tested is treated with from 5 to 20 parts of pus, and a drop of the mixture is added by means of a platinum loop to the serum tube, or, as Müller and Jochmann advocate, a plate of serum, which has the advantage of ease in working, and also the advantage that the same plate will serve for many experiments.†

If the serum shows liquefaction after incubation, then the proteolytic ferments of the leucocyte have not been interfered with, and therefore there is no anti-ferment present in the fluid. On the other hand, liquefaction may not occur, in which case one assumes that anti-ferment is present.

The pus must be freshly obtained, and must be sterilised by addition of toluol. The pus used must not be tuberculous, since tuberculous pus contains no proteolytic ferment.‡

Anti-ferment may be estimated, according to Ed. Müller, by ascertaining how many times fresh pus has to be diluted before the fluid to be tested will prevent it from liquefying the dried Löffler's serum. For blood-serum the normal limit is 7·5.

The method of detection of anti-ferment § may be applied to Eppenstein's method for detecting proteolytic ferment, which has the advantage of employing a readily prepared medium.

The fluid to be tested is mixed with 10 per cent. gelatine containing 1 per cent. soda, and a similar quantity of 0·85 per cent. saline is added to another portion of the same gelatine (to furnish

and 1908 will be found in the Bibliography on page 265 of this work, though only those names are included which have been mentioned in the text. Investigations are being made in order to be able to present as full evidence on this problem as possible.

* The serum is cow serum, and contains glucose, or 8 per cent. NaCl.

† The chief disadvantage lies in its manufacture.

‡ This fact may be of use for distinguishing tuberculous from other sources of suppuration.

§ It must be understood that these methods demonstrate an anti-ferment action without by any means indicating the existence of a specific anti-body. It will be remembered that even an antitoxin is acting in virtue of special colloidal properties without necessarily constituting an entity or a substance capable of being isolated.

a control). Both are incubated for twelve hours at body heat. On removal, both Petri dishes are set on ice until the saline sample (control) has set. If proteolytic ferment be present the gelatine will no longer set, while, if antiferment be present, or if there be no ferment at all, the gelatine will set.

To apply this test for detecting antiferments, it is obviously only necessary to add toluolated fresh pus to the fluid, and see the effect on the gelatine. It is, however, convenient to use ordinary leucocytes in place of the pus, and a small quantity of blood may therefore be collected, washed in citrate, and then in saline, exactly after Wright's method for getting leucocytes for opsonic index determinations. For the small quantity of fluid to be tested this will furnish readily, and at any time or place, the necessary means of carrying out this modified test.

SECTION II

THE PHYSICO-CHEMICAL EXAMINATION OF PUNCTURE-FLUIDS

CONTENTS.—(A) Osmotic pressure—Theoretical considerations on osmotic pressure; how to calculate the osmotic pressure; the degree of dissociation—Theoretical considerations on electroconductivity; corrections necessary for variations in temperature and in amount of proteid in the solutions examined; achloride electrolytes—Osmotic concentration—Theoretical considerations dealing with the effect of mixtures of many substances on the freezing-point depression and on electroconductivity—The relation of freezing-point depression to specific gravity—Methods of determining the freezing-point depression and the electroconductivity. —Results of examination in each case—The plasmolytic method; Hamburger's adaptation to the study of body-fluids; Wright's method—Other methods of determination of osmotic pressure. (B) The critical solution point. (C) The concentration of the hydrogen ions; theoretical considerations; meaning of the term acidity—The indicator method of estimating the reaction of a fluid—The inversion method—The methyl-acetate method—The dilatometer method—The diazoacetic-ether method—The concentration-chain method; the gas chain; a few details as to the method of carrying out this method; the results which have been obtained by Foà, and by Pfaundler—Significance of the results. (D) Viscosity—The viscosimeter of Hess; method of use; the theory of the instrument; results of examination of puncture-fluids. (E) Refractometry.

THE constant interchange of substance between the cells of the body and the fluids surrounding them depends to a large extent, if not entirely, upon a series of processes which come under the domain of physico-chemistry. Not only do we have to deal with a series of chemical reactions between living substance and the matter composing its outer world, but we have to deal with processes of diffusion and of osmosis. Osmosis is specially important in the case of puncture-fluids, as it accounts for so many of the phenomena that they exhibit. A consideration of the factors on which osmosis depends is therefore needed, although those theoretical considerations which deal with the question of pouring out

of fluids into the serous cavities, and of absorption from them, have come to be so special a subject that reference to it does not come within the objects of this work.

(A) **Osmotic Pressure.**—When a substance is dissolved in water the molecules of that substance are evenly distributed throughout the water, and exert, not only a pressure on each other, but also on the walls of the containing vessel. The pressure transmitted to the confines of the fluid in the endeavour of the molecules to occupy a larger space is called the *osmotic pressure* of the fluid.

The laws which govern the osmotic pressure of a solid dissolved in water have been found by van't Hoff to be identical with those which obtain when a gas is dissolved in water. In other words, a solid dissolved in water behaves like a gas dissolved in water. If there be several substances in solution, then the total osmotic pressure is equal to the sum of the osmotic pressures exerted by each substance taken separately.

The osmotic pressure of a solution varies according to the temperature of the solution as well as according to the number of molecules present. All solutions which contain the same number of molecules in the same volume of fluid exert the same osmotic pressure. In other words, all equimolecular solutions exert the same pressure.

$$\text{Pressure} \times \text{volume} = \frac{\text{pressure}}{\text{concentration}} = \text{constant.}$$

It is obvious that the only variable will be the weight of the molecules, so that if two substances be dissolved in a *given volume* of solvent, the osmotic pressure of each solution will be related to the other according to the molecular weights of the dissolved substances.

It is on this fact that one of the methods of determining the molecular weight of a substance depends,* but it also affords a

* The following laws have been found to hold good in the case of simple solutions: (1) A solution freezes at a lower temperature than its solvent; (2) the depression of freezing-point is proportional to the concentration of the solute (Blagden, 1783); (3) equimolecular solutions of the same solvent have the same freezing-point depression (de Coppet, 1871). In 1882-4 Raoult substantiated this law for many organic substances, and introduced the term molecular depression or freezing-point constant. At this time cryoscopy was invoked for determining the molecular weight of a substance. In 1888 Beckmann introduced his thermometer.

means of estimating the osmotic pressure of a given fluid. Just as the conversion of gas from liquid demands the expenditure of work, so work is needed in causing a solution to become more concentrated, that is, to acquire a higher osmotic pressure.

If we determine the amount of work (osmotic work) which is performed in just causing solvent and dissolved substance (a solution of known concentration) to separate, we can calculate the amount of pressure. If a solution be frozen, ice will separate out as the solution becomes colder and colder, while the remaining fluid is constantly becoming more and more concentrated. The molecules in this solution are thus being continually forced to occupy a smaller volume. In order to effect this change the osmotic pressure which they are exerting is being overcome by external work, and this necessitates the removal of more heat than is necessary to cause the pure solvent to freeze. The heat of fusion remains constant, so that the effect of the external work is to lower the freezing-point. In other words, the solution freezes at a lower temperature than that at which the solvent alone would freeze. It is this fact which underlies the use of *cryoscopy*, that is to say, the determination of the freezing-point of a fluid.

Suppose that x grams substance are dissolved in y grams solvent, and that the depression of freezing-point is then $z^{\circ}\text{C}.$,

Then 1 gm. substance dissolved in y gm. solvent will cause $\frac{z^{\circ}}{x}$ depression.

1 gm. mol. (M.)	"	y	"	"	$\frac{Mz^{\circ}}{x}$	"
1 " "	"	1	"	"	$\frac{Mzy^{\circ}}{x}$	"
and 1 " "	"	100	"	"	$\frac{Mzy^{\circ}}{100x}$	"molecular" depression.

The molecular depression is a constant (k).*

For our purposes we wish to know x , so that the equation becomes:

$$x = \frac{Mzy}{100k},$$

and supposing there were only one substance present in a given solution (usually water), we could readily ascertain how much substance was present, or, in other words, we should be able to

* K for water is 18.5.

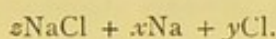
use this method for purposes of quantitative analysis of a fluid. Before this can be done we have, however, to discuss the effect on the above formula when we are dealing, not with one substance, but with several substances, in solution; and, more important still, we have to discuss the effect of solution on substances of various kinds. When several substances occur dissolved in water, van't Hoff's law tells us that the total osmotic pressure is equal to the sum of the osmotic pressures of each taken separately, and it is found that every 1.85° depression of freezing-point indicates the pressure* of one gram-molecule of substance in each litre of water.

The fact that the equation given does not hold good for every individual substance brings up the important question of *dissociation of salts* when in solution. A solution of salts such as is met with in urine shows a much greater freezing-point depression than its concentration would lead us to expect, and the same holds good for puncture-fluids where sodium chloride—though the most conspicuous—is not the only salt, but occurs associated with sulphates, phosphates, and carbonates of potassium, calcium, and magnesium. To take the case of NaCl alone, the formula above given would lead us to expect that a gram-molecular solution of sodium chloride (i.e. a solution containing 58.5 gm.—the molecular weight in grams—of sodium chloride in each litre of water) should have an osmotic pressure of 1.85×12.05 atmospheres, because all gram-molecular solutions in *water* freeze at 1.85° C., and each degree of depression of freezing-point means an osmotic pressure of 12.05 atmospheres (Raoult). As a matter of fact, the solution referred to has an osmotic pressure of 3.51×12.05 atmospheres, for its freezing-point is 3.51° C. This discrepancy was explained by Arrhenius, who showed that when a salt is dissolved in water it breaks up into electrically charged ions, without which the resulting fluid would never be able to conduct an electric current, and without which the fluid would not exert as great an osmotic pressure. It is clear that if a solution of NaCl contains not only NaCl but also Na and Cl *ions*, the resulting number of constituents present is greater than if all the NaCl has remained as NaCl. The conception that the in-

* Pfeffer found that if a gram-molecule of any substance be dissolved in 22.13 litres of water, such a solution will exert an osmotic pressure of one atmosphere at 0° C.

dividual ions act just as independent molecules and exert an osmotic pressure—which forms the pith of the theory of electrolytic dissociation—explains the fact that the freezing-point depression of the solution is greater than it should have been. As soon as sodium chloride is dissolved in water some of it dissociates into Na and Cl, each of which adds to the osmotic pressure, and also enables an electrical current to pass. In this way, then, the electroconductivity of a fluid is dependent on the same influences as is the freezing-point depression.* And the mention of this fact in this place will enable us to understand the link binding the electroconductivity method of research to the cryoscopic method which is dealt with in the present work.

To return to the solution of NaCl. Such a solution contains :



The osmotic pressure exerted by $x\text{Na}$ is the same as that exerted by $y\text{Cl}$, for though the ions have different atomic weights, it is the actual number of ions that is important.

The osmotic pressure of the ions will therefore be :

$$1.85 + 1.66 = 3.51.$$

The ions and molecules of the other salts present in the cited example of urine will add to the osmotic pressure, in accordance with van't Hoff's law, and the principle remains the same. The osmotic pressure, and with it the freezing-point depression, is produced by NaCl, sodium and potassium and calcium, etc., sulphates and phosphates, etc., *plus* Na and Cl and SO_4 , and PO_4 , and Ca and K, etc., etc.

If the solution of all these salts be rendered more dilute, the dissociation will increase, and the ratio between undissociated and dissociated salt will alter more and more until, theoretically, at infinite dilution, there will only be free *ions* present. We wish to know how the proportion between molecules and ions can be ascertained in a given solution. The method of calculating this can be indicated by reverting once more to the simple example of NaCl.

It has been stated that a gram-molecular solution of sodium chloride freezes at -3.51°C . instead of at -1.85° . This solution

* N.B.—Increase in "freezing-point depression" means a *fall* of temperature.

therefore behaves, not like a gram-molecular solution, but like a solution containing $\frac{3.51}{1.85} = 1.89$ gram-molecular solution.

In other words, by dissolving the gram-molecule of NaCl in water we have made it have the same osmotic pressure as if we had made a 1.89 gram-molecular solution of *undissociated* substance. In other dilutions the number will be different again, for the more a substance is diluted the greater will be the dissociation, but we can determine the ratio of the true to that of the theoretical (calculated) osmotic pressure in the way indicated. Putting the facts into a formula, we have :

$$\text{degree of dissociation " } \alpha \text{ " } = \frac{\frac{\text{freezing-point found}}{\text{freezing-point calculated}} - 1}{\text{number of ions in one molecule of salt} - 1}.$$

In the above example, for instance,

$$\alpha = \frac{\frac{3.51}{1.85} - 1}{2 - 1} = \frac{1.89 - 1}{1} = 0.89.$$

That is to say, 0.89 of the gram-molecule has dissociated, and only 0.11 has remained undissociated.

Unfortunately, however, the fluids under consideration are not solutions of NaCl only, but of a number of other substances, and it becomes a matter almost of impossibility to give a value for the amount of dissociation when we do not know how much there is of each salt present. Not only this, but there are substances present in a puncture-fluid which introduce the further complication that they have the power of preventing the salts from dissociating as fully as they otherwise would. They *inhibit* the dissociation of the constituents of the fluid.

However, by making use of the term *osmotic concentration* (C_o), which was introduced by Hamburger, we can obviate this difficulty to a certain extent, since every molecule or ion causes a freezing-point depression of 1.85. The fraction :

$$\frac{\text{freezing-point found}}{1.85}$$

gives the osmotic concentration of the *total number of molecules plus ions in each litre* * of fluid.

* The unit of fluid should be given in grammes and not in cubic centimetres. It will be evident that 1,000 cc. of fluid weigh more than 1,000 cc. of water by the weight of dissolved substance. If the specific gravity be g, 1 litre

It is now necessary to refer to the subject of *electroconductivity* in order to give a more correct idea of the constitution of the puncture-fluids, which forms the subject of the present studies.

A fluid will only conduct electricity provided that there is some dissociated substance (called an *electrolyte*) present in it. Absolutely pure water would not conduct electricity at all (i.e. it is a non-electrolyte). For this reason we may employ a determination of the electroconductivity as a means of ascertaining the number of ions which are present in the fluid. We could speak of an "ionic concentration" and then endeavour to make out a relation between the actual figure representing the electroconductivity and a figure representing successively increasing degrees of ionic concentration. As a matter of fact, this is, however, not possible, and we have to arrive at our deductions in a roundabout way, by ascertaining the conductivity possessed by different strengths of solutions of given salts, such as sodium chloride and sodium carbonate. If we determine the conductivity of a 1-per-cent. solution of sodium chloride and that of 1.5, 2, 2.5, 3, 3.5, 4.0, 4.5, 5.0, etc., per cent. solutions of NaCl, and then compare that of the fluid which is being examined, so as to find with which strength of NaCl that fluid agrees in conductivity, we can say that the fluid contains a certain concentration of salts, expressed as NaCl. If the chief constituent of the fluid be NaCl, we shall not be making a serious error, other things being equal, in comparing a fluid at one time with a similar fluid at another. It is a method such as this which has been employed throughout the present work.

But there is another use for the conductivity method. Suppose that a certain solution of sodium chloride has a conductivity which can be represented by the number 185, and suppose that we now dilute this same fluid of unknown strength a definite number of times. We shall find that the conductivity may have risen to 196, say. If the fluid be still further diluted, we shall

of fluid weighs 1,000 g grams; if the substance in solution weigh s , then the water in a litre of fluid is $1,000 - s$ grams. The osmotic concentration, if given in terms by volume, would be $\frac{1,000 - s}{1,000}$ too little. Of course, if s is very little, this fraction is practically negligible.

find ultimately that we come to a point at which, no matter what the dilution may be, we do not alter the conductivity any further. The figure remains constant. This means that the sodium chloride present has been as completely ionised as possible. If the figure representing the conductivity be now 209, we can ascertain the degree of dissociation of the NaCl in the original fluid from the simple relation :

$$\frac{\text{Conductivity of original fluid}}{\text{Conductivity at infinite dilution}} = \frac{185}{209} = 0.89,$$

which means that of every gram-molecule present at first, 0.89 part of this molecule was in a dissociated state.

It is obvious that we are arriving at the same result as we were doing with cryoscopy, when we were calculating the ionised portion of a fluid on page 96, with the exception that by the conductivity method we arrive at our result in a very short space of time. All that it is necessary to do is to ascertain by experiment the conductivity of the original fluid at a *given temperature*, and then dilute the fluid, say, four times. Multiply the new conductivity figure by 4, and this gives the conductivity of the fluid as it should have been when undiluted. Repeat this for ever-increasing dilutions until the final conductivity remains the same or attains a maximum.

The following example will explain this procedure :

TABLE XII
THE DISSOCIATION OF A PLEURAL FLUID

Distribution of Pleural Fluid.	Observed Conductivity.	Conductivity \times times diluted.
Undiluted.	843	843
2	537	1074
4	296	1184
8	173.3	1386.4
16	94.2	1507.2
32	45.1	1443.2
64	28.33	1829.1
128	14.43	1847.0
256	9.01	2296.5
512	4.351	2227.7

The last values for observed conductivity, \times times diluted,

show that the maximum has been attained, so that we have approximately arrived at "infinite" dilution.

The degree of dissociation is therefore $\frac{843}{2227} = 0.38$.

It is needful here to pause to consider how far the deductions described are really justified. We have taken a specimen of pleural fluid and diluted it with water, and estimated the conductivity of the successive dilutions. Now, pleural fluid often consists largely *—by volume, if not by weight—of albumen, which is a viscid substance, besides being a non-conductor of electricity. To put it in other words, we have in serum a mixture of electrolytes and non-electrolytes. Now, a non-electrolyte is a substance which has the power already referred to of *inhibiting* the dissociation of salts with which it may be associated. The albumen in the fluid will therefore interfere with the conductivity, and if the fluid be diluted a little reflection will show that although the non-electrolyte serum-albumen is being diluted, yet it does not dissociate, while the salts (electrolytes) present are dissociating in the water. The presence of the still undissociated molecules will exercise more retardation than ever on the dissociated molecules—number for number. That is to say, the conductivity will be ever less than it should be, and the degree of dissociation will work out less than it should be. The figure 0.38 in the above example would then be much too small.

We are able to correct this error, by the aid of researches by Bugarsky and Tangl, who found that for every degree per cent. of albumen the conductivity of the electrolytes was diminished by 2.5 per cent. If we make the needful correction for the original conductivity and divide that by the conductivity of serum at infinite dilution, we shall get a degree of dissociation of 0.41. But we have not corrected for the interference of the diluted serum on the diluted electrolytes. The needful formulæ for this are not to be had, so that in this case we have to content ourselves with a statement that the degree of dissociation of pleural fluid lies between 0.38 and 0.41. This is unsatisfactory.†

* Depending on the variety of the effusion (whether exudate or transudate).

† Fortunately, however, the value of a knowledge of the degree of dissociation of various body-fluids is not apparently of much importance, and probably depends entirely on the composition (relations between salts

Not only is there the interfering influence of non-electrolytes on electrolytes present in the case of serum, but there is also the interference resulting from friction between the ions. The friction is much greater when the serum is undiluted than when it is diluted, because there is so much albumen present. The very dilute serum exhibits no noteworthy degree of ionic friction. However, we may sum up the retarding influence as that due to the albumen as a whole, and by making use of the following formula,

$$\text{Corrected conductivity} = \frac{\text{original conductivity} \times 100}{100 - (\text{percentage of albumen} \times 2.5)},$$

correct for all errors together.

But there is one more point to consider in connection with this part of the subject, and that is as regards the actual electrolytes present. Is it justifiable to express the concentration of electrolytes in terms of NaCl, or should it be expressed in terms of any other body? This question must be answered in favour of the latter in some cases, and of the former in others.

The following figures will illustrate this conclusion:

A given pleural fluid contained 0.062 gram-molecule per litre of NaCl. Its conductivity * was 1068, which, for the temperature, is equivalent to that of a solution of NaCl of 0.123 gram-equivalent per litre. The difference, 0.061, gives the concentration of the achlorides in terms of NaCl. The ratio of chlorides to achlorides is then $0.062 : 0.061 = 1.016$. This is a simple calculation.

and albumen), so that we get no further by this method of study than by an ordinary chemical examination.

Thus, the following values have been obtained in the case of bile:

Fluid.	Number of Specimen.	Degree of Dissociation.
Bile	1	0.23
	2	0.34
	3	0.48
	4	0.51
	5	0.54
	6	0.41
	7	0.25

* Throughout this account "conductivity" means "specific conductivity," and is expressed in terms of 10^{-5} .

The other method gave these results :

The chlorides amounted to 0.062 gram-molecule per litre. The degree of dissociation of this (calculated by Arrhenius' formula) is 0.861. The number of molecules plus ions (above formula) is 0.1153. The conductivity of such a strength of NaCl subtracted from that of the fluid gave a conductivity which is possessed by a solution of Na_2CO_3 containing 0.062 gram-molecule per litre, with a degree of dissociation of 0.570, and 0.1342 molecules plus ions per litre.

The ratio $\text{NaCl} : \text{Na}_2\text{CO}_3$ is now 1.16.

This calculation is obviously much more prolonged.*

In the case of certain transudations, and in the case of urine, the preponderant electrolyte present is undoubtedly sodium chloride, but in the case of exudations sodium chloride does not occupy such a conspicuous position, and sodium carbonate takes its place to a certain extent. In blood-serum the sodium and the chlorine are the two most abundant elements, the potassium, calcium, phosphates, and carbonates are the least abundant, as shown by the following analysis of pus-serum by Hammarsten :

NaCl	5.39%	or,	Chlorides	5.39%
Na_2SO_4	0.31		Carbonates	1.13
Na_2HPO_4	0.46		Sulphates	0.31
Na_2CO_3	1.13		Total phosphates	0.94
$\text{Ca}_3(\text{PO}_4)_2$	0.31					
$\text{Mg}_3(\text{PO}_4)_2$	0.12					
PO_4 (in excess)	0.05					

This shows the preponderance of carbonates over phosphates and sulphates. It must be admitted that this is not invariable, but it has been found that even if the phosphates be in excess and the mode of expression of chlorides be altered accordingly, the ratio still remains similar. It is obvious that the only way of avoiding errors at all would be by complete chemical analysis. The present method is an attempt to obtain useful results by the simple method of conductivity determination.

The method of study of the particular fluid would therefore consist in the following observations and calculations. In the first place, we ascertain the freezing-point depression, which,

* An error may lie in these calculations in the values used for the conductivity of NaCl and Na_2CO_3 solutions, which have been estimated in *watery* solution, whereas they occur in *albuminous* solution in the body. The degree of dissociation is vastly different.

when divided by 1.85, will give the total number of molecules plus ions, the osmotic concentration.

Secondly, we ascertain the electroconductivity at a given temperature.

Thirdly, we ascertain the amount of albumen present, if any. Then the value for the conductivity can be corrected by the use of formula on p. 100. There are now two courses open—one can ascertain what strength of sodium chloride has the same conductivity by calculation, or one can ascertain the actual concentration of the chlorides in this fluid by means of chemical analysis and ascertain how much of the conductivity (or of the freezing-point depression) is represented by them. We shall then have the concentration of the electrolytes as a whole *expressed in terms of NaCl*, and the concentration of the chlorides alone, the difference between the two giving us the concentration of the "achloride" electrolytes—that is, the electrolytes other than chlorides, expressed, however, as if they were NaCl.

Or, again, we can express the concentration of achlorides in terms of *carbonates*, by ascertaining what strength of solution of Na_2CO_3 has the same conductivity as "total conductivity—conductivity of the given concentration of NaCl."

In order to ascertain the concentration of carbonate according to a given conductivity it is necessary to ascertain the conductivity of a series of solutions of sodium carbonate of known strength. This is best performed in a number of specimens prepared by oneself, the intermediate values being either obtained by Lagrange's interpolation formula :

$$y_x = \frac{(x-b)(x-c)\dots(x-n)}{(a-b)(a-c)\dots(a-n)} y_a + \frac{(x-a)(x-c)\dots(x-n)}{(b-a)(b-c)\dots(b-n)} y_b + \dots$$

where y_a is the lowest value for conductivity, y_b the next value (determined by experiment in each case), and y_x the value for conductivity at any strength of Na_2CO_3 desired. a is the concentration of Na_2CO_3 of conductivity y_a , b that of y_b , and so on; n is the concentration of the strongest solution whose conductivity one has determined, and x is the strength of the solution whose conductivity one seeks,

or by drawing a curve, on which the intermediate values can be read off.* It is important, as will be shown presently, that all the observations be made at one temperature, say 18°C .

* While the graphic method is the simpler, it is also the less accurate.

Not only is it desirable to know the concentration of the carbonates and chlorides in a given solution, but it is desirable to know *the number of molecules plus ions of each class of electrolyte per litre of fluid*. This demands a more lengthy calculation, which will be found to have been adopted in the series of analyses on puncture-fluids described in the section on differential diagnosis (IV.).

The method of calculation is as follows :

Starting with the percentage of chlorides in the fluid, we ascertain the same value in terms of "concentration"; i.e. gram-molecules per litre. From this value the degree of dissociation is ascertained from the formula $\frac{\Lambda}{l_{Na} + l_{Cl}}$ where Λ is the *equivalent* conductivity of the solution of NaCl, and l_{Na} , l_{Cl} the rate of migration of the ions Na and Cl at infinite dilution. Now $l_{Na} + l_{Cl} = 44.4 + 65.9 = 110.3$. The equivalent conductivity can be ascertained for any strength of solution at 18° C. from the tables given in the Appendix.

Having ascertained the degree of dissociation, its value is put into the formula of Arrhenius : $(1 + a)$ gram-equiv.

The result gives the number of molecules plus ions in the given concentration of NaCl.

The following formula will be found to give the result desired without any further trouble :

$$\text{Mols. + ions} = 1 + \left(\frac{\Lambda_0 - \left\{ \frac{a - C_0}{C_1 - C_0} \times (\Lambda_0 - \Lambda_1) \right\}}{110.3} \right) a$$

where a = gm.-equiv. solution of NaCl in question

C_0 = " " in Kohlrausch's table less strong than a .

C_1 = " " " " stronger than a .

Λ_0 = equivalent conductivity of solution C_0 .

Λ_1 = " " " C_1 .

Having subtracted the value for the conductivity of the fluid being examined from that for the given strength of chlorides (estimated by analysis), the difference gives the conductivity of the achloride electrolytes. Reading off either in one's diagram, or in the list of conductivities which one has prepared from a series of strengths of Na_2CO_3 , we ascertain to what concentration of Na_2CO_3 this conductivity corresponds, so that it is now possible

to calculate the number of molecules and ions in this strength of Na_2CO_3 . Proceeding in the same way we get this formula :

$$\text{Mols. + ions Na}_2\text{CO}_3 = 1 + 2 \left(\frac{\Lambda_0 - \left\{ \frac{a - C_0}{C_1 - C_0} \times (\Lambda_0 - \Lambda_1) \right\}}{113.4} \right) a$$

where

a = gram.-equiv. solution of Na_2CO_3 in question.
 C_0 = " " " in Kohlrausch's table less strong than a .
 C_1 = " " " " " stronger than a .
 Λ_0 = equiv. conductivity of solution C_0 .
 Λ_1 = " " " C_1 .

Finally, number of molecules plus ions of chlorides *plus* number of molecules plus ions of carbonates represents the total number of molecules plus ions in each litre of the fluid under consideration ; i.e. $C_{\text{elect.}}$, and this value subtracted from the osmotic concentration of the fluid gives the concentration of the non-electrolytes ($C_{\text{non-elect.}}$).

The *osmotic pressure of the non-electrolytes* is given by the formula :

$$\left(\text{Total freezing-point depression} - \frac{\text{f-pt. dep. of sol}^n \text{ of NaCl}}{\text{of same conc. as fluid}} \right) \times 12.05$$

where NaCl is assumed to be the only electrolyte.

We have now arrived at the following facts: A given fluid possesses a certain *osmotic concentration* (C_0), which is made up of

$C_{\text{ne.}}$ the concentration of non-electrolytes.

$C_{\text{elect.}}$ the total concentration of electrolytes: that is,

$C_{\text{chlor.}}$ the concentration of chloride electrolytes plus

$C_{\text{achlor.}}$ the concentration of achloride electrolytes.

The first is deduced from the cryoscopic determination, the second by direct analysis, and the last two by the aid of a conductivity determination.

There are yet two considerations which must be borne in mind in order to estimate the correctness of this process of calculation with justness.

(1) When we are dealing with an albuminous fluid with a view to ascertaining the quantity of sodium chloride with *absolute accuracy*, we are in two dilemmas which do not seem to have been realised in their full extent. There is firstly the question, Does all the chlorine exist in combination with sodium

only? and secondly, Can one separate albumen completely from a fluid without altering the amount of sodium chloride which remains in the de-albuminised fluid? A candid opinion will certainly answer both these questions in the negative, though it would be admitted that the amount of chlorine which is not united with Na, but with some other metal, is very insignificant, and not likely to lead to any error that is worthy of consideration in what is, after all, only a rough method of analysis (speaking, that is to say, of electroconductivity). The methods which may be adopted for the estimation of chlorides in puncture-fluids have already been dealt with in Section I., and it was there pointed out how necessary it is to decide how much of the Cl is free and how much in combination *with the albumen*, and the advantage of simplicity which an estimation of chlorine titrimetrically after removal of the albumen by boiling has.

(2) The effect of variations of *temperature* on the conductivity of the fluids examined.—As would be expected, the warmer the fluid, the better will be its conductivity. This is due to the increased rate of migration of the ions with the increase in the temperature.

In practice, the author has found it most convenient in the end to make all determinations of every kind at a temperature of 18° C., which is the temperature at which the determinations of conductivities of various substances by Kohlrausch, Holborn, etc., have been made. The advantage lies in the fact that one has now no correction to make for temperature variations. It is true that there are correction tables to be had, by which one can calculate the conductivities of salt solutions at, say, 37° , or body heat, but the formulæ do not give quite accurate results, and are only really approximate.

However, as there is always the risk that one may not be able to make an observation exactly at 18° C., either from the thermostat being out of gear (changing the water, for instance), or from a desire to make an observation at a moment's notice, it becomes of more than mere academic interest to know how the error may be corrected.

As there is a rise of conductivity for each degree rise of temperature, one might expect that there were some mathematical relation between the two. For instance, if the conductivity be known at 18° and also at 25 and 30 and 37° C., one should be able

to work out the intermediate values with tolerable accuracy by aid of the differential calculus. This is so. But this involves actual determinations at each of these temperatures for that particular strength of solution. It would be necessary to do the same for every variation in strength of solution, a procedure which would become most irksome. It is true that one might proceed on similar lines, and deduce a formula by which one could calculate the conductivity of successively increasing strengths of salt solution. But then we should be having two variables, or two series of only approximate values, a condition which is undesirable.

The increase in conductivity which would occur with each degree rise in temperature has been called the temperature coefficient, and has been worked out for various substances.

The temperature coefficient is calculated as follows:

For a difference in temperature of $t_2 - t_1$, where t_2 is the higher temperature, the conductivity is $k_2 - k_1$, so that for 1 degree the conductivity would alter by

$$\frac{k_2 - k_1}{t_2 - t_1}$$

The relation between the rise per degree to the total conductivity, i.e. the temperature-coefficient, will therefore be:

$$\frac{k_2 - k_1}{t_2 - t_1} = \frac{1}{k} \cdot \frac{k_2 - k_1}{t_2 - t_1}$$

$$\text{i.e. coefficient (c)} = \frac{1}{k_0} \times \frac{k_2 - k_1}{t_2 - t_1}$$

$$\text{or, } k_2 = k_1 (1 + ct),$$

where t is the number of degrees rise of temperature.

If, therefore, one's observation be made at 22°C . instead of at 18° , one substitutes for the conductivity found the result of multiplying this conductivity by $(1 + \text{difference in temperature})$, viz., 4 multiplied by the coefficient, which might be .020.

The formula is more likely to hold good the less the rise of temperature. That is to say, the correction in the cited example will be more correct than if the higher temperature had been 37°C .

From observations by Bugarsky and Tangl we may correct

for temperature in the experiments with puncture-fluids by the aid simply of the following formula :

$$k_{18} = k_t - \frac{k_t \times (t - 18) \times 2.21}{100},$$

where t is the higher temperature, k_t is the conductivity ascertained by experiment at this higher temperature.

We are now in a position to discuss the effect of mixture of electrolytes and non-electrolytes on the freezing-point depression and on the conductivity, especially in cases in which van't Hoff's law does not hold.

The simple conceptions already detailed do not hold for a wide range of dilutions, but the osmotic pressure will show deviations from the law as one passes to high or to weak dilutions. A solution of cane-sugar will show a more rapid increase of osmotic pressure on dilution than would be the case if the simple law held good. In the case of electrolytes the deviation is even more decided.

This question is worthy of consideration.

The interaction between them may take three forms: (1) inhibition of dissociation of electrolytes; (2) chemical interaction leading to the formation of a number of larger and more complex molecules; (3) polymerisation. It has been found that the latter does not occur.

This question has been carefully gone into by Ernst Tezner, and he made up accurately weighed solutions of mixtures of this kind, in order to discover, if possible, whether there were any relation capable of mathematical expression. The results obtained showed that when the concentration in watery solution is increased the osmotic pressure and other colloid properties increase much more rapidly than van't Hoff's law would have. This fact is noticeable in even very dilute solutions (more than $\frac{n}{0.5}$). If there are several different substances present in the solution, the total depression of freezing-point is *less* than the sum of the components.

Starting with van't Hoff's formula :

$$\sum \Delta = \frac{RT^2}{100 W} (k + k_1),*$$

* $(k + k_1)$ = osmotic concentration of the dissolved substances, W = latent heat of fusion, T = absolute temperature, R = constant.

where W and T remain constant whether the substances are mixed or whether they remain separate, Tezner's experiments show that—

$$\frac{RT^2}{100 W} (k + k_1) < \frac{RT^2}{100 W} k^1 + \frac{RT^2}{100 W} K^1,$$

or $k + k_1 < k^1 + k^1$.

By mixing components, the osmotic concentration becomes diminished.

By adding non-electrolytes, further, the friction between the ions is increased, and their rate of migration altered; there is increased viscosity. These effects are manifest in the change of the electroconductivity of the solution. The equivalent conductivity Λ comes to be diminished because of the change in the viscosity V and in the diminution of dissociation D .

$$d\Lambda = f(dV) + f'(dD)$$

Since the change in the osmotic pressure is a simple function of dD this equation will enable $f(dV)$ to be calculated as increase in viscosity (not an actual change of viscosity).

The degree of dissociation is the expression of the state of equilibrium which results from the force of separation of the ions acting against or with the force of electrostatic attraction between oppositely charged ions; and the degree of dissociation is an expression of the dissociating power of the electrolytes. The force of electrostatic attraction prevents new ion-combinations from forming, and may be expressed by the dielectricity-constant, a constant which is diminished the greater the concentration of the non-electrolyte.

Tezner ascribes the entire effect of adding a non-electrolyte to an electrolyte to diminution of the ionisation of the fluid.

The introduction of several new considerations into the above arguments shows, however, how complex the problem is with which we have to deal when we wish to form some conception of the actual ionic or other constitution of such a fluid as a puncture-fluid.

We are indebted to Arrhenius for further considerations about ionisation in mixtures of electrolytes from the point of view of electroconductivity. He correlates the following: (1) the degree of dissociation in a mixture of electrolytes is represented by the formula:

$$\text{Conc. } X A = \text{const. } (\text{conc. } X)^{\frac{3}{2}},$$

— X and A being different electrolytes. (2) In a mixture of salts, the

salts are dissociated to nearly the same extent as the molecular concentration, and the fraction which remains undissociated is proportional to the product of the valencies of the two ions; (3) if the number of ions per cubic centimetre be the same in the two solutions (isohydric), the degree of dissociation will not alter.

$$\text{Conc. } XA = \text{const.} \times \text{conc. } X \times \text{conc. } A.$$

If a be the conductivity which the solution would have if only one substance were dissolved, and a_1 , that which it would have if the other only were dissolved, then on mixing the two the water may be supposed to be divided into two parts, each being isohydric. If v, v_1 be the respective volumes of the solutions

$$\text{conductivity of mixture} \times (v + v_1) = av + a_1 v_1;$$

(4) the friction between the ions has also to be considered, a correction being especially necessary in the case of solutions above 0.1*n*, and may attain 2 to 3 per cent. even at this degree of dilution. Taking into account this factor, Arrhenius suggests the formula:

$$\text{Conc. of } XA = \frac{\text{const. (conc. of ions } X^+ \text{) (conc. of ions } A^- \text{)}}{\sqrt{\text{total conc. of ions } X^+ + X_1^+ + \dots + A^- + A_1^- + \dots}}$$

X, A , etc. representing different salts.

Many attempts have been made to draw some relation between the freezing-point depression of a fluid and its specific gravity. Fuchs considered that the specific gravity of urine multiplied by 0.075 will give the freezing-point depression. Other factors have been devised by different observers. If, however, we reflect upon how many conditions the freezing-point depression depends, we shall at once see that any factor of this kind must needs fail.

The following figures from cases in the Leeds General Infirmary will show the incorrectness of such formulæ:

TABLE XIII

Specific Gravity.	Freezing-point observed.	Freezing-point calculated from the Specific Gravity.
1.024	1.459	1.80
1.017	1.025	1.27
1.032	1.910	2.38
1.033	2.006	2.47
1.015	1.061	1.126
1.014	0.990	1.047
1.010	1.002	0.750
1.013	0.919	1.013
1.022	1.352	1.625
1.029	1.775	2.161

In the case of puncture-fluids the calculated results are hopelessly incorrect.

We now come to the *methods of performing cryoscopy and electroconductivity*. As regards the former, the widespread knowledge of the method renders any exact account superfluous. As regards the second, there are several details worthy of consideration, especially from the standpoint that the method plays an important part in the examination of puncture-fluids as advocated in this work.

1. **Cryoscopy.**—SOURCES OF ERROR.—The first possible source of error lies in an incorrectly graduated thermometer. The calibre of the tube may not be absolutely uniform, so that each degree of the scale may not have the same value. This error can be allowed for by the ordinary methods of calibration. The construction of the reservoir may be at fault: the sides should slope quite gradually towards the capillary.

The zero-point may have been incorrectly determined. The best way of ensuring the correctness of this is to make several determinations at the outset, and take the mean. Subsequent extreme care in handling the instrument, with occasional controls of the zero after days or weeks, according to the frequency of its use, will suffice. It is essential to use absolutely pure water (or distilled water which has been boiled to liberate gases) for the purpose of fixing the zero-point. It is, however, best to use water which has been twice distilled, and once with glacial phosphoric acid, reboiled, and preserved in absolutely scrupulously clean (boiled with acid, etc.) Jena glass flasks.

A further control is obtained by estimating the freezing-point of a 1-per-cent. solution of pure sodium chloride in distilled water after *each observation of a fluid*. It is much easier to obtain the freezing-point of a salt solution than of pure water, and this gives a control on the accuracy of the instrument.

The next source of error is *undercooling*, because the more the fluid is frozen the more ice separates out, and the more concentrated becomes the residual salt solution. If only half a degree of undercooling is allowed, then only $1/60$ of the bulk of the fluid has separated out as ice, and the resulting error is negligible. The freezing-point may be reached by this time if the mixture be suddenly energetically stirred as soon as so low a temperature has been reached.

The error which may arise from using too powerful a freezing-mixture is similar, but the means of avoiding this have already been explained. The temperature of the freezing-mixture should not be more than 3°C . below that of the freezing-point of the fluid to be examined.

Any error which may arise from the fluid not being cooled uniformly is avoided by careful enclosure of the apparatus in cotton-wool and the use of a mechanical stirring device.

Hamburger recommends that at each determination the reading of the thermometer should be taken for a 1-per-cent. solution of NaCl ,* and also for distilled water. If this be done before and after each series of observations one can control the thermometer itself, as it occasionally happens that the readings vary during the same day, either from variations in barometric pressure or from changes in the glass following exposure to cold.

2. The Determination of Electroconductivity.—The apparatus which is necessary for determining the electroconductivity of a fluid is usually described sufficiently fully in text-books of practical physics. The following account will, however, save reference to such works, and include certain practical details which have been found useful.

The following diagram will indicate the various parts of the apparatus.†

The *vessel* which receives the fluid to be examined demands special consideration. It consists of a specially shaped tube, wide above and narrow below. It is very easily cleaned. As it is made of Jena glass there is no risk of interaction between glass and fluid to be examined. Every time it is used it is thoroughly washed out with distilled water, and wiped dry with absorbent non-medicated cotton wool. Every now and then it should be cleaned by soaking in weak nitric acid overnight. If these precautions are followed there is no need for errors to arise.

* To prepare a 1-per-cent. solution of NaCl , Hamburger recommends that 10 grams of pure NaCl which has been strongly heated in a porcelain basin, to drive out HCl and water, be dissolved in 1 kilogram of pure distilled water. By weighing the water there is no risk of errors from temperature of the water or of graduations in the measure. The specific gravity of such a solution is 1.0076 at 0°C ., and there are 9.970 grams per litre, or .1704 mol. per litre, and the freezing-point is -0.589°C . By adding a little thymol, this solution will keep a long time.

† Obtained from Fritz Köhler, Universitäts Mechaniker, Leipzig.

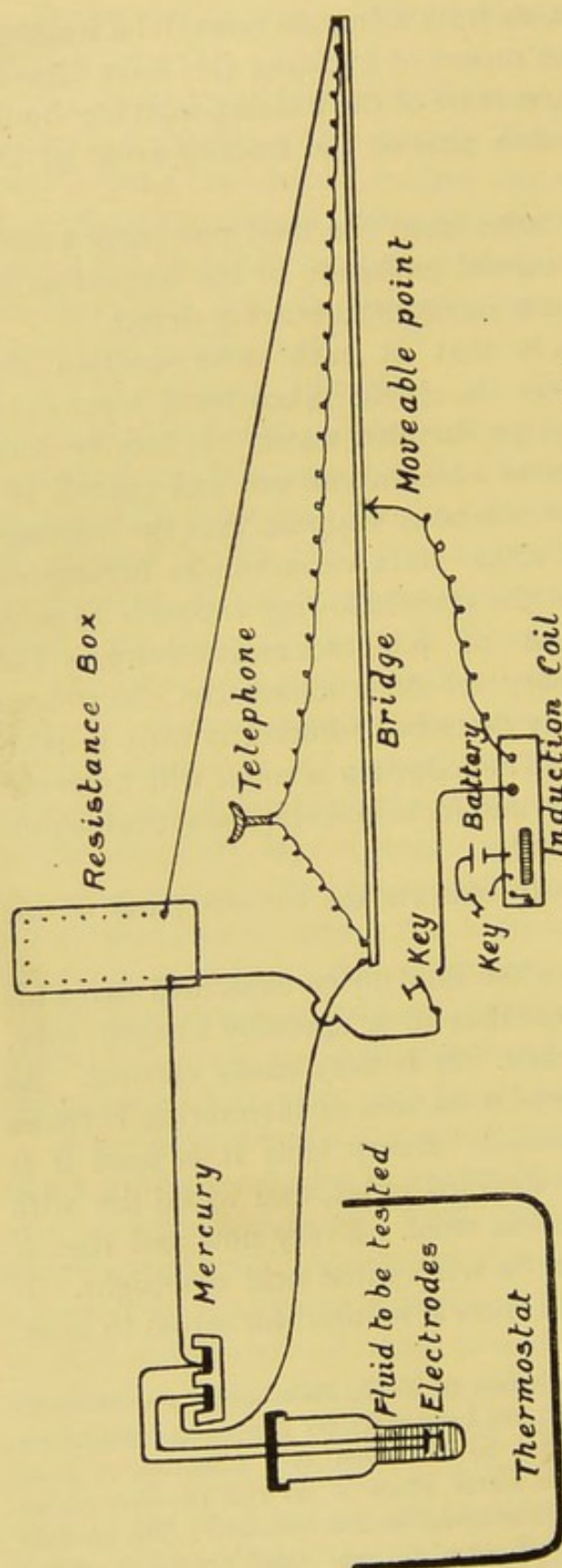


FIG. 4.—Scheme of Apparatus for Determining Electro-conductivity.

The adoption of this form of vessel also avoids any consideration of the many varied forms of vessel that different authors have used and described, for none of them are so readily cleaned and so little liable to break.

The electrodes for this vessel are arranged as in the drawing which shows the apparatus. One electrode passes through the other, and they are formed of strong glass tubing; into the free end of each the electrode is fused. They are kept in position by being fixed into an ebonite disc grooved to allow them to sit firmly within the tube, and be absolutely vertical in it. The tubes are three parts filled with mercury, and receive thin copper wires, a layer of hard paraffin being added, so as to completely fill the tube above the

mercury. This has been found most useful for preventing any spilling of the mercury should the apparatus be

accidentally upset, and the copper wire is also kept firmly in place.*

THE TREATMENT OF THE ELECTRODES.—It is necessary to platinise the electrodes thoroughly before they can be used. This increases the conducting surface very greatly. The electrodes are soaked in soda solution (placed in the corresponding vessel) for a few hours, and then repeatedly cleaned with water. They are now placed in aqueous platinum tetrachloride (the usual strength purchasable), to which has been added lead acetate to the extent of 0.025 per cent. and a strong current passed through. Four Daniell cells will serve the purpose. The current flows for five minutes, and is then interrupted, and the connections altered so as to reverse the current, which flows for a further five minutes. This is repeated till both electrodes are found thickly coated with platinum black. The electrodes are now left in distilled water for some hours before use, in order to soak out impurities entangled in the platinum black.

The electrodes are to be kept in pure water, and must never come in contact with the skin or be allowed to dry up. In the latter case they would have to be cleaned and reblacked. To remove the excess of water before dipping the electrodes into the fluid to be examined, all that is necessary is to hold a piece of clean filter-paper against them, when they become almost dry. They are then rinsed in some of the fluid to be examined, and subsequently placed in the vessel containing this fluid.

It will be found essential for rapid work to have several vessels, which should be marked in successive numbers with commercial hydrofluoric acid.†

As regards the size of the electrodes it is only necessary to say that the larger the electrode the more accurate the results, because otherwise polarisation occurs, especially with a low resistance. The result is shown by not being able to find a spot on the bridge where the sound is entirely lost in the telephone. The smaller the electrode the more careful must be the platinising.

* It should be mentioned that in case of breakage it is best to send the electrodes and vessel straight back to the makers, since there is so much difficulty in obtaining hard glass and fusible glass of exactly the same melting-point as each other and as the platinum. It is, however, wise to have two sets of electrodes in case of accident.

† It is also convenient to etch the "capacity" of the vessel (see below) on the glass.

The use of fairly large electrodes is therefore the most simple and the most accurate.

The vessel is maintained at a definite temperature correct to $\frac{1}{100}$ degree by means of a thermostat.

This thermostat consists of an enamelled vessel of suitable size covered with felt and raised on a stand sufficient to allow a small gas-jet to stand beneath. It is filled with water to within an inch or two of the brim. Into the water dips a specially accurate thermometer registering to 60°C. , and graduated into tenths of a degree. A lens will allow hundredths to be read off. A toluol regulator also dips into the water. This form of regulator is the most sensitive and satisfactory in all ways. A mechanical stirring arrangement is necessary to keep all parts of the water at the same temperature.

THE METHOD OF CARRYING OUT THE DETERMINATIONS.—In the first place it is necessary to determine the resistance-capacity ("C") of the vessel into which the fluid is placed. By this is meant the resistance presented when a conductor of conductivity 1 is placed in the vessel. The value will vary with the dimensions of the vessel used.

The standard solution of known conductivity is decinormal KCl, and the vessel receives enough to well cover the electrodes. The vessel is placed in the thermostat at, say, 18°C. , and the connections are made as shown in the figure, the induction-coil started, and a resistance, say 100, is interposed by means of the box. The movable point is moved about till there is silence in the telephone. A few controls may be made, and then a higher resistance is interposed, and another reading taken. Again, a third resistance is used, so as to bring the reading on the scale as nearly 500 mm. as possible, since most accurate results are obtained near the centre of the bridge.

The capacity is equal to the value for the known conductivity multiplied by the mean resistance found necessary in the box.

The mean resistance is obtained thus :

Suppose at 100 ohms the reading is	498
and at 120 "	"	"	453
and at 90 "	"	"	523
Then the resistance of the fluid will be:					
as $x : 100 :: 498 : 1000 - 498 = 99.20$					
as $x : 120 :: 453 : 1000 - 453 = 99.384$					
as $x : 90 :: 523 : 1000 - 523 = 98.636$					

Mean ... 99.086

Capacity = conductivity of n/10 KCl at 18° ($\cdot 01119$) + $99.08 = 1.10$

When this has been once determined for a particular vessel the value should be noted, and it is convenient to make a table showing the correction that will have to be made for every resistance that will be found to be possessed by any fluid that will be subsequently examined. This will be found to save a great deal of calculation. The procedure with the fluid to be tested is exactly the same. The fluid to be tested replaces the decinormal KCl in the vessel after the latter has been cleaned in the manner already described. The little piece of apparatus is placed in the thermostat at the same temperature, and then the estimations carried out exactly as before, a mean reading being taken.

We have now the following relations :

The resistance to be estimated: resistance in box :: $a : b$, where a is the length from zero on scale to movable point when the telephone is silent, and b is the distance from the latter to the end of the metre.

$$\frac{a}{b} = \frac{a}{1000 - a}$$

A ready reckoner has been provided by Obach for reading off the values of all these fractions without labour (see Appendix).

Now, the conductivity of the fluid is found when the resistance-capacity of the vessel is divided by the resistance of the fluid.

This is *specific conductivity*, or the conductivity possessed by a volume of fluid 1 square cm. in area, and 1 cm. deep. This is a convenient mode of expressing the conductivity, and will be found to be adopted in most of the papers published on the subject abroad. The letter k is used to express it in brief.

Another mode of expressing the conductivity is in terms of the number of gram-equivalents of electrolyte in a vessel whose electrodes are 1 cm. apart, no matter how large the electrodes. This is the *equivalent conductivity*, represented by the symbol Λ .

The *molecular conductivity* is the value when the result is expressed in terms of number of gram-molecules of electrolyte in the vessel. It is the same as the former with monovalent electrolytes.

The equivalent conductivity is calculated by dividing the specific conductivity by the number of gram-equivalents per cubic centimetre. The objection to this mode of expression is that in the fluids under consideration we do not know the gram-equivalent per centimetre, and it is not wise to express it in terms of NaCl for reasons already fully dealt with.

RESULTS OF EXAMINATION OF THE FREEZING-POINT AND OF THE CONDUCTIVITY.—Since both these modes of study practically form the basis of the work which is herein recorded, it is unnecessary to specially collect together the values obtained in the case of various puncture-fluids, since these will be found under the appropriate headings throughout the book, and especially in Sections III. and IV.

We may refer to Table XVI. for the values obtained in many pleural and peritoneal fluids, and Tables XVI. to XIX. for the electroconductivity of fluids. Further details are also given in the special cases reported in Section VI.

The application of these methods of study to other problems which are of interest in the study of puncture-fluids is illustrated by the detection of autolytic phenomena as recorded in Table XI., by the examination of the osmotic concentration of the blood at the same time as that of the puncture-fluid * (Section III.), and of the urine secreted at the time of tapping the effusion to be examined (Section VI.).

The following examples illustrate the value of this method of study. Allaria made out that the conductivity of the blood is *not* increased in uræmia, showing that the cause of uræmia must lie in an accumulation of the *organic* decomposition products.

Sasaki also employed the method for studying the ascitic fluid in cases of experimentally induced nephritis, and concluded that there was no *retention* of electrolytes in the fluids or tissues in cases of uræmia.

Again, Asher employed the method in order to determine whether blood-serum were a solution or a mixture.

Its application to the detection of adsorption-phenomena was gone into in Section I.

OTHER METHODS OF DETERMINING OSMOTIC PRESSURE.—*Hamburger's red-cell method*.—When a vegetable cell is placed in watery salt solution of a different concentration to itself, water will pass either out of or into the protoplasm until the two fluids

* Cohn elaborated this idea.

are at the same concentration. The two solutions are now isotonic. If water passes out of the cell into the water the protoplasm will contract and vacuoles appear. De Vries ascertained that there are definite relations between the molecular concentration of salt solutions and the appearance of this phenomenon, and Hamburger found that if red cells be used in place of the vegetable cells, similar rules will hold good, with the difference that in this case absence of tonicity will be shown by the passage of hæmoglobin out of the red cell.

In 1906 Hamburger published his method of estimating the osmotic pressure on this principle. *The volume of the red cells depends on the osmotic pressure of the solution in which they lie.*

The fluid to be tested is placed in a special tube, like that indicated in the accompanying figure, funnel-shaped above, and calibrated below. Into other similar tubes are placed salt solutions of different strengths (0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6 per cent. NaCl). To each tube is now added 0.02 to 0.04 cc. of defibrinated blood, and after mixing each, and allowing to stand for half an hour, the tubes are all centrifuged till the deposit of red cells ceases to alter in amount. The osmotic pressure of the fluid will be the same as that of the solution of NaCl in which the volume of the deposit is the same as that in the fluid tested. The graduation of the tubes enables this volume to be read off accurately.*

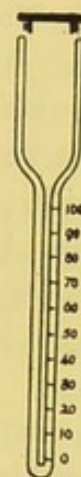


FIG. 5.
Hamburger's
Pipette.

The sole objection to the method is that it involves the use of a very powerful centrifuge, an apparatus which is out of the question for the practitioner, and probably few clinical laboratories can find accommodation for an instrument which is constructed to hold twelve of the tubes and revolve 3,000 times a minute.

Besides this difficulty the method fails in those cases in which the fluid to be tested causes hæmolysis at the outset, and it also fails in the case of fluids which contain substances devoid of any influence on the volume of the corpuscles in

* Obtainable from J. J. Böhm, Groningen.

spite of their influence on the osmotic pressure. Urea, for instance, which divides itself evenly over corpuscles and medium, will not show any appreciable change by the hæmolytic method.

Another difference between the results obtained by the red-corpuscle method and those obtained by the freezing-point-determination method lies in the fact that in the former case one ascertains the strength of a solution of sodium chloride with which blood-serum, say, is isotonic, whereas in the other case one learns the total concentration of the solution, no matter what salts are present. Blood-serum, or ascitic fluid, etc., does not contain NaCl only, but carbonates, phosphates, sugar, urea, etc., so that one is not strictly accurate in expressing them all in terms of NaCl. Then, again, in diluting an albuminous fluid like blood-serum with water in order to test its tonicity, one does not diminish the osmotic pressure in the same proportion as the dilution, because one not only increases the ionisation of the molecules each time,* but the resistance to the movement of the ions is lessened by the pressure of the relatively less colloid matter.

A further error arises from the fact that red corpuscles are permeable to different ions in different degree, and not only that, but the permeability of red cells for ions varies with the actual condition (the "health," as it were) of the red cells themselves. The tonicity of serum tested in this manner and expressed in terms of NaCl will not correspond precisely to that expressed in terms of Na_2CO_3 , where sodium carbonate was used instead of sodium chloride; and so also the tonicity would not correspond with that expressed in terms of such a substance as cane-sugar.

The error which might arise in not using absolutely fresh red corpuscles is not important, provided they are used with aseptic precautions and not kept unduly long.

For these reasons, then, the method cannot replace cryoscopy, but it forms a valuable adjunct as affording information about such substances as urea in a fluid. (See under Amniotic Fluid, Section III.).

* Dilution of 8 per cent. NaCl with equal quantity of water does not halve the osmotic pressure, but leaves the latter only a little less than it was before, since there are now more free ions present than before.

The following table will show the application of the method (from Hamburger's paper, *Biochem. Zeit.* I.).

Fluids.	Volume of Deposits of Red Cells after Centrifugalising.						
	$\frac{1}{2}$ hour.	$\frac{1}{2}$ hour.	$\frac{1}{2}$ hour.	$\frac{1}{2}$ hour.	$\frac{1}{2}$ hour.	15 min.	10 min.
Lymph	58	51	48	47	46	46	46
0.9 % NaCl	55	50	47	47	46	46	46
" " " " " " " "	58	54	50	49	49	49	49
0.95 % NaCl	59	54	52	50	49	49	49
" " " " " " " "	58	49	48	47	47	47	47
1 % NaCl	55	51	48	47	47	47	47
" " " " " " " "	56	50	47	46	45	45	45
1.05 % NaCl	54	49	47	46	45	45	45
" " " " " " " "	50	46	44	43	43	43	43
" " " " " " " "	51	46	44	43	43	43	43

The objections mentioned also apply to the method devised by Sir A. E. Wright, although in this case the apparatus has the advantage of extreme simplicity. For comparing the osmotic concentration of blood-serum and urine of a patient it is very useful, because so small a quantity of fluid is needed. In this method the familiar pipettes are employed, and a mark being made on the stem of the capillary, different dilutions of blood, with a standard solution of NaCl ("N"), until the particular dilution is found that just causes hæmolysis. Suppose two volumes of a N/35 NaCl solution cause hæmolysis of one volume of blood, i.e. two volumes of a 0.167-per-cent. NaCl cause hæmolysis of one volume of blood. The urine is then diluted in a similar manner, using distilled water in this case until it is found that two volumes of the diluted urine when mixed with one volume of blood just cause hæmolysis. Suppose the dilution of urine is twelve-fold. Then the twelve-fold dilution of urine = N/35 saline = 0.167 per cent. NaCl. The urine is therefore equivalent to 2.004 per cent. NaCl. Finally, the serum to be tested is diluted in the same way, till a dilution is found which just causes hæmolysis of one volume of blood. The equivalent of the serum in terms of NaCl is again calculated, and may be compared with that of the urine.

The clinical utility of the method it is not possible to over-estimate, but as an exact means of determining osmotic pressure it is unfortunately of no avail, for the same reasons that the

hæmolytic method of Hamburger fails. On the other hand, the method may be used in place of Hamburger's to amplify the values ascertained by cryoscopy, and throw light on the abundance or otherwise of substances in a fluid which are permeable to red cells.

Limbeck's method consists in placing 1 cc. of increasing (by 0.03 per cent.) concentrations of NaCl into each of sixteen small tubes and adding a trace of blood to each. After six hours it is noted in which tube hæmolysis has occurred. The method takes into account the resistance of the red cells—quite another subject.

The *differential tensimeter* of Friedenthal has the advantage of enabling the variations in osmotic pressure *to be watched*, but necessitates the study of the body-fluid *without its gases*, since these will be removed by the mercury pump belonging to the apparatus.

B. The Critical Solution-point.—Quite recently (February 1908) W. R. Gelston Atkins published a new physico-chemical method of examination of urine, which he advocated in place of cryoscopy for the diagnosis of the functional efficiency of the kidney. Without discussing the value which is to be attached to this mode of diagnosis, one may refer to the method as one likely to be of interest in the study of those puncture-fluids which do not contain much albumen.

The method depends on the fact that if phenol and water be shaken together at room-temperature, they will not completely mix, whereas on raising the temperature a point will be found at which the two fluids just become completely miscible. This temperature is the critical solution-temperature, and is a constant for the particular mixture. Any deviation from the critical temperature causes an opalescence to appear (blue by reflection and brown by transmission). A series of mixtures of phenol and water present a series of increasing critical solution-temperatures, a maximum temperature (the critical solution-point) being reached on the curve so obtained. In some cases, however, it is the opalescence which is the guide to the critical solution-point, and not the maximum temperature on the curve. The fact that the addition of a third substance to the mixture raises the critical solution-temperature to a degree depending on the concentration of the added substance has led to the suggestion of the method for the purposes indicated above.

The procedure is as follows: some crystalline phenol (m.p. $40^{\circ}\text{C}.$) is placed in a dry test-tube and distilled water poured in till the phenol is covered. The tube is then warmed till the contents are homogeneous. On cooling a fog appears, and with certain dilutions this fog is preceded by an opalescence. If opalescence does not appear, more water is added till it does so on cooling. The temperature is then noted. Further addition of water again will prevent the opalescence from appearing. The temperature in which a fog appears in the mixture having the

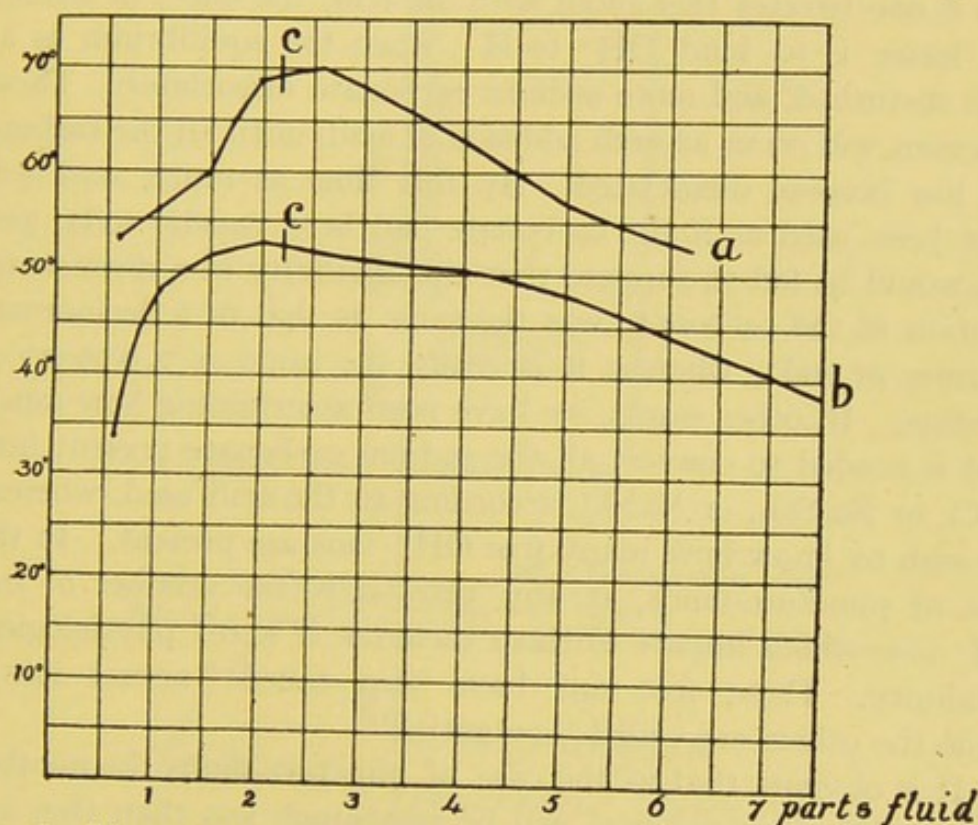


FIG. 6.—The critical solution-point (*c*); *a*, the curve showing the critical solution temperatures at different mixtures of phenol with a specimen of ascitic fluid (case of cirrhosis of the liver); *b*, the curve in the case of phenol and distilled water.

best opalescence is the critical solution-point and is constant for each sample of phenol. Having ascertained this, the *fluid to be tested* is treated with phenol in the same way, and the difference in the temperatures in the two cases is observed. This difference is the rise in the critical solution-point required.

For instance, in a case of peritoneal fluid the rise was found to be $16^{\circ}\text{C}.$

The accompanying figure serves to explain the principle of the method (Fig. 6).

C. **The Concentration of Hydrogen Ions.**—It is now well known that the simple method of testing the reaction of a fluid by means of some "indicator" does not give a correct idea of its real acidity or alkalinity. This is because the reagent with which one titrates is entering into chemical combination with the indicator as well as with the fluid to be tested.

Thus, it has been pointed out that a decinormal solution of sodium carbonate, being partly dissociated (by 3.17 per cent.), contains as many hydroxyl ions as a 0.00317 *n* solution of soda, and if one titrates this alkali with an acid, the effect of adding the latter is to bind OH^- to H^+ , when the equilibrium is at once disturbed, and more sodium carbonate dissociates. These processes will occur at each addition of acid, until all the carbonate has become dissociated. By this time as much acid will have been used as if the carbonate had been caustic soda, and one would be led to suppose that the alkalinity of a decinormal solution of the carbonate was the same as that of a decinormal solution of soda, whereas it is really the same as a 0.00317 *n* solution. In other words, we have been ascertaining how much acid is needed to convert all the sodium carbonate present into NaCl , or Na_2SO_4 , or NaNO_3 according to the acid used, whereas we wish to know how many free OH^- ions are present. In the case of puncture-fluids, at any rate, it is the number of *free* OH^- ions which impart to them their (as it were) physiological alkalinity. These free ions have been called "actual ions," while the others are called "potential."

It is obvious that in the case of puncture-fluids the number of free OH^- ions per litre will be very much less than they are in a decinormal or even a centinormal solution of sodium carbonate. In fact, if we take the number of grams of H^+ ions present in each litre of puncture-fluid we shall find it to be only 5 or 6×10^{-8} (i.e. 0.00000005 gm. per litre), which is at first sight so small an entity as to be negligible.

The quantity is, nevertheless, of very great importance, as can be readily shown from a reference to the ionic concentration of *water* itself. In the case of water there is very little dissociation present, and there is a definite relation between the amount of H^+ and OH^- , which is expressed by:

$$C_{\text{H}^+} \times C_{\text{OH}^-} = 0.64 \times 10^{-14}$$

good in all cases. The more the C_H preponderates the less will be the C_{OH} .

It follows, then, that, given either C_H or C_{OH} , one can calculate the other.

The diagram (Fig. 7) has been devised to illustrate these considerations graphically. The curve represents the varying concentration of H ions as one passes from a highly acid solution at A to one which is alkaline at B. In this curve the ordinates correspond to fractions of normal solution of HCl, and it will be observed that as one passes from the neutral point in the direction of A, the concentration of ions in terms of 10^{-8} is constantly diminishing, though the curve does not rise much from the horizontal. That is to say, the distance travelled longitudinally becomes greater and greater with each increment in the strength of acid. On the other hand, near the neutral point a slight journey to the left means little change in concentration of ions, but is commensurate with a considerable loss of acidity, because the curve rapidly turns down to the zero-point. At this point $C_H = C_{OH}$.

When the curve has passed the neutral point and comes to represent an alkaline solution, the reverse takes place, and a small distance to the right means a small change in concentration of H ions, but a considerable degree of increase of alkalinity, and as one passes towards B one traverses a considerable distance before one reaches any increase in the strength of alkali.

Put in other words again, the stronger the acid is, the more rapidly does the concentration increase with each increment of strength: the stronger the alkali is, the more effect on concentration of OH does a slight increase in strength of alkali produce. The higher the curve rises above the line, the more acid is the fluid, while the lower it falls below the line the more alkalinity does it represent.

The reaction of the fluids of the body is all focussed round the "neutral point," with the sole exception of the gastric juice,* which appears well on the left end of the curve, and it is just about the neutral point that the fluids come to be almost completely dissociated. A solution of HCl which is so weak as to

* Pancreatic juice will be well on the right end of the curve.

be nearly neutral may be looked upon as completely dissociated, so that its ionic concentration is exactly the same as the gram-equivalent of H^+ . The position of the various fluids which appear in the table on page 133 is indicated on the curve as far as the size of the drawing permits, and the degree of acidity which is necessary to produce any effect on the common "indicators" has been marked on the curve in order to bring these conceptions more into line with the old conception of acidity or alkalinity.

We now come to the curve indicated by dotted lines. In this case, position below the horizontal line means relative diminution of C_{OH} , and when the curve rises above the line it indicates absolute alkalinity of the fluid. It will be at once evident that the further we go towards B, the greater the C_{OH} and the less the C_H , since the C_{OH} curve is constantly rising and the other falling. Just as a slight increase in acidity makes the curve travel a long way horizontally towards A (corresponding great increase in concentration of H ions), so a slight increase in alkalinity is accompanied by a very great decrease in concentration of OH ions, since the curve elongates in exactly the same way in the other direction. When we come to the neutral point the OH curve actually passes above the line, because the solution is definitely alkaline, and with the increase in alkalinity the C_{OH} curve constantly rises higher and higher towards infinity.

The only substance which coincides with the neutral point is water, and it is at this point that the two curves cross, showing that $C_H = C_{OH}$. The symmetrical character of the curves shows that the product $C_H \times C_{OH}$ remains constant, and the dotted lines drawn between the two curves at the various points representing the special body-fluids demonstrate their reaction. Thus the upper limit of acidity of normal urine is indicated by a line which passes from the C_H line to the C_{OH} line, the point on the former lying as much above "zero" as does the corresponding point on the C_{OH} curve lie below it.

The varying degrees of H^+ ion concentration which are necessary before given "indicators" will react, are shown in the following scheme, quoted by Höber from the work of Saleesky and Fels.

Indicator.	Colour-change.	Concentration of H ions.
Tropæolin 000 ...	Orange to Red ...	0.000063
Phenolphthalein ...	Red	0.174
Curcumin W ...	Red	0.24
Lacmoid ...	Blue to Red ...	1.1
p.-nitrophenol ...	Yellow	1.8
Methyl Orange ...	(Yellow	59.0
	(Red	5000.0
Congo Red ...	Blue	1738.0
Methyl Violet ...	Violet	41690.0

This table shows how low a concentration suffices to produce a blue coloration with lacmoid, whereas methyl orange is turned red with a concentration of 3×10^{-7} .

There are various means by which the number of hydrogen ions present in a given fluid may be ascertained. The following are the most important:

DETERMINATION OF C_H AND C_{OH} BY THE USE OF AN INDICATOR SERIES.—The observation which has been made that different indicators vary in their sensitiveness to acids and alkalis, so that the colour change is produced only by a definite concentration of H, has led to the idea that a scale of indicators such as is given in the table will enable the concentration of H ions in any particular fluid to be estimated. For instance, blood-serum will not redden with phenolphthalein, so that it must contain at least 0.1 to $0.3 \times 10^{-7} H^+$, a value which agrees with that found electrometrically. In the case of fluids which are so highly coloured that one cannot use an indicator, it is possible to make one's observations by noting the disappearance of absorption bands in the spectrum (Fels). In the method of Sir A. E. Wright the fluid is diluted so many times with a standard solution of acid, until the mixture ceases to redden litmus paper.

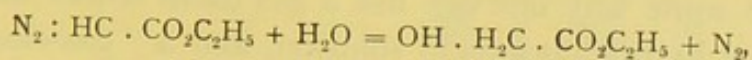
THE INVERSION METHOD.—This is only available for the study of gastric juice. It depends on the inversion of cane-sugar by HCl. The H^+ acts as a catalyst and undergoes no change in the process. The velocity of inversion is proportional to the concentration of the free H ions, and the reaction is a monomolecular one. The polarimeter is used, and the degree of rotation observed before the action of acid, and after. The ratio of the velocity of the fluid tested to that of a standard solution of HCl is made out in this way. This method has already been referred to in Section I., Sub-section "Ferments."

THE METHYL-ACETATE METHOD requires no complicated apparatus, and the calculations are much simpler. Here also there is a catalytic process, and is only applicable to such a strongly acid fluid as gastric juice. A given quantity of fluid is incubated with a given quantity of methyl acetate, a similar quantity of $n/20\text{HCl}$ being treated in the same way as a control. After four hours each is titrated. The presence of neutral salts interferes with this reaction (chlorides and nitrates accelerate, sulphates depress the velocity of reaction).

The *digestion of egg-white* depends for its velocity on the concentration of the H ions, so that one could estimate this by noting the degree of digestion (weight before and after). Here again free acid is essential to the process.

THE DILATOMETER METHOD.—This method is unfortunately not available for puncture-fluids because of the small number of OH^- ions present, and is uncertain in the presence of neutral salts, while the presence of ammonia renders the method useless because it enters into combination with the acetone, and thus inhibits the velocity of reaction. The method depends on the conversion of diacetone alcohol $\text{CH}_2 \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{C}(\text{CH}_3) \cdot \text{OH}$ into acetone, a change which is associated with an increase in the volume of the fluid.

THE DIAZOACETIC-ETHER METHOD.—Bredig has pointed out that this method forms a very convenient one for ascertaining the concentration of the H ions. When in contact with acid the following reaction occurs with great rapidity, and nitrogen is liberated (read off) :



so that the progress of the reaction is very readily watched. Since the velocity-constant of the reaction is proportional to the C_H it becomes a very useful method, and is quite sensitive even at the temperature of the room. This reaction is also monomolecular. Bredig states that it will measure C_H even in $\frac{1}{10000}$ to $\frac{1}{100000}$ n dilution with exactitude, that is $\frac{1}{100000000}$ gm. hydrogen per 1,000 cc., so that it is applicable to puncture-fluids.

It is evident that these methods are either inapplicable to puncture-fluids, or that they are liable to be rendered inaccurate by the presence of salt, which certainly occurs—sometimes in considerable amount—in these very fluids.

The only method which does not present these difficulties is that in which "gas chains" are employed, and unfortunately labours under the insuperable objection that it is too complicated and lengthy for general use. However, it is worthy of a brief consideration, as some important investigations by C. Foà have been made on various physiological fluids which have gone far to show that ionically the fluids of the body are universally practically neutral.

THE CONCENTRATION-CHAIN METHOD.—Suppose that in each of two small vessels containing zinc sulphate solutions (each of different strength) there is placed a zinc electrode. In each case there will be a tendency for zinc ions to pass into the solution. This happens with a certain force depending on the concentration of the zinc sulphate solution. If the osmotic pressure of the zinc ions in the solution be less than the force with which ions tend to leave the electrodes, ions will pass from electrode to solution, while, if the pressure in the solutions is greater, the reverse will occur, and if the two forces are equal, nothing will occur.

In the first case there are positively charged ions passing into solution, leaving a negative charge in the electrode; in the second case the reverse holds good. Therefore, if the two electrodes be joined by a wire, and the two vessels be joined by a tube of fluid, a current will pass from the strong solution of zinc sulphate to the weak, until equilibrium is reached. The electromotive power of this current will depend on the relative strengths of the solutions.

In the same way one can use a *gas chain* where the fluid is, say, strong hydrochloric acid solution, and a "gas-electrode" * (e.g. hydrogen), while the other vessel contains a weak acid and another hydrogen electrode. A current will again pass, whose force depends on the difference in the strengths of the two fluids. The ions concerned in this form of apparatus are *hydrogen* ions instead of zinc ions, so that by this method we can calculate the concentration of the hydrogen ions. Suppose that in one of the vessels there is a slightly acid fluid (body-fluid), and in the other known strength of HCl, we can calculate the acidity of the fluid in terms of hydrogen ions. If we deal with an alkaline fluid

* A gas electrode consists of a platinum electrode coated with platinum black, and saturated with a gas, with which the electrode is also surrounded.

such as blood, and use oxygen electrodes,* and a known strength of sodium hydrate, we can calculate the concentration of the

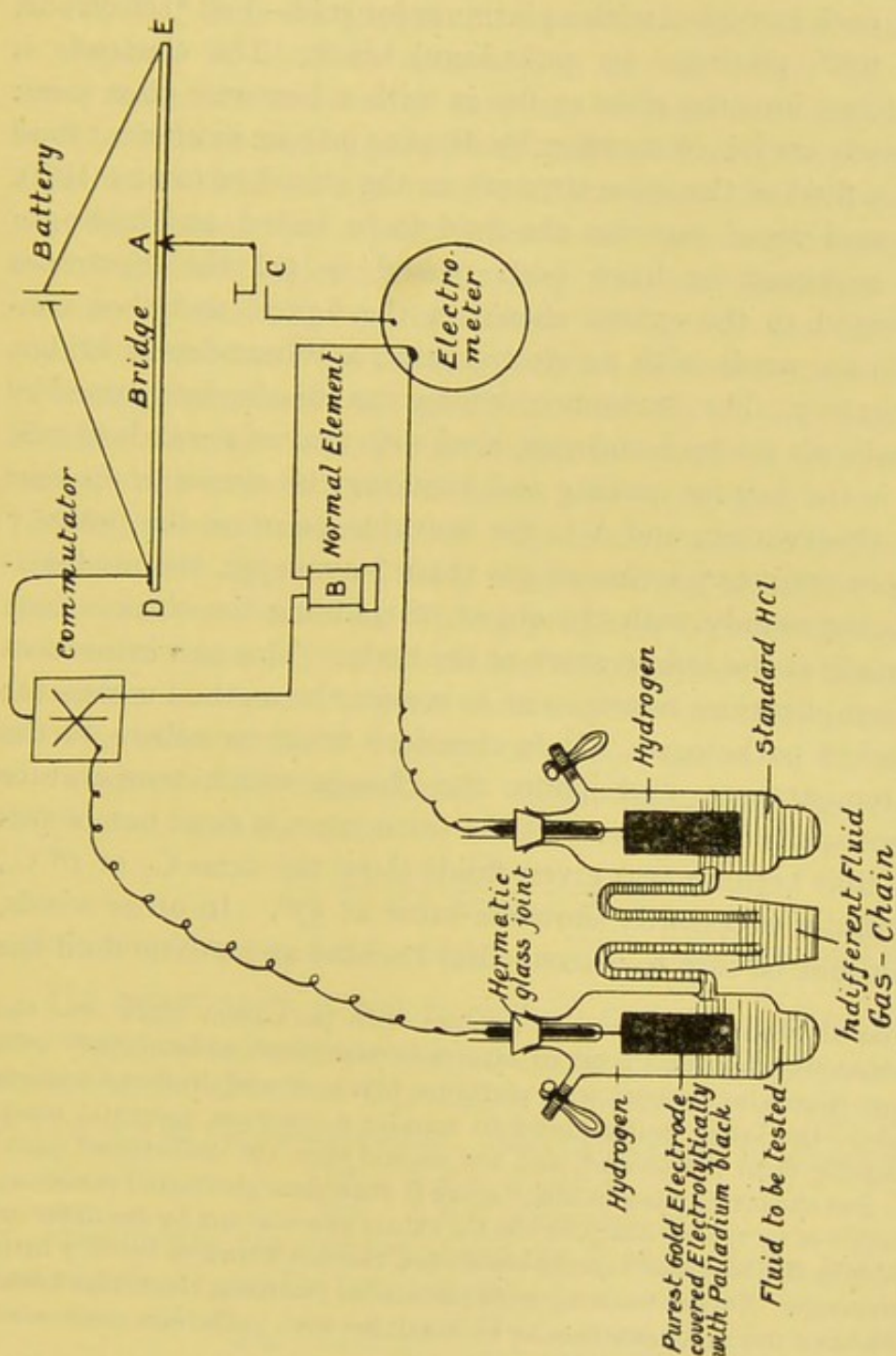


FIG. 8.—Diagram showing the Apparatus for Determining the Concentration of Hydrogen Ions in a Puncture-fluid.

hydroxyl ions, and so ascertain the degree of *alkalinity*. The use of oxygen electrodes is permissible for acid fluids also when we wish to determine CO_H in an acid fluid.

* If one uses oxygen electrodes, and soda solution in one vessel, with an *acid* fluid in the other, we shall also learn the concentration of the hydroxyl ions.

Fig. 8 will explain the way in which the apparatus is set up. The gas chain is seen to be made up of two specially shaped vessels, each furnished with a platinum (or gold—Foà *) electrode, coated with platinum (or palladium) black. The electrode is either fused into the glass or fits in with a hermetic glass joint. The vessels are joined together by dipping into an indifferent fluid or into a fluid of the same strength as the standard (0.01 *n* HCl). The second vessel contains the fluid to be tested, and hydrogen gas is supposed to have been passed in till the electrodes are covered to the extent shown in the figure, and then connections are made with an electrometer, a Wheatstone's bridge, and a battery. The electromotive force can then be determined by Poggendorff's method, and compared with that of a standard cell. B. C is the key for making and breaking the circuit *at the time* of the observation, and A is the movable point on the bridge.†

There are many forms of gas chain ‡ available, the modifications being mainly with the object of enabling the observations to be made at the temperature of the body. This aim introduces many complications which come to render the method useless for the clinical pathologist. It is therefore wiser to adhere to the room temperature, and ignore the change which temperature will necessarily introduce. At the same time, it must not be forgotten that because two given fluids show the same C_H at 18°C., they will not necessarily show the same at 37°. In other words, it would not be safe to assume that because any ascitic fluid has

* Foà showed that the gold covered with palladium black was the more sensitive; but this type of electrode compared unfavourably with platinum electrodes covered with platinum black, or with iridiated iridium electrodes—the latter being found to acquire a constant potential much more rapidly than the second, and the second than the palladiated palladium. But the latter adsorbs much more H than does platinated platinum. As a matter of fact, if we compare the C_H values as made out by the different electrodes in the hands of various observers, there is found to be very little real difference. Farkas working with platinated platinum electrodes found blood to have the same reaction as Fränkel did with palladium electrodes, C_H in each case being 1×10^{-7} .

† Hamburger recommends the use of two similar resistance boxes in place of a bridge, as the enormously increased length of the wire so obtained ensures more delicate observation. The use of these boxes and full details of the method as a whole are given in the second volume of Hamburger's "Ionenlehre."

‡ An "all-glass" gas chain seems to me to be more advantageous than any in which indiarubber corks are integral parts of the gas chain.

a concentration of 7.8×10^{-8} at 18°C. , which a given specimen of pleural fluid also happens to have, that therefore in the body, and *during life*, they are identical in reaction. Relative accuracy, as opposed to absolute accuracy, is one to which the reader needs to become accustomed here, as everywhere throughout this work. It is enough to say that if the distinction between the value at 18° and its significance at the temperature of the body be remembered, the results become at once legitimate. The most important objection to making the observations at 37°C. , however, is that the various factors which enter into the subsequent calculation of the value C_H depend on measurements which have only been made out at 18° and 25°C. , so that correction formulæ (always to be avoided) become essential, and it is far from certain how far these formulæ may be trusted.

The *procedure* in an actual experiment may be outlined as follows: The apparatus being connected up as shown in the figure, and the various parts being prepared for use,* the commutator is arranged so that the current will flow through the normal element. The movable point A is adjusted until a momentary closure of the circuit at C produces no movement of the column of mercury in the electrometer. The reading on the bridge is then noted, and the ratio

$$\frac{DE}{DA} = \frac{\text{E.M.F. of battery.}}{\text{E.M.F. of normal element.}}$$

Since DE is 1000 mm., E.M.F. of battery = $\frac{1000 \times \text{E.M.F. of normal element.}}{DA}$

The commutator is now altered, so that the gas chain is in circuit and the movable point is again adjusted, so that momentary closure of the circuit at C produces no movement of the column of mercury in the electrometer. The ratio again holds good, and

$$\frac{DE}{DA'} = \frac{\text{E.M.F. of battery}}{\text{E.M.F. of gas chain.}}$$

or, substituting the symbol π for the E.M.F. of the gas chain, and inserting 1,000 for DE as before,

$$\pi = \frac{1000 \times \text{E.M.F. of normal element.}}{DA'}$$

or we may still more simplify our procedure by inserting the value for the E.M.F. of the battery, when

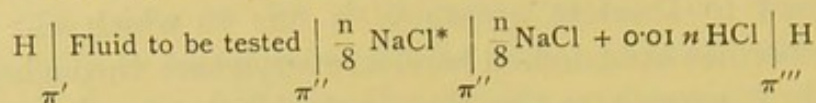
$$\pi = \frac{DA'}{DA} \times \text{E.M.F. of normal element,}$$

which is the same thing as saying that the E.M.F. of gas

* It is this preliminary preparation that makes the method so tedious.

chain : E.M.F. of normal element :: resistance of gas chain : resistance of standard cell.

The coupling of the gas chain is indicated thus :



The symbols beneath indicate the various contact potentials which have to be added up (algebraically) in computing the total E.M.F. of the chain.

However, it is not necessary to enter into the details of the calculation and discuss the mode of introducing π' , π'' , π''' , into the formula :

$$\pi = 0.0575 \log. \frac{c_2}{C_H},$$

which will enable the concentration of the H ions in the given fluid to be calculated, provided the observation is made at about 17°C .† c_2 is the concentration of the H ions in the solution of $0.01 \text{ } n \text{HCl}$, while C_H represents the desired concentration of the fluid. Or we may state that

$$\log. C_H = \log. c_2 - \frac{\pi}{0.0575} = -2 - \frac{\pi}{0.0575}.$$

If desired, the result obtained in this way may be controlled by ascertaining C_{OH} in the same way, using oxygen electrodes in an atmosphere of oxygen, and $0.01 \text{ } n \text{NaOH}$ in place of acid.‡ The formula already given,

$$C_H = C_{\text{OH}} = 0.8 \times 10^{-7},$$

will enable one to see if the result for C_H has been arrived at correctly.

A reference to the two formulæ—(a) one for estimating π ; (b) for estimating velocity of ferment-action—is of interest, since the graphic representation of change of reaction (acidity) in Fig. 7 shows a remarkable resemblance to that representing the velocity

* The exact strength of this can be arranged by ascertaining the strength of NaCl, which has the same conductivity as the fluid to be tested, but this procedure greatly lengthens the time occupied in the determination of C_H .

† For other temperatures the formula from which the above is derived would need alteration.

‡ The electrodes must not be the same as those used for the acidity determination, since the whole of the O or the H cannot again be removed, and mistakes will occur in the subsequent use of the apparatus.

of ferment-action (Fig. 3). Comparison of the two formulæ shows that while one has several *symbols*, representing constants, the other has similar constants expressed in *figures*. The rate at which a fluid alters its acidity with alteration in concentration follows exactly the same mathematical law as does the change of reaction produced by the agency of a ferment. This is rather a suggestive fact.

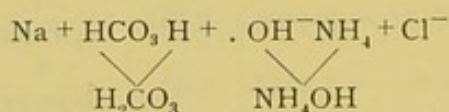
The following table shows some of the results obtained by C. Foà. The first column of figures gives the E.M.F. of the gas chain in volts, the second column gives the value obtained for $\log C_H$ from this π , and the third column gives the corresponding value of the concentration of H ions in terms of 10^{-8} . The remaining columns are added for comparison of the ionic acidity with that shown by titration (in terms of potash), since these values are more familiar.

Table showing some of the results that have been obtained by study of the hydrogen concentration of fluids (Foà).

Fluid.	π EMF (volts).	$\log C_H$	$C_H \times 10^{-8}$	Reaction in terms of KOH.	Reaction as given by Titra- tion.	Indi- cator used for Titra- tion.
Water	...	-7.0969
Blood-serum (dog)	...	-7.1501
Endocellular Fluid	...	-6.0065	9.852
Peritoneal (horse)	.1543	-7.4219	3.785	$\frac{n}{430,000}$ KOH
Pericardial (horse)	.1553	-7.4400	3.681	$\frac{n}{410,000}$ KOH
Cerebrospinal (dog)	.1428	-7.2234	5.97	$\frac{n}{690,000}$ KOH	$\frac{n}{50}$	litmus
Amniotic Fluid1362	-7.1072	7.813	$\frac{n}{900,000}$
Aqueous Humour (horse)	.1328	-7.049	8.93
Vitreous Humour (horse)	.1299	-6.998	1.005
Bile1251	-6.9185	12.18	$\frac{n}{1,000,000}$ HCl
Pancreatic Juice...	.248	-9.0515	0.08882	$\frac{n}{300,000}$ KOH	$\frac{n}{10}$	dimethyl amido- azob.
Urine (human)...	.06	-5.7820	16	$\frac{n}{2,500,000}$ HCl
	.094	-6.3733	42.3	$\frac{n}{1,000,000}$ HCl
	.081	-6.1472	71.2	$\frac{n}{600,000}$ HCl

The most conspicuous result of this kind of study is that practically all the normal fluids are at the neutral point, except the secretions, which show striking deviations from the rule. Urine, for instance, is moderately acid, gastric juice is markedly acid, and pancreatic juice is markedly alkaline. The fact that in nephritis the urine shows a very much higher degree of ionic acidity is not only of interest, but may be of use as a means of clinical pathological research.

Some theoretical considerations on the reaction of blood-serum which have been entered into by Höber deserve notice here in relation to the reaction of puncture-fluids. The electrolytes which occur in such fluids are either strong acids combined with strong bases, or consist of strong bases combined with weak acids, or, lastly, of weak bases combined with strong acids. In the first cases, hydrolysis will produce an acid fluid, and in the other cases, a mixture of the two kinds of salt in varying proportions would produce a medium which was either alkaline or neutral. The proteids of the body-fluid are also amphoteric electrolytes, and act either as acids or as bases, according to circumstances. Höber has pointed out that it is a great advantage to the organism to have these various groupings of acid and base, because it secures a possibility of addition of more acid or more alkali to the medium without any corresponding risk of an actually acid or alkaline medium being produced. This he illustrates by the following consideration: suppose that the fluid contains Na_2CO_3 and NH_4Cl , then, when dissociated, there will be:



H^+ being equal to OH^- . Addition of a strong acid will prevent the dissociation of the H_2CO_3 and liberate OH^- by acting on the ammonia, so that the fluid will remain neutral. The more weak acid there is, and the more free weak base there is, the more rapidly will the addition of acid or alkali be prevented from disturbing the equilibrium. The fact that according to Friedenthal 70 times as much soda has to be added to blood-serum as to water in order to produce the same colour-change with phenolphthalein, and that 327 times as much acid has to be added to produce the same colour-change with methyl orange shows how great the adaptability of the blood-serum is to the variations

in reaction which might be produced by disorders of metabolism, and of course the same applies to transudates and *especially to exudates*.

The reflection in an exudate of the physico-chemical characters of the blood-serum justifies a brief reference to Pfaundler's work. He showed that in children the serum is practically neutral, while with advancing age the reaction becomes more and more alkaline; on the other hand, the alkalinity reaches a high degree if an infant suffer from chronic nutritive disease. The presence of OH^- is necessary to many of the phenomena of vital processes; the deficient OH^- content of the blood of the infant must therefore have some relation to the inactivity of their ferments. Again, the fact that arterial blood is much richer in OH^- * throws some light on the function which the trace of free OH^- plays in normal vital processes.

The results of Benedict's study of the OH^- content in the blood of diabetes lead one to think that the coma of diabetes is not due to the presence of acid in the blood so much as to variations in the equilibrium mechanism already mentioned, while, on the other hand, the possibility of acid being masked by this mechanism renders it difficult to draw conclusions.

D. Viscosity.—Recent months have witnessed the introduction of a number of new forms of viscosimeter, designed to provide the clinician with a type of instrument which is much more handy and easy of manipulation than that hitherto available (Ostwald's). Of these new instruments, it is proposed to describe that of Hess, as from practical experience it has been found to fully answer all the needs of the clinician, being simple, rapidly used, and small in bulk. This instrument has been employed in the examination of the cases in the Leeds General Infirmary.

The *viscosimeter of Hess* consists of two capillary tubes, *a* and *c* (Fig. 9), of like bore and length, coupled together at one end by a T-tube, *e*, to which a hard indiarubber ball, *g*, is attached. This ball can be used for either suction or expulsion of air; by means of closing the hole at *g'* with the finger; fluid in *a* (water) can thus be drawn through at the same time as a fluid (the material to be tested) in *c*. By observing the distance through which the water has passed during the time that the fluid to be tested passes from

* Höber.

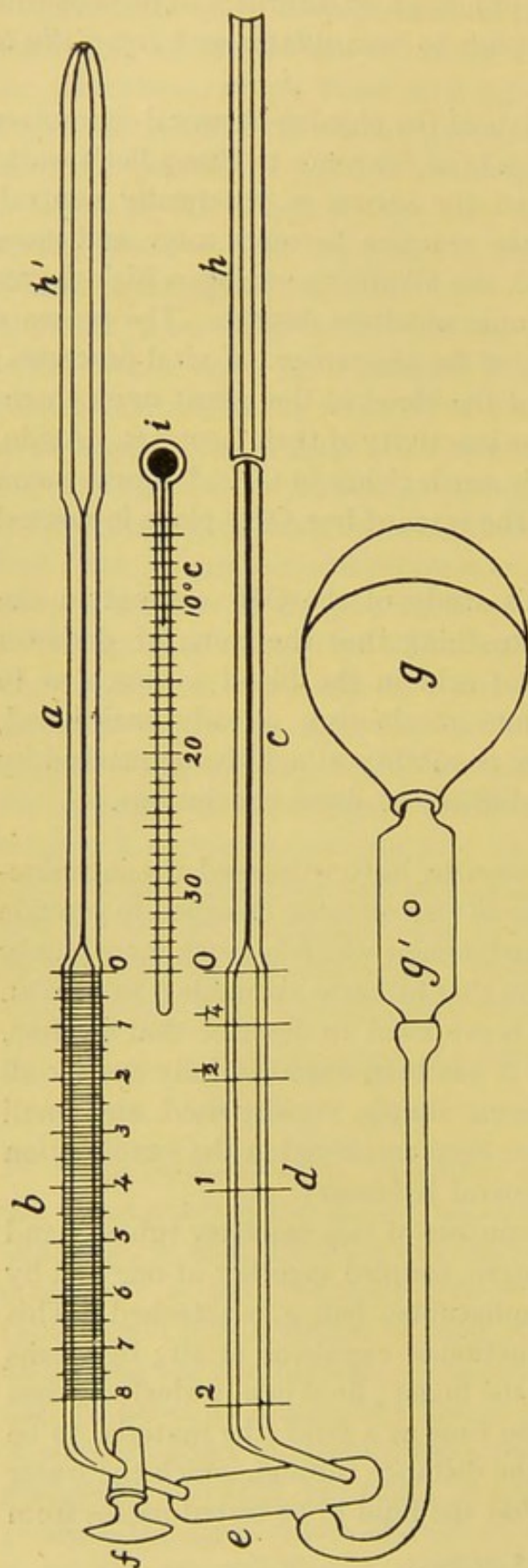


FIG. 9.—The Viscosimeter of Hess.

one arbitrary mark to another, the rate of flow of the latter is compared with that of the water.

It will be seen from the diagram that the capillary tube, *a*, leads into a wide tube, *b*, which is provided with a tap, *f*, before it joins the T-piece. In the same way the capillary tube, *c*, is continuous with a wide tube, *d*, of the same diameter as *b*. This tube, however, has no tap. It will also be noticed that there is a wide tube, *h*, joined on to the capillary, *a*. This is simply a reservoir for the water which is used for comparison, and is undetachable. On the other hand, the tube, *h*, which fits on in a corresponding situation, is readily detachable, and is used to receive the fluids to be examined. A large number of these tubes are supplied with the instrument, so that a new clean one can be used for each new case.

The thermometer

serves as a convenient means of reading the temperature exactly at the time of the observation.

Method of Procedure.—The ammonia which lies in the system, *cd*, is expelled by the use of the bulb, and air is blown through for a few moments, in order to dry the capillary. The stopcock, *f*, is now opened, and the position of the latter column adjusted until it stands at zero. Turning off the tap and applying the tube, *h* (containing the fluid to be examined), to the free end of tube, *c*, taking care that no air-bubble intervenes, suction is applied until the column of this fluid is on the zero of the corresponding scale.

The tap is once more opened, and the two fluids drawn on by an appropriate suction, the tube *d* being carefully watched so as to stop the suction as soon as the fluid reaches mark 1. The reading of the water column is now taken, as also the temperature. If desired, the fluid may now be drawn up to mark 2, and a second reading taken, but this is not usually necessary.

This done, the two fluids are forced back till the water is once more at zero, when the tap is turned off and the other tube is entirely emptied. Strong ammonia is then at once used for washing out this tube, and unless a second experiment is about to be undertaken at once, some clean ammonia is left in the tube.

A little experience with the instrument would soon show that unless there is plenty of fluid in tube *h*, there would be very great risk of having the fluid suddenly shot into the reservoir *e*, owing to the capillary having been passed before the advancing edge of the fluid has reached either mark. The capillary must be full right up to the time that mark 1 or even mark 2 has been reached by the fluid. To ensure this, the little tubes, *h*, provided should be *quite* filled before they are applied to the free end of capillary *c*.

It is essential that the instrument be kept scrupulously clean, so that the fluid being examined should not be allowed to remain any longer within the tube than is absolutely necessary. It is also advisable to clean out the tube occasionally by the aid of strong nitric acid.

Other precautions needed are: the readings must be taken with the eye directly over the meniscus in the tube. The water should be replaced from time to time. The free end of the tube *c* must be closed with the indiarubber cap provided when the apparatus is not in use. The stopcock must be gently lubricated.

*The Theory of the Instrument.**—When a fluid travels through a narrow glass tube the particles which are nearest the glass move more slowly than do the particles in the centre of the flowing stream, and the velocity of the particles will be greater the farther they are from the walls of the tube. The effect of the different velocities of the various particles according to their position within the stream of fluid is that the more slowly moving particles tend to retard the velocity of the more quickly moving particles next to them, which, however, are at the same time accelerated by the action of the layer still more internal (which is moving still faster). Each particle of fluid is under two forces, one accelerating and one retarding, and the balance of the two forces is shown as the viscosity of the fluid.

If the fluid be escaping from a long narrow tube of radius r and length l , the coefficient of velocity η will be given by the formula,

$$\eta = \frac{\pi pr^4}{8lV},$$

where V is the volume of the liquid which escapes in one second of time, and p is the difference of pressure between the two ends of the tube.

This formula affords a very convenient method of estimating the viscosity of a fluid, for in Hess's instrument we have two tubes of given length, l , and of given radius, r , and we subject the fluid in each tube to the same pressure, p , *during a constant time*. The difference in coefficient of viscosity, n , in the case of water and that (η_1) in the case of, say, ascitic fluid will be expressed by the ratio :

$$\frac{\eta}{\eta_1} = \frac{\frac{\pi pr^4}{8lV}}{\frac{\pi pr^4}{8lV_1}} = \frac{V_1}{V}$$

That is to say, the volume of fluid which passes through the tube containing the ascitic fluid is to the volume of water which passes through the tube as the viscosity-coefficient of the water is to that of the ascitic fluid. In the instrument, it amounts solely to a determination of the volume of water which has passed

* The inventor of the instrument has published an account of the theory in a journal unfortunately inaccessible to me, but it is not difficult to work out the mathematics.

through while the fluid to be examined passes from scale mark 0 to 1. Taking water as 1, we now know the fluid viscosity of the fluid as compared with the water.

If we refer to the theory of the ordinary viscosimeters which are available, we find that the viscosity is measured by noting the time which is occupied by the passage of a given quantity of fluid from one mark to the other. In this case the time is a variable and the volume a constant. However, it will be admitted that it is much easier to measure off a volume than it is to measure off so many seconds or fractions of a second—which necessitates the possession of a stop-watch.

In this case the formula becomes :

$$\eta = \eta_1 \frac{st}{s_1 t_1}$$

where η is the coefficient of friction of the fluid to be tested, s is its specific gravity, η_1 is the coefficient of friction of the fluid with which it is to be compared, and s_1 is its specific gravity; t and t_1 are the flowing times of the two fluids in seconds. Now, as it stands, this formula demands the determination of the specific gravity of both the fluid to be tested and a standard fluid; and it requires a knowledge of the coefficient of viscosity of the standard and a correct observation of the times of flow. Freshly distilled aniline has been found to be practically equal in viscosity to that of *blood*, so that if aniline be used,

$$\eta = \eta_1 \frac{t}{t_1}, \text{ or ignoring } \eta_1, \eta = \frac{t}{t_1}$$

which means that one is always comparing the fluid with aniline—a very artificial standard.

There is only one other point to consider, and that is the *influence of temperature* on viscosity.

Water at 0° C. has a coefficient of viscosity of 0.0178 c.g.s. units.

"	10	"	"	"	0.0131	"
"	30	"	"	"	0.0081	"

Obviously one can avoid the necessity for correction for temperature by always studying the viscosity at the same temperature, as could be arranged by the use of a water or air bath. But it is more convenient to dispense with this and correct for results afterwards, since the temperature of the room may be maintained correct within small fluctuations.

On the other hand, water will not be influenced by temperature to the same extent as would the viscosity of a fluid such as a body fluid. Hess, however, made out from a number of experiments that there is not a greater variation than 4 per cent. within 5° above or below 17° C. Every degree difference from 17° requires a correction of 0.8 per cent.

From a large number of observations published by Hess, the blood from different cases showed the following variations in viscosity:

Normal Blood	5.0 to 5.4
Chlorosis	4.4
Carcinoma Uteri	3.2
Tubercle	4.95, 5.15, 5.4
Pleurisy	5.2
Peritonitis Traumat.	5.8
Tubercular Meningitis with Coma				7.65

The following table (XIV.) shows the results of examination of various puncture-fluids by this method. It has been found that the higher the albumen-content, the higher the value for the viscosity, so that in exudates, as a rule, the viscosity is greater than in transudates. As might be expected, ovarian cyst fluids and purulent fluids have a greater viscosity.

According to Rossi, the viscosity of blood-serum runs parallel with the conductivity. Ascoli came to think that the variations of conductivity met with in sera of different degrees of alkalinity might be due to variations in the viscosity of the sera, although an assumption of formation of albumen-salt compounds would afford a more satisfactory explanation.

Herz found that the viscosity exerts an influence on the velocity of enzymatic reactions, and that the velocity of reaction is an exponential function of the viscosity.

An application of the use of viscosity for estimating the amount of pepsin has been referred to in the preceding section.

E. Refractometry.—The study of the refractometric characters of various fluids has led to results which are not only interesting but of value in the differential diagnosis of the nature of fluids. The appliances necessary for the work are, however, too expensive to enable the method to be widely used. Since the subject is mainly of interest in the differential diagnosis of exudates from transudates, the description of the results obtained has been placed in Section IV.

TABLE XIV
VISCOSITY OF PUNCTURE-FLUIDS

	1.0 — 1.5	1.5 — 2.0	2.0 — 2.5	2.5 — 3.0
Pleural ...	Adt. Pericardium ... 1.1 Cardiac Failure ... 1.18 Cardiac Failure ... 1.16 Effusion (assoc. with Subphrenic Abscess) 1.48	Tuberculous Pleurisy... 1.76 Postpneumonic Gangrene... 1.58	Tuberculous Pleurisy... 2.05 Empyema ... 2.41 Empyema (diplococcal) 2.46	
Pericardial ...	Hæmorrhagic Pericarditis 1.35			
Peritoneal ...	Adt. Pericardium ... 1.09 Cirrhosis of Liver ... 1.22 Cirrhosis of Liver ... 1.09 Atrophic Cirrhosis ... 1.2 Chronic Nephritis ... 1.09 Cardiac Failure ... 1.31 Œdema sine Albuminuria 1.18	Carcinoma Ventriculi... 1.79 Carcinoma Peritonei ... 1.63 Tuberculous Peritonitis 1.87	Tuberculous Peritonitis 2.08	
Subcutaneous Œdema ...	Cardiac Failure ... 1.06			
Cysts ...	Hydatid 1.12		Unilocular Cyst ... 2.499 Ovarian ... 2.75	Unilocular Cyst ... Ovarian ... 2.75

SECTION III

THE CHARACTERS POSSESSED BY VARIOUS PUNCTURE-FLUIDS

CONTENTS.—The lymph—Pus—Pleural fluids—Peritoneal fluids—Opalescent or turbid effusions—Effect of repeated tapping—Chyle—Pericardial fluid—Synovial fluid—Hydrocele fluid—Aqueous humour—Amniotic fluid—Cerebrospinal fluid—Cysts: ovarian, pancreatic, thyroid, liver, kidney, spleen, lymphatic, lacteal, parotid, bone, spermatocoele, hydatid.

HAVING passed in review the main methods by which the analysis of puncture-fluids may be accomplished, we have now to describe the chemical and other characters of each of the classes of fluid which may be met with. The close resemblance which ordinary lymph bears to the fluids which are poured out into serous cavities as a result of back-pressure, for instance, renders it advisable to prelude the description of special fluids by one of the main characters of lymph, in so far as they concern us.

THE LYMPH

This alkaline fluid varies considerably in composition according to the part of the body from which it is collected and according to the state of nutrition, besides being influenced in other ways (blood-pressure, digestion, etc.). We are chiefly concerned with the amount of proteid, and perhaps especially with the inorganic constituents. An analysis by Gubler and Quevenne, quoted by Hammarsten, to which I have added some other records, gives :

Water	939.9 %	
Solids:	60.1	(may drop to 35 during fasting*)
Total nitrogen	0.12%	†
Fibrin	0.5	
Albumen	42.7	(albumen : globulin = 2.4 or 4 : 1)
Fat, cholesterin, lecithin...	3.8	
Extractives: xanthin, hypo-		
xanthin, guamin, leucin,		
uric acid, tyrosin	5.7	
Salts	7.3	
NaCl	5.83	} †
Na ₂ CO ₃	2.17	
K ₂ HPO ₄	0.18	
Ca ₃ (PO ₄) ₂	0.28	
Mg ₃ (PO ₄) ₂	0.09	
FePO ₄	0.025	

The following ratios are worthy of notice : $\frac{\text{NaCl}}{\text{achlorides}} = 2.13 : 1$, $\frac{\text{NaCl}}{\text{P}_2\text{O}_5} = 10.22 : 1$, and $\frac{\text{carbonates}}{\text{phosphates}} = 3.83 : 1$, as they show that though chlorides are abundant, they may not be very much in excess of the carbonates or achlorides, while carbonates are the most conspicuous of the achloride salts.

The *gases* found in the case of a dog ‡ amounted to 37.4 to 53 per cent. CO₂, and 1.6 per cent. N (at N.T.P.), the CO₂ being mostly "fixed."

The other constituents of lymph, such as would be obtained in puncture of the legs in cases of dropsy, are apparently similar to those of the serum, and will vary greatly according to the disease causing the dropsical accumulation. Thus, the *specific gravity* varies from 1005 to 1011, the *amount of proteids* is generally less than 1 per cent., and is less in cardiac than in renal dropsies. Blister fluid will become solid on boiling, however.

The *fat-content* rises greatly during starvation (1.4 per cent. instead of 0.75 to 0.85).

The *proteid-quotient* in a case of cardiac dropsy was 3.25, and in a case of renal dropsy 2.35 §. The globulin is increased in renal dropsy.

Urea is often fairly abundant (1 to 2 per cent.).

* Munk and Rosenstein.

† Pickardt.

‡ Hammarsten.

§ From analyses made by Halliburton.

The *osmotic concentration* of such a fluid varies greatly, being often less than that of the blood, especially in cases of cardiac disease,* where the freezing-point depression is 0.54°C ., while in renal cases, as one would expect from the retained salts, the osmotic concentration is greater than that of the blood (freezing-point depression = 0.57°C .). In a case of dropsy due to cirrhosis of the liver, the fluid from the legs had a freezing-point depression of 0.557°C .

The *nitrogen-content* of dropsical fluid is of considerable interest, especially in cases of renal disease. Estimation of the nitrogen which is not bound in the proteid molecule ("filtration-nitrogen" of v. Noorden), and is mainly (80 per cent.) composed of urea, shows that there is a relation between the filtration-nitrogen of the dropsical fluids and that of the blood, and that the filtration-nitrogen in renal disease is much greater than that of health, especially if uræmia is impending or actually existent. To this statement the ever-present exceptions will be found, and uræmic cases may show no unusual amount of filtration-nitrogen. However, the *general* rule is to find an excess of this nitrogen stored in the tissues, and in a case of chronic nephritis v. Noorden found the following daily variations:

FILTRATION-NITROGEN IN RENAL DISEASE

Date.	Right Leg.	Left Leg.	Remarks.
June 26	0.0928%	0.084%	...
" 27	0.0897	0.0792	...
July 23	0.0716
" 24	...	0.0710	...
Aug. 26	0.0144	...	Uræmia
Sept. 27	0.0748

and that as much as 70 grams of nitrogen could be stored up in the dropsical fluid within five days, so that the subcutaneous tissues form a very effective depository for waste nitrogen.† Such a change would escape a cryoscopic and filtration-nitrogen

* Zangemeister.

† Reference to these observations is made in order to draw attention to the relation between the subject of this work and the study of metabolism.

determination of the *blood*, and account in part for erroneous results in diagnosis of functional renal disease by the cryoscopic method.

We may therefore find much urea, uric acid,* and chlorides stored up in dropsical fluids of nephritis, and glucose may also occur to the extent of 0.05 to 0.15 per cent, (apart from diabetes).

Electrochemical examination of lymph shows that it has a slightly acid reaction :

π	$\log C_H$	$C_H \times 10^{-8}$
0.729	-6.0065	98.52

The special observations shown in the table on this page, have been made on cases in the Leeds General Infirmary.

PUS

The chemistry of pus affords an explanation of the properties of certain exudations; the physical properties need no comment. When pus has an acid reaction, this may be ascribed to the presence of lactic or of glycerophosphoric acid.

Hoppe-Seyler gives the composition of *pus-cells*, and I have added marginal notes from other authors.

* 0.006 to 0.009 per cent. (Pickardt).

Nature of case.	ii. Fluid contains chlorine g. aeq. in litre.	iii. Degree of dissociation "a."	iv. No. of molecules + ions	v. Specific conductivity of fluid corrected ($\times 10^{-3}$).	vi. Conductivity of ii.	vii. Conductivity of achlorides (v. - vi.).	viii. No. of molecules + ions pro litre of a solution of Na_2CO_3 with conductivity in column vii.	ix. Total concentration of electrolytes (v. + viii.).	x. viii. - iv.
Fluid from leg in renal case with Cirrhosis of Liver ...	128	827	2338	1420	1100	320	0658	2988	0.28
Fluid from leg in Atrophic Cirrhosis of Liver...	124	837	1910	893	855	38	0990	2900	0.51

Proteids	13.7 %	(mostly nucleo-albumen; also albumose, peptone and fibrin ferment *)		
Nuclein	34.26			
Insoluble substance	20.56			
Lecithin	7.5			
Fats and soaps	7.5			
Cholesterin	7.4			
Cerebrin	5.2	(also pyosin and pyogenin †)		
Extractives	4.4	(xanthin bodies; leucin ‡)		
Glycogen in living pus-cells						
Salts—Miescher gives:						
Potassium phosphate	12	parts
Sodium phosphate	6.1	"
Earthy phosphates and iron phosphate	4.2	"
NaCl	1.4	"
Organic phosphates	3.14 to 2.03	"

As bearing on the differential test fully described in Section IV., attention may be directed to the salts of pus-cells. § The ratio of chlorides to achlorides is $\frac{1.4}{25.4} = 1 : 18$, and the potassium salts forms by far the larger proportion of the achlorides. The exact composition varies, however.

In tuberculous abscesses there will be found leucin, tyrosin, free fatty acids, volatile fatty acids (formic, butyric, and valeric), and urea.

The *serum* of pus is similar to that of the blood, though its relative bulk, as compared with the volume occupied by the pus-cells, is small; the result is that the composition of the latter will have much influence on the total composition of the pus.

Hoppe-Seyler gives:

Water	913.7 — 905.6		
Solids	86.3 — 94.35		
Organic solids	78.57 — 86.58		
Proteids	62.23 — 77.21	$\left\{ \begin{array}{l} \text{NaCl} \\ \text{Na}_2\text{SO}_4 \\ \text{Na}_2\text{HPO}_4 \\ \text{Na}_2\text{CO}_3 \\ \text{Ca}_3(\text{PO}_4)_2 \\ \text{Mg}_3(\text{PO}_4)_2 \end{array} \right.$	5.22
Lecithin	1.5 — 0.56		0.40
Other organic matters	14.84 — 8.81		0.98
Inorganic salts	2.51 — 2.38		0.49
NaCl	5.22 — 5.39		0.49
			6.19

The amount of lecithin may be noted, in reference to its appearing in certain inflammatory effusions.

* Halliburton.

† Kossel and Freytag.

‡ Fränkel.

§ I am indebted to Prof. B. Moore for directing my attention to the composition of the inflammatory cells, which will explain the electrolytic character of exudates as compared with transudates (Section IV.).

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The salt ratios may again be referred to : $\frac{\text{chlorides}}{\text{total salts}} = 1 : 1.4$,
 $\frac{\text{carbonates}}{\text{chlorides}} = 1 : 10.7$ $\frac{\text{chlorides}}{\text{achlorides}} = 2 : 1$.

The pigments in pus are separable by ether and are due to micro-organisms. Crystalline pigments are met with, and also a yellow pigment (pyoxanthin).*

The osmotic concentration is usually high, being considerably above that of the blood. In a case of pyopneumothorax, for instance, I found a freezing-point depression of 0.867 (osmotic concentration 0.468), and in the accompanying list are observations by Zangemeister, who noticed that bacterial growth increases the osmotic concentration.† This would satisfactorily explain the high concentration of pus. Sterile pus will be isotonic with the blood. v. Rzentkowski, however, attributed the high freezing-point depression to the presence of the pus-cells.

OSMOTIC CONCENTRATION OF PUS

Nature of Case.	Freezing-point Depression.	Osmotic Concentration.*	Pressure in Atmospheres.*
Pneumonic Empyema... ..	.684°	.369	8.2
Tubercular "828	.447	9.9
" (three other cases)	.840	.454	10.1
Psoas Abscess539	.290	6.5
" " " " " " " "	.580	.313	6.9
Puerperal Peritonitis642	.346	7.7
Mastitis " " " " " "	.586	.316	7.0
" (recent abscess)709	.383	8.5
Empyema (Herzfeld)47 and .55	.254 and .297	5.6 and 6.6

* These values are my own.

A case of empyema ‡ examined gave the following results : specific gravity 1.025, albumen 3 per cent., chlorides 1.4 per cent., concentration of electrolytes .092, of achlorides .068. Globulin was abundant. Urea and glucosamin were not found. The ferment-content is recorded in another place.

In another case of empyema which was examined,§ the fluid contained 2.45 per cent. chlorides, 8 per cent. proteid, and had a

* Fordos and Lücke.

† Herzfeld denies that the molecular concentration is usually increased by *Bacilli coli*, *staphylococci*, or *Bacilli tuberculosis*.

‡ Register No. 7334 (Leeds General Infirmary).

§ Reg. No. 8349.

specific gravity of 1022. Urea was present in large amount, but purins could not be found. Protalbumose and hetero-albumose were present. The tryptophane and glucosamin radicles were not identified.

PLEURAL AND PERITONEAL FLUIDS

The similarity in the physical and chemical characters of these two fluids will make it more convenient to consider them together. Those properties, which enable the pleural and peritoneal exudates to be distinguished from the transudates, will be found dealt with fully in Section IV.

These fluids are generally alkaline and of straw-yellow colour. The brown-stained fluids usually owe this character to the presence of blood, but in some cases hæmoglobin-colouring is met with (familiar in tuberculous and carcinomatous effusions). An intensely blood-stained fluid was recently met with in a purely cardiac case.*

Pleural fluids from cases of new-growths of the pleura are well known to be frequently hæmorrhagic, and they may become darker in colour the more frequently they are tapped—up to a certain point, after which they become paler † (see also the cytological characters of these fluids, Section V.). Probably this phenomenon is due to the relief of tension on the delicate tumour vessels.

Sometimes there is a peculiar opalescence, or even actual milkiness, a feature which calls for special consideration.

The odour and colour of a fluid give some indication as to the bacterial infection in a given case of peritonitis.

We may compare the composition of pleural with that of peritoneal fluids (transudates).

					Pleural (C. Schmidt).	Peritoneal (Hoppe-Seyler).
Water	966.24	952.9
Solids	33.76	46.38
Organic solids	{ Proteids Extractives }			...	26.82	34.9
						4.28
Inorganic solids	7.64	7.2
Sugar	0.05%*	present
Uric acid	0.0015%*	.0012—.0078%*

*. Pickardt.

* Record No. 10173.

† v. Starck.

Analyses which have been made by the same authorities show that the different effusions met with in a given case of Bright's disease may show slight differences in chemical composition. The most striking point about the analyses which are obtainable is the absolutely uniform salt-content in each fluid (pleural, peritoneal, œdema). The proteids are, as one would expect, uniformly less in the dropsical fluid, and the water is always greater in that fluid than in the case of the pleural and peritoneal effusion. The pleural fluid usually contains the largest percentage of proteid and the least water. The variations, then, are mainly in the organic constituents.

From analyses of various pleural fluids recorded by Halliburton it is seen that acute pleurisies have a specific gravity of more than 1020, while the hydrothorax fluids (whether renal or cardiac) are below 1020. The total proteid is 3 to 5 per cent. in the pleurisies, and only 1 or 2 in the transudates. The proteid-quotient shows that globulin may be more abundant in exudates, while it is practically always less in amount than albumen (proteid-quotient 2.2 to 2.9) in transudates.

GLOBULINS.—In three different specimens of ascitic fluid, Freund and Joachim found the following globulins :

Peritoneal Fluid.	Euglobulin.	Pseudo-globulin.	Para-globulin.	Parapseudo-globulin.	Nucleo-globulin.
Cirrhosis of Liver	+	Trace	+	+	+
" "	+	Trace	+	Small amount	...
Carcinoma "	+	Merest trace	Moderate amount	Trace	...

Associated with the globulin, there may be a considerable quantity of *lecithin*,* as we shall have to refer to fully presently when dealing with the cause of opalescence of puncture-fluids. The presence of globulins in excess in exudates will have a physiological value, since the association—in some unknown way—of globulin with antibodies will serve to protect or help to protect a patient from the dangers of bacterial peritonitis, for instance.

AMINOACIDS have been described as occurring even to the extent of 0.062 per cent. in cases of peritoneal effusion associated with Banti's disease. Glycocoll was found amongst them. Considering how difficult it is to reach a high degree of accuracy in the estimations when 30 per cent. or more of leucin may escape

* Jolles, Joachim. See also page 159.

in the process of quantitative analysis, we may conclude that the quantity of aminoacids may be much greater than 0.0627. Leucin and tyrosin occur in peritoneal fluid in cases of cirrhosis of the liver, according to Hammarsten.

RESIDUAL NITROGEN.—This is relatively high in transudates (22.77), as well as being absolutely increased, 2.83 gm.*

AMMONIA.—More than .9 mg. per cent. was met with in a case of cirrhosis of the liver.†

BENCE-JONES PROTEID.—The presence of this substance has been described by Ellinger as occurring in the ascitic fluid in a case of multiple myeloma of the bones.‡

SUGARS.—*Dextrose* has been described as occurring in the ascitic fluid in a case of renal disease.

Fructose § appears in ascitic fluid in cases of carcinoma and in some cases of granular kidney, if given by the mouth. It was found in pleural fluid in cases of multiple lymphoma, though no levulose was given by the mouth. Pickardt states that the total reducing substance in ascitic fluid may amount to 0.06 per cent. The following table of analyses made by Pickardt will illustrate the variations in composition which may be met with :

Disease	% N.	% Albumen.	% Uric Acid.	Rotation.	% Reducing Substance.
Cirrhosis of Liver ...	0.14	0.87	0.0036	dextro-	0.084
" " ...	0.46	2.875	trace	laevo-	0.070
" " ...	0.22	1.375	"	"	0.106
Carcinoma of Peritoneum ...	0.59	3.69	0.0048	dextro-	0.029
Tubercular Peritonitis ...	0.65	4.06	0.003	laevo-	...
Pleurisy ...	0.906	5.663	0.0025	"	0.050
Nephritic Œdema ...	0.188	1.175	...	dextro-	...
Cardiac Failure (Œdema)	0.09	0.563	0.006	"	0.113

* Friedrichsen, Vages, and Husches.

† H. Strauss.

‡ Though this substance has been known for nearly sixty years, its exact nature remains problematical. Perhaps the most recent view is that it is an albumen (Mann, Parkes Weber) which can be obtained in crystals, and contains neither glycocoll nor phosphorus.

The chief properties are these :

1. It coagulates at 55° C. and redissolves at a higher temperature if ammonia salts or urea are present. It recoagulates on cooling.
2. Digestion with pepsin converts it into albumoses and peptones, but not into hetero-albumose. This proves it to be a true albumen.
3. It is not soluble in 66 per cent. alcohol.

§ Neuburger and Strauss.

Reference to the nitrogen and albumen percentage will show the striking fact that if the peritoneal cavity contain 10 litres of fluid at 2 to 4 per cent. of albumen, there will then be 625 to 1,250 grammes of proteid—equivalent to more than 2 pounds of meat—within the peritoneal cavity!

In serous fluids the dry matter and total nitrogen are, as one would expect, less than in the blood-serum, but the number of extractives is still less than those in the serum.*

ALLANTOIN has been found in the transudate in a case of cirrhosis of the liver.†

MUCINS.—“Serosamucin” occurs in certain exudates. The subject is dealt with in Section I.

PIGMENTS.—Bile-pigment and urobilin may occur. There may be an undue quantity of uric acid associated with urobilin.

A special method of study of body fluids was devised by Landolf, which is of interest, though somewhat complicated. The description of the method ‡ does not seem complete. The total proteid is first estimated by Kjeldahlising, and hydrolysis is then carried out by means of strong hydrochloric acid. After hydrolysis, polarimetric observations are made, allowing for the lævo-rotation of peptone, which may be present in the final fluid. This author discusses (a) the ratio of the insoluble albumens to the degree of rotation of the hydrolysed fluid, (b) the ratio of the insoluble nitrogenous bodies after hydrolysis to the total nitrogen of the hydrolysed fluid, (c) the ratio of the rotation of this fluid to the total nitrogen, (d) the ratio of the dry residue of the hydrolysed fluid to the insoluble nitrogenous substances and the total dry residue, (e) the ratio of the total nitrogen of the fluid to the nitrogenous bodies estimated directly, *plus* rotation of the filtrate after estimating these nitrogenous bodies.

The chief result of these laborious researches is to show that the albumen of puncture-fluids is much more resistant to hydrochloric acid than is serum-proteid.

The following analyses are selected from Landolf's paper :

Fluid.	Albumen %	Dry Residue	Ash.	Urea %	NaCl %	Reducing Substance.	Insoluble Albumen other N.	Fat.
Pleural ...	5·861	6·896	0·844	0·55	0·58	0·102	2·5:1	...
" ...	0·947	1·832	0·804	...	0·62	0·02	1:3	...
" ...*	4·03	5·09	0·87	...	0·56	0·2	...	0·62
Abdominal*	2·17	3·47	0·84	...	0·57	0·24	...	0·90

* Opalescent.

* Rzentkowski.

† v. Jaksch.

‡ *Biochem. Zeit.*, vi. 61.

INORGANIC CONSTITUENTS.—Chlorine is retained in the peritoneal fluid, not only in nephritis, but also in other conditions, such as hepatic or cardiac back-pressure.

While a peritoneal fluid is accumulating the salts will increase in amount ; Cl, N, and P, come to be retained, showing a deficient power in the organism of disassimilation. On the other hand, if the fluid is undergoing absorption these elements are excreted in considerable quantity, and there is P- and Cl-loss. If the nitrogen of the food does not meet the demand, the albumen of the tissues comes to be attacked.*

Gases.—Transudates contain CO₂ and traces of nitrogen and oxygen. The tension of the CO₂ is greater than that of blood in transudates, especially if pus is present (Hammarsten).

PHYSICO-CHEMICAL FEATURES.—As compared with the blood, the physico-chemical characters are usually found similar,† though the concentration of the non-electrolytes is hardly ever the same in the two cases.

Osmotic Concentration.—This has been studied by various observers, with a view to the detection of the nature of an effusion, and in the hope that prognostic indications might be obtainable. Thus it has been said that the lower the osmotic pressure as compared with the blood, the more likely is the fluid to become absorbed;‡ but this is disputed by Herzfeld, who holds that absorption does not depend on the osmotic pressure. From personal observations one is inclined to agree with Herzfeld, but it is difficult to be dogmatic on such a point, and it is most likely that the factors influencing absorption are more complicated.

Rzentkowski thought that tuberculous pleural exudates have generally a much higher osmotic concentration than the blood, especially if there be pus present. But this observation, which raised a hope that tuberculous pleurisy could be readily diagnosed from the cryoscopic examination, was soon found to be faulty.

The value of the determination of the osmotic concentration of an ascitic or pleural fluid has been stated to lie in the determination of the ratio $\frac{\Delta}{\text{NaCl}}$ of the *urine* ; if that is increasing, the fluid may be said to be increasing in amount.

* Marischler and Ozarkiewicz.

† Bodou.

‡ Ketley and Torday.

TABLE XV. CONSTITUENTS MET WITH IN SOME OF THE FLUIDS EXAMINED *

	Nature of Case.	Sp. Gr.	Chlorides %	Urea %	Conductivity.	C. elect.	C. achlor.	Lecithin.	Prot-albumose.	Hetero-albumose.	Tryptophane.	Glyco-proteids.	Albumen %	Globulin %	Remarks.
8334	Simple Inflammatory	1020	2.6	trace	1169	.126	.082	trace	o	+	+	o	5.1	0.8	Purins, trace
8342	Tuberculous	1022	3.75	trace	1269	.138	.124	o	+	+	+	o	6.5	1.8	C ₀ = 0.306
7313	Tuberculous	1019	1.46	o	742	.108	.084	4.0	+	No Purins
8343	Tuberculous	1021	2.5	+	1118	.121	.079	o	o	o	+	+	2	...	
8346	Cardiac Failure	1015	4.75	+	1116	.120	.040	o	o	o	+	+	0.67	0.04	
8337	Post-pneumonic	1022	2.1	+	1195	.261	.180	...	+	+	+	+	8.0	0.87	
8353	Idiopathic	1023	3.6	+	1046	.124	.057	+	+	4.65	1.84	Cholesterin
8358	Renal	...	5.1	o	1558	.177	.090	1.65	...	
7486	Renal	1022	...	o	+	+	+	+	+	7.75	...	No Leucin or Tyrosin
7617	Idiopathic	1024	0.65	trace	1071	.231	.173	...	+	+	+	+	2.4	1.36	C ₀ = 0.296
6196	Cardiac Failure	1012	0.8	...	1065	.246	0.42	0.5	...	
4518	Renal	1022	+	...	
6603	Colloid Cancer of Stomach	...	4.0	...	1102	.02	.052	2	0	
5939	Portal Thrombosis	1011	6.3	o	5	...	C ₀ = 0.360
7472	Polyrrhomenitis	1019	7.5	...	1215	.284	.065	+	
5903	Cirrhosis of Liver	1012	5.5	.02	1187	.143	.049	8	...	C ₀ = 0.360
7456	Cardiac Failure	1018	+	+	+	+	
7553	Chronic Peritonitis	1013	2.5	+	1311	.267	.187	...	+	+	+	+	
6839	Sarcoma of Omentum	1017	6.3	o	1165	.259	.173	+	+	7.75	...	
6417	Tubercular Peritonitis	1015	6.4	o	1171	.265	.159	8.0	...	
6425	Toxic Nephritis	1018	6.6	o	1326	.284	.065	2.6	0	
5838	Sarcoma of Ovary	1020	6.6	...	1165	.259	.173	0.02	+	
8350	Cardiac Failure	1021	2.5	+	3.0	...	
8355	Tubercular	1022	3.5	?	+	+	5.4	0.25	
7559	Cirrhosis of Liver	1010	5.2	...	974	.212	.042	+	trace	o	6.7	0.568	
8340	Cirrhosis of Liver	1016	2.5	+	965	.211	.133	...	+	+	+	+	1.7	4.5	
8344	Peritoneal Cancer	1021	2	+	1102	.242	.051	1.8	...	
6261	Cardiac Failure (Tubercular)	1014	6.3	...	1121	.241	.096	...	+	+	...	+	2.99	...	C ₀ = .311

* Leeds General Infirmary. Other specimens just examined for special points are recorded in the appropriate places throughout this work.

The following table (XVI.) shows the observations which have been made on cases in this hospital :

TABLE XVI
OSMOTIC CONCENTRATION OF PLEURAL AND PERITONEAL FLUIDS

Nature of Case.				Freezing-point Depression.	Osmotic Concentration.	Pressure in Atmospheres.
PERITONEAL.	Exudates.	Tuberculous Peritonitis569	.299	6.83
		Carcinomatous " (stomach)520	.274	6.24
		Chronic Peritonitis (gastric ulcer)...		.752	.406	9.0
		Carcinomatous Peritonitis... ..		.664	.358	8.0
		Tubercular Peritonitis569	.307	6.8
		Suppurative Tuberculous Peri- tonitis				
	Transudates.	Thrombosis of Portal Vein595	.313	7.13
		Cardiac Failure667	.360	8.0
		Multilobular Cirrhosis576	.310	6.9
		Cardiac Failure566	.298	6.80
		" " and Cirrhosis of Liver649	.341	7.78
		Renal Ascites574	.302	6.89
		Cirrhosis of Liver750*	.395	9.0
		Cirrhosis of Liver667	.360	8.0
		" "558	.301	6.7
		" " Chronic Perito- nitis667	.360	8.0
		Cardiac Failure544	.294	6.5
		Renal Ascites586	.316	7.0
		Acute Nephritis672*	.361	8.0
		Interstitial Nephritis676*	.364	8.1
				.75*	.405	9.0
PLEURAL.	Tuberculous Pleurisy563	.303	6.78
		" "488	.257	5.86
		Simple Pleurisy549	.297	6.6
		Tuberculous Pleurisy562	.303	6.7
		" "552	.298	6.6
		" "520	.297	6.6

* Markedly hypertonic.

The molecular concentration is thus seen to vary very greatly, and to be, as a rule, much more marked in peritoneal than in pleural fluids, and, as a rule, greater in transudates than in exudates.

In nephritic transudates the osmotic concentration is the same as, or a little less than, that of the blood of the patient.

The chlorine retention which occurs in transudates will explain this feature in part. The reference which has been made to the other constituents in transudates affords additional information.

As regards the *electro-conductivity* and the concentration of the electrolytes, both chloride and achloride, reference need not here be made, as the application of this method to diagnostic purposes is gone into at length in the following section. Some experiments of Sasaki may, however, be referred to, in which a nephritis was artificially induced in rabbits, with a resulting decided increase in the electrolyte-content of the peritoneal fluid.

Concentration of Hydrogen-Ions.—The concentration of the hydrogen-ions in ascitic fluid has not been frequently determined. The reader may be referred back to the table in Section II.

FERMENTS, ANTIFERMENTS, AND TOXINS.—The ferment-content of various fluids has been discussed on a preceding page, where the results of one's own observations are tabulated. These observations may be amplified by a reference to Marshall's work, who found that both pleural and peritoneal fluids have the power of hæmolyzing blood other than human, pig, rat, or goose blood, a fact which goes to show that these fluids contain *complements* which can activate various amboceptors.

The content in complement of some fluids for a given lysin varies independently of the content in complement for other lysins.

Fibrin-ferment is frequently present in exudates.

The ascitic fluid in carcinomatous cases is hæmolytic, just as the extract of these tumours possesses a hæmolytic power.

ANTIFERMENTS.—Müller found that the amount of anti-ferment rises with the albumen-content and varies in amount with the degree of destruction of the leucocytes. The following results were obtained:

Case.	Antiferment-Content.
Ascites due to passive congestion	In excess
Hydrothorax	"
Hydrocele	"
Pure Tubercular Peritonitis	Lessened
Acute Peritonitis	Lost

Antiferments to congenital syphilis have been found in the peritoneal fluid in three cases by Hans Bab.

Autolytic-ferments are said not to occur in puncture-fluids.*

POISONOUS EFFECT OF EXUDATES AND TRANSUDATES WHEN INJECTED INTO THE BLOOD.—In cirrhosis of the liver the transudate is no more poisonous than the effusion in pleurisy, showing that the portal blood is not more toxic than the blood of the general circulation.

THE EFFECT OF REPEATED TAPPING ON THE COMPOSITION OF PLEURAL AND PERITONEAL FLUIDS

That a change in composition resulted from repeated tapping was noted by Halliburton and discussed by him in some detail. He found that the total proteid first rises in amount and finally falls, the globulin-content increasing more than the albumen. This increase in amount of proteid is very frequently noticed, though it is not an invariable rule, since in an analysis of peritoneal fluid by Hoppe-Seyler there was a decided fall, both in total solids and in the proteid- and salt-content.

The fact that the composition of puncture-fluids does not always necessarily vary during the progress of a case has been several times demonstrated in the series studied in the Leeds General Infirmary. In some cases there was merely a slight fall in the concentration of the electrolytes. Thus, in a case of peritoneal carcinomatosis secondary to disease of the omentum, the only change noticed during three weeks was a fall in the achloride-content from '03 to '01.

A peritoneal fluid due to cirrhosis of the liver showed a fall in the proteid-content during five weeks. The achloride electrolytes remained constant, while the chlorides showed a slight rise. The total osmotic concentration remained practically constant throughout.

In another class of case the chloride-content remained constant during six weeks, while the achloride electrolytes became increased. In this case one may set off the change in concentration against the fall in proteid-content which occurred. The fluid was associated with thrombosis of the portal vein. The diminution of proteid-content would naturally allow an increased ionisation of the electrolytes present, and there would be a diminished inhibitory effect on the conductivity. Since,

* Zock.

however, the diagnosis of increased achloride electrolytes is based on the cryoscopic result, it becomes evident that a difference in albumen of 1·4 per cent. would not materially alter the values obtained. Translated into ordinary language, we may say that after a peritoneal collection had existed for six weeks in a case of portal thrombosis, the chief change which occurred was a deposition of salts such as phosphates and sulphates, while the transudatory character of the fluid became more decided, and inflammatory changes did not occur.

A case of ascites due to disseminated peritoneal cancer (ovarii) was examined on four occasions during as many months. During all this time the specific gravity and the albumen-content remained identical—except for a slight loss of albumen. The chlorides steadily diminished, though not to a great amount (·137 to ·046 gm. equiv.). The conductivity diminished steadily as well, so that the concentration of the achlorides in this case remained constant.

One other case may be referred to as of special interest. The patient was a woman of middle age who had suffered from ascites and anæmia for a considerable time. The fluid was removed on several occasions, and was at first perfectly clear and subsequently opalescent. Numerous bacilli of the *Bacillus lymphagogen* type were met with in the subsequent tapplings. There was no fat present. The variations in character of the fluid were very slight, and the most remarkable fact was the almost complete absence of coagulable proteid in it. The following results were obtained :

	Sp. gr.	Albumen.	Chlorides gm. equiv.	C. elect.	C. achlor.	Urea.	Globulin.	Glucosamine.	Colour.
Aug. 21.	1008	0·25	·113	·284	·065	...	+	0	Opalescent
Sept. 29.	1010	0	·121	·141	·020	"
Nov. 8.	1009·5	Trace	·132	·170	·042	0	+	...	White
" 9.	1009	0·25	·155	+	...	Golden-yellow, with flakes of lymph

Post mortem, the liver was found to be in an extreme state of fatty degeneration, the cells having practically disappeared.

The pancreas was normal. The kidneys showed an extreme cloudy swelling in the convoluted tubules, but no increase in the interstitial tissue, nor any leucocytic infiltration. An unprejudiced observer would hardly regard the tissue as really abnormal; in the absence of any other abnormality in other parts of the body, one must regard this case as one of *toxic* "nephritis." The table shows a progressive increase in the chloride concentration.

The other cases which came in for repeated tapping showed little noteworthy change, and they are fully recorded in Section VI.

TURBID, OPALESCENT, AND MILKY EFFUSIONS

Considerable discussion has arisen on the nature of the turbidity which certain effusions possess; and while in some cases the cause is not far to seek, there are other cases whose turbidity is even now inexplicable. One may classify the cases as follows:

A. True chylous ascites (or pleurisy or pericardium).

B. Pseudo-chylous ascites—

(1) Due to bacteria.

(2) Due to the presence of globulin.

(3) Due to a lecitho-globulid.

(4) Due to a mucin.

(5) Due to the physical properties of the proteid.

(6) Due to other proteids than globulin.

A. The TRUE CHYLOUS EFFUSIONS are most simple to explain. In this case the turbidity is due to microscopic particles of fat, and an examination of an unstained slide will clear up the case at once, or the fact that ether will render the fluid clear also explains the class of case with which one has to deal.

An analysis by Hoppe-Seyler gave:

Water	904.8	fibrin, albumen, globulin	70.8
Solids	95.2	fat, lecithin, cholesterin	9.2
		fatty acids, soaps, and other organic substances	10.8
		salts	4.4

The dry residue of an ethereal extract contained:

Cholesterin	11.3 %
Lecithin	7.5 %
Olein and palmitin	81.1 %

There was no peptone or proteose.

The content of fat varies in different specimens from 0.9 to 0.7 per cent. A case was recently reported by Hammerfahr, in which the patient

received a kick in the neck from a horse, with a double chylothorax as a result; here the fat-content was higher (2.95 per cent.). Lothersin collected twenty-three cases, of which eleven were due to trauma, and twelve to compression by tumour.

The fat may be *identified* by the use of the microscope, when it is seen in the form of minute granules like micrococci, which do not stain and are soluble in potash and ether. Sudan III. may be used. Sometimes, however, the fat is enclosed in cells, which may be of very large size.

The osmotic concentration of chyle is slightly lower than that of the blood, being 0.291.*

Contrasting the characters of chyle with those of lymph, we find that the former is much richer in solids, three times as rich in fats, but much poorer in salts. The extractives are high in each case.

See also "Lacteal Cysts."

B. PSEUDO-CHYLOUS ASCITES.

(1) Due to *bacteria*.—The presence of enormous numbers of bacteria is occasionally met with even in *non-septic* effusions. The use of a Chamberland filter will then procure a clear filtrate. Hamburger's *Bacillus lymphagogen* is occasionally met with in these cases, and Plate I. Fig. 1. shows an example.

(2) Due to the presence of *globulin*.—Micheli and Mattiolo state that the cause of turbidity in a case which they report was a molecular alteration of the globulins present. The molecular alteration may be really a combination of globulin with a fat, forming—

(3) A *lecitho-globulid*, as one might term it.—Bernert drew attention to the fact that globulin can combine with lecithin and produce a turbid fluid; but a much more complete study of a case of this kind was made by Joachim (1903), who discovered that it is the *pseudo-globulin* fraction that has an affinity for lecithin. After removal of the pseudo-globulin the fluid became clear, the action of boiling in the presence of acetic acid having removed the source of the turbidity in the first place. He found 0.368 part of lecithin pro mille. This observation was corroborated by Gross, Micheli and Mattiolo, and Christen. The latter author, however, does not think that lecithin is the sole offender in respect of causing a milky effusion. From one's

* Strauss and Grossmann.

own observations this explanation was found to hold good in a few cases, the source of turbidity being shown by its disappearance either on boiling or on $\frac{1}{2}$ saturation with ammonium sulphate.

(4) Due to a *mucin*.—The presence of excess of mucin in a fluid may cause a milky or opalescent appearance, especially if the electrolyte conditions do not allow of its complete solution.

(5) Due to the *physical properties of the proteids*.—The progress which has been made in colloid chemistry enables us to form some conception of a change that may possibly take place in an effusion so as to render it turbid. It is sometimes very striking that a first tapping brings out a clear, translucent, typically straw-coloured fluid, while a subsequent tapping brings a perfectly milky fluid to light. A striking example of this was met with in a case in which the only post-mortem change was extreme cloudy swelling of the convoluted tubules of the kidneys. What change had taken place between the two tapplings? When, as in the above case, there is no explanation forthcoming that chyle, bacteria, or lecithin can account for the phenomenon, may it not be assumed that a change has taken place in the physical properties of the colloids? If a change in the electrical charge takes place by which the attraction between colloidal particles and ions is altered, then the former may form aggregates of a size sufficiently different from those in the first tapping to make the fluid turbid. That is to say, an alteration in the electrical charge of the colloid particles of the effusion, an alteration in the number of particles, and an alteration in the size of the particles is all that is needed to evoke such a striking macroscopic change in the fluid on different occasions. The ultramicroscope would show evidence of this change, but apparently it has not, so far, been impressed for elucidating this particular problem. Raehlmann, however, in 1903, applied this instrument to clinical medicine, and in his account of the appearances produced by various albuminous solutions he refers to glycogen, which in dilute solution has a bluish-white opalescence. In very dilute solution extremely minute particles of glycogen, of varying size, can be detected throughout the solvent, and show a characteristic type of very energetic vibratile movements.

We have, however, to seek the cause of the alteration in

electrical charge, but this is not very difficult, for it is well established that addition or subtraction of various electrolytes may completely reverse or nullify the electrical charge possessed by proteid substances.

In connection with this line of thought, the behaviour of globulin solutions when dropped into distilled water may be referred to. As is well known, this clinical test for globulin (in urine, *e.g.*) is due to the fact that in the absence of electrolytes globulin cannot remain in solution. The addition of a few drops of a fluid rich in globulin to a 100-cc. measure of distilled water is sufficient to produce a turbid, milky fluid of very similar appearance to these pseudo-chylous effusions. A deficiency of salts and an excess of globulin would thus suffice to produce the effect of nature. The case of toxic nephritis referred to might be explained in this way, because it is a remarkable fact that there was practically no albumen present, but much globulin, and the salt-content was low. Of course these factors produce their effect by changes in the electrical charges referred to, and it is well known that, after tapping, the globulins may increase at the expense of the albumen. The question is one of very great interest, and is bound up with the explanation of the phenomena of salting-out of proteids.*

(6) Due to *other proteids than globulin*.—The observation of Fuld and Levison that when the vegetable proteid edestin is treated with dilute hydrochloric acid, a strongly opalescent solution is obtained, suggests that this may throw some light on the causation of turbidity in some cases. Quincke regards the turbidity as sometimes due to an emulsion of albuminous granules.

A milky fluid is described by Poljakoff as occurring in a case of syphilitic cirrhosis of the liver (verified post mortem) associated with tubal nephritis. The fluid contained 1.625 per cent. proteid (chiefly serum albumen), 1.42 per cent. urea, 6.26 per cent. fat and extractives. He considers that the amount of fat is much too small to account for the milkiness, and also remarks that the fluid did not clear up on shaking with ether.

PERICARDIAL FLUID

As regards colour, specific gravity, and similar characters, pericardial fluid falls in line with pleural and peritoneal fluids.

* See Neisser and Friedemann, "Studien über Ausflockungserscheinungen," *Münch. med. Woch.*, 1903, 11; Bechhold, *Zeitsch. für physik. Chemie*.

Thus, in a case of pneumonia intercurrent in the course of spinal caries* the pericardial fluid was found post mortem to be a brown, turbid fluid, and the pericardium showed no trace of inflammatory change. The albumen amounted to only 1.8 per cent., the chlorides were very abundant (0.137 gm. equiv.), and the conductivity was 1093 at 18° C., showing that the achloride electrolytes amounted to 0.019, and the ratio achlorides : chlorides was only 0.72, as usual in transudates. Tryptophane was present.

According to Hammarsten, the chemical composition of pericardial fluid is:

Water	96%						
Solids	40%	{	proteids	29	{ fibrin ... 0.5
									{ globulin ... 6.0
									{ albumen ... 22.5
					NaCl	7.2	
					other soluble salts	1.4	
					insoluble salts	0.4	
					extractives	2.0	

The proteid-quotient is thus 3.74, and the proteid-extractive ratio 14.3. The analyses given by Friend and Halliburton, Gorup-Besanez, Wachsmuth, Hoppe-Seyler, and others, show that there is considerable variation in the composition in different specimens.

An analysis in a case of tuberculous pericarditis, made by Bockelman, showed that this fluid, which was hæmorrhagic, contained a considerable clot, and had a specific gravity of 1.024 at 15° C. Its freezing-point depression was 0.51° C., indicating an osmotic concentration of 0.275 and a pressure of 6.1 atmospheres (at 0° C.). The chlorides amounted to 7 gms. per litre, which means a concentration of 0.12, so that the concentration of the achlorides would amount to 0.155 (expressed as NaCl). The albumen, estimated by Esbach's instrument, was 4.1 per cent., and the total nitrogen was 9.268 gms. per cent.

A case of chylo-pericardium, recorded by Hasebrock, contained 10.36 per cent. solids, 7.3 per cent. albumen, 1.07 per cent. fat, 0.33 per cent. of cholesterin, 0.177 per cent. lecithin, and 0.93 per cent. of salts.

Concentration of Hydrogen Ions.—The normal fluid gives for $\log C_H - 7.4400$, meaning an alkalinity of $\frac{n}{6}$ mill. potash.

* Reg. No. 8072.

SYNOVIAL FLUID

The chief peculiarity of this fluid is the special variety of mucin which it contains. This mucin does not reduce Fehling, and behaves like a nucleo-albumen or nucleo-proteid. It has been analysed by von Holst, and by Salkowski, who called it synovin. Synovial fluid is alkaline, of yellowish colour, and may be turbid even in health. The following analyses form a useful comparison:

	Normal (Hammarsten).	Synovitis (Hoppe-Seyler).
Water	948 *	928
Mucin	2	7
Proteid	39	51
Extractives and fat	5	4
Salts	2	9
Sodium chloride	6	?

Uric acid may occur in synovial fluid, especially in gouty effusions.

ANTIFERMENTS.—Suppuration joint fluid was found by Müller to contain no anti ferment.

As regards the clinical value of examination of synovial fluid, one must admit that the interest here lies mainly in the bacteriological characters, and perhaps no less important are the cytological characters.

HYDROCELE FLUID

The colour varies from straw-colour to a dark brown or greenish colour. The specific gravity of hydrocele fluid varies between 1016 and 1026. An analysis given by Hammarsten gave 938 per mille water and 61 per mille solids, which consisted of fibrin (0.59), globulin (13.25), albumen (35.94), ether extractives (4.02), salts (9.26), sodium chloride (6.19).

Lecithin, and traces of reducing substance and of urea are found.

Metalbumen † and paralbumen have been found in hydrocele

* For convenience, the nearest whole number is given.

† Devillard.

fluids. Old-standing cases, as with all cysts, may present abundance of cholesterin. The possibility of finding filaria in hydrocele fluid may be just mentioned.*

Succinic acid is said to be present in some examples † and occasionally inosite.

FERMENTS AND ANTIFERMENTS.—In syphilitic cases the hydrocele fluid may give a positive reaction with the serum test (Wassermann's).

Aqueous Humour.—Lohmeyer gives the following analysis: Water 968.87 per mille, solids 13.13 per mille, consisting of proteids (serum-albumen, and globulin 1.22), extractives 0.21, NaCl 6.89, other salts 0.81. There were no cellular elements present. The specific gravity varies between 1003 and 1009.

Grünhagen found a reducing substance in it, which was not sugar; he also found urea and sarcolactic acid ("paralactic acid").

The *absolute acidity*, determined by C. Foà in the case of a horse, gave:

π	$\log. C_H$	$C_H \times 10^{-8}$
1328	— 7.049	8.93

which shows it to be practically ionically neutral.

AMNIOTIC FLUID

Amniotic fluid has been studied very closely in order to decide whether it is to be regarded as a transudate or as a urinary fluid. ‡ The specific gravity is usually low (1008) and the solids only amount to about 11 per cent.

* Salm.

† Hammarsten.

‡ Hamburger studied the amniotic and allantoic fluids in order to decide this question by determining the freezing-point depression, in association with his blood-corpuscle method (which informs about those substances for which the red cells are not permeable). He found the freezing-point of the two fluids identical, while the allantoic fluid requires less dilution than amniotic fluid in order to cause hæmolysis; the allantoic fluid therefore contains bodies which do not exert any osmotic pressure on the red cells (urea). This means that allantoic fluid is a kind of foetal urine. Jacqué had an additional argument for his belief, namely, that the ratio between NaCl and soluble salts varies greatly, whereas if the allantoic fluid were a transudate the ratio would be constant and the same as the blood. The same arguments hold for amniotic fluid, though the evidence is less convincing in this case.

The substances which have been found in hydramnios fluid * are :

Nitrogen-content †	0.094%	Urea	0.111%
Globulin	0.273%	Uric Acid	0.0635%
Albumen	1.272	Allantoin	present
Sugar (<i>not</i> levulose) ‡			Ash	7.836%
A mucin §			{ Cl, CO ₂ , P ₂ O ₅ , SO ₃ (Na ₂ O, K ₂ O, CaO, MgO, Fe		

OSMOTIC CONCENTRATION.—The normal *freezing-point depression* is 0.510° || or 0.475° , † which corresponds to an osmotic concentration of 0.275 or 0.256, or a pressure of 6.1 or 5.6 atmospheres at 0° C. In cases of hydramnios* it is less, being found to be 0.395° C., and in cases of eclampsia it is increased || (0.610° C.).

Hamburger finds it isotonic with the blood-serum.

In cases of ectopic gestation Zangemeister found the osmotic concentration 0.301, and that the longer the time that has elapsed since the death of the foetus, the more concentrated does the fluid become, owing to the salts passing out from the intestine into the sac.

The *electro-conductivity* in a case observed by me was 1070 at 18° C., and the chloride-content 0.507 per cent., so that the achloride electrolytes amounted to 0.030, the ratio of achlorides to chlorides being therefore 0.35.

CONCENTRATION OF HYDROGEN IONS.—Foà obtained the following values :

π	$\log. C_H$	$C_H \times 10^{-8}$
1362	— 7.1072	7.813

Scipiades and Farkas found for $CH \times 10^{-8}$ 9.0.

The fluid is practically ionically neutral.

FERMENTS.—Bonoli examined the ferment-content in amniotic fluid and found that trypsin, chymosin, autolytic-ferment and glycolytic-ferment are never present, while diastase, pepsin, fibrin-ferment and lipolytic-ferment are always present. A ferment which can split up salol is also always present. He found a catalytic action.

ANTIFERMENTS.—Bab failed to find antibodies to syphilis in an amniotic fluid in a case of congenital syphilis.

* Stozyzowski.

† Scipiades and Farkas.

‡ Grube and Grünbaum.

§ Schere and Weyl.

|| Vicarelli and Cappone.

CEREBROSPINAL FLUID

The utility of a study of this fluid has come to be widely recognised, so that lumbar-puncture* is a frequent occurrence, and regarded as of very great value. While much stress has been hitherto laid on the cellular characteristics, it may be safely said that it is chemistry which will afford us full realisation of the diagnostic information which lumbar-puncture can supply. In recent literature tests have been given which are simple to perform, and promise well for clinical diagnosis.

It has been asserted that even if the cerebrospinal fluid served no diagnostic purpose it would still be of use to perform lumbar-puncture as a therapeutic measure. v. Bókay has shown that a therapeutic effect can be expected, owing to the relief of pressure in the cerebrospinal canal, and to the removal of bacteria or their toxins, so that lumbar-puncture should be performed two or three times in a bad case. The pressure of the fluid may be measured as it comes out of the cannula. It may be found to vary in amount almost from hour to hour.

Cerebrospinal fluid is always slightly alkaline, and has a low specific gravity (1003-8), which disease does not appreciably alter, except in the case of meningitis, where the specific gravity is increased.†

NAKED-EYE CHARACTERS.—*Colour*.—Normally the fluid should be perfectly *clear*, although, according to Pilcz, it is not invariably turbid in disease, since in a case of subacute tuberculous leptomeningitis it was clear. As a rule, however, a clear fluid indicates that there is no suppurative meningitis.

* *Lumbar-puncture*.—The requisites are: (1) A trocar three to four inches long, of the diameter of an antitoxin syringe-needle. (2) Means for sterilising. (3) Sterile test-tubes. (4) Culture media. The puncture is made a third of an inch to right of middle line between third and fourth lumbar vertebræ (= a line drawn between the upper points of the iliac crests). The patient should lie on his left side, with his knees drawn up. After the usual aseptic procedure (no chemicals must be used) the trocar is taken up and entered (at the point marked with the left forefinger), forwards, slightly upwards and slightly inwards, using steady uniform pressure. It may be necessary to withdraw the needle, owing to intervening bone. The needle having been correctly introduced, the fluid will run out, and is allowed to run into the sterile test-tube, the first drops being rejected. It may be allowed to run straight into the culture-tubes also (human blood serum, agar, glycerine-agar).

† Sahli.

A *yellow* colour has been met with in tuberculous meningitis where red cells were not abundant,* and in a case of cerebral hæmorrhage which had perforated into the meningeal cavity.†

A *red* colour has been met with in a case of subarachnoid hæmorrhage. If the blood is accidentally present the colour is paler.‡

Turbidity is generally due to a great increase of pus-cells.

The following table shows some of the characters possessed by the cerebrospinal fluid in cases of *epidemic cerebrospinal meningitis*, as observed by H. v. Bennecke :

No. of Case.	Naked-eye Characters.	Albumen.	Neutrophiles.	Large Mono-nuclears.	Lymphocytes.	Leucocytosis.
1	Very turbid; flakes and threads on standing	99%	Trace	0	+
2	Very turbid; flakes	68	2	30	+
3	Turbid; flakes ...	Moderate	99	Trace	0	...
4	Slightly yellow; only a suspicion of flakes ...	$\frac{1}{4}\%$ (Esbach)	8	8	84	+
5	Fairly turbid	99	Trace	0	...
6	Turbid; flakes	80	Trace	21	...
7	Slightly turbid; a few flakes	...	90-95	0.5	4-4.5	+

PERCENTAGE COMPOSITION.—From some analyses by Halliburton we learn that cerebrospinal fluid contains about 99.0 per cent. of water and 1 per cent. of solids, of which 0.8 per cent. is formed of extractives and salts, the remaining 0.2 per cent. consisting of proteid matter.

PROTEID CONSTITUENTS.—Much dispute has arisen as to whether albumen is present or no, but it may be assumed, from the investigations of Quincke, Riecken, Gumprecht, Lenhartz, Nawratzki, Babesch, and others, that cerebrospinal fluid normally contains 0.2 to 1 per mille of albumen, while the amount rises higher than this in meningitis, in tumour cerebri, in apoplexy, and in paralytics (Esbach's method).

Frenkel-Heiden examined the cerebrospinal fluid from various cases of nervous disease, especially as regards the amount of albumen and the total nitrogen-content. The albumen was precipitated by adding 10 to 15 times the bulk of absolute alcohol,

* Nissl.

† Sicard.

‡ Siemerling.

and allowed to stand 24 hours. The following results were obtained : *

ALBUMEN-CONTENT OF CEREBROSPINAL FLUID (FRENKEL-HEIDEN)

Below 1%	1 to 2 %	2 to 2·8%	Above 2·8 %
Dementia Paralytica Tuberculous Meningitis Pontine Tumour Amaurotic Idiocy Normal †	Tuberculous Meningitis (2 cases) † Tubercle of Brain (3 cases) Glioma Cerebri Serous Meningitis Pachymeningitis Hæmorrhagica Acute Amentia	Tabes Dorsalis Tuberculous Meningitis (2 cases) † Tubercle of Brain	Dementia Paralytica Above 3% indicates a definite inflammatory condition ‡

The maximum values are found in a case of general paralysis of the insane and in one of tuberculous meningitis, but no constant-values for particular classes of fluid were obtained. He lays the chief stress on the variations in *residual nitrogen*, which was always conspicuous in amount, and a comparison of the distribution of the nitrogen over the albumen or the urea showed that in a case of positive tumour there was sixteen times as much nitrogen attached to the urea as there was to the albumen, while in other cases the reverse condition was noted.

The following ratios are obtained § from the analyses made by this author :

Disease.	Total Nitrogen	Total Nitrogen	Urea-nitrogen
	Albumen-nitrogen.	Urea-nitrogen.	Albumen-nitrogen.
Tubercular Meningitis ...	1·8	2·56	0·7
Simple Meningitis ...	3·04	1·22	2·52
General Paralysis ...	1·02	1·2	0·85
Pontine Tumour ...	16·5	1·62	16·0

The precise indications as to particular diseases from the albumen-content such as are given by Rieken are too artificial.

* The classification is my own.

† Quincke.

‡ Fürbinger says that more than 1 per mille of albumen means tuberculous meningitis.

§ I do not reproduce the actual figures, as the ratios—which the author of the paper does not work out—seem more interesting and striking.

|| The value for total nitrogen in these cases is less than that for albumen-nitrogen + urea-nitrogen. Surely an error.

Globulin is always present, and the amount of it which is present has been utilised by Nonne and Apelt for diagnostic purposes. They employ ammonium sulphate as a precipitant, using the abundance of the precipitate as a measure for the amount of globulin. The fluid is mixed with an equal quantity of saturated ammonium sulphate. If a turbidity appears in three minutes, the reaction is positive. (Phase I. reaction.)

THE GLOBULIN-REACTION IN CEREBROSPINAL FLUID *

Nonne's Cases.		Literature.		Disease.	No. of Cases.	% of Cases where Phase I Reaction was Positive.
No. of Cases Examined.	% of Cases in which Lymphocytes were Present.	No. of Cases.	% of Cases in which Lymphocytes were Present.			
56	97	331	98	Dementia Paralytica	22	100
76	95	95	95	Tabes Dorsalis	17	93
36	75	14	80	Lues III.	15	92
5	40	76	40	Lues II.	5	20
2	100	15	100	Lues Congenita	2	100
35	33	18	44	Healed Lues	18	0
19	4	17	6	Alcoholism	12	0
13	15	21	15	Idiopathic Epilepsy	10	0
12	33	15	23	Apoplexia Sanguinea
14	23	15	24	Sclerosis Multiplex
5	40	14	65	Tumor Cerebri	3	33
20	0	37	0	Neurasthenia, Hysteria	12	0
5	0	6	0	Health	12	0

It is convenient to add acetic acid to the *filtrate* resulting from ammonium sulphate; boiling will bring down the *albumen*, and it may be estimated by weighing.†

Siemerling uses *magnesium sulphate* as an indicator of the character of cerebrospinal fluid. If a saturated solution of magnesium sulphate be added to normal cerebrospinal fluid, and the mixture filtered and boiled, the filtrate will remain clear in normal cases, while if from a case of dementia paralytica. it will become turbid. The addition of acetic acid causes a precipitate of serum-globulin.

PROTALBUMOSE.—Deutero-albumose has been occasionally met with by Halliburton and by Donath.

Variations in Composition on Successive Tapping.—Halliburton

* From *Münch. med. Woch.*, 1907, p. 42.

† Nissl.

found the specific gravity to rise and the amount of proteid to rise from '045 per cent. to '069 per cent. and '072 per cent. in a case of chronic hydrocephalus which was tapped at intervals. He found the reducing substance (see below) also to increase in amount under these circumstances, being only present in trace to begin with.

COMPOSITION OF CEREBROSPINAL FLUID IN HYDROCEPHALUS

Dry Substance.	As ¹ .	Albumen.	Author.	Remarks.
0.94	0.84	0.12	Gröber	Mean of 17 specimens
1.09	0.78	0.31	"	" 11 "
0.97	0.78	0.18	Neumeister	...

Nucleo-albumen is found only in tubercular meningitis,* and it is convenient to precipitate it when searching for tubercle bacilli, as they will become entangled in the precipitate.

Reducing Substance.—Halliburton extracted this substance by using alcohol as a precipitant. The residue is dissolved in water and extracted by the use of neutral lead acetate, when, after removal of the lead by H_2S , the filtrate can be shaken with ether. He considers the substance to be pyrocatechin, since it does not give a compound with phenylhydrazin. Iron perchloride gives a green colour, and potash gives a brown colour.

Lannois and Boulard state that *glucose* is present to the extent of 0.4 to 0.5 per cent. in normal cerebrospinal fluid, and that it may disappear from the fluid when meningitis develops.

Galactose was found in hydrocephalus fluid by Langstein.

Lactic Acid has been found to be always present in cerebrospinal fluid by Lehndorff and Baumgarten, though it is specially abundant in cases of meningeal inflammation.

Carbaminic acid was found in a case of eclampsia.†

Bile-pigments and bile-acids have been found in cases of jaundice.‡

Cholin.—The discovery of cholin in cerebrospinal fluid by Halliburton and Mott in cases of organic nervous disease, as opposed to functional disease, is the most important advance in our knowledge of the chemistry of cerebrospinal fluid. The

* Sahli.

† N. B. Kofmann.

‡ Gilbert and Castaigne.

cholin is derived from the breaking down of nervous tissue (see Section I. under Lecithin).

In order to detect it, the fluid is treated with absolute alcohol, and the extract evaporated to dryness. Alcohol is again added, and the process repeated several times, the last alcoholic solution being treated with an alcoholic solution of platinum tetrachloride, when a yellow precipitate results. This is washed with alcohol and dissolved in 15 per cent. alcohol. On concentration yellow crystals of the double chloride of cholin and platinum separate out. To distinguish these microscopic crystals from a similar potassium double salt, a strong solution of iodine in potassium iodide is added, when dark brown dichroic plates of cholin periodide ($C_5H_{14}NOI \cdot I_8$) result, while the potassium salt does not alter. On standing, the cholin periodide breaks up into oil-globules. A simpler method of differentiation is to look at the crystals with the polarising microscope, when the cholin salt comes out bright and the potassium salt becomes invisible (Rosenheim's recent method of identifying cholin by the use of alloxan is apparently not reliable).

The presence of excess of potassium salts in cerebrospinal fluid is, however, regarded by Halliburton and Mott as in itself evidence of nervous-tissue destruction.

Other Tests.—(1) Make a saline solution of the alcoholic extract and inject it into an animal. If cholin be present, the arterial pressure will fall, a phenomenon which will be prevented by a preliminary dose of atropine.

(2) Dissolve in alcohol and treat with concentrated alcoholic picrolonic acid. A precipitate of cholin picrolonate is produced.*

FERMENTS.—A *diastatic ferment* has been described by Cavazzani and Gröber as present in the fluid of chronic hydrocephalus.

Antiferments have been found in tuberculous meningitis by Müller, while they are absent in cerebrospinal fever. In syphilitic nervous disease Weygandt found that antibodies are present—that is to say, certain albuminous substances in the normal spleen interfere with hæmolysis, especially when the cerebrospinal fluid of a tabetic is added. According to Merù and Levaditi, 73 per cent. of cases of general paralysis of the

* Otori.

insane contain these antibodies, and 66 per cent. of cases of tabes contain them.

INORGANIC CONSTITUENTS.—Sodium chloride, sodium carbonate, sodium phosphate, and earthy phosphates are met with. The potassium salts are increased in amount in organic nervous disease according to Halliburton. The ratio between Na and K has been made out by various authors to be 1 : 2.43 (C. Schmidt), 1 : 21.6 (Yorn), 1 : 21.5 (F. Müller), 1 : 33.3 (Halliburton). It must be remembered that this excess of potassium salts which has been described is only relative; the sodium salts are absolutely in excess in all cases.

PHYSICO-CHEMICAL CHARACTERS OF CEREBROSPINAL FLUID.

The *Osmotic Concentration*.—Observations by Fuchs and Rosenthal in different diseases have given the following results :

Disease.	Freezing-point Depression.	Osmotic Concentration.*	Pressure in Atmospheres.*
Tubercular Meningitis	0.46	.248	5.5
Dementia Paralytica	0.54	.291	6.5
Chronic Alcoholism	0.54	.291	6.5
Epilepsy	0.52	.281	6.2
Various Cases	0.53	.286	6.3
3898 † Tubercular Meningitis ...	0.337	.181	4.0
3899 † Old Cerebellar Abscess ...	0.524	.283	6.3

The Electrolytes.—Observations of cases in the Leeds General Infirmary have given the results shown in the table on next page.

These results show that the achloride electrolytes are always very scanty, whether the meninges be affected or no. The amount of chlorides is usually high and the total concentration of electrolytes is also considerable. The last column shows a very great uniformity in the different cases.

The electroconductivity varies with the freezing-point depression, except in cases of meningitis.

Ionic Acidity.—Foà records the following values in a case of normal cerebrospinal fluid :

π	log. C_H	$C_H \times 10^{-8}$
.1428	7.2234	5.97 or 6.35

Titration against tornasole gave an alkalinity of $\frac{n}{50}$ KOH.

* I have added these values.

† Own observations.

Viscosity.—Too few determinations have been made to allow any deductions to be made.* In a case in this hospital the viscosity was found to be 1.22, taking water as 1.

CYSTIC FLUIDS

Ovarian Cysts.—At the present day the composition of ovarian cysts is a subject of comparatively little importance. From the theoretical standpoint, however, their colloidal contents possess very great interest. It does sometimes happen, nevertheless, that a fluid supposed to be a peritoneal effusion is really derived from an ovarian cyst, so that the chemical and other characteristics of this class of fluid demand consideration. Among cystic fluids, many varieties of which call for study, the ovarian cysts are the most frequent and possess the most decided characters.

The numerous varieties of ovarian cysts which have been described by gynæcologists frequently show perfectly distinct varieties of contents. The unilocular cysts, as a rule, contain perfectly clear and watery fluid, while the multilocular cysts may contain

TABLE XVII

No. of Case.	ii. Name of Case.	iii. Fluid contains chlorine g. aeq. in litre.	iv. Degree of dissociation "a"	v. No. of molecules + ions.	vi. Specific conductivity of fluid corrected ($\times 10^{-7}$)	vii. Conductivity of iii.	viii. Conductivity of achlorides (vi. - vii.).	ix. No. of molecules + ions pro litre of a solution of Na_2CO_3 with conductivity in column viii.	x. Total concentration of electrolytes (v. + ix.).	xi. ix. - v.
6668	Cerebrospinal Fluid (Tumour)	1294	1260	34	.0990	.3910	0.33
6658	" " (Syphilis)	1259	1240	19	.0990	.3837	0.34
8437	" " (Tub. Peritonitis)	1177	1157	20	.0990	.3460	0.36

* Fuchs and Rosenthal.

different forms of extremely mucinous material in the different loculi; thus, in one cavity there may be a jelly-like mass, another may contain a mucilaginous clear fluid which will just pour, another may contain a bright green solid jelly, and so on. This is a rather remarkable fact, and a section through a cyst of this kind consequently furnishes a very striking appearance. The explanation of such features may be found in the character of the lining cells, for in some cases there is an abundance of goblet-cells in the epithelium, while in others there is relatively little. In other cases, again, the epithelium is perfectly intact and stains beautifully in the histological preparation (Fig. 10), while in other cases the epithelium is practically universally dead, the only indication of the structure of the original cyst being the connective-tissue strands which separate the locules.

It seems so remarkable that there should be these absolute differences in the physical characters of the fluid in different loculi of the same cyst that the question must be worth discussing. In order to arrive at any opinion, we shall have to discuss certain properties of colloids, and discuss also the histological appearances in the cysts. In the first place, the problem may be merely one of a conversion of a sol into a gel (in this instance, of course, a hydrosol into a hydrogel), but if we endeavour to ascertain what explanation there is to be had for the change from sol to gel in general we find that there are abundant theories, but that very little is really definitely understood. Perhaps the simplest explanation is one which takes into account the surface tension. We have already mentioned, when discussing the difficulties of removing albumen completely, that when mastic is gradually added to an albuminous solution, the particles of mastic become distributed over the particles of albumen (hydrosol) without any visible change occurring until a certain point has been reached, when the amount of mastic causes the separation of the proteid, the particles of the latter having become exceeded in number by the former, so that no more distribution of mastic can take place. The same may hold in the colloid of ovarian cysts. The addition of electrolytes, for instance, from the circulating blood may result in the conversion of the hydrosol into the hydrogel, the process being one of modification of the electrical charges origin-

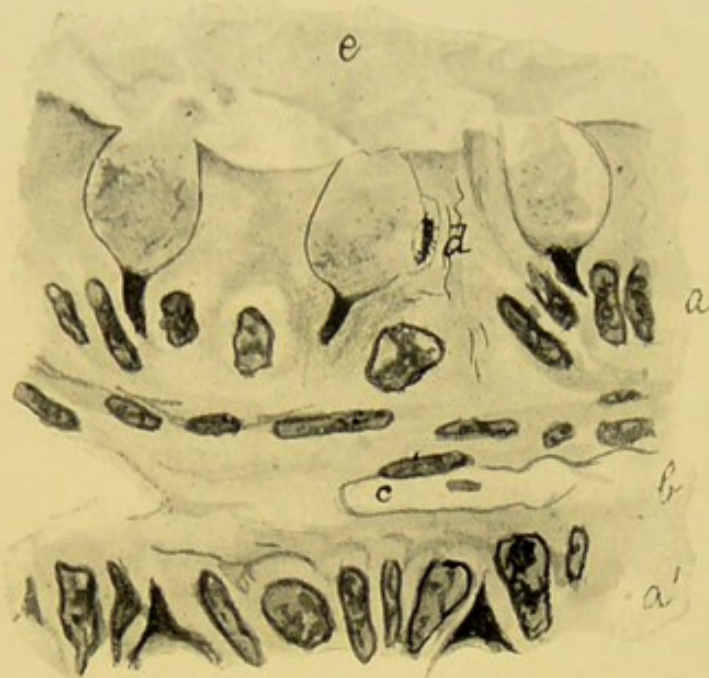


FIG. 10.—From a Cysto Adenoma Papilliferum Ovarii,
to show actively secreting goblet-cells; the secretion here encounters no undue pressure.
a, a, epithelial cells; *b*, supporting tissue of the filamentous process; *c*, capillary;
d, mitotic figure in a goblet-cell, partly beneath the plane of the section; *e* lies in the
cavity of the cyst. Only the basal portions of the cells of *a'* are to be seen.—Zeiss
Homogen Imm., Oc. 4.

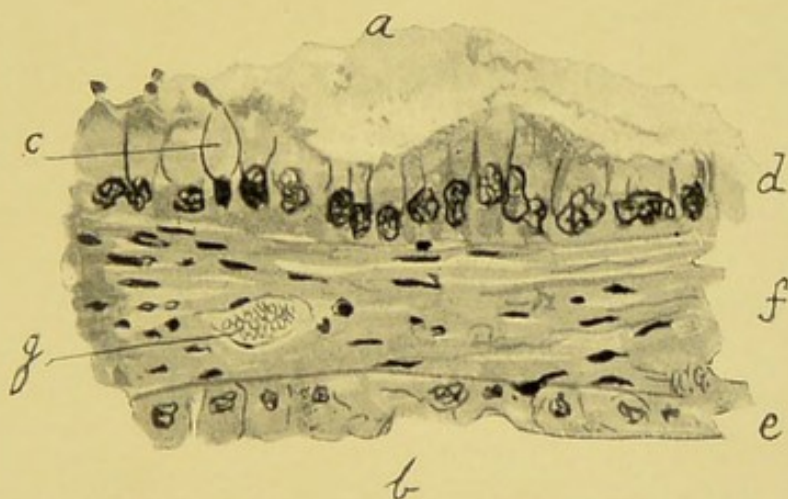
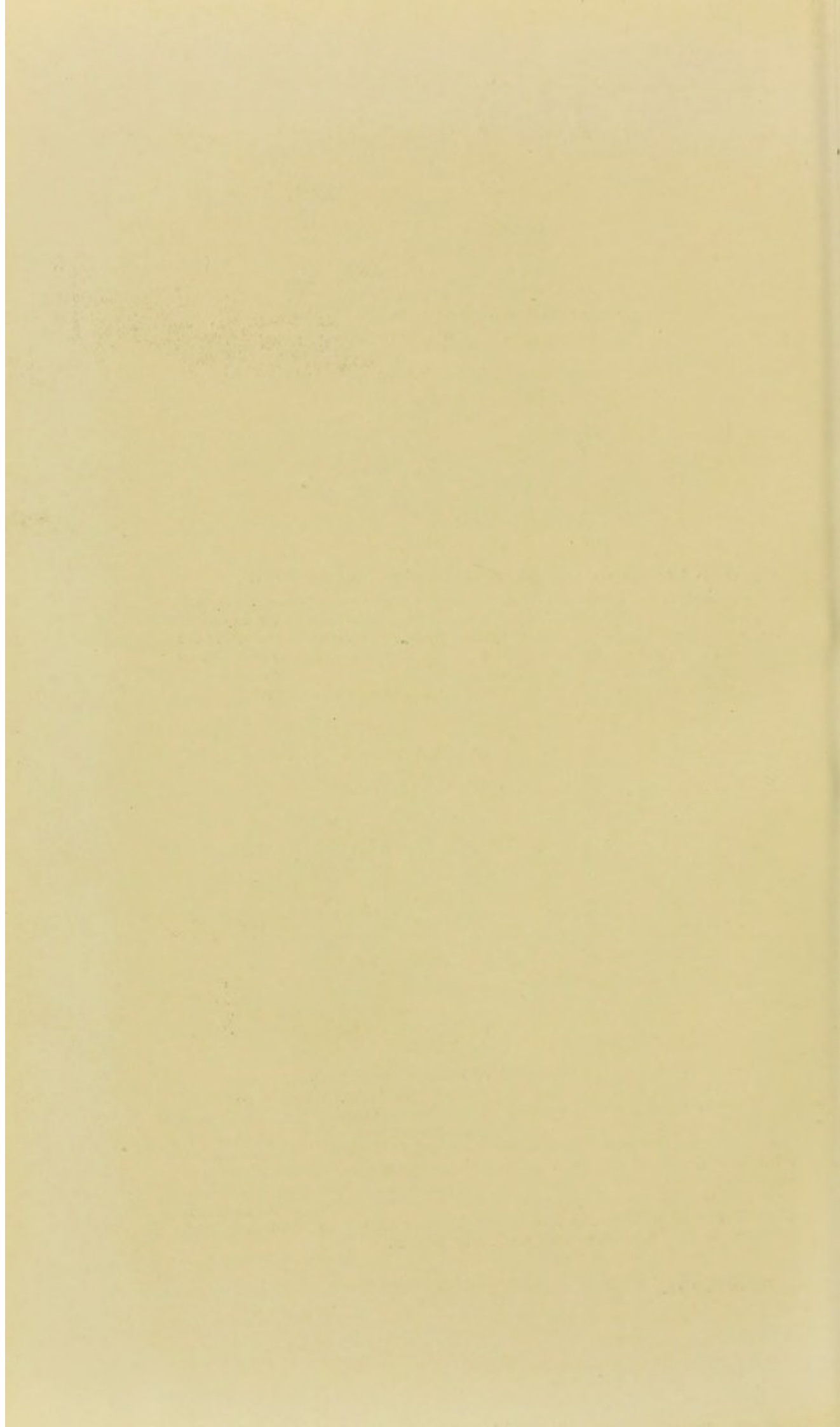


FIG. 11.—From a Multilocular Ovarian Cyst.

The drawing shows the wall limiting loculus *a* from loculus *b*, and on each side there is an epithelial covering, *d* and *e*. The former is composed mainly of goblet-cells in a single row, and the latter of flattened cubical cells, also in a single layer. The stroma *f* is fairly dense, and a capillary *g* is seen in it. Among the goblet-cells, *c* is conspicuous by its sharp contour, and by the exuded drops of secretion. The difference in the character of the secretory cells of the two cysts is striking, and may be used as showing the effects of pressure from excessive secretion in the loculus *b*, although it is in juxtaposition with loculus *a*, where there is no evident internal pressure. In both cases there were gelatinous contents.



ally possessed by the particles of "colloid," * now become so large that they aggregate into a solid substance.

In the case of the capillary electrometer, where the electrical charges of surface tension come into play, there is at the junction of mercury with acid a double electrical layer, which is influenced by an incoming electrical current (change of potential) when the instrument is used. A loss of the positive charge of the mercury meniscus causes its surface to tend to assume a smaller size. So with colloids: according to Bredig, the particles of colloid are surrounded by water, and the junction line possesses an electrical double charge. If the negative charge of the colloid particle be neutralised the surface of the particle will endeavour to shrink from the surrounding water, and, meeting with other particles will tend to associate into complex groups, till by a continuation of the change in electrical sign we ultimately arrive at a point at which the aggregations of particles have attained so large a size that they fall out as a gel.

This would merely be part of what seems to be a general fact, and has much to do with the elucidation of problems of immunity, namely, that a continuous change can be produced in a colloid without the slightest indication to the naked eye, as it were, until a certain point is reached, when the conditions are so strained that coagulation, or precipitation, or what not, takes place.†

The fact that electrolytes have something to do with this change of sign demands no more detailed consideration in this place, because the process is the same as described above, and it is speculation to consider whether more chlorides or less, or more phosphates or less, or more or less of any other salts, do at any particular time enter into the contents of loculi.

The most evident feature of the walls of these cysts on microscopic examination is the goblet-cells to which we have referred already, although they deserve a more careful consideration. There is a decided secretory activity going on in all quarters

* It is a pity that the term "colloid" should be applied to the material in ovarian cysts at the same time as it is applied to *matter* in a definite physical state.

† In boiling egg-white, for instance, one is disturbing the electrical charge of the particles. This again illustrates what I contend is the difficulty in chemical study of fluids. We do not realise that even simple boiling may be exerting very far-reaching effects.

—cells nearly all laden with secretion, others emptied, others showing active mitotic figures. The secretion is presumably fluid, even though viscid. Can this secretion become solid (gel) as a result of the processes detailed above, or may there be another cause? For instance, there is a possibility that the secretion may continue to such an extent within a limited sized cyst that enormous pressure is mechanically exerted by the non-expanding cyst wall on its contents. Such condition could well vary in different loculi, and a difference in viscosity of adjoining cysts would hinder or allow expansion of the neighbouring cysts. In this connection, then, the first problem is that of discovering whether enormous pressure can alter the state of a colloidal substance, and if so, by what means, and the second is a histological one, namely, as to whether there is any evidence of pressure in these cysts.

In answer to the first question, we may refer to the observation made by Pauli, that the delicate layers of intercellular material which surround cartilage cells may come to exert enormous pressures when they absorb or gradually give off water, as is shown in the process of formation of compact bone. Thus, some proteid matter was forced into steel tubes under enormous pressure, and became so ivory-like that it could be worked with tools.

Again, it has been computed that in order to remove the merest suspicion of water from gelatine which has taken up 8.4 parts in the 100 of water, would require a pressure of more than 200 *atmospheres*.*

We have, then, to take the factor of imbibition into account as a possible one in the production of the jelly-like matter of ovarian cysts. That is to say, the colloidal matter secreted by the goblet-cells may take up water, and in so doing become so swollen up in a given space that it has become of the consistence of a jelly. Under this supposition, the particles of colloid take up water like bibulous paper, for instance: each molecule not only becomes increased in size, but there is less water remaining in solution.

The laws governing the process of imbibition of water by a colloid such as agar have been expressed in the following mathematical formula by Hofmeister: Let W be the weight of water

* Nagel.



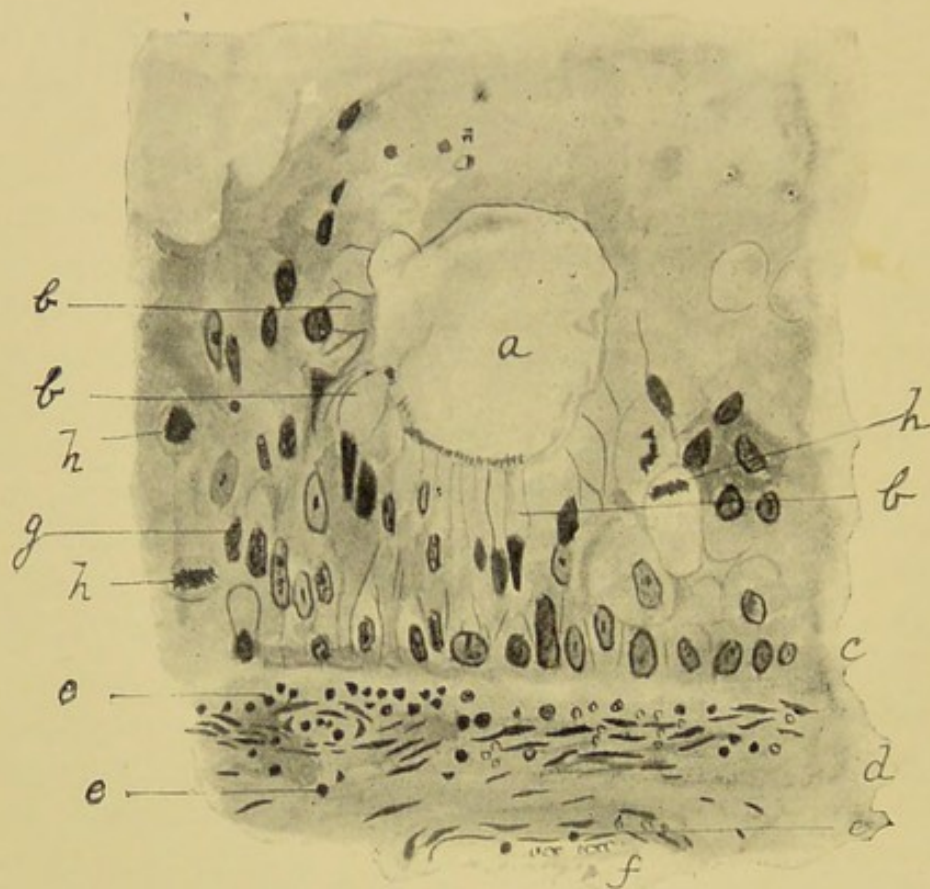


FIG. 12. — From a Cysto Carcinoma Ovarii.

The drawing shows the epithelium covering one side of the fibrous trabecula, which separates one loculus from another. The growth is not papilliferous.

The minute cyst *a* is enclosed by goblet-cells *b, b, b*, in varying stages of secretion. Some are empty, others swollen, and the exuded secretion can be seen adhering round the stomata of the cells. In some of the cells there appear to be minute channels (rather too dark in the drawing), through which the secretion has to pass before entering the cavity *a*. *c*, basal cells, probably about to form goblet-cells; *d*, fibrous trabecula supporting the epithelium. This trabecula consists mainly of short spindle cells with slender nuclei, and lymphocytes *e, e, e* (exuded from the walls of the capillary *g*), infiltrate the fibrous tissue. *g*, mesial cells of the stratified epithelium. *h*, mitotic figures.

The specimen shows the commencing formation of a cyst, which is going to expand not only by excess of secretion, but also by the actual growth of the epithelium. In this case the jelly-formation is due to a subsequent change in the original mucinous secretion.

absorbed during a time t , and P be the maximum amount of turgescence possible at the given temperature, and D the thickness of material which is taking up the water in millimetres, then—

$$W = P \left(1 - \frac{1}{1 + k \frac{t}{D}} \right)$$

where k is a constant.

In order to work this formula out for the conditions which exist in the case of ovarian cysts, several experiments would be necessary. We will, however, pass on to consider the second question—whether there is any evidence of pressure within the cysts.

In many examples with which one meets, the pressure exerted by the contents makes itself very evident by the spurt which results on making an incision into a cyst, and it is remarkable how flaccid are the walls in the case of some jellies, while in other cases the material at once swells out on cutting the tumour open.

Microscopic examination shows a remarkable richness in goblet cells, which consist of clear cytoplasm, and relatively large triangular nucleus at the lower end of the cell (Fig. 10), while the upper end is widely dilated and contains granular matter presenting somewhat the arrangement of a network thickened here and there by granules. Some of the contents can be seen oozing out of the free end of the cell, although in other cases (Fig. 12) there seems to be a cap of striated substance, as if there were numerous minute channels through which the secretion had to pass. Observations on living goblet cells by Merk showed that movements may occur within the goblet, the granules becoming darker or lighter from time to time, while granules come to the surface and explode, as it were, becoming discharged into the surrounding medium, and lost in it, "like smoke from a chimney." This is well seen in Fig. 10, and it indicates that when the secreted substance comes outside the walls of its manufactory, it undergoes imbibition with water, and so alters its physical characters.

All these cells are large and conspicuous, though here and there there are smaller cells, apparently empty (Fig. 12), and in other places again (Fig. 11e), the cells seem to be devoid of

secreting activity altogether. The presence of numerous mitotic figures and the full distension of the goblets, however, show that a very striking degree of secretory activity is present throughout the epithelial lining of these cysts.

Changes in the nucleus may also be observed, and it has been supposed by Krause that it is concerned directly with the process of secretion. Thus, in the salivary glands of cephalopods he finds that the nucleus exerts a ferment action during secretion, the ferment being of an albuminolytic nature. During secretion the nucleus lies near the base of the cell, and becomes increased in size by nearly 20 per cent., besides losing some of its staining power and showing granulations to a much more noticeable extent than is the case in the resting cell. Then, again, the nucleolus may have something to do with the process, being more conspicuous and even multiple or showing a spiral thread-like structure during secretion (Nussbaum).

The substance secreted is a *mucin*, and one of its characters is still seen in the case of jelly-containing cysts, where so much glucosamine is found. We may suppose that the glycoprotein of the colloid matter in the cyst only differs from ordinary protein in having relatively many more glucosamine radicles per molecule, and if this be so, it is not so difficult to understand how the cytoplasm may give rise to mucin. We may, for instance, say that cytoplasm of goblet-cell = x albumen + y glucosamine, but secretion of goblet-cell = $(x-z)$ albumen + $(y-z)$ glucosamine, where x and y are definite but not known quantities, and z is a variable, and not necessarily the same in the case of the albumen as it is in the glucosamine.

That pseudo-mucin as met with in ovarian cysts with fluid-contents is exceedingly like "paralbumen," or paramucin in chemical composition, was shown by Otori, who found that both possessed identical decomposition products. We may illustrate this by the following:

Substance.	C	H	N	S	O	Author.
Mucin of saliva	48.84	6.80	12.32	0.84	31.2	Hammarsten
" sputum	48.17	6.91	10.8	1.42	31.7	Müller
Pseudo-mucin of ovarian cysts	49.8	6.9	10.27	1.25	31.78	Hammarsten
Paramucin of ovarian cysts ...	51.76	7.76	16.7	1.09	28.69	M tjukoff

The paramucin contains more C and H and less O.



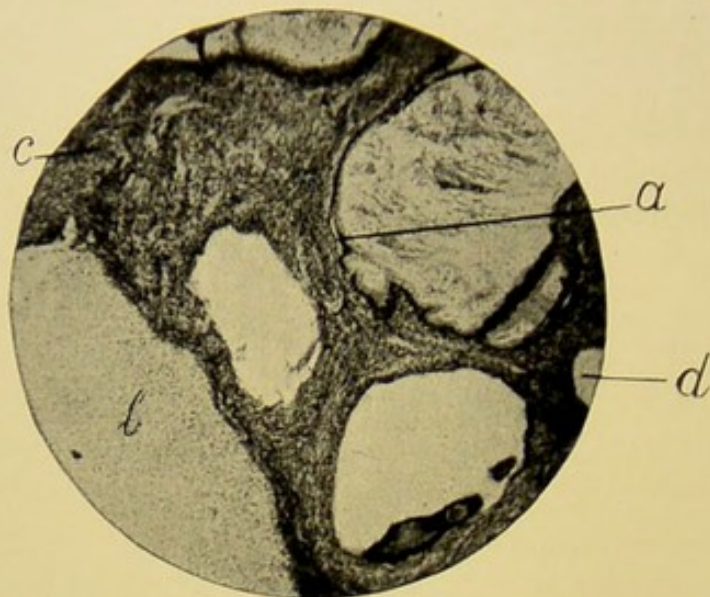


FIG. 13.—From a Multilocular Ovarian Cyst.

The specimen shows several of the loculi, which are of different sizes. *a*, intact lining epithelium of decided cubical shape. This cyst contains semi-transparent contents; *b*, a larger cavity filled with granular matter (shows *débris* of cells with high power)—the lining membrane has disappeared; *c*, interstitial tissue densely infiltrated with mononuclear cells (lymphocytes, connective-tissue cells, etc.); *d*, a minute cyst containing homogeneous material, and devoid of lining membrane.

(The details of the original micro-photograph have suffered greatly in this reproduction.) *Mag. 80 diam.*

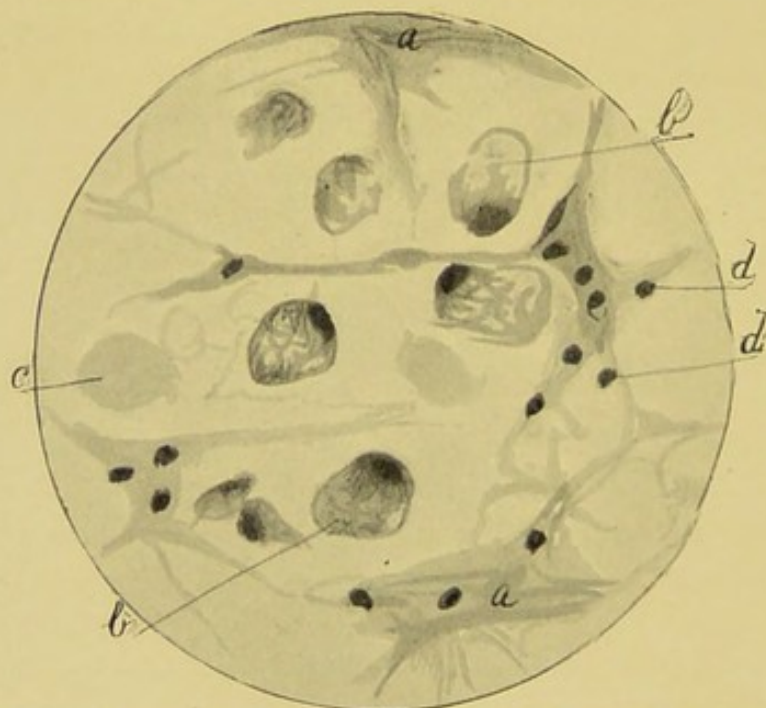


FIG. 14.—Dead and Dying Cells from a Colloid Carcinoma.

This illustrates another method of formation of "colloid" material—direct metamorphosis of the necrosed cells.

a, a, remains of the trabeculae supporting the cells of the growth; *b, b*, cells whose nuclei are still visible, though their shape is lost; *c, c*, "shadows" of carcinoma cells; *d, d*, living cells (connective tissue cells, and other wandering cells).—*Zeiss Homogen Imm. Oc. 4.*

To face page 179.

It must, however, be admitted that the substances which may be present in ovarian cysts cannot be limited in number to the two indicated on this table. It is probable that there are many varieties of "pseudo-mucin," which may be shown by very slight, but nevertheless distinct, variations in chemical properties.

The well-development of these goblet-cells seems to cast doubt on the existence of pressure within the cyst, whose wall they line, but it nevertheless seems to be a fact that even tense cysts may show a perfectly well-formed goblet-cell epithelium. This is contrary to the well-accepted idea that cavities containing fluid under pressure have a flattened or cubical lining. This may be so in many cases, but it is certainly not so in all. In Fig. 11 it will be seen that on one side of a small loculus the cells are large and conspicuous, while on the other side they are low and cubical. In the latter loculus the contents may have been tightly packed, but in both cases there was "colloid" material. (See also Fig. 12.)

In a section prepared from a cyst whose contents were entirely gelatinous in consistence (Fig. 13), it will be seen that the cells are large and well formed. In another specimen, where the fluid spurted out on opening the cyst, and there was an adenomatous growth (papillary) all over the walls, the goblet-cells are seen to be peculiarly well formed (Fig. 10). We must therefore conclude that the gelatinous material is due perhaps to an excess of mucinous material in the secretion, but, nevertheless, to some physical change which the secretion has subsequently undergone. This change will be either one of imbibition or will be due to a change in the electrical properties of the material, under the influence either of water or of some electrolyte, as already referred to.

The question as to whether the mucin is ever *derived from broken-down cells* may be considered for a moment. It is certainly the fact that if we search through the material in the microscopic cysts, we shall find many desquamated cells. It may be presumed that in the colliquative process which has taken place (Fig. 14), special mucinous bodies are liberated, which may have something to do with the question. The fact that nucleoproteid such as is met with in the substance of the nucleus may have some chemical relation to the production

of mucin is of interest, though a comparison of the formulæ of the two substances shows that unless there are many carbohydrate radicles in the nucleoproteid in question, there will not be a possibility of its conversion into mucin. The relative abundance of degenerating cells, still less of shed cells, as compared with those which are evidently actively secreting, is, however, so small, that this factor does not demand much serious notice. The formation of "colloid" material must be referred to physical changes occurring in the mucinous *secretion* of the goblet-cells so characteristic of these ovarian cysts.

While the mucinous substance contained in ovarian cysts is usually a glycoproteid of the type fully described in Section I., Halliburton has described a case in which true mucin is present. Hammarsten says that no mucoids occur in the cyst if it be derived from the Wolffian body.

The following analyses by Halliburton will show the percentage composition of the fluids from various ovarian cysts in a convenient form :

Kind of Cyst.	No. of Specimens examined.	Sp. gr.	Total Solids.	Proteids.	Peptone.	Mucin.	Salts.
Colloid ...	24	1010-1038	28-75%	8.8-108.3%	0	+	6-8.7%
Papillary ...	2	1036	116.4	102.67	...	±	-
Hydrops. folliculate ...	2	1009	-	-
Hydrops. tubæ ...	2	1008	10.5-11	1.2	...	-	6-7
Fibrocystic ...	1	63.056*	...	-	-

The following details about cases which have come under one's own observation will illustrate the characters of different types of ovarian cyst.

A specimen of multilocular papillomatous ovarian cyst† showed the following varieties of fluid in different loculi: (1) a thin watery opalescent fluid having a conductivity of 1233 (at 25° C.); (2) a clear straw-coloured fluid having a conductivity of 1244; (3) a viscid, grumous-looking material with a conductivity of 1216, and (4) a jelly of dark green (fluorescent) colour whose conductivity could not be measured with the apparatus. There

* Fibrin 3.58 per cent.; globulin, albumen.

† No. 6643.

was the merest trace of chlorides in the watery fluid (0.06 per mille). The specific gravity of this type of cyst-fluid is usually 1020 and the amount of solids 2 to 5 per cent. The colloid matter is of course a glycoproteid in chemical reactions, but cholesterin, urea, and fat may be present.

A specimen of *parovarian cyst* * contained a clear, highly albuminous fluid (2.9 per cent.) of watery consistence which was moderately rich in chlorides. The conductivity at 18° was 1168, corresponding to an electrolyte content of 0.241. The specific gravity is below 1010 and the solids amount to only 1 or 2 per cent. This type of fluid contains only a trace of pseudo-mucin.

A unilocular *broad ligament cyst* which had been known to exist for 33 years † contained a brown muddy fluid remarkably rich in cholesterin. There was only a moderate amount of albumen, and the conductivity was low. The ferment-content was considerable (see Table X.). Leucin was found in this fluid; a trace of levulose; the α -naphthol test gave no definite test, while the glucosamine test produced a well-marked reaction. The chlorides amounted to 3.6 pro mille. These intraligamentous cysts usually have a fairly high specific gravity (1035) and contain 9 or 10 per cent. of solids.

A unilocular cyst with dark brown fluid-contents is referred to under the appropriate headings in Sections I. and II.

An *ordinary multilocular ovarian cyst* ‡ in a patient aged 21, contained a clear watery (slightly mucilaginous) fluid, of specific gravity 1009. Proteids were scanty in amount, and were mainly albumen, protalbumose and hetero-albumose being absent. There were marked metalbumen reactions. There was no urea, no purin bodies § or lecithin. The osmotic concentration was 0.286, the chlorides were abundant (0.145 gm. equiv.), and the concentration of the electrolytes 0.264. No ferments were detected. One or two mononuclear cells were alone seen, and some stellar phosphates were found.

A case of *papillomatous ovarian cyst* with an opalescent fluid contained 2 per cent. of albumen, 6 pro mille of chlorides, and a

* No. 8177.

† No. 8364.

‡ No. 7618.

§ *i.e.* detectable in the quantity of fluid obtained.

conductivity of 1075, so that the electrolyte-content was 0.243. The osmotic concentration was .560. The usual glycoproteid reactions were obtained.*

Lastly, special reference may be made to a case † of a multilocular ovarian cyst which contained cholesterin, the typical cellular elements, and had a remarkably small chloride-content (.05 pro mille), with a considerable amount of albumen (2.5 per cent.). The concentration of the electrolytes was .146.

The contents of dermoid cysts are well enough known. Among many other substances they contain oleic, stearic, palmitic, and myristic acids, cetylalcohol, and cholesterin.‡

PHYSICO-CHEMICAL CHARACTERS.—The osmotic concentration is similar to, or most frequently less than, that of normal blood. The following values will be of interest :

TABLE XVIII
OSMOTIC CONCENTRATION OF OVARIAN CYST-FLUID

Fluid.	Freezing-point Depression.	Osmotic Concentration.	Osmotic Pressure in Atmospheres.	Author.
Cystoma Ovarii543	.293	6.5	Zangemeister
" "531	.286	6.3	"
" "546	.294	6.5	"
Parovarian Cyst552	.298	6.6	"
Multilocular Cyst531	.286	6.3	O. C. G.
Papillomatous Cyst560	.302	6.7	"

The electrolyte-concentration has been studied by me in several cases where the cyst-contents were sufficiently fluid. The results are given in the table on opposite page.

Reference may here be made to a case reported by Nassauer in which an abundant foul-smelling fluid was discharged per vaginam, having the appearance of clotted milk. The fluid was of a mucoid character and contained flakes of coagulated material, and stiffened linen. He states that this fluid was derived from a *tubo-ovarian cyst* which had ruptured into the tube. There was no examination of the fluid itself, beyond the points which have been mentioned.

* 6151.

† 6179.

‡ Ludwig and Zeynek.

TABLE XIX
ELECTROLYTES OF OVARIAN CYST-FLUIDS

No. of Case.	ii. Nature of Case.	iii. Fluid contains chlorine g. aeq. in litre.	iv. Degree of dissociation "a"	v. No. of molecules + ions.	vi. Specific conductivity of fluid corrected ($\times 10^{-9}$)	vii. Conductivity of iii.	viii. Conductivity of achlorides (vi. - vii).	ix. No. of molecules + ions pro litre of a solution of Na_2CO_3 with conductivity in column viii.	x. Total concentration of electrolytes (v. + ix.)	xi. ix. - v.
6643	Multilocular Cyst ...	·001	·970	·0019	1042	20	1022	·1442	·1461	6·01
6151	Papillomatous Cyst ...	·040	·880	·0752	1075	360	715	·1680	·2432	2·23
7618	Intraligamentous Cyst	·145	·821	·2640	1080	1080	0	0	·2640	0·00
6179	Multilocular Cyst ...	·001	·970	·0019	1104	20	1084	·1442	·1461	6·01
8177	Parovarian Cyst ...	·058	·865	·1082	1168	551	617	·1333	·2415	1·23
8364	Unilocular Cyst ...	·061	·861	·1150	1064	584	480	·1066	·2216	0·93

Hæmatometra.—Zangemeister records a case of atresia of the hymen in which a fluid was obtained having an osmotic concentration identical with that of the blood (0·301).

Gelatinous masses have been described as occurring in the peritoneum as the result of rupture of an ovarian cyst.

An interesting case of this kind is recorded by Hueter as occurring in a male subject. The cyst had arisen from the rupture of an appendix which had undergone extreme cystic distension, and a quantity of sterile mucilaginous matter was found in the peritoneal cavity. Two other cases have been recorded, one by Fränkel and the other by Merkel.

The *pseudo-myxoma peritonei*, when it bursts into the peritoneum, may give a characteristic appearance to the puncture-fluid, either from the presence of gelatinous masses, or from the presence of a large amount of mucinous matter. In this case the fluid will be tenacious, homogeneous, yellowish or whitish in colour, and give strong mucin reactions, being completely precipitated by acetic acid. Boiling scarcely alters the appearance of the fluid, since there is very little albumen present. There is no pseudo-mucin, but paralbumen has been described as abundantly present (Marchand). The presence of very granular cells may also be noted in the material in the peritoneal cavity. This is owing to the liberation of the so-called compound granule cells out of the cyst cavity into the peritoneal cavity.

Pancreatic Cysts.—The most recent account of these cysts is that which is given in the work on Diseases of the Pancreas by A. W. Mayo Robson and P. J. Cammidge. They describe pancreatic cysts as being either true or false (distension of lesser peritoneal sac, localised collection of fluid in the vicinity of the pancreas). The true cysts are due either to retention of secretion, to hydatid, to new-growth, to hæmorrhage, or are of congenital origin.

The fluid is of varying appearance and consistence, being sometimes watery and sometimes of mucous or gelatinous consistence. The colour may be brownish (as is usual),* or greenish, or the fluid may even be purulent. The hæmorrhagic fluids probably indicate that the cyst is false.† The odour is stale.

* Lillenstein, also my own cases.

† Schmidt.

Normal pancreatic juice may contain :

	Zilwa.	Schumm.	Glässner.
Specific gravity	1009	1007
Freezing-point depression	0.61°	...	0.46°
Water	98.5%	98.5%	98.7%
Dry matter... ..	1.5	1.5	1.3
Albumen	0.0	0.1	0.17
Nitrogen	0.07	0.08	0.1
Ash... ..	1.0	0.85	0.56
Organic matter soluble in alcohol	0.56	0.51
Alkaescence in terms of soda	0.49%	0.45%	...

The fluid contained in a pancreatic cyst, on the other hand, may have the following composition :

Specific Gravity ... 1010—1020—1060

Solids	24.1—23.8%	Chlorides	5.8
Ash	6.2—9.2	Phosphates	0.16
Organic Matter	17.9—18.5	Sulphates	Traces
		Calcium and Magnesium	0.5
		Iron	Traces
		Copper }	Traces
Albumen	5.2%		
Globulin... ..	0.6		
Mucin	present		
Albumose	3% (no peptone)		
Urea	Traces 0.14%		
Uric acid	Trace		
Sugar	None		
Acetone	0.05		
Fat and Cholesterin	0.16		

Several cases of pancreatic cyst-fluid which have been examined gave the following analysis :

TABLE XX
PANCREATIC CYST-FLUIDS

No.	Reaction.	Colour.	Sp. gr.	Albu- men.	Ferments.	Freezing- point Depression.	Chol- esterin.	Blood.	Mucin.
8309	Alkaline	dark							
		brown	1022	5.98%	invertase,	...	++	+	+
8347	"	dirty			trypsin	...	++	+	+
		brown	1022	2.6	trypsin	...	++	+	Trace
5744	"	dark	1012	10.0	trypsin	...	0	0	...
5919	"	straw	1010	0.401	0	0	...
4157	"	bile- stained	trypsin	...	0	0	...

The solid material from two specimens was found to consist

of fatty acids or their salts, cholesterin, and potassium carbonate.

Another specimen, having a specific gravity of 1012, contained a maximum quantity of albumen, but neither sugar nor cholesterin. This fluid coagulated spontaneously. Proteolytic ferment was detected.

The ferment-content of such fluids is the most characteristic property which they possess. The following ferments may be found :

Trypsin is generally present, but may be absent, especially in cystic adenoma of the pancreas.*

Pepsin has been found (Schmidt).

Diastase.—Equal parts of starch paste and fluid will give a reaction with filtering in an hour (Schumm). This ferment is, however, present in many fluids (see Section I. Table X.) and was *not* found in a case described by Lillenstein.

Lipase has been looked upon as characteristic of this class of cyst, though reference to Table X. will show that it has been met with in other fluids.

The method of testing for these ferments has already been gone into sufficiently fully in Section I.

OTHER CONSTITUENTS.—Chlorides are scanty; phosphorus is not present.†

Hæmin crystals have been obtained from the deposit in these fluids, and cholesterin may be readily detected on evaporation of an ethereal extract ‡

* Robson and Cammidge.

† Schumm.

‡ Tests for *Cholesterin*.

(1) *Conc. H₂SO₄* and a trace of iodine turn the crystals violet, then blue, through green to red.

(2) Hesse-Salkowski.—Dissolve in chloroform and let *conc. H₂SO₄* run in. Shake a little. The chloroform turns blood-red, then cherry-red, and the acid becomes fluorescent (moss-green). Pour the chloroform into a watch-glass, when the colour passes through blue and green to violet.

(3) Denigés.—Dissolve in chloroform and add $\frac{1}{2}$ vol. *conc. H₂SO₄*. Now add a few drops of acetic anhydride to the chloroform layer. A brilliant red turns to a blood colour in *H₂SO₄*.

(4) Liebermann.—Dissolve in acetic anhydride. Add *conc. H₂SO₄* drop by drop when cold. The solution becomes rose-red after a time and then turns blue, and finally green.

(5) Treat the alcoholic solution with δ -methyl furfural solution and run in *conc. H₂SO₄*. A rose-red ring appears at the junction line. On

Glucose and Glycoproteids were not present in Schumm's case.

Proteids and their Derivatives.—While albumen and albumoses are present in varying amount, the derivatives leucin and tyrosin are frequently conspicuous * and may be found by simply allowing the fluid to evaporate in a small watch-glass.

The variations in composition have been suggested by Lillenstein recently to be due to the anatomical structure of the cyst. If there be a lining epithelium the contents will be different from those cases in which the fluid has been extravasated (no definite lining membrane).

The Osmotic Concentration.—Pinkussohn has determined the freezing-point depression of normal pancreatic juice, and found it to vary from 0.58 to 0.66°C .

It will be seen from Table XX. that the fluid from a pancreatic cyst showed a depression of 0.401°C ., corresponding to an osmotic concentration of 0.217 . Another fluid examined from the physico-chemical standpoint showed a conductivity of 1430 at 24.4°C .

Thyroid Cysts.—A report of Hoppe-Seyler's shows that one may expect to find mucin, 7 per cent. proteid, cholesterin, and calcium oxalate. The dark colour of these fluids may be due to methæmoglobin or even to altered bile-pigment.

Abdominal Cysts.—This loose clinical term refers to all

shaking, the fluid turns a deep red, giving an absorption band beginning just before E and ending at b. (Neuberg and Rauschwerger's reaction.)

(6) Schiff's reaction.—Gently warm with a little FeCl_3 and HCl in a porcelain capsule till nearly dry. The edges turn violet-red. Cool. Add chloroform and some hydrochloric acid. Evaporate to dryness and heat. The whole of the capsule becomes purple-red, then blue-violet, then dirty green.

(7) Evaporate with HNO_3 in a porcelain dish. A yellow colour appears, turning red with ammonia.

(8) Dissolve in glacial acetic acid. Add acetic anhydride and zinc chloride, a rose-red colour with a greenish-yellow fluorescence appears, especially after a 5' boil. This test is more delicate than test 4.

(9) If mixed with fat, put the substance in a sealed tube with benzoic anhydride. Boil with alcohol and crystallise out the residue with ether. Rectangular plates will be found.

(10) Hirschsohn's reaction.—A solution of trichloroacetic acid in HCl gives a red colour.

(11) Obermüller's reaction.—Melt the crystals with a few drops of propionic anhydride. On cooling, violet colour appears, turning green, orange, and then copper red.

* Schumm, Zeehuisen.

those cysts which are met with in the abdominal cavity, and whose nature has not been determinable at the operation.

(1) Two specimens which have been examined were of this undetermined origin. One was associated with a carcinoma recti, and had a specific gravity of 1006, was of straw colour, neutral in reaction, and contained only a trace of albumen. Mucin, blood, and cholesterin were present, and microscopic examination showed groups of rounded cells. In the other case * cholesterin crystals also formed a characteristic feature, though the chief examination made was of the cells, which were mainly red cells and polynuclear cells (streptococcic infection).

One would be inclined to regard both these cases as having been mesenteric cysts, though in the absence of post-mortem examinations it is not certain that they were not retroperitoneal.

(2) Here may be mentioned a case of cystic lymphangioma of the peritoneum which has been recorded, and which is referred to by Hueter as being probably a mistaken diagnosis for pseudomyxoma peritonei (see p. 184), with gelatinous masses in the peritoneum.

(3) Another class of abdominal cysts is formed by *mesenteric cysts*, which form the subject of an inaugural dissertation by Theodor Hein (Leipzig, 1907). In one cyst of this kind Zangemeister found an osmotic concentration of 0.299.

A case of mesenteric cyst was described in 1907 by Niosi, who points out that these cysts are very important, from their occasional simulation of ovarian cysts. There are several possible varieties: hæmorrhagic, chylous, serous, dermoid,† hydatid, and angiosarcoma,‡ and congenital cysts (from omphalo-mesenteric duct). Other forms have been supposed to arise from softening of the mesenteric glands as a result of such infectious diseases as typhoid, tuberculosis, etc. The very extensive literature is fully quoted by Niosi. The case which he describes at great length was one of a cyst containing a turbid, tenacious, dark chocolate-brown coloured fluid of specific gravity 1040, and neutral reaction. Its composition was 89 per cent.

* No. 5905.

† S. Kostlivy.

‡ See Pfennig—über retroperitoneale Dermoidzysten. "Inaug. Diss." Bonn, 1907. See also H. Max: Zur Kasuistik der Mesenterialzysten (causing pernicious vomiting). "Inaug. Diss." Leipzig, 1907.

water, 10 per cent. organic substances, and nearly 1 per cent. salts. Traces of mucus and peptone were found, and 0.298 per cent. of fat, the remainder of the organic matter consisting of albumen and globulin. There was neither urea nor sugar present. The salts comprised NaCl 6.76 per cent., traces of S and P, Ca and Fe. The cellular elements were granular corpuscles and fatty epithelial cells, and there were crystals of cholesterin. Niosi assigns to this cyst a congenital origin, connected with the Wolffian bodies. There was no adrenal tissue present.

(4) Another cystic *retroperitoneal* tumour is described by Heyrovsky. The tumour was the size of an adult head, and contained mucoid material. It had existed for seventeen years, and was found to be a papilliferous cyst containing goblet-cells which formed mucus and then broke down into a colloidal carcinoma. Retroperitoneal cysts are usually pancreatic or hydatid, but may be due to softening of a sarcoma.

(5) Dermoid cysts, arising by implantation from a ruptured ovarian dermoid.

(6) Hæmorrhagic cysts, arising after traumatism.

(7) Hydatid cysts.

(8) Omental cysts, which may be either hæmorrhagic or dermoid.

(9) Peripancreatic cysts, due to closure of the foramen of Winslow by peritonitic adhesions.

(10) Urachal and allantoic cysts due to congenital malformations—all these belong to the class of abdominal cysts. Ovarian cysts, pancreatic cysts, and the swelling of a hydro-nephrosis also, strictly speaking, come under this head. The characters of the fluids need not be enlarged upon in this place.

Cysts connected with the Liver.—These have been found to contain cholesterin if they are connected in any way with the biliary passages. Bile-pigment may therefore be expected as well. Such characters are also noted when the cyst is connected with the *gall-bladder* itself. Probably these cysts are too small to come under the notice of the clinician, and are usually post-mortem findings.

Other liver cysts are, however, met with, which are due to the *breaking down of new growths*. Hosch records such a case, which he says was secondary to a cancer of the stomach.

The only case of the kind which I have observed was one

of breaking down angiosarcoma of the liver of enormous size (No. EE46, Pathological Museum, University of Leeds). The patient was aged fifty-six, and he had had pain in his left side for four months before admission to the Infirmary, and noticed a swelling in the hepatic region only during three months. An orange-sized tumour could be felt, which fluctuated in the centre. A diagnosis of hydatid cyst was made, but at the operation masses of blood-clot were found in the cavity, and these, on microscopic examination, were found to consist of spindle and round cells. It was not till the patient's death, which occurred soon after, that the real nature of the case could be appreciated.

BILE.—In this connection it may be interesting to mention that *bile* has an electroconductivity, ranging from 110 to 1600 10^{-5} at 35°C., the conductivity having no relation to the viscosity or to the intensity of the coloration. On the whole, however, the golden-coloured biles have a low conductivity, while the dark brown and dark green biles have a high conductivity.

The degree of dissociation varies from 0.25 to 0.51, which shows that no constant rule can be made from a study of this question.

The fact that the osmotic and electrolyte concentration of bile may vary with the time of day shows it to be a fluid with very variable characters. Thus, Engelmann found a steady rise of osmotic concentration from 0.308 at 7 a.m. to 0.329 at 2 p.m., after which there was a fall to 0.310 at 6 p.m. and again a low rise to 0.319 at 8 p.m. The conductivity was 0.131 at 7 a.m., and rose to 0.138 at 2 p.m. and then fell to 0.133 at 6 p.m.

Dilute acetic acid does not precipitate any body like the serosamucin of Umber, and the α -naphthol test, which has been applied, has, however, given a positive reaction, though the glucosamin test of Ehrlich failed to give any reaction. A body giving the reactions of pseudo-mucin has not been found.

Renal Cysts.—A few of those which have been examined have shown varying characters. The chief point of note has been the absence of urea or uric acid, which shows that these substances cannot be relied on for making a diagnosis of renal origin as opposed to cysts of other organs. Indeed, unless the "cyst" be really a distended pelvis, i.e. a hydronephrosis, and unless there is some functioning kidney substance as well, so as to allow much urea to be present, one cannot distinguish a

renal cyst. In a cyst,* which proved to come from a hypernephroma, there was found a considerable quantity of albumen, which was probably due to the presence of much blood; there was an excessive amount of cholesterin, which had appeared in the urine. The content in chlorides was very small, but there were copious oxalate crystals, and also cystin. Both these substances were sufficient to prove the *probability* of the cyst being connected with the kidney. There was neither urea nor uric acid. There were no cellular elements to form a guide.

Another specimen was diagnosed as from a cystic kidney † on these characters: specific gravity, 1011; albumen, 1 to 4 per cent.; urea, trace; chlorides, 4.75 per cent.; conc. elect., 0.168; conc. achlor., .087. In a case of hydronephrosis opened by the surgeon the fluid contained 1 per cent. urea.

Reference may be made to a *paranephric cyst* which was due to rupture of the pelvis of the kidney, recorded by Hildebrand.

It is impossible to pass in review the chief points which will enable a fluid to be distinguished as URINE. But it is needful to mention that there is occasionally (apart from the cases described above) a necessity for this. Thus, a fluid was drawn from a cystic swelling ‡ in the posterior vaginal wall, and it was only from examination of this fluid that its origin from a dilated ureter that had become malplaced was possible. There was an abundant effervescence, with sodium hypobromite, the reaction was *acid*, the chlorides amounted to 3.9 per cent. and the conductivity at 23° C. corresponded to a concentration of electrolytes of 0.158.

The acid reaction of such a fluid makes the diagnosis practically undoubted.

In cases of *hydronephrosis* the fluid will vary in composition according to the degree of functional activity of the kidney relics. The specific gravity is generally less than 1010, the amount of albumen may vary from mere presence to 8 per cent., and the same is true of the salts. The presence of creatinin and of urea or uric acid would not necessarily exclude, say, hydatid cyst in the abdomen. §

* No. 4484.

† No. 6685.

‡ Record No. 6047.

§ Cf. Nos. 5869 and 6987.

In other words, there are none of the characteristic bodies present which would lead one to identify the fluid as renal.

On the other hand, if there is some outlet to the flow of urine, and if the kidney is excreting tolerably well, in spite of its functional power being much diminished, there will be some urea and other urinary constituents present. The *amount* of urea, however, is of more importance than its mere presence, because urea may be found in many other fluids; and to base a diagnosis of hydronephrosis fluid or urine on the presence of urea might lead to fallacy. However, the quantity of urea will settle the question, if abundant. A large quantity of urea will not occur except in urine, although it must be remembered that "no or very little urea" does *not* mean "no renal origin" for the fluid.

A case of congenital cystic kidney which is of interest was described by Freund, at the Medical Congress in Halle (December 11, 1907). The case occurred during labour, and it was a question as to whether the source of obstruction were ascites, distended bladder, or kidney. At a time such as this, the elaborate details which are referred to throughout these pages are obviously useless. The author of this case considers the tumour to have been a multilocular cyst of congenital origin, but gives no details as to the nature of the cyst-contents.

Spleen Cysts, which are very rare, are described by Lascialfara as including (1) hydatid, 2 cases, (2) serous, (3) sero-hæmorrhagic—one case of traumatic origin, but he gives no details of the characters of their contents.*

Cysts connected with the Lymphatics.—Toyosumi describes a cystic lymphangio-endothelioma papilliferum which was situate in the abdominal wall, but does not supply any information as to the chemical or other characters of the contents.

Dencks describes a lymphangioma of the neck, which contained a whitish, milky fluid, with a yellowish tinge like lymph. He states that other cysts of similar nature may contain a brownish fluid owing to hæmorrhage into them. He gives no other details about these fluids.

Albrecht refers to a case of lymphangiectasis, in which numer-

* See also Zieglwallner F., *Über multiple seröse Zysten der Milz*. "Inaug. Diss." München, 1907.

ous little cysts appeared in the skin. They contained a milky-white fluid, which contained some blood-cells, lymphocytes, and a considerable quantity of *fat* in the form of very minute drops. He states that if fat was given by the mouth it rapidly appeared in these cysts.

Lacteal Cysts.—An example of these cysts was accidentally found by me during a post-mortem examination in a case of suicide. The small intestine for the greater part of its length showed numerous white swellings in its walls, varying in size, and exuding an opaque milky-white fluid when cut open. The size varied from that of a pea to about half an inch in diameter, and they projected into the lumen of the intestine.

Parotid Cysts.*—If these cysts are connected with the gland itself we should expect the fluid to be alkaline, very poor in salts (0.2 per cent.), and a low osmotic concentration (0.104). The salts include chiefly carbonates. Whether sulphocyanide is present in these fluids or no does not seem to have been investigated. The chief feature will be the presence of diastatic ferment.

Cysts of Bone.—Rumpel refers to cysts of bone, which are usually due to the breaking down of tumours, such as sarcomas. In one case described by him he comes to the conclusion that the cyst, which contained clear fluid, was formed from a softened enchondroma.

The cellular elements are the chief characters on which he lays stress, though the interest to him is mainly surgical. Such cysts are not, as a rule, likely to come under the notice of the clinical pathologist. But it would be of great interest to have an analysis of such a fluid, since it would afford excellent ideas as to the variety of decomposition products of new-growth proteid, which is different from normal tissue-proteid.

A large **cyst** was found **in the cerebrum** in a case by Fahr of Hamburg (December 3, 1907). The walls were very delicate, the size was that of the left hemisphere. There was no evidence of hydatid. No mention is made of the contents, the sole interest taken in the case apparently having been its clinical

* Unfortunately the only specimen which I had the opportunity of examining, cannot now be traced, and the record of the result of examination cannot be found. I have not had the opportunity of examining the fluid from a ranula.

symptoms. One would have preferred evidence as to the *causation*.

Cysts in connection with the Cerebellum.—Cysts in this locality can hardly ever come under the notice of the clinical pathologist, but for the sake of completeness they may be referred to. Henschen states that the following varieties may occur: dermoid cysts, simple serous cysts (which have an ependymal lining and are related to the fourth ventricle), cystic tumours, hæmorrhagic cysts (due to hæmorrhage and softening), parasitic cysts, and serous cysts, which are due to developmental anomalies.

Spermatocele. This fluid is colourless, thin, and watery. The specific gravity varies between 1006 and 1010. Solids, 1·3 per cent. It is not spontaneously coagulable. Apart from the presence of spermatozoa, which is the diagnostic feature, a reaction was devised by Florence for distinguishing spermatocele from similar fluids. A solution of iodine (1·65) and potassium iodide (2·54) in water (30 cc.), added to prostatic secretion, results in dark brown rhombic crystals (plates, fine needles, and rosettes) soluble in excess of water, ether, alcohol, acids, and alkalies. Spermatocele fluid does not give the reaction.

Parasitic Cysts.—Hydatid cysts alone call for consideration, though their characters are so well known that nothing new can be added. The specific gravity is low (generally not more than 1015); proteids are usually absent, while salts are abundant.* Sugar is occasionally present, and traces of urea may be expected, as well as of creatin. Succinic acid† is characteristically present.‡ Microscopic examination of the deposit is, of course, the most conclusive evidence.

* In case No. 6897, 6 per mille of chlorides were found.

† To detect succinic acid in hydatid cysts (Salkowski).

In Table A, No. 6, the concentrated alkaline fluid is treated with alcohol, and alkaline salts of succinic acid will separate out. These are dissolved in water, the solution filtered and squeezed. They are obtained pure by adding equal parts of alcohol and ether with HCl.

Characters.—Four-sided needles, melting at 182° C., soluble in water and alcohol, very soluble in ether.

Heated in a glass tube, it will sublime. Heated on platinum foil, it gives off irritating vapours.

Neutral lead acetate gives a heavy crystalline precipitate of lead succinate.

‡ Also present in hydrocele fluid (see p. 164).

Mourson and Schlagdenhauffen found a poisonous ptomaine in the fluid of a hydatid cyst.

If in a suspected hydatid cyst one finds an alkaline reaction, no urea or sugar, and abundant chlorides, this will not decide between hydatid and hydronephrosis, unless hooklets are also found. So that the only really reliable sign is the presence of hooklets. It must be remembered, however, that absence of hooklets does not, *ipso facto*, exclude hydatid.

SECTION IV

THE DIFFERENTIAL DIAGNOSIS OF EXUDATES FROM TRANSUDATES

CONTENTS.—Deductions to be made from: (a) specific gravity, (b) amount of total proteid, (c) refractometry, (d) viscosity, (e) presence of serosamucin, (f) Rivalta's test, (g) presence of fructose, etc., (h) presence of ferments, (i) effect of oral administration of drugs, (j) reaction with immune serum, (k) evidence furnished by the chloride *versus* achloride electrolytes, (l) cytodagnosis.

THE attempts at differentiation between exudates and transudates have occupied a very important place in clinical pathology, and opinions as to their value have varied from time to time according to failures in diagnosis or to improved methods which prevented recurrence of such failures. As has been insisted in former pages, it is not possible to experience uninterrupted successes in diagnosis by these means any more than they can be expected in any other branch of medical science. The evidence furnished by the methods to be detailed must always be made subservient to either physical signs or to the medical history of the case concerned.

The various methods which are to be advocated are not equally well known, and are deserving of wider use, and from the experience in all but the biological test (reaction with immune serum) that has been obtained from cases in the Leeds General Infirmary one is justified in collecting the methods and offering criticisms. The idea of using the electrolyte-determinations has been the result of the discovery that exudates differ essentially from transudates (*as a rule*) in their achloride-electrolyte-content.

The following tests will be considered *seriatim*:

Specific gravity; amount of total proteid; presence of serosamucin, and Rivalta's test; certain special tests (for ferments, for acetone, the reaction in tubercular pus, the reaction

with immune serum, the effect of administration of drugs) ; the relation of chlorides to achlorides.

A practical note on the collection of pathological fluids may be useful. The fluid should be collected into a perfectly clean bottle, sterilised if necessary by thorough heating in an oven. The instruments used for tapping must have been boiled in plain water, and the skin as thoroughly disinfected as for an operation (not only patient, but operator). The needle for puncturing is conveniently kept inside a glass tube of suitable diameter, plugged at each end with cotton wool, so that the whole can be readily sterilised with hot air (D'Este Emery). If only a bacteriological examination is needed the fluid can be sent to the bacteriologist in a small piece of glass tubing which has been drawn out at each end and sealed up after filling.

(a) **Specific Gravity.**—The most well-known characteristic of transudates is that their specific gravity is usually much lower than that of exudates. Thus, transudates usually have a specific gravity of less than 1020, while exudates have one of more than 1020. This is shown in the table on the following page.

Reference to the cases will show that both pleural and peritoneal fluids of transudatory origin have a low specific gravity.

The significance of these variations may be attributed mainly to the amount of albumen present in the fluid. The more albumen the higher will be the specific gravity ; and albumen is more abundant in exudates than in transudates. The relation between amount of albumen and specific gravity has been the subject of a very large amount of study, especially by Reuss and by Bernheim.

According to Reuss, the specific gravity may be taken as a rough index of the amount of proteid,* and he devised the following elaborate formula, which should enable one to estimate the amount of albumen from the specific gravity :

Percentage of albumen = $\frac{3}{8} (S - 1000) - 2.8$, S being the specific gravity.

* Reuss made out that specific gravity of

1018 means more than 4 per cent. of proteid.

1015 " less " 2.5 " "

1012 " " " 1.5-2 " "

1010 " " " 1.0-1.5 " "

1008.8 " " " 0.5-1.0 " "

A series of relations which has far more practical utility, even if not strictly accurate, than any elaborate and necessarily artificial formula.

TABLE XXI
SPECIFIC GRAVITY

	1010.	Below 1020.	Below 1030.	Above 1030.
Pleural	{	Transudate. Toxic Nephritis	Transudate. Renal Disease " Simple Pleural Effusion (3 cases)	Exudate. Tuberculous Pleurisy.
		Exudate. Associated with Carcinoma Ovarii	Exudate. Tuberculous Pleurisy, 4 cases " Associated with Carcinoma Ovarii " Empyema " Tuberculous Pleurisy	
Peritoneal	{	Transudate. Toxic Nephritis	Transudate. Cardiac Disease	
		" Cirrhosis of Liver	"	
		" Cardiac Back-pressure	"	
		Exudate. Chronic Peritonitis with Gastric Ulcer	Exudate. Carcinomatosis, 2 cases " Tuberculous Peritonitis, 2 cases	
Edema Fluid		Cirrhosis of Liver		
Cysts		Cyst round Uterus	Parovian Cyst Unilocular Ovarian Cyst	

This comparatively simple relation * was controverted, as might be expected, by Bernheim, who had, by the aid of a mathematician and very numerous observations of albumen *versus* specific gravity, been able to issue the formula :

Percentage of albumen = $4.9446S + 126.0467S^2 - 131.5$ for exudates, or $275.116S - 1.74256S^2 - 275.216$ for transudates, a relation which is obviously so artificial that though the formula meets the observations of Bernheim, one cannot expect it to fit in with every case that will ever occur.

Bernheim came to a totally different conclusion, namely, that the specific gravity is no definite index to the nature of a fluid. The presence of several exudates in the column "below 1020" of the table will illustrate the truth of Bernheim's contention. At the same time, it must be admitted that in a fair proportion of cases the specific gravity *does* help in forming an opinion.

As a matter of fact, the specific gravity depends on other bodies besides albumen, and the two cannot be brought into relation with each other, even if albumen play the most important part in the question. Thus, the total nitrogen and the residual nitrogen vary with the specific gravity, and so also do the ammonia and the purin nitrogen. The parallelism between specific gravity and the amido-nitrogen and the nitrogen of the uric acid prevents these substances from helping in the diagnosis, only that alteration in circulatory conditions such as occurs in transudates from cardiac disease increases the amount of urea and amidoacids.†

(b) **Amount of Total Proteid.**—This has long been regarded, and rightly so, as a safe index to the nature of an effusion into either abdominal or pleural cavity. Here, as everywhere, *it is the borderline cases that present difficulty*. The explanation of the exceptions is suggested by Stähelin, who found that the amount of albumen varies with the nature of the disease causing the effusion, with the state of nutrition of the individual, as well as other factors which need to be taken into consideration before making any conclusion.

* Compare Christen's formula for giving the percentage of albumen (a) from the weight of a litre of fluid in grams at 15° C. (p).

$$(a) = 4 (p - 1006.8)$$

This formula he asserted to be correct within .47 gm. per litre (.04 per cent.).

† Otori.

A most important series of observations have been made during recent years on the albumen-content of peritoneal effusions by Engländer of Vienna. The albumen was estimated by weighing, and not by Esbach's method. This author, whose results were only published in full last year, found that in hydræmia the amount of albumen varies between 0.3 and 0.5 per cent., while in cases of portal stasis it varies between 1 and 1.5, rising to 3 per cent. in old exudates, or falling to 0.4 per cent. in cachexia. In the last group of cases, those due to portal stasis, the albumen was not more than 2 per cent. in 50 cases. On the other hand, cases of carcinoma show a high albumen percentage (up to 7 per cent.), and in chronic exudative and tuberculous peritonitis the *minimum* is 3 per cent. The chief service afforded by this author is his insistence that the amount of albumen must be considered in relation to clinical facts. Thus, in a case of cirrhosis of the liver, if the albumen in the peritoneal fluid is 2, or less than 2 per cent., there is no peritonitis, while if there is much more than 2 per cent. an explanation must be found; and if the general nutrition is not good, or if the ascites has not existed for some time, or if the abdominal walls are not tense, then some inflammatory condition in the peritoneal cavity must be looked for (either carcinomatous, serositic, or syphilitic). Whereas if the albumen-content be much more than 3 per cent., and carcinoma can be excluded, one may be sure that there is tuberculous peritonitis superadded to the liver disease.

The possibility of excluding tubercle by finding the percentage of albumen to be less than 3 is an exceedingly useful point.

Such conclusions were expressed by Runeberg, though not to the same extent of completeness, when in 1897 he stated that 4 to 6 per cent. of proteid signified an inflammatory exudate (tubercle or carcinoma), 1 to 3 per cent. signified a transudate from passive congestion, and 0.1 to 0.3 (*maximum*) meant a purely hydræmic transudate. The more chronic the condition, the less proteid will there be in the fluid.

By bearing such considerations as these in mind, it becomes possible to diagnose the appearance of dissemination of carcinoma over the peritoneum in a case where the primary growth has merely at first caused portal stasis. And further, these considerations will serve to emphasise the fact that it is not necessarily by

a single exploratory puncture that we shall be able accurately to decide on a diagnosis, but rather is it by seeing what changes occur in the fluid *after an interval of time* that we come to be able to form a reliable opinion. If example were necessary one would cite a case in which there was found at first a low albumen-content (2.5 per cent.) which did not exclude carcinoma, while the exact diagnosis remained doubtful. The cytological character pointed to stasis, as it was not certain whether endothelial or carcinoma cells were present. A subsequent puncture, however, showed a rise in albumen to 6 per cent., diminished chloride-content, and alteration in the cellular characters. At the necropsy there was found a growth in the omentum which had at first caused merely back-pressure, but had subsequently come to the surface and commenced dissemination over the surface of the peritoneal cavity.

So much has been said as to the methods of estimating the amount of albumen, that there should be no need to refer to them again. Runeberg's method may, however, appeal to the clinician for its simplicity. It is carried out as follows :

A few drops of nitric acid are added to some of the fluid in a test-tube. If a thick heavy plaque forms that rapidly sinks, this means inflammatory or tuberculous effusion. If there are abundant large flocculi which sink less rapidly, this points to a congestive transudate, whereas if there is only an opalescence, resulting small flakes appearing only after a long time, this signifies a hydræmic transudate. The limitations of this method will be readily made out from what is being said.

The table on the following page from one's own cases will illustrate the varying amounts of proteid present in different effusions.

As regards the *proteid quotient*, which is obtained by dividing the percentage of albumen by that of globulin, there is little to be said, as the ratio cannot be said to be characteristic of any particular class of effusion, even if all the effusions in a given patient show the same ratio as Halliburton first described. The following variations were found :

Peritoneal fluid from a case of Monolobular Cirrhosis	0.38
Pleural fluid, Chronic inflammatory	3.5
Tuberculous	2.6
Simple effusion...	0.8

The ratio varies according to the amount of globulin present where this body bears relation to the disease in virtue of its association with immunisation-processes. Antitoxin becomes attached to globulin, so that where protective bodies are formed

TABLE XXII
PERCENTAGE OF ALBUMEN IN VARIOUS FLUIDS

		Below 1%.	At or below 2.	Below 3.	Below 4.	Below 5.	Over 5.
Pleural	Transu- dates	Simple Pleural Effusion	Simple Pleural Effusion Hydro- thorax (Renal)	Simple Pleural Effusion		Cardiac	Chronic Nephritis, 7.77
	Exu- dates		Assoc. with Carc. Ovarii	Assoc. with Carc. Ovarii		Tuberculous 2 (cases)	Tuberculous (3 cases), 6-8 Assoc. with Carc. Ovarii, 5.11 Empyema, 8.8
Perito- neal	Transu- dates	Renal Cardiac Alc. Cirrho- sis of Liver Toxic Nephritis Syphilitic Cirrhosis (Halliburton)	Thrombosis of Portal Vein Alc. Cirrho- sis of Liver (2 cases) Syph. Cir- rhosis of Liver (Poljakoff)	Chronic Nephritis (2 cases) Portal Ob- struction (Runeberg)			Cardiac, 5.44 Alcoholic Cirrhosis of Liver, 8.8
	Exu- dates		Carcinoma- tosis (2 cases) Tuberculous Peritonitis	Carcinoma- tosis (2 cases) Polyorrhomenitis Chylous ascites (McHey)	Carcinoma- tosis (Runeberg)	Tuberc. Peritonitis	Simple Chronic Peritonitis, 7.75 Carcinomatous Peritonitis, 10 Tubercular, 6.75
Œdema Fluid							Cardiac cases
Pericar- dial			Tuberculous				
Cysts				Ovarian Cyst			Pancreatic Cyst

in the course of an effusion excited by bacterial agency, they will enter into combination with the globulin, and so affect the proteid quotient.* These changes have been noted chiefly in regard to the composition of the blood, but inasmuch as the proteid quotient is constant both for blood and any effusion that occurs in the given individual, the same conditions will hold in the case of

* Glassner.

the effusion. But when we reflect that the urine of cases of nephritis may show a varying proteid quotient * from day to day, the value of such a quotient for diagnosis in effusions comes to require substantiation. If the urinary composition varies in this way, a renal ascites may be expected to show similar daily changes. The relation, too, between proteid and molecular concentration cannot be regarded as having any prospects of being of practical importance.

The total proteid may also be compared with the extractive nitrogen, and these two together may be compared with the proteid-extractive ratio of blood-serum. Rzentkowski utilised these ratios in order to decide whether a fluid were a transudate or the result of endothelial proliferative processes. If the ratio were much less than that of the serum he would argue against a transudatory origin. He states that while they are accumulating the fluid mainly consists of salts, while when the serous membrane becomes active the proteid element enters into the effusion (an autochthonous process). In an exudate we have the following relations: the dry matter is less than that of the blood-serum, the total nitrogen is also less, but the extractives amount to still less than those of the blood-serum.

Be these statements correct or no, they cannot be of much assistance in the differential diagnosis, merely from the fact that they involve tedious methods of research which cannot conveniently be employed for diagnostic purposes.

(c) **Refractometry.**—The refractometric observations which have been made by Engl and advocated strongly by him as a means of distinguishing between the two main classes of effusion are really nothing more than a refined method of albumen-estimations, since the refractive index of a fluid depends very largely upon the amount of albumen which the effusion contains. It will not be necessary to enter into any details of the methods of refractometry, simply because a suitable instrument is very expensive, and cannot reasonably be added to the instrumentarium of the clinical pathologist. Still, it cannot be doubted that the method—given the instrument—is a very handy one, but the results are not so uniform that general use of the method is advisable. In the following table, which is adapted from a

* v. Noorden, Noel-Paton, Patella, Moram.

long series of observations by Engl, the numbers represent the refractive coefficients of the fluids. (Note the thickened figures.)

REFRACTIVE COEFFICIENTS OF VARIOUS FLUIDS (ENGL)

		Pleura.	Abdomen.	Peri- cardium.
Nephritic Transudates	Subacute Tubal	1·3379	1·3371	1·3371
	Chronic Tubal	1·3362	1·3872	1·3397
	Interstitial	1·3372	—	—
	Average:	1·3375	1·3374	1·3398
Cachectic Transudates	Pernicious Anæmia ...	1·3378	1·3380	1·3408
	Tuberc. Abscess of Chest ...	1·3378	1·3365	1·3374
	Average:	1·3385	1·3382	1·3398
Passive Congestion Transudates	Cardiac	1·3410	1·3405	1·3401
	Cirrhosis of Liver	—	1·3368	—
	Emphysema	—	1·3378	—
	Aortic Valve Disease ...	—	1·3401	1·3390
	Average:	1·3392	1·3398	1·3405
Exudates ...	Acute Pleurisy	1·3436	—	—
	Tuberculous Pleurisy ...	1·3474	—	—
	Purulent Pleurisy	1·3480	—	—
	Tuberc. Peritonitis... ..	—	1·3443	—
	Purulent Peritonitis ...	—	1·3479	—
	Carcinomatous Peritonitis...	—	1·3430	—
	Carcinoma Ovarii	—	1·3442	—
	Hæmorrhagic Pericarditis...	—	—	—
	Average:	1·3446	Average: 1·3445	1·3459
Various ...	Chronic Hydrocephalus ...	1·3347 to 1·3350	—	—
	Cerebrospinal Fluid ...	1·3350	—	—
	Hydrocele	1·3429	—	—
Œdema Fluid	Cachectic Nephritis	1·3351	—	—
		1·3362	—	—
		1·3359	—	—

The advantages of the method are that the refractive coefficient, which is proportional to the albumen-content, (1) is not affected by suspended particles, (2) necessitates the least quantity of fluid, and (3) is much less tedious than a determination of the dry residue according to Runeberg or a Kjeldahl estimation. Besides this, a comparison of the refractive coefficient of the effusion can be readily made with that of the blood. The only sources of error are that certain substances other than albumen

influence the coefficient and that different forms of proteid cause variations. While 1 per cent. albumen has a coefficient of .0018, 1 per cent. of globulin is .0020-.0023 (according as it is globulin, pseudo-globulin, or crystallin).

(d) **Viscosity.**—The investigations which one has made as to the viscosity of the different fluids that have come under observation have shown very strikingly how much lower the value (as compared with water) is in the case of transudates than it is with exudates. Inasmuch as the viscosity will be influenced by the amount of albumen present, a natural explanation for the fact is found. Probably there are other factors to be considered, but the full treatment of the subject will be found in Section II., last sub-section. The clinician can hardly be advised to purchase a viscosimeter in order to assist him in such a diagnostic problem as is under consideration, so that more detailed reference to the values found is out of place in this section.

(e) **Presence of Serosamucin.**—The chemistry of this substance, and of the bodies to which it is allied, has already been fully entered into. It is merely necessary to repeat that the presence of serosamucin in a peritoneal fluid is evidence in favour of its being an exudation,* that is, a fluid associated with inflammatory processes in the peritoneum.† This fact is the basis of—

(f) **Rivalta's Test**, which consists in adding two drops of glacial acetic acid to 100 cc. of distilled water, and then one drop of the fluid to be tested. The result, if the reaction is "positive," is that a white cloud appears in the trail of the descending drop, the trail being of varying degrees of whiteness, according to the severity of the inflammatory process. Janowski tested this in a number of cases and obtained uniform results. The results obtained in the Leeds Infirmary series have also given precisely concordant results, and it is hardly necessary to tabulate the findings under such uniform circumstances. Only one point would one emphasise, and that is that unless there be a decided white cloud, which *increases as the drop descends*, the result must not be regarded as definitely positive. Often a faint turbidity

* Rivalta.

† Stähelin reports fourteen cases in which he examined the fluid for serosamucin and found its presence in those fluids which were of an inflammatory nature. Eleven cases were pleural fluids; the others were from the abdomen.

will be produced with transudates, but it will be found to disappear as the drop descends. In a "positive" case the whiteness will be very distinct, and the appearance of ever-increasing numbers of white specks which tail out during the descent of the drop is very striking. The precipitate is, of course, one of serosamucin, though the proof of such by chemical analysis would involve precipitation from large quantities of fluid. The globulin will not separate out, owing to the presence of the acetic acid.

(g) **Presence of Fructose.**—Neuburger and Strauss found this body in the fluid in cases of peritoneal cancer, and in ascites due to granular kidney. They also found it in pleural fluids when not given by the mouth. The oral administration of fructose usually causes its reappearance in the effusion.

ACETONE is said to occur constantly in exudates.

WIDEROE'S TEST.—Wideroe devised a test which should inform the clinician whether a given effusion were due to tubercle or no. The method may be carried out thus: in a watch-glass a few drops of Millon's reagent are placed, and on to the surface a single drop of the fluid to be tested is allowed to fall from a glass rod. A film at once forms where the drop meets the reagent. If a platinum wire be now passed beneath the drop, and an attempt made to lift it from the reagent, the drop will readily lift up if the fluid be of tuberculous nature, less readily possible in the case of inflammatory effusions, and quite impossible (owing to the breaking up of the film) in the case of transudates. In the case of the inflammatory effusions, it will generally be found that a few stabs with the loop will break up the film if the cases be non-tuberculous.

From a number of specimens examined I can recommend this test, but absolute reliance cannot be placed on it, and in the case of other fluids than peritoneal and pleural, it is not at all reliable. The discoverer of the reaction admits that the presence of blood interferes.

MOLECULAR CONCENTRATION.—According to Rzentkowski, this is greater in transudates than in exudates, but v. Ketly and v. Torday deny this.

(h) **Presence of Ferments.**—It seems reasonable to suppose that we shall have different ferments in different fluids, varying with their causation. In the case of transudates, where there may not be many cell-elements, there will not be the cell-ferments

characteristic of those cells. On the other hand, in a severe inflammatory exudate with many blood-cells there will naturally be the ferments associated with leucocytes, a point which has already been entered into at some length (Section I.).

Umber has advanced proof of the existence of ferments in *exudates* by the fact that perfectly sterile exudates treated with toluol showed breaking-down of the proteid during incubation. Total nitrogen-determinations were made, as well as determinations of the different forms of nitrogen. These are shown in the following table, which is taken from Umber's original paper :

	a	b	c	d	e
	Total N.	Coagulable Proteid (a - c × 6.25)	Dissolved N.	Ammonia N.	% of Ammonia N. from total N.
Before Autodigestion	0.857 gm.	5.137	.0352	.0234	2.25
After "	0.857 "	5.037	.0595	.0448	4.12

It is seen that the total nitrogen remains unaltered, while the uncoagulable proteid is decidedly diminished and the dissolved non-coagulable nitrogen is increased. This dissolved non-coagulable nitrogenous matter consists of primary and secondary albumoses, leucin, and tyrosin, which are therefore increased during autodigestion. The ammonia is seen to be increased almost twofold.

Müller found that proteolytic ferment only occurs in the deposit of a puncture-fluid if the causation is inflammatory ; this is to be ascribed to the presence of proteolytic ferment in the leucocytes. The amount of *antiferment* which occurs (see Section I., p. 86) depends on the degree of destruction of the leucocytes, as otherwise it is saturated by the free ferment. Not only does the antiferment-content not depend on these conditions, but probably the *kind* of albumen in the fluid will have something to do with the reaction. If antiferment is present, the fluid may be regarded as a transudate. If there be only a slight inhibitory action the fluid may be assumed to be due to a chronic inflammatory process and to contain no bacteria. If there be no antiferment (*i.e.* the leucocytes can digest the gelatine used for the test), this indicates an acute suppurative process, and will answer even though the leucocytes in the deposit be thoroughly disorganised.

The diastatic and inverting ferment are often present, but do not show any regular relation to the cause of the effusion (p. 80). But it may be that we must go rather by quantity of ferment than by its mere presence or absence. The methods for estimating the various ferments will be found on p. 70.

LIPASE has been found by Mammi to be more abundant in exudates than in transudates.

(i) **Effect of Oral Administration of Drugs.**—Some interesting observations were made by Landolfi at Naples, which showed that if certain drugs were administered by the mouth they could be subsequently detected in the effusion, and that if such drugs be introduced into the serous cavity they will reappear in the urine.

It was found that while such bodies were found in either the pathological fluid (in the one case) or in the urine (in the other) in both exudates and transudates, the *quantity* of drug which reappeared was very considerably greater in the case of transudates than in exudates. The following list shows the effect of various drugs :

Nature of Fluid.	Sodium Iodide.	Antipyrin.	Salicylic Acid Pyramidon.
Purulent exudations	—	—	—
Hæmorrhagic exudations	—	—	—
Tuberculous serofibrinous exudate	—
Non-tuberculous serofibrinous exudate	—
Transudation from passive congestion	+	+	+
Transudation from hydræmia	+	+	+
	Appearing in effusion: administered per os.		Appearing in urine: injected into effusion

+ means present; — means negative result.

The reaction with potassium iodide was noticed by Mammi. Fructose given by the mouth may appear in the exudate (see p. 150).

(j) **Reaction with Immune Serum.**—Tedeschi of Genoa found that if an effusion were tested with immune serum a precipitin reaction * would occur if the fluid were a transudate,

* See also Forssner.

while no reaction occurred in the case of an exudate, presumably owing to the latter containing the necessary complement, because of the cells in the exudate.

However, if thickening of the serous membrane (such as the pleura) occur, a transudate may fail to give the reaction. Again, if the exudation be examined in an early stage the reaction may come off owing to the anti-bodies not having had time to appear.

Whereas the reliability of the reaction is likely to be called into question, it must be admitted that the practical difficulties in the test, involving as they do a vivisection licence, will prevent its use.

The distinction between an exudate and a transudate is made a very sharp one by such a reaction as this, and it cannot be emphasised too much that the demarcation line where exudate ends and transudate begins does not exist. In well-marked cases the distinction is easy, but there are and always will be many cases which are not strictly either the one or the other.

The fact that lecithin plays a part in immunity reactions, and the fact that lecithin is associated with pseudo-globulin or with euglobulin, would throw some light on the observations of Tedeschi, and only brings us back to the original fact that the amount of albumen (including globulin) may form an index as to the variety of the fluid being studied.

(*k*) **The Chloride versus the Achloride Electrolytes.**—In the course of studies on the electro-conductivity of the various fluids which have been examined, it was first noticed that the chloride-content of these fluids varied very greatly, according to the causation of the effusion. As one would expect, the renal dropsies contain a relatively large percentage of chlorides, while the exudates due to severe inflammatory change contain but few. This observation led one to endeavour to ascertain whether the other inorganic constituents of the effusion underwent any change. The comparison of the actual conductivity of the fluid with the conductivity which the fluid would have if only chlorides were present to the amount found by quantitative analysis formed the basis of the method adopted for investigating this point. The principles involved in this type of "chemical analysis," and the difficulties in the way of a just interpretation, with indications as to how the errors can largely be obviated, have already been dealt with fully (Section I., under the problem of adsorption of chlorine by egg-albumen; Section

II., sub-section 2). It therefore becomes only necessary to give an account of the results obtained.

Before proceeding with the relations existing between the amount of chlorides compared with that of achloride electrolytes, it will be advantageous to call attention for the moment to the variations in chloride-content alone which obtain in different classes of fluids. Reference to Table IX. will show the striking fact that in transudates the concentration of chlorides expressed as gram-equivalent (gram-molecule per litre) is always, or nearly always, up to 0.1, while in fluids associated with varying degrees of inflammatory change, or with disseminated malignant disease, the figure barely reaches 0.07. In many cases the fluid contained in ovarian cysts falls to a still lower chloride-concentration, as will be seen on reference to Table XIX. The subcutaneous fluids resulting from cardiac failure or from renal inadequacy follow the transudates in respect of their chloride-content, though in some cases the fluid is remarkably free from inorganic matter. Again, in the case of *hydatid cysts*, it is usual to find a high chloride-concentration, a feature which has so far been also met with in cases of *cerebrospinal fluid*. It is thus evident that an estimation of the chlorides is a considerable help in assigning to a fluid the diagnosis of its source. That is to say, a fluid obtained from the abdomen by exploratory puncture may conceivably be a transudate, an exudate, an ovarian cyst, or a hydatid cyst, for each of these conditions may be confused with each other even by experienced diagnosticians. The clinical pathologist must utilise every little possible factor which will help him to decide such questions when a puncture-fluid is handed over to him to issue a report upon it.*

Turning now to the Table (XXIII.) showing the electrolyte-content of exudates and transudates, the first column is seen to give the chloride-concentration, the second column gives the degree of dissociation possessed by such a solution of NaCl, from which value it becomes possible to deduce the number of molecules, *plus* ions, in that solution. The next column (vi) gives the specific conductivity, the values being given as whole numbers raised to the power of 10^{-5} , since such values are more

* Experience of the bulk of the precipitate in the 100-cc. flask enables one to judge roughly of the quantity of the chlorides before the actual titration has been carried out.

tangible.* This value has been arrived at by correcting the conductivity found both for temperature (all stand at 18° C.) and for the percentage of albumen.† The corresponding conductivity of a solution of NaCl of the same strength as the fluid is given in the next column, and the difference between the two gives that portion of the total conductivity which is to be ascribed to the achlorides. Regarding these as Na_2CO_3 we can express the number of molecules, *plus* ions, per litre of achlorides in terms of Na_2CO_3 . The last column but one gives the total concentration of electrolytes, and the last column expresses the ratio of achlorides to chlorides.

Comparing the different classes of fluid, the striking feature will be found to be that in the case of exudations the ratio named is nearly always greater than unity, and, indeed, is frequently 2, 3, or even up to 6. The transudates, on the other hand, show a ratio which is never more than 0.9. That is to say, the transudates contain relatively few achlorides.

Before we can assert that such a series of observations may be relied on for differential diagnosis, we have to consider whether an explanation for the findings can be found. This must be sought in the essential difference between a *typical* exudate and a *typical* transudate. The most conspicuous difference will be found in the albumen-content. The presence of so large an amount of albumen might interfere with the estimation of the chlorides by adsorption or other circumstances, or the fact that nearly all the fluid disappears when so highly albuminous a fluid is boiled might lead one to suspect an intrinsic error in the estimation. Both of these explanations can be excluded, however, the former on the grounds detailed on page 18, and the latter on the ground that there is an undoubted difference in the chloride-content in exudates.

Another consideration is as to the permeability of the inflamed serous membrane for the different ions. The difference cannot have any relation to the relative tonicity of blood-serum and fluid, since we have to deal with a difference in the content of various salts. On the other hand, just as with questions of absorption of lymph from a serous cavity there are three factors

* This notation has been employed uniformly through this work.

† The formulæ available for this correction will be found on pp. 100 and 107.

TABLE XXIII.—CHLORIDE *v.* ACHLORIDE ELECTROLYTES IN EXUDATES AND TRANSUDATES

i	ii	iii	iv	v	vi	vii	viii	ix	x	xi
No. of case *	Nature of case	Fluid contains chlorine g. aeq. in litre	Degree of dissociation "a"	No. of molecules + ions	Specific conductivity of fluid corrected ($\times 10^{-7}$)	Conductivity of iii	Conductivity of achlorides (vi-vii)	No. of molecules + ions pro litre of a solution of Na_2CO_3 with conductivity in column viii	Total concentration of electrolytes (v+ix)	$\frac{\text{ix}}{\text{v}}$
8337	Pleurisy secondary to pneumonia	.064	.860	.1190	1496	550	946	.1804	.2994	2.23
8338	Tubercular Pleurisy	.035	.885	.0639	1169	325	844	.1830	.2469	2.86
7313	"	.024	.901	.0456	825	205	620	.1334	.1790	2.92
6662	"	.062	.861	.1153	1053	550	503	.1141	.2294	0.98
6677	"	.062	.861	.1153	1068	550	518	.1146	.2299	0.99
6325	Pleurisy secondary to abdominal cancer	.024	.901	.0456	1096	205	891	.1813	.2269	3.97
7617	Pleurisy secondary to pneumonia	.031	.891	.0586	1071	245	826	.1733	.2319	2.95
8358	Pleurisy in a renal case (old tubercle)	.087	.846	.1621	1677	779	898	.1813	.3434	1.09
7334	Empyema	.024	.901	.0456	616	205	411	.0866	.1322	1.93
6603	Peritoneal Cancer (colloid)	.068	.858	.1263	1183	589	594	.1400	.2663	1.10
6712	" Sarcoma	.040	.880	.0752	1188	345	843	.1758	.2510	2.33
6838	" Cancer (ovary)	.046	.874	.0863	1165	425	740	.1733	.2596	2.00
8334	"	.014	.922	.0269	971	145	825	.1813	.2082	6.74
8344	" (?)	.104	.837	.1910	1102	855	247	.0516	.2426	0.27
6417	Tuberculous Peritonitis	.056	.866	.1055	1171	505	666	.1599	.2654	1.51
8911	"	.059	.862	.1100	1001	566	435	.0980	.2080	0.89
7553	Chronic Peritonitis (gastric ulcer)	.043	.877	.0800	1311	425	986	.1875	.2675	2.34
8182	Chronic Peritonitis	.078	.852	.1444	1432	720	712	.1663	.3107	1.15
8340	"	.042	.878	.0788	965	380	585	.1330	.2118	1.68

* In Register of Clinical Laboratory.

TABLE XXIII.—*continued*
CHLORIDE *v*. ACHLORIDE ELECTROLYTES IN EXUDATES AND TRANSUDATES.

i	ii	iii	iv	v	vi	vii	viii	ix	x	xi
No. of case *	Nature of case	Fluid contains chlorine g. aeq. in litre	Degree of dissociation "a"	No. of molecules + ions	Specific conductivity of fluid corrected ($\times 10^{-3}$)	Conductivity of iii	Conductivity of achlorides (vi-vii)	No. of molecules + ions pro litre of a solution of Na_2CO_3 with conductivity in column viii	Total concentration of electrolytes (v+ix)	$\frac{\text{ix}}{\text{v}}$
Pleural.	Pleural fluid (cardiac)	.107	.837	.1965	1065	875	190	.0424	.2389	0.21
	"	.061	.861	.1150	1037	584	453	.0100	.1250	0.87
	Peritoneal fluid (cardiac)	.100	.838	.1838	1281	840	441	.0990	.2828	0.53
	" (renal)	.113	.834	.2035	1326	915	311	.0658	.2693	0.31
	"	.128	.827	.2338	1220	1183	37	.0001	.2339	0.99
Peritoneal.	" (cardiac and renal)	.059	.862	.1100	1088	566	522	.0141	.2241	0.77
	" (cirrhotic liver)	.094	.842	.1731	1187	802	385	.0868	.2599	0.50
	" (thrombosis of portal vein)	.111	.834	.2035	1283	900	383	.0851	.2886	0.41
	" (multiple effusions)	.111	.834	.2035	1215	900	315	.0658	.2693	0.32
	" (cirrhosis of liver)	.113	.834	.2035	993	915	78	.0140	.2175	0.59
7559	" (cirrhosis of liver)	.088	.845	.1623	974	780	194	.0426	.2049	0.26

* In Register of Clinical Laboratory.

to consider—the endothelial layer, the capillaries, and the connective tissue canals—so we shall have to consider the influence which each of these factors can have in allowing certain salts to pass through and not others. But more than this, we have to consider the effects of inflammation of the serous membrane, whereby not only are the perivascular lymph-spaces filled with fluid of abnormal composition and the endothelial cells undergoing proliferation, but we have to remember that the endothelial cells are being cast off into the fluid, and that angiogenic cells as well as histogenic cells are making their way into the fluid in question. In the case of carcinomatosis of the peritoneum, too, there is also the discharge into the fluid of the carcinoma cells with the products of their disintegration or the products of metabolism of those carcinomatous cells which have not been shed.

According to experiments made by Hamburger, artificially induced inflammation or injury short of inflammation of a serous membrane does not cause any change in the phenomena of absorption from that cavity, so that we may presume that the problem to be solved is the same whether we are dealing with the ordinary three factors above referred to or with the same factors in a state of disease. Such experiments, however, as Hamburger performed (injection of strong salt solutions after chemical and thermal injury to the peritoneum) do not affect the probability that the cellular elements which are discharged into the peritoneal cavity when the membrane is inflamed will cause a change in its composition.

Hamburger, in a series of experiments on the influence of pressure on absorption from the connective-tissues, came to the conclusion that increased pressure does not allow proportionately as much NaCl to pass out from the blood into the tissues as does a lower pressure. The process seems to him to be purely physical and to depend on the lower chloride-concentration in the tissues to that in the blood. If other salts are present in the tissues, these will pass into the blood, while the NaCl passes out. This is probably in accordance with Hamburger's principle that unless an ion passes out of, say, a red cell, another ion cannot enter.

PERMEABILITY OF CELLS TO IONS.—Urea, sodium chloride, and dextrose were found by Lazarus-Barlow to pass through an artificial membrane with distinctly varying velocities, and Roth proved that the same fact holds good for the living peritoneum, urea passing through most rapidly, while dextrose passed through least rapidly of the three. Following on these

observations, an extensive series of researches on the permeability of red cells for the various ions were made by Hedin, Gyns, and Overton, and then by Hamburger. Leaving the organic substances to which red cells are permeable or no out of count for the present, we find that red cells are quite *impermeable* for Ca'' , Sr'' , Ba'' , Mg'' , but *permeable* for NH_4'' , free acids, and free alkalies. Leucocytes are permeable for Cl' , SO_4'' , and NO_3 , and if the solution in which they lie contain CO_2 they will lose CO_3'' . This is an important fact, for it shows us that if CO_2 is present in an effusion the result will be the passage of carbonates from the cellular elements in it into the fluid, and the absorption of Cl and achlorides other than CO_3 into the cellular elements from the fluid. The result will be that the concentration of the carbonates will rise, and that of the chlorides fall. The analysis of pus-cells (see p. 146) shows that the achloride salts other than carbonates are not abundant, so that the passage of carbonates out will have more effect on the electrolyte-content than will the passage of the other electrolytes into the cells (loss from the fluid). This, then, in such a fluid as a purulent exudate would account very well for the finding of preponderance of achloride over chloride electrolyte. In those inflammatory fluids where actual pus is not present, there may be nevertheless enough cellular elements to account for the phenomenon, and in the case of malignant effusions and tuberculous effusions where there is an out-pouring of red cells (limited in amount, it is true), the permeability properties of the latter will also assist in the production of the ratio which is under discussion.

It may be urged that we do not know that CO_2 is present in a peritoneal fluid, except in so far as may be assumed from the alkaline reaction.

When we come to consider the influence which the *endothelium* will have, we come upon less known ground. As far as the researches of Höber show, Cl is much more readily taken up than any other salt, but this will not afford any explanation of the preponderance of NaCl in a transudate. The rate of migration of the ions will not help here, excepting that CO_3 has a rather slower rate (65) than the others (70-73).

Just as there is a difficulty in assigning a proper place to the endothelial layer, so there is a difficulty in discovering what may be the influence of the endothelial *walls of the capillaries*, or,

indeed, the *intercellular substance* of the endothelial lining of the peritoneum. The influence which the *connective tissue canals* exert is the same as that exerted by the endothelium, according to the experiments of Starling. That is to say, they readily take up isotonic NaCl solutions. If we consider a large vessel containing NaCl and albumen, separated from another smaller vessel by a permeable membrane containing a similar salt solution, but no albumen, then, according to Starling's simile, the albuminous fluid will attract water from the non-albuminous side, with a resulting increase in the concentration of that fluid. Some NaCl will therefore diffuse into the larger cavity, and the osmotic pressure of the smaller will fall. The large cavity represents the blood, and the small one represents the lymphatics and the peritoneum. The difference in the amount of proteid will cause a difference in the water-attraction of the two sides, and cause NaCl to leave the fluid and enter the blood. In exudates, where the albumen-content will about equal that of the serum, there will therefore be no passage of NaCl from exudate into the blood; in transudates, on the other hand, there should be a passage of NaCl into the blood, which will bring us to the conclusion that in the exudate there is more NaCl in the fluid than there is in the transudate. This apparent contradiction is, however, to be explained thus: that (1) in the effusion there has been a reverse of a normal process, for filtration with diffusion and a change in osmotic pressure causes fluid to be poured out into the serous cavity. The movement of ions is in the opposite direction. So the result is that in a transudate there will be more ready passage of NaCl *into the cavity*. In the exudate, on the other hand, where there is a process of secretion on the part of the serosa, this reverse flow of fluid has nothing to do with mere filtration or diffusion. (2) In the renal transudate it is the excess of NaCl which is causing outpouring of fluid into every available space, in order to procure its solution isotonicly with the blood.

Probably it is here that the explanation of the differences in chemical composition is to be found: in inflammation there is a secretion, in transudation there are merely physical factors. And added on to that we have the excess of cellular elements in the exudate as compared with the transudate, a fact which affords full play for the varying permeability of ions to cells to

take place. It may, however, be argued that in the process of inflammation the endothelium of the vessels and of the serosa as well as its permeability for ions is altered, while the increased pressure in the capillaries of the inflamed peritoneum will also affect the osmotic processes.

To what extent the *permeability of carcinoma cells* may have an influence in the question of carcinomatous effusions having an increased achloride-content it is not possible to decide, in the absence of any experiments on the permeability of such cells for the various ions. But probably their influence is rather in the direction of their chemical composition, since so many of them are shed and in a state of disintegration. Here the fact that we have more potassium salts than sodium salts, and more phosphates than chlorides in the cells, will serve to indicate a possible explanation. The chemistry of carcinoma and sarcoma tissue is, however, not well enough known to enable us to base any opinions upon this aspect of the question.

In conclusion it may be said, then, the difference in electrolyte-content of transudates from exudates is sufficiently fundamental to allow of its forming a basis of diagnosis in practical work. At the same time it must be insisted that this is not necessarily an infallible test, and exceptions are sure to be met with; indeed, in some of the tabulated cases there are such exceptions recorded (Nos. 8344, 6677, 6662). An interesting example of this kind is afforded by No. 8358, pleural fluid. This was a case of apparent hydrothorax and hydroperitoneum due to renal disease. The ratio is seen to be 1.09, which would be an exception to the rule, since the renal fluid should be transudatory. The explanation is that the pleural cavity had been filled with an inflammatory fluid owing to a complicating inflammation with adhesions. In 6667 and 6662 the inflammatory character of the pleural fluid is not certain. But the occurrence of such exceptions may be courted, from the scientific standpoint, as, in all probability, when the explanation for such exceptions is found we shall have obtained a deeper insight into the constitution of body-fluids under certain conditions than was before possible.

(*l*) **Cytodiagnosis.**—This section of the differential diagnosis problem is dealt with in the succeeding section.

SECTION V

CYTODIAGNOSIS

Sources of failure in practical diagnosis—Methods of examination—The results afforded by cytodiagnosis—Heinz' researches—Lymphocytes, pseudo-lymphocytes, polynucleosis, endothelial cells, large mononuclear cells, eosinophile cells, mast cells, red blood cells, carcinoma cells—The special features of hydrocele fluid, of joint fluids, of cerebrospinal fluid—Ovarian cysts—Special findings in the deposit of puncture-fluids—Artificial scheme for cytodiagnosis—The chemistry of the cell elements.

THE study of the cellular elements in exudates and transudates was not prosecuted systematically until after the publication of the work of Widal and Ravaut at so recent a date as 1900. Their observation, among many others, that tuberculous effusions were associated with an excess of lymphocytes in the fluid aroused instant and widespread interest in the subject, and the literature on "cytodiagnosis" has steadily come to assume exceedingly formidable proportions.

It may perhaps be said that the enthusiasm which ascribed to this study an almost pathognomonic importance has now simmered down into a more sober realisation that lymphocytes may be in excess in other fluids than those excited by tubercle bacilli, while even the latter may at certain stages be associated with excess of polynuclear leucocytes in the fluid.

A possible explanation for the undoubted discordance in the results obtained by cytological examination of puncture-fluids may be found in the objection made by Sahli that the fluid obtained by aspiration does not necessarily accurately represent the cellular characters of the fluid, owing to the inevitable influence of gravity on the deposition of the cells whose specific gravity shows variations. The cells in the deepest layers would escape the exploratory puncture.

Again, when the cellular elements become entangled in a fibrinous coagulum, it does not necessarily follow that all the varieties of cells will become entangled in equal proportions.

Lastly, it must be admitted that the cellular characters found to exist in a fluid really only represent the condition of that fluid at the moment of tapping, and a subsequent observation may reveal an entire change in the cytological picture.

In view of the fact that so much has been written on the facts of cytodiagnosis, it is not proposed to enter at all fully into the various phases of the subject, and it is rather with the view of suggesting that the cellular characters of a fluid may throw light on its chemical characters that the subject is at all approached. The quite recent (1908) publication of a monograph on cytodiagnosis by Königer renders a detailed description the more superfluous, as that work can be thoroughly recommended for its clearness and comprehensiveness to all who are interested in the subject.

METHODS OF PREPARATION OF THE CELLS

The deposit from a fluid may be examined both unstained, and after staining by various methods. It is advisable to combine the two methods, as much can be learnt from the appearance of the cells in a fresh condition that would be lost during the process of staining, especially as regards carcinoma cells. As regards staining methods, Jenner's stain, answering as it does all purposes, is most to be recommended. The exact formulæ, etc., of this or other stains will be found in several exhaustive works that have appeared on blood-cell technique.

The Collection of the Deposit.—In the case of fluids which do not coagulate spontaneously, the cellular elements separate out on standing, and are readily pipetted off. It is, however, advisable to prevent any risk of decomposition of the very delicate cells, by centrifugalising the fluid as soon as possible. Dilution with citrate of soda before centrifugalisation, pouring off the supernatant fluid, and shaking up again with saline, with re-centrifugalisation (after the method of Sir A. E. Wright for white cells), can be highly recommended, since the removal of the albumen in this way greatly facilitates staining. The risk, however, of breaking up degenerate endo-

thelial cells or vacuolated carcinoma cells has to be borne in mind.

In cases where a coagulum has formed before the fluid comes to hand for examination, it may suffice to take a small snipping of the coagulum and examine it fresh (no diluting fluid to be used). If the result is not satisfactory, some of the coagulum may be stirred about in a small quantity of fluid placed in a watch-glass, using a platinum wire as stirrer. The fluid may then be centrifugalised. The risk is, of course, that the deposit will not then exactly represent the characters of the deposit, owing to uneven separation of the cells. The unstained snipping may then be used as a control.

Preparation of the Film.—For fresh specimens a drop is simply covered with a cover and examined with the $\frac{1}{4}$ th. For stained preparations a thin film is spread on a slide,* preferably albumenised, and allowed to dry *on* a shelf arranged on the paraffin oven. A saturated solution of Jenner's stain (Grubler, e.g.) in anhydrous acetone-free methyl alcohol (Merck) is prepared *in a well-fitting stoppered* bottle, and a couple of drops are allowed to fall on the film. A Petri dish is convenient as a cover for the ordinary 3-inch slide. After two minutes † the slide is gently washed in distilled water till the pink colour appears, and dried by the application of "fluffless" blotting-paper, followed by a momentary warmth in the hollow of the hand over a Bunsen flame. The preparation is now ready for examination with an oil-immersion lens. The staining effects are illustrated on accompanying plates.

Though special works on blood-cell technique will mention the precautions needed in the use of Jenner's stain, besides explaining the chemistry of the stain, it may be mentioned (1) that the stopper of the bottle must be "turned off" when not in use; (2) that no time must be lost in applying the Petri dish after pouring on the stain; (3) that the time of staining must not be

* Slides are much preferable to cover-glasses for cytological and bacteriological work of all kinds. The whole slide is covered with deposit, save a strip at one end, which is left free for the fingers. One slide will then have as much on as many coverslips, and a sliding stage enables the work to be done with much less labour than is involved in using several coverslips.

† A two-minute sand time-glass is most handy.

exceeded ; (4) that the washing must be carefully watched till the colour *just* appears ; (5) that no time must be lost in drying.

Another method, recommended by Jagri, is to add 2 per cent. formalin to the fluid whose cells are to be examined, centrifuge off the deposit after a few hours, and then stain with a freshly prepared Giemsa solution in glycerin and methyl alcohol. The cells should be examined while in the stain.

The Results afforded by Cytodiagnosis.—The cells of a puncture-fluid may be either enumerated in a counting-chamber or may be subjected to a "differential count." Sometimes it is advisable to perform both forms of counting. The second count is, however, much more generally useful, especially as by its means we can watch the progressive changes in the cellular constitution of an effusion—changes which occasionally afford valuable prognostic indications. The insight into diagnosis is indicated by the following considerations: If inflammation supervenes during the course of a passive accumulation of fluid, the cytological picture will become completely changed, as will be shown later ; or if recovery is taking place from an inflammatory effusion another change will be found to take place in the relative proportion of the cells ; or, yet again, if inflammation arises in the neighbourhood of a serous membrane into which there is already an effusion from passive causes, the cellular elements will be altered, even though the inflammation has not affected the serous membrane itself.

It will be necessary to refer to these points again, but to avoid repetition it will be more satisfactory to discuss each class of cell that may occur in a puncture-fluid, and its relations to disease, than to discuss the diseases, with reference to their cytological characters, or, as it is termed, their "cytological formula."

A series of researches made by Heinz in 1900 throw much light on the occurrence of different cytological formulæ, although no particular notice seems to have been hitherto taken of his work. This observer studied the effects of artificially induced inflammation of the peritoneum in rabbits (injections of emulsions of turpentine and of iodine). The result was an accumulation of leucocytes *beneath* the endothelium at the spots at which the irritation came in contact. Little raised masses consisting of fibrin filaments (due to coagulation of outpoured serum) in-

filtrated by these leucocytes, appeared, and subsequently fibroblasts replaced the coagulated material, which was still covered by an intact serosa. As the inflammation proceeded, however, the endothelial cells began to proliferate and assume a more cubical shape, their cell-body became more granular, and their nucleus larger and richer in chromatin, with development of mitotic figures. Delicate strands of cells and membranous structures made their appearance still later, until the tag-like processes of a *cor villosum* were produced, and these tags were also covered by several thicknesses of cubical epithelial cells showing numerous mitotic figures. These young embryonic cells formed part of a new mucoid tissue which was vascularised from the serosa vessels. The liberation of such cells, as well as a liberation of polynuclear cells, would give a distinctive character to the exudate, varying according to the stage at which the inflammatory change had reached.

The exact course which the inflammatory processes is going to take, the intensity of the inflammation, and the degree of proliferation of the endothelium of the serous membrane will all influence the cytological formula, and the significance of the findings in a given exudate or transudate will depend on a due consideration of all these factors.

THE CELLS WHICH OCCUR IN EXUDATES AND TRANSUDATES

1. **Lymphocytes.**—The preponderance of lymphocytes in an effusion under certain conditions, especially tuberculous conditions, was the first fact about cytodagnosis to which attention was directed.

If we inquire into the possible *source* from which the lymphocytes of an effusion are derived, we shall find that they may be classified into angiogenic, endotheliogenic and histiogenic lymphocytes, though it is not possible to decide between them as they are met in an ordinary preparation. The angiogenic lymphocytes are those which have wandered, by their intrinsic power of wandering, from the blood stream or from the lymphatics. The endotheliogenic lymphocytes include those forms which are really pseudo-lymphocytes, the result of degeneration of polynuclear or of endothelial cells. The histiogenic lympho-

cytes have come from the lymphatic tissues that enclose the blood-vessels supplying the serous membranes. Signorelli supposes that lymphocytes may be derived from proliferation at the site of effusion.

It may be said that lymphocytes in an effusion are a response to a stimulus of low activity, the reaction on the part of the tissues in the immediate locality of a disease which does not seriously implicate the rest of the organism. They are therefore most frequent in effusions of a chronic nature, and if they do occur in the course of acute disease, it is because there are reparative processes going on in the damaged tissues.* A weak stimulus spread over a long space of time constitutes two factors, which, existing as they do in tuberculous disease, serve to explain the frequency with which lymphocytotic effusions occur in tubercle.† Where exceptions to this rule occur, the explanation must be sought in some superadded acute condition, or in greater toxicity of the micro-organisms, or in the presence of other micro-organisms as well, unless there be deficient resistance on the part of the individual.

The preponderance of lymphocytes is a feature not only of pleural tuberculous effusions ‡ (especially at the end of the second week), but also of tubercle of the peritoneum, joints, tendon sheaths, and any serous cavity. It may be said that if lymphocytic effusion be of *acute* onset, it is certainly tuberculous, and that the appearance of lymphocytosis after a polynucleosis indicates a favourable prognosis. When polynucleosis gives place to a lymphocytic phase in the course of secondary tubercle of the serous membranes, there will be an absolute increase in the number of cells per cubic millimetre.

Lymphocytes are also met with in considerable number in transudates of mechanical origin,§ but in nephritic transudates they number less than 20 per cent.|| According to Signorelli,

* Signorelli.

† This fact has been verified by many observers. It is only necessary to mention Widal, Ravaut, Wolff, Quincke, Ehrlich, Grawitz, Raubitschek, Köster, Vargas-Suarez, Stassewicz, Jacobsohn, Steinbach, v. Ketley and v. Torday. The percentage of lymphocytes varies from 70 to 95.

‡ Attention may be drawn to the fact that the connective tissue of tuberculous lesions is often densely infiltrated with histiogenic lymphocytes.

§ v. Ketley and v. Torday, Bunting.

|| Stassewicz.

lymphocytes in excessive amount may be expected in effusions associated with disease of the lymphatic system.

ERRORS OF DIAGNOSIS OF LYMPHOCYTES

1. *Dead endothelial cells* (Plate 2, Fig. 4) may assume the aspect of lymphocytes.* 2. *Dead polynuclear cells* may also simulate them. 3. *Sarcoma cells* differ from lymphocytes because the former stain differently and their nuclei contain less chromatin. 4. *Lymphocytes* may themselves undergo necrotic changes, when fat droplets and vacuoles appear. 5. *Pseudo-lymphocytes* are probably of various kinds. They include Nos. 1 and 2 of the above list, and also new-formed embryonic connective tissue cells (young fibroblasts).

Pseudo-lymphocytes are most frequent in those tuberculous exudates which are associated with lymphocytosis (Königer).

The following are distinguishing features: the size is that of a lymphocyte, the nucleus is relatively large, round, rich in chromatin, and stains deeply. The cytoplasm is moderately abundant and sometimes contains neutrophile granules. Often they have much resemblance to nucleated red cells.

2. **Polynucleosis.**—The appearance of polynuclear cells in an exudate may be looked on as the expression of a general reaction on the part of the individual against a more severe infection. The more virulent the infection the more decided will the polynucleosis be. It is met with in empyema, pyopneumothorax, in the initial stage of tubercle, especially when starting in another organ, and especially in tuberculous pericardial effusions or when tuberculous disease in the lung is undergoing caseation.

As a general rule, the number of these cells diminishes as the case progresses towards recovery, and lymphocytosis takes its place. On the other hand, if polynucleosis takes the place of a lymphocytosis in a pleural effusion in a cardiac case, one should suspect the development of an infarct. The state of preservation of these cells occasionally gives a clue to the nature of the case.

ERRORS OF DIAGNOSIS OF POLYNUCLEAR CELLS.—They may be simulated by *degenerate cells* of other kinds, owing to fragmentation of the nucleus. Careful focussing will assist in distinguishing them.

* Patella.

Pseudo-lymphocytes have been referred to (p. 224).

Polynuclear cells may themselves undergo *degenerative* changes. There is then fragmentation of the nucleus, disappearance of granules, swelling of the cell-body ("hydrops"), loss of staining power of the nucleus, and even bursting of the cells. Such degenerate cells may be found as cell-inclusions in endothelial cells. Shrinkage of polynuclear cells, with fragmentation of the nucleus, occurs in tuberculous pleurisy secondary to pulmonary disease.

3. **Endothelial Cells.**—(Plate I, figs. 2 and 4.)—The presence of a large number of endothelial cells in a fluid is sufficient to enable one to exclude an ordinary inflammatory process. They are especially characteristic of transudations from back-pressure and may then form 99 per cent. of the total cells. As one would expect, they are derived from proliferation of the endothelium of the serous membrane, the proliferated cells becoming shed. They are more likely to appear as a result of repeated tapping of a serous cavity, presuming that the effusion is purely of mechanical origin. In cases of inflammatory effusion there will be no change in the variety of the cellular elements, a fact which is of great importance in differentiating doubtful cases of tubercle, for instance.

A large number of endothelial cells in an effusion of traumatic origin may be looked on as a good prognostic sign, especially if any polynuclear cells that may co-exist are in a good state of preservation. It must be borne in mind that some cases of *tubercle* show even as much as 60 per cent. of endothelial cells in the early stages of an effusion, while they disappear later on.

The presence of endothelial cells in a peritoneal fluid in cases of simple ovarian tumour has been noted by some observers.

ERRORS IN DIAGNOSIS OF ENDOTHELIAL CELLS.

1. *Leucocytes.*—If endothelial cells, in virtue of their phagocytic powers, have taken up leucocytes, they may come to be mistaken for the latter.

2. *Carcinoma Cells.*—It has often been said that one cannot distinguish between these two,* but so definite a negation must

* Lately, Sawyer, 1908.

be disputed. The characters of endothelial cells and of carcinoma cells are shown on Plate 1, figs. 2 and 4, and Plate 2, fig. 3, the use of colour serving to accentuate the features. We may say that (a) the *endothelial cell* has *usually* a very large cell-body, with a regular oval nucleus (Plate 1, fig. 2), which stains but feebly with Jenner. While the cell-body may show vacuolation, it has usually a perfectly uniform structure. (b) The cells have all a similar appearance. Contrasting *carcinoma* cells, we find that here there are hardly two alike, (i) the cytoplasm never stains uniformly, and is (ii) often vacuolate and contains fat granules and cell-inclusions ("bird's-eye-like" structures—Erben); (c) the nucleus under favourable circumstances stains deeply, and shows mitotic figures, often of *heterotype* character, besides frequently containing several nucleoli (Plate 2, fig. 3); (d) the adhesion of many cells in a bud-like fashion is frequent. Some of the cells are multinucleate. (e) It is hardly fair to cite, in exemplification of the ability to diagnose cancer-cells, the appearance of the phenomenon depicted in Plate 2, fig. 1, for an opportunity of this kind must be rare. It is, however, conclusive. The fluid in this particular case was turbid, and the turbidity was found to be due to particles formed of clusters of cells of perfectly distinctive character even in the unstained preparation. The drawing is prepared from a paraffin section made from a mass of the deposit that was dehydrated and embedded. The columnar shape of the cells, which are rather degenerate, and the central stroma, are shown. It is of course not certain that this was from a peritoneal fluid rather than from an ovarian cyst, and, unfortunately, neither operation nor post-mortem examination was permitted. (f) The usual type of carcinomatosis consists of a diffuse growth scattered all over the peritoneum and shedding its cells into the fluid that its presence has excited. These cells are often degenerate, and cannot be distinguished from similarly degenerate endothelial cells. A comparison of all the cells of the effusion and a search for some better-preserved types may reveal the nature of the case. The difficulties are of course insuperable where there is much endothelial hyperplasia as well as the carcinomatosis. Solid fragments of tissue would settle the diagnosis, but even in their absence the diagnosis is not as hopeless as some would have us believe. The drawings referred to have

been made from specimens obtained in the Leeds General Infirmary, and are from cases verified post mortem, so that there can be no plea that carcinoma cells were not present in, say, Plate 2, fig. 3. There were enormous numbers of these cells, and there was also pleural carcinomatosis absolutely widespread.

3. *Large Mononuclear Cells*.—These will be considered under the next heading.

4. *Large Mononuclear Cells*.—These cells are best distinguished in unstained preparations. Changes in the osmotic conditions of the fluid after tapping tend to alter their characters greatly, so that early examination becomes particularly important. They are depicted in fig. 4 of Plate 1. They possess phagocytic properties, and are apparently derived sometimes from connective tissue cells, sometimes from endothelial cells, and sometimes from the blood-stream, while, according to Marchand, they may come from the perivascular lymphatic tissue (leucocytoid cells). The phagocytic cells are very large in size, and often have included fragments of degenerate cells. If they are increasing in number they indicate a favourable prognosis, while if they are absent from the deposit in an inflammatory effusion, and bacteria appear, their absence indicates approaching suppuration. In early tuberculous effusions they may number 1 per cent. of the total cells (Bunting). They have been met with in the pleural effusion associated with enteric fever.

5. *Eosinophile Cells*.—Two classes of eosinophilia may be described—a relative and an absolute eosinophilia. The latter exists when the cells number from 10 up to 74 per cent. of the total count. The significance of so high a count is unknown, though it has been described as occurring in cases of acute rheumatism, in nephritis, and in convalescent cases of tubercle. Burnet regards the phenomenon as indicating diminution of the virulence of the organism, while Bibergeil ascribed it to chemotactic influence of broken-down endothelial cells.

There is generally eosinophilia of the blood at the same time, though this is not met with in infective cases, with the exception of syphilitic cases, as Widal and Ravaut record.

Eosinophilia has been described in several cases of carcinoma, thus, by Erben, by v. Starck, by Kröniger. When it appears in

an effusion which developed since a growth was noted in another part of the body, it may be assumed that metastasis has taken place.

In v. Starck's case there were also enormous cells which stained badly, were much vacuolated and contained peripherally situated nuclei. He found no tumour elements. The fluid became darker in colour with repeated tapping, up to a certain point, after which it became lighter in colour.

6. **Mast Cells.**—These may often be seen, especially if the fluid be examined rapidly. They occur in chronic effusions chiefly, and are pathological, since they are never found in the normal fluid bathing the serous membranes.

7. **Red Blood Cells.**—Apart from accidental contamination, importance has always been attached to the presence of blood in an effusion as indicating either tubercle, carcinoma, or renal disease. It may occur, however, in metapneumonic empyema * and in rheumatic cases.† A large amount of blood is met with in those rare cases, of course, where a thoracic aneurism is gradually leaking into the pleural cavity. In such a case the utmost importance will be attached to the finding.

8. **Carcinoma or Sarcoma Cells** (Plate 2, fig. 3).—The appearance of these cells in an exudate may be looked on as of diagnostic value to an extent which does not exist in the diagnosis of carcinoma of any solid organ. The presence of carcinoma cells in the sputum, in the stomach contents, or in the fæces or urine must be looked upon as mainly mythical, as it is out of the question to distinguish between malignant cells and the endothelial or epithelial cells of the passages. We can only hope to diagnose the condition from the presence of *cell-masses*. But in serous cavities freer conditions obtain, and although fragments of tissue may occur, the individual cells are much more capable of accurate study. The chief features of diagnosis have been gone into fully on page 226, and need not be again described.

Quincke described a glycogen reaction in carcinoma cells, granules visible on staining with iodine-gum being met with

* Raubitschek; also a recent case in the Leeds General Infirmary.

† Earl.

in such cells ; but little importance can be attached to this from the diagnostic point of view, in the same way as the glycogen reaction of pus cells so often proves misleading.

ERRORS IN DIAGNOSIS.—(a) *Endothelial cells*, when entangled in fibrin, may simulate carcinoma cells. (b) *Vacuolated cells* may occur in ascites connected simply with an ovarian cyst, or in benign tumours of the ovary. The most serious difficulty, however, is that there may be no free tumour cells at all in the exudate, or that those which were shed have become dissolved. Grenet and Vitry, too, describe two cases in which the peritoneal fluid in a case of carcinomatosis contained only lymphocytes, large mononuclear cells, and red corpuscles.

Certain Special Fluids.

HYDROCELE FLUID.—Flakes of endothelial cells or isolated cells are met with in large numbers in simple hydrocele, and sometimes they are abundant in chronic hydrocele. As a rule, the other cellular elements follow the familiar types. Lymphocytes preponderate in tuberculous hydrocele, or in any chronic inflammatory process. Polynucleosis, on the other hand, is generally (not always) associated with acute inflammation—usually gonococcal. The irritation produced by tapping, and especially by injecting such remedies as iodine, will result in the appearance of endothelial cells and lymphocytes (Julliard).

JOINT FLUIDS.—In the effusions into joints resulting from various causes the various types of cell-elements give similar indications to those of other serous membranes. Thus, polynuclear cells predominate in gonorrhœal or acute suppurative cases, though, if there be no pyrexia, there may be only lymphocytes present. The same polynucleosis occurs in acute rheumatism, and in irritation after tapping. Lymphocytes indicate a chronic affection, much less frequently tuberculous than with other serous membranes, and a small proportion of endothelial cells is frequently met with. Rice-bodies are associated with lymphocytosis. Red cells are likely to be found in fair number in tuberculosis of joints. Tabetic joint effusions contain very few cells, and these are mostly lymphocytes and large mononuclear cells.

CEREBROSPINAL FLUID.—On reviewing the enormous accumu-

lation of literature of the cytology of cerebrospinal fluid alone, we strike upon one great fact, namely, that in functional disease the lumbar puncture will show few, if any, cellular elements at all, while if there be organic disease of the brain or spinal cord, we may expect to find a considerable number of cellular elements, a differential count of which will assist in the diagnosis of details with similar limitations to those that obtain in the case of pleural and peritoneal fluids. Probably in this kind of fluid the real significance of lymphocytosis or polynucleosis, or of a mixed type, is similar to that which obtains for pleural and peritoneal fluids, namely, that it is not so much a question of tubercle or other organisms, as that in the one case the inflammatory change is of a much less active kind, demanding fewer phagocytes.

The varieties of cytological formula which may be met with may be grouped under the following headings :

(a) *Absence of cellular elements*, or, fewer cells than two per cubic millimetre. This is the normal condition, as shown by many investigators (Schwarz, Bronstein, Verzeanu, Devaux, Schlesinger, and others). Cells are absent in such purely mental diseases as mania, paranoia, melancholia, imbecility, acute alcoholism, delirium, senile dementia (Merzbacher).

If there be no cellular elements present, or if their number be not greater than two per cubic mm., this is sufficient to exclude any meningeal inflammation, syphilitic meningitis, and such diseases as tabes, superficial gummata, tumours, hypertrophic pachymeningitis. A similar condition is met with in the cerebrospinal fluid in cases of herpes.

(b) *Lymphocytosis*.—This term denotes either a relative excess of lymphocytes over the other cell-elements or it means that lymphocytes constitute the only cells present.

Lymphocytosis occurs, to a moderate extent only, in some cases of dementia paralytica, of chronic alcoholic paralysis and of disseminated sclerosis, while it occasionally occurs in perfectly normal fluids (Schwarz and Bronstein). Speaking broadly, lymphocytosis occurs in any meningeal inflammation that is of *chronic* nature. It is therefore met with in tuberculous meningitis (especially in children), in syphilitic meningitis, whether congenital* or acquired.† It has been found in cases of herpes,

* Kretschmer.

† Raubitschek, Devaux, Donath.

of mumps,* and of tabes, as well as in epileptics † (showing that epilepsy is not purely a "functional" disease). It has been observed that when lymphocytosis occurs in a syphilitic case mercury causes the cells to disappear.

The view has been expressed ‡ that lymphocytosis is not characteristic of syphilitic nervous disease, but that it is only present if the meninges are involved.

There are one or two additional points to note about this type of cell-predominance: (1) its development during the course of a case indicates improvement. (2) If polynucleosis were present before, the supervention of lymphocytosis may be regarded as a favourable sign. (3) It is not present during the initial stages of tuberculous meningitis. On the other hand, in the later stages of this disease one may expect to find as many as 10,000 cells per cubic millimetre.

(c) *Polynucleosis*.—This generally signifies an acute suppurative meningitis, or, at any rate, acute meningeal disease.§ It may develop in the initial stages of tuberculous meningitis, and is often continuously present in the tuberculous meningitis of adults. It is met with in cases of commencing poliomyelitis, and in cases of herpes. In cerebrospinal fever there is an excess of polynuclear cells at the outset of the disease, and at each exacerbation of the fever. Sawyer gives: polynuclear cells 84 per cent., lymphocytes 15.6 per cent., degenerate cells 0.4 per cent. It may occur during the congestive attacks of hemiplegia.

It is important to note the state of the preservation of these cells, for if transient aseptic disease, such as during syphilitic nervous disease, is present, the cells will be found particularly well preserved.

The number of cells per cubic millimetre in a case of suppurative meningitis may reach 100,000, of which 53 or more per cent. will be polynuclears. The number diminishes as the case progresses towards recovery. In a case of cerebral abscess || there were 97 per cent. of polynuclear, 2.4 per cent. of lymphocytes, 0.4 per cent. of degenerate cells, and 0.2 per cent. of endothelial cells. (One would dispute, from one's own experience, whether endothelial cells can be identified in cerebrospinal fluid.)

(d) *Other Cellular Elements*.—Blood may be present in cere-

* Raubitschek.

† Merzbacher.

‡ Funke.

§ Verzeanu, Devaux, Donath.

|| Sawyer.

brospinal fluid, apart from accidental contamination, in cases of intracranial hæmorrhage where the blood has made its way into the meningeal cavities. In a case of this kind, Sabrazes and Muratet found round or oval polyhedral isolated cells or masses of such cells, containing fragments of red corpuscles, or hæmatoidin granules. These they regarded as "macrophages," derived from the endothelium of the subarachnoid spaces. Such cells may contain fatty granules, fragments of myelin, and pigment.

The presence of *large mononuclear cells* with basophile protoplasm, and eccentric nucleus, has been described as occurring in tubercular meningitis, but they also occur in tabes and cerebrospinal syphilis.

Tumour cells have been described as occurring in cerebrospinal fluid by Sahli.

Degenerate cells in cerebrospinal fluid have been explained as due to decomposition having set in; but it is satisfactory to learn that Pappenheim has offered the view that the degenerate character is evidence of pathological processes, and that it is the result of a toxic action exerted by the diseased cerebrospinal fluid on the polynuclear cells. This type of cell occurs most frequently in paralytics, and the proof adduced is that warming the fluid to 56° C. causes it to cease to have any deleterious action.*

The presence of organisms such as spirochætes or trypanosomes does not come within the scope of this work.

The *rate of flow* of the fluid from a lumbar puncture has been laid undue stress on by some authors, but to adduce arguments for believing so is out of place here.

OVARIAN CYSTS.—The cellular elements in these cases are almost pathognomonic, and are so well known as hardly to call for more than mere mention. Columnar epithelial cells, ciliated epithelial cells, and squamous epithelial cells may be met with, according to the character of the growth. Cells with fatty granules

* One may emphasise this idea of Pappenheim's by calling attention to the fact that degenerate appearances which are observed are only too readily ascribed to imperfect technique: thus, a liver which becomes almost fluid on removal from the body is often regarded as an example of early post-mortem decomposition. So with the suprarenal gland. As a matter of fact, most livers and suprarenals do not break down readily, even if the necropsy is delayed 48 hours. The explanation of the undue friability must therefore be sought, as there must be a reason for such changes apart from "accident."

are also characteristic, especially in multilocular cysts. Colloid masses may appear in the fluid in cases of colloid carcinomata. The presence of cholesterin crystals is a frequent feature in ovarian tumours, and has even been met with in a simple par-ovarian cyst (in this Infirmary), though such cysts have usually perfectly clear watery contents.

Special Findings in the Deposit of Puncture-Fluids.

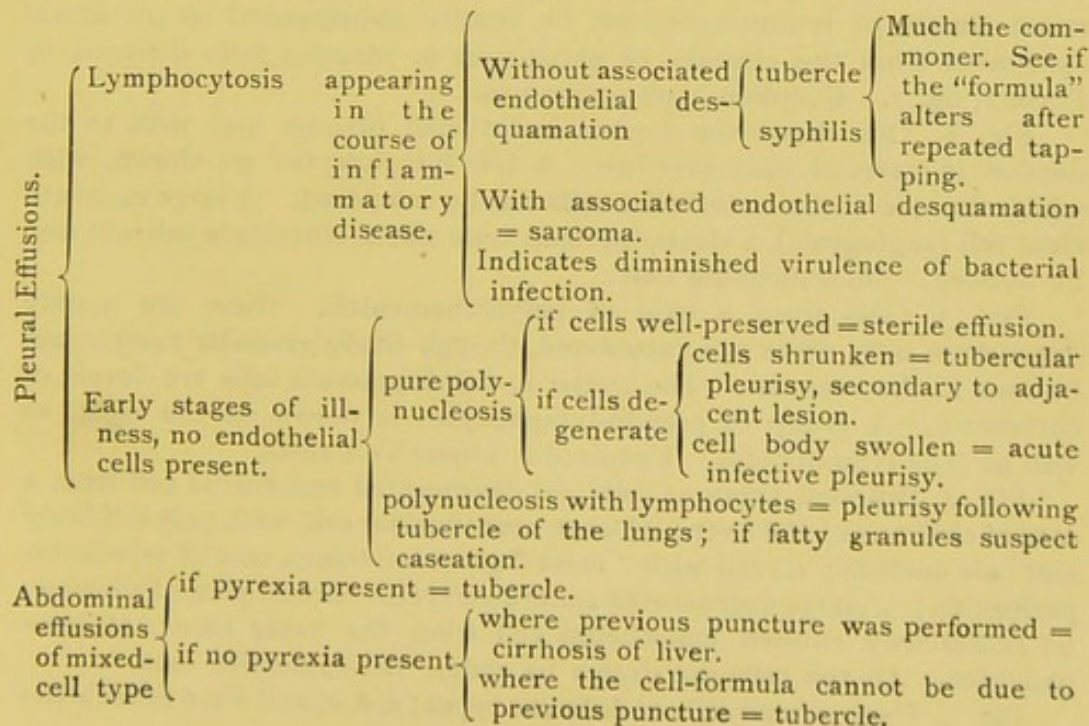
—*Hæmatoidin crystals* occur in empyema, subchronic abscess, pulmonary abscess, and especially in old suppurating hydatid cysts. They indicate antecedent hæmorrhage.

Food particles may be found in the peritoneal fluid after perforation in the course of the alimentary canal. *Tumour fragments* may be met with. *Myelin bodies*, composed of protagon, are referred to by Sahli as being abundant in tumour of the lung which has invaded the pleura. *Fatty acid crystals* occur in putrid collections of pus. *Triple phosphate crystals*, calcium, carbonate, and phosphates may be seen in purulent collections. *Hydatid hooklets* or scolices may be found, and *renal casts* will occur in the corresponding cysts.

Spontaneous coagulation of a fluid is an indication that fibrin ferment was present.

Scheme for Differential Diagnosis in Special Cases.

—The following scheme has been devised from the data supplied by Königer, as it may be found useful :



The Chemistry of the Cell-elements present.—This subject does not come under cytodiagnosis, but reference is made to it in order to draw attention to the fact that upon the composition of the cells present in an effusion the chemical characters will to a certain extent depend. The degeneration of the cells will naturally lead to the substances of which they were composed appearing free in the fluid. The exact substances present will be found on reference to the appropriate headings in the preceding sections, but especial attention may be drawn to the facts made out by Müller and Jochmann, which go to show that the granules in polynuclear cells, not to mention the eosinophile granules, are probably of the nature of zymogen granules, and that they are intimately connected with the proteolytic ferment which exists in leucocytes. In this manner it will be obvious that the granular appearance of a polynuclear cell must be placed on a par with the appearance of gland cells (such as salivary gland cells) at different stages in their activity.

EXPLANATION OF THE PLATES

PLATE I

Fig. 1. The bulk of the cells in this figure are lymphocytes, many of them having a granular appearance. In the middle is a large mononucleate cell, also very granular, and of endothelial origin. Just below it will be seen a large mononuclear cell of angiogenic origin. The small cluster of lymphocytes will be readily distinguished (in an actual specimen) from the clusters of carcinoma or sarcoma cells depicted in Plate 2, fig. 2. Eyepiece 2, objective $\frac{1}{4}$ in.

Fig. 2. This shows the typical endothelial plaques met with in the deposit in cases of back-pressure. A few lymphocytes are shown, with which the size of the endothelial cells can be compared. A large mononuclear cell (angiogenic), a cluster of dead cells and a binucleate cell will also be noticed. Oil-immersion lens.

Fig. 3. Cells from a case of polyorrhomenitis. These are mainly degenerate cells, some are vacuolated, though finely granular matter can be seen in the vacuoles. The nuclei in the degenerate cells are devoid of chromatin. A group of pseudo-lymphocytes is shown on the right, as well as true lymphocytes. Eyepiece 2, objective $\frac{1}{4}$ inch.

Fig. 4. Different types of cells, (a) degenerated endothelial cell from a case of cirrhosis of the liver; (b) large mononuclear cell, with pale cell-body and pale nucleus; (c) cell with "mast" granules, from a case of tubercular peritonitis; (d) large degenerated endothelial cell—chronic pleural effusion; (e) enormously swollen endothelial cell from the same case; (f) lymphocytes. All the cells are drawn to scale. Oil-immersion lens.

Fig. 5. Degenerated cells of different types: a, b, c, and d are endothelial



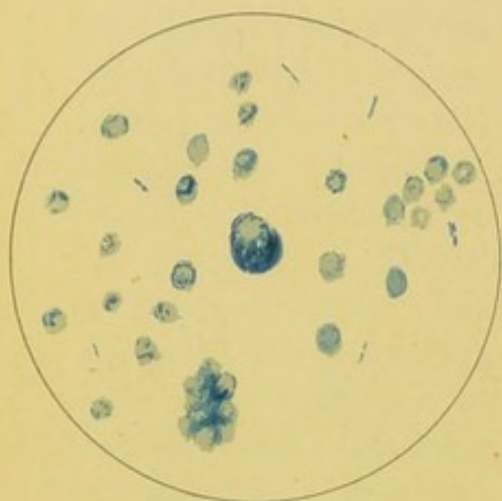


Fig. 1.

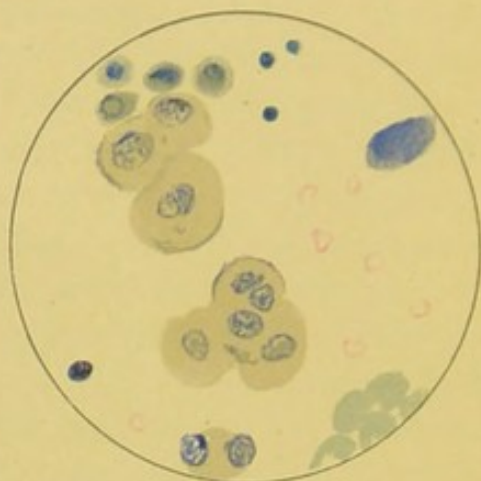


Fig. 2.

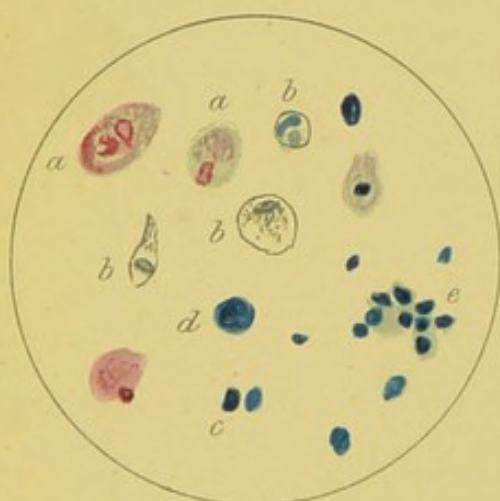


Fig. 3.

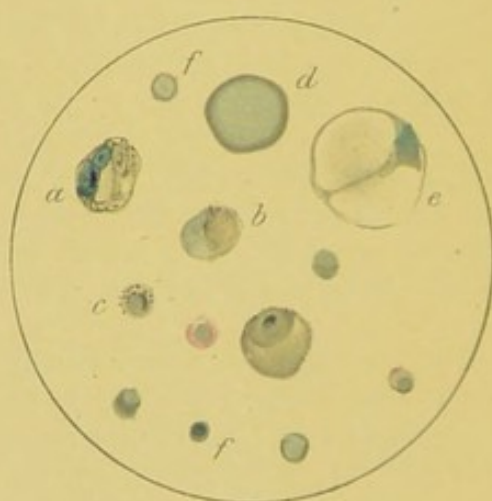


Fig. 4.

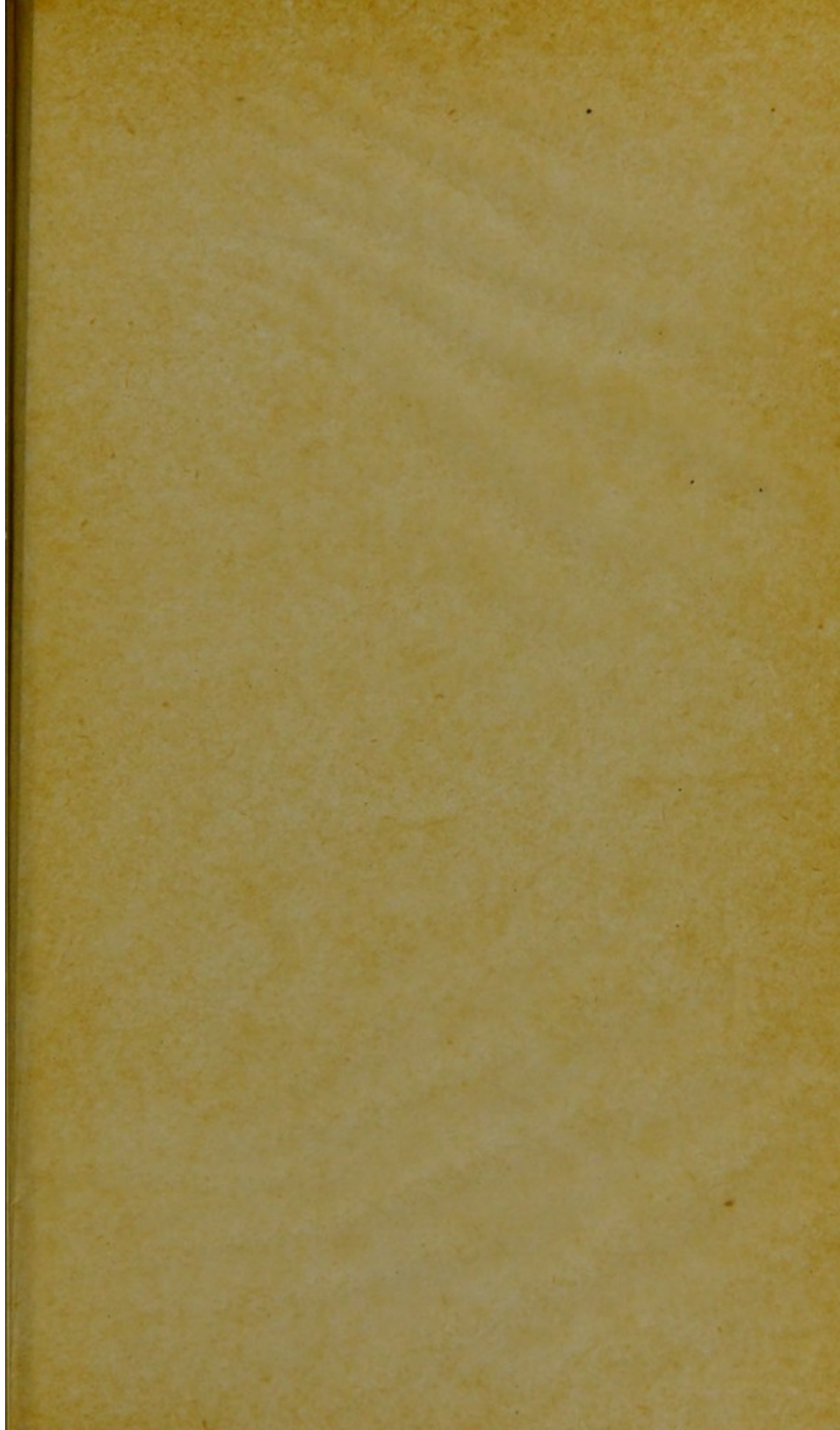






Fig. 1.

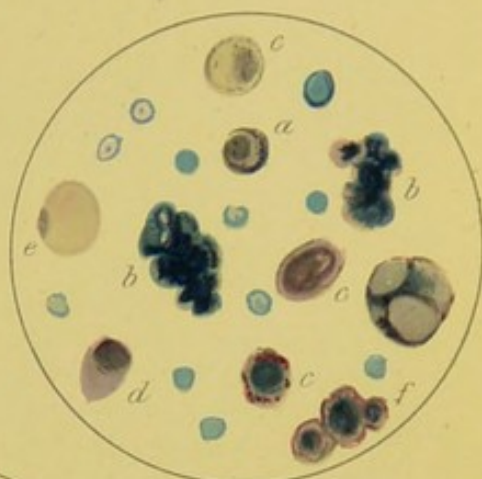


Fig. 2.

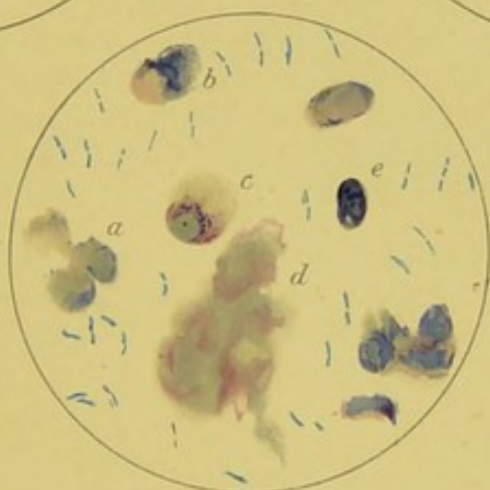


Fig. 5.

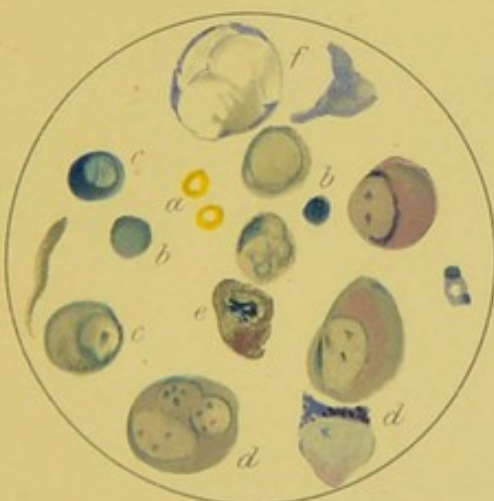


Fig. 3.

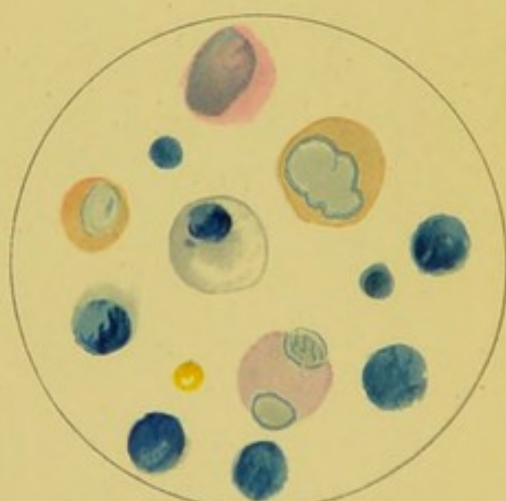


Fig. 4.



cells; *c* shows nucleus, with nucleolus, granules, and degenerating cytoplasm; *d* is a structureless mass; *e* large mononuclear cell. The bacilli are *Bacillus lymphagogen* (Hamburger). Oil-immersion lens.

PLATE 2

Fig. 1. Fragments of tissue from the peritoneal fluid in which there was dissemination of carcinoma. Columnar cells are seen on a basement membrane. There are indications of structure within the globules of tissue. The spherical appearance was well seen in the fresh specimen. Eyepiece 2, objective $\frac{1}{8}$ inch.

Fig. 2. Cells from a case of sarcomatosis of the peritoneum. (*a*) Sarcoma cell whose nucleus has two nucleoli, and large granules are seen in the cytoplasm; (*b*) typical clusters of cells with deeply staining nuclei. There were extremely large numbers of these clusters present in each film. (*c*) large mononuclear cells; (*d*) lymphocytes (the relative sizes of the cells are readily seen from these); (*e*) swollen degenerate cell; (*f*) a few sarcoma cells in juxtaposition, showing deeply staining nuclei, and deeply staining cytoplasm. Eyepiece 2, objective $\frac{1}{8}$ inch.

Fig. 3. Endothelial and carcinoma cells. (*a*) Red cells; (*b*) lymphocytes; (*c*) endothelial cells; (*d*) swollen cells containing several nuclei and nucleoli; (*e*) carcinoma cell showing mitotic figure; (*f*) excessively swollen endothelial cell. Oil-immersion lens.

Fig. 4. Cells from peritoneal carcinomatosis. Shows large vacuolated cells, some large multinucleate cells, some binucleate cells and large deeply staining mononuclear cells. Oil-immersion lens.

SECTION VI

SPECIAL CASES

THE application of the facts recorded in previous pages and of the various methods of diagnosis which have been advocated is best illustrated by the relation of certain special cases which have been examined. The various observations made on the specimens of fluid received for examination are here recorded, and the deductions which have been made as to the nature of the fluid are introduced. The post-mortem diagnosis subsequently obtained is introduced, and affords an opportunity of commenting on the diagnosis made in those cases where one's conclusions proved to be at variance with the actual truth.

The greatest difficulty is undoubtedly attached to the diagnosis of the effusions in either chest or abdomen, especially in medical cases, and it becomes more useful to refer solely to these two kinds of fluid.

The cases are grouped therefore into (*a*) pleural, and (*b*) peritoneal, but no other order is observed. Only those which presented features of particular interest or of difficulty are considered in this section.

(A) PLEURAL FLUIDS

I. G., aged 40.—The fluid was straw-coloured, with blood-staining of the deposit. The specific gravity was 1012. There were no endothelial cells in the deposit, which contained a few lymphocytes and some red cells.

The chemical examination showed an abundance of chlorides (8 gm. per litre), and a very small amount of albumen (0.5 per cent.). There was no glycoproteid present.

The concentration of electrolytes was 0.2460, of which 0.0424 consisted of achlorides. The ratio achloride/chloride was 0.21.

The post-mortem examination showed cardiac incompetence due to valvular disease (aortic and mitral). There was also ascites, cirrhosis of the liver, infarcts in the lung, spleen, and kidney.

The findings in the pleural fluid point to a passive accumulation of fluid (transudation), partly because there was an increase in the chloride-content, and partly on account of the achloride/chloride ratio. All the facts made out fall in line with the diagnostic indications which were mentioned in Section IV.

II. RICHARD H., aged 26.—The straw-coloured fluid had a specific gravity of 1.032, and showed a considerable deposit, mainly of lymphocytes.

The *chemical* examination showed a maximal quantity of albumen, and a considerable quantity of chlorides (7.25 per cent.). The presence of other substances was not investigated.

The electroconductivity examination showed the amount of total electrolytes to be 0.2319, and the achlorides were as much as 0.1533 (total concentration in terms of Na_2CO_3). The achloride/chloride ratio was therefore above unity, 2.95.

On the strength of these facts, high albumen-content, high "salt" ratio, and the cytological characters, the diagnosis of inflammatory effusion of tuberculous origin was made.

III. ALFRED B., aged 15.—A green, fluorescent fluid, having a specific gravity of 1.021, contained 2 per cent. albumen.

The concentration of the chlorides was 0.043 gram-mol., while the total concentration of the electrolytes amounted to 0.113. The achloride electrolytes therefore amounted to 0.070 gram-mol. and the achloride/chloride ratio was 0.16.

The osmotic concentration was 0.281, so that the non-electrolytes amounted to 0.168.

The chemical examination showed a trace of urea, no purins, a trace of glycoproteid, a trace of invertase, but no diastase.

The α -naphthol test was tardy, but the glucosamine test instant and intense.

The cellular elements consisted mainly of lymphocytes.

The examination on three different occasions gave the following results :

Observation.	April 29.	June 7.	June 8.
Specific Gravity	1021	1023	...
Albumen	2%	6.31%	6.95
Urea... ..	+	+	...
Purins	+	...
Albumoses	0	...
Monamino-acids	0	...
Lecithin	+	...
Glycoproteids	+	0	0
α -naphthol test	+	+	+
Glucosamine test	+++	+++	+
Concentration of Electrolytes ...	0.113	0.116	0.123
" Chlorides	0.043	0.042	0.070
" Achlorides	0.070	0.074	0.053
Achloride/chloride ratio	0.16	1.7	0.75

It will be seen that only on June 7 did the "salt" ratio fall in with the idea of an inflammatory effusion. This case was only of "idiopathic" pleural effusion, and was *presumably* tuberculous.

Comment.—There is a certain amount of difficulty in weighing up this case. The chlorides being relatively low indicates an inflammatory process, although the total concentration of electrolytes was also low, so that the "salt" ratio is not constant. On the whole, the diagnosis is in favour of the pleural effusion being of inflammatory origin, and probably tuberculous.

IV. WILLIAM A., aged 42.—A milky fluid showing no spontaneous coagulum or any deposit beyond a few granular cells.

The total proteid amounted to 4.65 per cent., and the chlorides to 3.6 per cent. The concentration of the achloride electrolytes was almost equal to that of the chlorides. There was a moderate amount of urea. There was no glycoproteid. Cholesterin was present. The tryptophane reaction was positive. As regards ferments, there was a trace of diastatic and of inverting action.

Comment.—The microscopic characters and the "salt" ratio are in favour of this fluid having a transudatory origin, although the proteid-content is rather high. The diagnosis is that this fluid is a chronic effusion in which there may at first have been inflammatory processes, but that these are now quiescent.

V. MARY A., aged 34.—The pleural fluid was examined on two occasions, but with an interval of only a week between them.

The turbid greenish fluid (later, blood-stained), with a small amount of coagulum, had a specific gravity of 1020, and contained 6 per cent. of total proteid (only 0.58 per cent. globulin).

The chloride electrolytes showed a concentration of 0.035 on one occasion, the total electrolytes amounting to 0.2469, and the achloride electrolytes to 0.1830. The achloride/chloride ratio was therefore 2.86.

The chemical examination showed the presence of a trace of invertase, urea, no protalbumose, no glycoproteids, no mono-amino-acids. The α -naphthol test and the glucosamine test were positive but slight. The diazoreaction, on the other hand, gave a positive result.

The cellular elements were mainly lymphocytes.

This fluid was from a case in which there was carcinomatosis of the peritoneum, the primary growth being in the ovary. The pleural effusion was of inflammatory, but not malignant origin. The high salt ratio and other points will be noted.

The examination on the two occasions, when compared, showed no appreciable difference; not enough to justify reproduction of the findings in each case.

VI. ALFRED K., aged 28.—An amber-coloured fluid with an abundant coagulum, had a specific gravity of 1022, and contained 8 per cent. of total proteid, of which only a small proportion was globulin.

The concentration of chloride electrolytes was 0.064 gram-mol., the total electrolytes amounting to 0.261, and the achloride electrolytes to 0.180. The achloride/chloride ratio was therefore 2.23.

The chemical examination showed the presence of invertase, urea, albumose, but no purins; no serosamucin. The α -naphthol and the glucosamine reactions were immediate and marked.

The cells consisted mainly of lymphocytes and red cells.

The fluid was an inflammatory effusion of tuberculous nature.

VII. WILLIAM L., aged 21.—A clear yellow fluid with a considerable coagulum. The specific gravity was 1024 and 1 per cent. of albumen.

The concentration of the chlorides was 0.031, while that of the total electrolytes was 0.2319; the achloride electrolytes amounted to 0.1733 and the achloride/chloride ratio was 2.95.

The osmotic concentration was 0.296 gram-mol. per litre.

The chemical examination showed 0.004 per cent. purin N,

albumoses, but no monamino-acids. The α -naphthol and the glucosamine reactions were immediate and intense. Globulin formed a large percentage of the total proteid.

The cellular elements consisted mainly of lymphocytes.

This was an inflammatory fluid, occurring four weeks after an attack of pneumonia.

VIII. HILDA B., aged 6.—A bright yellow-coloured fluid, having specific gravity of 1022, contained 8 per cent. albumen.

The concentration of the chlorides was 0.042 gram-mol., while the total concentration of the electrolytes amounted to 0.122. The achloride electrolytes therefore amounted to 0.080 gram-mol. and the achloride/chloride ratio was 1.91.

The chemical examination showed the presence of much urea, but no albumoses, peptones, monamino-acids, or purins.

The cellular elements consisted mainly of degenerate pus-cells.

This was a fluid from an empyema associated with mastoid disease.

(B) PERITONEAL FLUIDS

I. FLORENCE W., aged 27.—The fluid is alkaline, and has a specific gravity of 1012. It does not coagulate spontaneously, but on standing deposits some flaky lymph, which entangles a large number of cells.

The *cells* are chiefly granular mononuclear leucocytes, and there are relatively fewer polynuclear leucocytes. Besides these, there is a not inconsiderable number of large cells with abundant cytoplasm and relatively very small nucleus. Some multinucleate cells—some having three or four, others several nuclei—were also observed.

The *chemical* examination has shown that the fluid is comparatively rich in chlorides (0.66 per cent.), and that there is about 0.1 per cent. of urea. The albumen is present in considerable but not in excessive amount (1.95 per cent.).

The *osmotic* pressure of this fluid is higher than that normal for blood (equivalent to 9 atmospheres instead of 7).

The urine secreted at the time of tapping was examined, and found to have as special characters a high specific gravity and an increased quantity of urea. It was practically free from chlorides, while the osmotic pressure was high, and caused largely by non-electrolytes.

The results of examination are tabulated below.

	Peritoneal Fluid.	Urine Secreted at time of Tapping.	Peritoneal Fluid 5 weeks later.	Urine Secreted at time of Tapping.
Specific gravity ...	1012	1030	1011	1017
Reaction ...	alk.	acid	alk.	acid
Deposit ...	{ granular leucocytes, large bi-nucleate cells, mononuclear cells }	red urates	0	urates
Urea ...	0.01 %	3.2 %	0	1.9 %
Albumen ...	1.95 %	0	0.5 %	nil
Chlorides ...	6.5 %	0.05 %	6.35 %	15 %
Freezing - point depression ...	0.591	1.950	0.666	1.150
Osmotic Concentration	319	1.052	0.360	0.644
Osmotic pressure in atmospheres ...	7.1	23.4	7.9	14.3
Conductivity (22° C.)	1161	1772	1181	1105
Conc. of electrolytes	0.3047	0.201	0.128	0.120
Conc. of achlorides ...	0.0851	0.196	0.020	0.095
Conc. of non-electrolytes ...	0.014	0.851	0.232	0.524
C elect. }	2.16	0.23	0.55	0.24
C non-elect. }	0.41	0.97	0.15	0.79
C achlor. }				
C chlor. }				

Comment.—From these figures, showing the changes that have taken place during five weeks, it will be seen that while the two specimens show a similar composition as regards the most essential constituents, the urine shows slight changes. The amount of area has diminished, and that of the chlorides has increased. The concentration of electrolytes has also diminished. On this change there can be laid little stress, owing to the fact that urine is so variable. The constancy of the composition of the peritoneal fluids is, however, important, as indicative of a stationary condition. The ratio of electrolytes to non-electrolytes and that of achlorides to chlorides, following the rules formulated, indicates that this fluid is transudatory in nature.

The question as to whether there was or was not tuberculous peritonitis was raised, and the answer to that was as follows: Against tubercle (no tubercle bacilli found): less than 3 per cent. albumen, low specific gravity, absence of spontaneous coagulability, high achloride/chloride ratio. Alone in favour of tubercle was the presence of many mononuclear cells in the deposit. The

fact that this preponderance was very slight did not influence one's opinion much in favour of tubercle.

Post-mortem diagnosis: thrombosis of the portal vein.

II. ELLEN D., aged 50.—A milky fluid of specific gravity 1018–1019–1020. The fluid was not spontaneously coagulable.

There is a large amount of albumen present (3 per cent.). The chlorides amount to 8 gm. per litre (0.137 gram-mol.).

The osmotic concentration is 0.272, while the urine secreted at this time showed only a concentration of 0.156. The urine had a specific gravity of 1005, contained no albumen, and only 3 per cent. of chlorides; the urea was 0.25 per cent.

The electrolytes consisted almost entirely of chlorides, the total electrolyte concentration being only 0.139 gram-mol. per litre.

The deposit was considerable, and consisted of numerous very large mononuclear cells (some bi- or tri-nucleate); also red cells, and some polymorphonuclear leucocytes. Some of the cells contained refractile non-fatty granules.

The percentage of albumen indicates that the fluid is either of a chronic exudative nature (associated with either hepatic cirrhosis or with peritoneal new growth). While the percentage of albumen just comes within the limit for tuberculous effusions, it is so high that this form of peritonitis may be excluded.

	April 25, 1906. Abdominal.	June 12, 1906. Abdominal.	June 19, 1906. Pleural.	July 19, 1906. Abdominal.
Specific gravity ...	1020	1020	1018	1019
Albumen	3%	1.7	0.2	2
Glycoprotein	present
Chlorides	0.137 gm.-mol.	0.113	0.097	0.046
Freezing-point depression ...	0.502	0.679
Osmotic Concentration	0.272358
Conc. of Electrolytes	0.139	...	0.115	.223
Conc. of Achlorides	0.002	...	0.018	.177
Conc. of Non-electrolytes ...	0.133135
Achloride/chloride ratio	0.01	...	0.34	2.62
Deposit	? Sarcoma	Enormous cells (sarcoma?) and red cells	Endothelial cells and lymphocytes	Numerous cells and amorphous coagulated proteid

The fluid is an exudate, not a transudate.

This case was tapped several times, and was found to show fairly constant composition.

In the pleural fluid the achloride ratio was low, just as at first in the abdominal fluid. However, the latter subsequently showed the normal ratio of inflammatory conditions. We might assume that the pleural fluid was not of an inflammatory origin, and that the peritoneal had steadily become inflamed till eventually it was actually invaded by the growth.

Post-mortem Diagnosis.—Round-celled sarcoma of ovary with peritoneal dissemination.

III. JOSEPH B., aged 36.—The clear straw-coloured fluid had a specific gravity of 1012, and contained 0.8 per cent. of albumen. There was a small amount of urea present, and the chlorides amounted to 0.55 per cent. (0.094 gram-equiv.). The cells in the deposit were mainly endothelial.

Physico-chemical Examination.—The osmotic concentration was 0.360, and the concentration of the electrolytes 0.135. The achloride electrolytes amounted to 0.041 gram-mol. per litre, so that the achloride/chloride ratio was 0.435.

The urine secreted at the same time as the fluid was tapped was concentrated, and contained a copious deposit of red urates.

The fluid was examined again a month later, and found to still have practically the same characters, and the urine also had very similar characters, as will be seen from this table of results.

Observation.	May 2, 1906.		June 9, 1906.	
	Fluid.	Urine.	Fluid.	Urine.
Specific Gravity	1012	1036	1013	1035
Albumen	0.8%	none	1.0%	none
Urea	0.02%	3.3%	none	3.65%
Chlorides	5.5%	3.5%	6.85%	0.7%
Freezing-point Depression ...	0.667	2.405	0.658	2.400
Osmotic Concentration	0.360	1.326	0.355	1.316
Concentration of Electrolytes ...	0.135	0.266	0.131	0.254
" Achlorides	0.041	0.207	0.014	0.243
" Non-electrolytes	0.225	1.060	0.224	1.062
Achloride/chloride ratio	0.435	3.5	0.012	4.54
Deposit	large mono-nuclear cells and lymphocytes and endothelial cells	urates	same as before	no deposit

From these facts one arrives at the conclusion that the accumulation is transudatory, and that there is no evidence of inflammation having occurred since the first tapping. The high chloride-content suggests that a considerable degree of back-pressure is taking place.

The necropsy showed a cirrhosis of the liver in an advanced stage and no sign of peritoneal inflammation.

IV. MARY G., aged 33.—A watery and colourless slightly milky fluid was received for examination at different times. Post mortem, the fluid was found to be *golden-yellow* in colour.

The results of examination are best tabulated :

Observation.	Aug. 28, 1906.	Sept. 29, 1906.	November 8, 1906.	November 9. Yellow Fluid.
Specific Gravity	1008	1010	1009.5	1009
Albumen ...	0.25%	0	0.0250%	2.5
Urea	0	...
Chlorides ...	0.113 gm.-mol.	0.155	0.132	0.155
Concentration of Electrolytes	0.135	0.164	0.170	...
Concentration of Achloides ...	0.022	0.009	0.038	...
Achloide/chlo- ride ratio ...	0.19	0.017	0.119	...
Deposit ...	Sporulating bacilli	Amorphous	b. lymphogogon	...
Chemical notes	Excess of glo- bulin. No glycoproteid	...	Excess of Glo- bulin	...

The evidence is in favour of a transudate, since the chloride-content is high and the achloide-content very low. The exact nature of the case is difficult to explain. The following account of microscopic examination of three of the organs obtained at the necropsy will sum up the case :

Liver.—Microscopic examination shows an extreme degree of fatty infiltration and degeneration. In some parts the liver cells have almost disappeared. The parts in which there is no change are few and far between. The capillaries are dilated.

Pancreas.—There is no increase in the amount of interstitial tissue. The islands are normal, and there is no evidence of chronic interstitial pancreatitis.

Kidney.—The only abnormal condition is to be seen in the convoluted tubules, where the epithelium is extremely cloudy and the nuclei are in most places lost. In other parts the tubules are apparently quite healthy. The glomeruli cannot honestly be said to show any abnormality. There is no increase in the amount of interstitial tissue, and there is no leucocytic infiltration. The straight tubules are perfectly normal. The convoluted tubules are filled with finely granular detritus, and in some places this matter is so abundant as to form what would be hyaline casts. In some parts the epithelium of these tubules is much swollen, while in others it is low. In the former place the nuclei are generally present, while in the latter they are often absent. Here and there the capillaries are widely dilated, but this feature is inconspicuous. In short, an unprejudiced observer would not diagnose any serious renal disease, and the only conclusion to be drawn is that the kidney has been subjected to some serious toxic agent, which has been excreted through the epithelium of the convoluted tubules and damaged it during its passage.

V. HARRY E., aged 30, was tapped several times, and yielded a clear amber-coloured fluid of specific gravity 1010, and containing only 1.5 per cent. albumen.

The chloride electrolytes amounted to 0.088 gram-equiv., and the total electrolytes amounted to 0.2121 gram-mol. per litre, so that the achloride/chloride ratio was 0.26.

The acidity, tested by Wright's method, was equivalent to $\frac{n}{1000} \text{H}_2\text{SO}_4$.

The chemical examination showed the presence of much globulin, of serosamucin, of invertase, but no albumoses or peptones, no monamino-acids and no diastase.

The deposit contained very little blood,—of white cells mostly lymphocytes; a few endothelial cells, none being vacuolated.

The report on this case was that there was proliferation of the cells of the serosa, but no evidence of new growth or tubercle. Most likely it was due to hepatic cirrhosis.

The post-mortem showed the case to be one of monolobular cirrhosis of the liver.

The following table will show at a glance the condition of the fluid on different occasions :

Observation.	Jan. 23, 1907.	Feb. 26, 1907.	April 10, 1907.
Specific gravity	1010	1010	1011
Albumen	1.5%	1.7%	5.5%
Urea	trace	none	moderate amount
Purins	0.0021% N	...
Amino acids	none	none	...
Albumoses	none	+	...
Glycoproteid	+	none	trace
Ferments	trace invertase, no diastase	No diastase, trace invertase	trace invertase, no diastase
α -naphthol test	+	+
Glucosamine test	++	+
Chlorides (gram-equiv.) ...	0.113	0.088	0.094
Osmotic Concentration	0.300
Conc. of Electrolytes	0.168	0.212	...
" Achloride Electro- lytes	0.055	0.0426	...
Achloride/chloride ratio ...	0.47	0.26	...
Conc. of non-electrolytes	0.206

VI. ISAAC S., aged 23.—A turbid straw-coloured fluid of specific gravity 1015 and containing 2 per cent. albumen.

The chlorides amounted to 0.11 gram-mol. per litre, the electrolytes to 0.12, so that the achlorides were 0.01. The achloride/chloride ratio was therefore 0.01.

There was no globulin present. The cellular elements consisted of a few mononuclear leucocytes.

The "salt" ratio is *low* (exception to rule), and the low percentage of albumen is in favour of a tuberculous effusion. The case was one of tubercular peritonitis.

VII. WILLIAM D., aged 67.—A turbid straw-coloured fluid of specific gravity 1017, containing 8 gms. per litre of albumen.

The chlorides amounted to 0.107 gram-mol. per litre, the electrolytes to 0.118, so that the achlorides were 0.011, and the "salt" ratio 1.5.

Chemical examination showed no glycoproteid. The cellular characters were of most interest, and are shown in Plate I.

The findings indicate an effusion of inflammatory origin (low NaCl-content, high "salt" ratio, high albumen-content).

The case proved to be one of sarcoma of the omentum, with peritoneal dissemination.

VIII. JOHN K., aged 46.—The turbid fluid, in which large white particles were floating, contained 2 per cent. of albumen and 0.4 per cent. of chlorides (0.068 gram-mol.).

The electrolytes amounted to 0.132 gram-mol., so that the concentration of the achloride electrolytes was 0.064 gram-mol.; the "salt" ratio was therefore 0.74.

No globulin or glycoproteid was found, but triple phosphate crystals were present.

The cells included numerous large mononuclear cells, with abundant non-granular cytoplasm and also some large multinucleate cells.

The salt ratio pointed to a transudatory fluid, the low albumen, content likewise pointed against an inflammatory effusion, while the low chloride-content was decidedly against a transudation. On the other hand, the absence of globulin or of glycoproteid seemed to indicate a transudatory fluid.

The necropsy showed colloid carcinoma of the stomach, with extensive peritoneal dissemination; on the other hand, there *had been* a tuberculous *pleural* effusion.

IX. GEORGE W., aged 52.—A straw-coloured fluid, forming a small coagulum after a considerable length of time, had a specific gravity 1.014. The albumen only reached 0.5 per cent.

The chloride electrolytes amounted to 0.108 gram-mol. (fairly high), and the concentration of electrolytes was 1.121, so that the achlorides amounted to 0.013 gram-mol. The "salt" ratio was therefore 0.83.

The osmotic concentration was 0.311 gram-mol., so that the non-electrolytes amounted to 0.070 gram-mol.

The chemical examination showed abundant glycoproteid (not metalbumen).

The cellular characters were insignificant—a few lymphocytes.

In this case the low albumen-content and the fairly high chloride-content point to a transudation. The salt ratio also indicates a non-inflammatory collection. The presence of glycoproteid is peculiar.

The case proved to be one of back-pressure from tricuspid regurgitation and stenosis, and the presence of glycoproteid here falls in line with several other back-pressure effusions which have been examined and found to contain a reducing body after hydrolysis.

X. WILLIAM H., aged 19.—A highly coloured and slightly glistening fluid of specific gravity 1018, and containing a considerable amount of albumen.

The fluid deposited a thick layer of finely granular pus, with streptococci; the polynuclear cells were necrotic. No large cells or multinuclear cells were seen.

The precipitation limits for ammonium sulphate were 4.5 and 9, the maximum being at 6. There was a trace of heteroalbumose, and some protalbumose. Glycoproteid was found, and some lecithin. The α -naphthol and the glucosamin test gave a negative result. Leucin and tyrosin were absent.

Physico-chemical tests were not applied.

This was a case of ascites due to back-pressure in a case of adherent pericardium and endocarditis. Fat necrosis was found in the abdomen.

XI. WALTER S., aged 36.—A foul-smelling, reddish-brown fluid, containing much blood and a small amount of coagulum, had a specific gravity of 1013, and contained 7.75 per cent. albumen.

The chloride electrolytes amounted to 0.043 gram-mol. per litre, and the total electrolytes to .2675, the achloride electrolytes amounted to 0.1875 and the achloride/chloride ratio was 2.34.

The osmotic concentration was high, 0.406.

The chemical examination showed the presence of much urea, purins, of serosamucin, of protalbumose, and a trace of phosphates. The α -naphthol reaction and the glucosamine test were prompt and intense. Diastase and not invertase was found.

The cells consisted mostly of lymphocytes.

This was evidently a case in which there was some inflammatory condition of the peritoneum, and the post-mortem examination showed a chronic adhesive peritonitis with a coli infection.

XII. ARTHUR S., aged 42.—The amber-coloured fluid had a specific gravity of 1016 and contained 2.5 per cent. albumen.

The chloride electrolytes showed a concentration of 0.042, while the total electrolytes were .211; the concentration of the achloride electrolytes was therefore .133, and the achloride/chloride ratio 1.68.

The osmotic concentration was 0.293.

The chemical examination showed a trace of urea; the

α -naphthol reaction was slight, and the glucosamine test decided. Metalbumen or serosamucin was not found. There was a trace of invertase and no diastase.

The cells were mostly endothelial.

This was a case of monolobular cirrhosis of the liver. Note the high salt ratio.

XIII. EMMA N., aged 35.—The turbid straw-coloured fluid had a specific gravity of 1013, and contained 3 per cent. albumen.

The chloride electrolytes amounted to 0.119 per cent. gram-equiv. and the total electrolytes amounted to 0.149 per cent., so that the achlorides were 0.030 per cent. gram-mol. per litre, giving a "salt" ratio of 0.25 per cent.

Chemical examination showed the presence of much globulin, and of purins, and of albumose. No glycoproteid was found. The diazoreaction gave a positive result, as also the tryptophane test. There were large vacuolated endothelial cells and lymphocytes in the deposit.

This was a case of polyorrhomenitis, but the high chloride-content and the low "salt" ratio points to a transudation. Inasmuch as there was no direct evidence of inflammation in the serous membranes, one may conclude that this name "-itis" is not strictly accurate.

XIV. ALICE D., aged 44.—The deeply blood-stained and spontaneously coagulable fluid had a specific gravity of 1021 and contained a considerable amount of albumen (3 per cent.).

The chloride electrolytes amounted to 0.104 gram-mol. per litre, and the total electrolytes amounted to 0.119, so that the achlorides were 0.015 and the "salt" ratio 0.14.

The fluid contained 0.98 per cent. globulin; invertase; prot-albumose, urea, but no glycoproteid.

The deposit consisted mainly of lymphocytes. There were mast cells, but no endothelial or carcinoma cells.

This was a case of tuberculous peritonitis.

XV. JOHN T. I., aged 44.—The deeply yellow-coloured fluid had a specific gravity of 1016, and the scanty deposit was made up mainly of polygonal cells like endothelial cells.

The chemical examination showed 2.26 per cent. of albumen, 0.17 per cent. globulin, and 4.65 gm. per litre of chlorides. There was a large amount of urea. Amino acids were not found. Albumoses were not found, but a trace of mucin was noted.

This was from a case of effusions into all the serous cavities, associated with dilated heart and previous pneumonia. The effusion was of transudatory nature, as shown by the specific gravity, the albumen-content, and the considerable amount of chlorides. The other tests were not applied in this case.

XVI. ANTHONY S., aged 28.—A straw-coloured fluid, having a specific gravity of 1020, and containing 4.75 per cent. albumen.

The concentration of the chlorides was 0.037 gram-mol.

The osmotic concentration was 0.317.

The chemical examination showed the presence of urea, but no albumose, or glycoproteid, or ferments.

The α -naphthol test and the glucosamine test were positive, but slight.

The cellular elements consisted of large mononuclear cells and vacuolated cells.

The characters fit well in with the typical signs of an accumulation of fluid due to back-pressure; the case was one of uncompensated valvular disease of the heart.

XVII. FLORENCE W., aged 23.—A straw-coloured, blood-stained fluid, yielding a bulky coagulum, had a specific gravity of 1022 and contained 6.75 per cent. albumen.

The concentration of the chlorides was 0.059 gram-mol., while the total concentration of the electrolytes amounted to 0.128. The achloride electrolytes therefore amounted to 0.069 gram-mol., and the achloride/chloride ratio was 1.17.

The chemical examination showed the presence of stellar phosphates and oxalates; no monamino-acids; no ferments.

The α -naphthol test and the glucosamine test were positive, but slight.

The cellular elements consisted mainly of lymphocytes and red cells.

The characters are in accordance with the diagnosis of tuberculous peritonitis—low chloride-content, high "salt" ratio, absence of ferments.

XVIII. LUCY H., aged 23.—A turbid yellowish fluid of specific gravity 1020 and containing $2\frac{1}{2}$ per cent. albumen. The chlorides were very scanty, amounting only to 0.05 per cent.

Metalbumen was found on examining the precipitate after treating the fluid with thrice its bulk of absolute alcohol.

The concentration of electrolytes was 0.113 gram-mol. per

litre, the achlorides being 0.112 gram-mol., so that the electrolytes were almost solely achlorides. (Achloride/chloride ratio = 112.0.)

Examination of the deposit showed a very large amount of cholesterin crystals, and also a very considerable quantity of fatty acid crystals. There were some granular epithelial cells, but the bulk of the deposit was unorganised.

Report.—The fluid is most probably not ascitic, but derived from an ovarian cyst—to judge by the chemical characters.

Operation.—Multilocular ovarian cyst.

Comment.—The facts in favour of the fluid being ovarian are: considerable albumen-content, *very* low chloride-content, presence of metalbumen, the achloride/chloride ratio, as well as the cholesterin and fatty acid crystals.

APPENDIX

TABLE I

FOR READY RECKONING OF CHLORIDES FROM NO. OF CC. OF AMMONIUM
SULPHOCYANIDE SOLUTION USED

cc. used.	gm. per litre.	cc. used.	gm. per litre.	cc. used.	gm. per litre.	cc. used.	gm. per litre.	cc. used.	gm. per litre.
30	0'00	26'0	2'00	22'0	4'00	18'0	6'00	14'0	8'00
29'9	'05	'9	'05	'9	'05	'9	'05	'9	'05
'8	'10	'8	'10	'8	'10	'8	'10	'8	'10
'7	'15	'7	'15	'7	'15	'7	'15	'7	'15
'6	'20	'6	'20	'6	'20	'6	'20	'6	'20
'5	'25	25'5	2'25	21'5	4'25	17'5	'25	13'5	'25
'4	'30	'4	'30	'4	'30	'4	'30	'4	'30
'3	'35	'3	'35	'3	'35	'3	'35	'3	'35
'2	'40	'2	'40	'2	'40	'2	'40	'2	'40
'1	'45	'1	'45	'1	'45	'1	'45	'1	'45
29'0	'50	25'0	2'50	21'0	4'50	17'0	6'50	13'0	8'50
28'9	'55	'9	'55	'9	'55	'9	'55	'9	'55
'8	'60	'8	'60	'8	'60	'8	'60	'8	'60
'7	'65	'7	'65	'7	'65	'7	'65	'7	'65
'6	'70	'6	'70	'6	'70	'6	'70	'6	'70
'5	'75	24'5	'75	20'5	'75	16'5	'75	12'5	'75
'4	'80	'4	'80	'4	'80	'4	'80	'4	'80
'3	'85	'3	'85	'3	'85	'3	'85	'3	'85
'2	'90	'2	'90	'2	'90	'2	'90	'2	'90
'1	'95	'1	'95	'1	'95	'1	'95	'1	'95
28'0	1'00	24'0	3'00	20'0	5'00	16'0	7'00	12'0	9'00
27'9	'05	'9	'05	'9	'05	'9	'05	'9	'05
'8	'10	'8	'10	'8	'10	'8	'10	'8	'10
'7	'15	'7	'15	'7	'15	'7	'15	'7	'15
'6	'20	'6	'20	'6	'20	'6	'20	'6	'20
'5	'25	23'5	'25	19'5	'25	15'5	'25	11'5	'25
'4	'30	'4	'30	'4	'30	'4	'30	'4	'30
'3	'35	'3	'35	'3	'35	'3	'35	'3	'35
'2	'40	'2	'40	'2	'40	'2	'40	'2	'40
'1	'45	'1	'45	'1	'45	'1	'45	'1	'45
27'0	1'50	23'0	3'50	19'0	5'50	15'0	7'50	11'0	9'50
26'9	'55	'9	'55	'9	'55	'9	'55	'9	'55
'8	'60	'8	'60	'8	'60	'8	'60	'8	'60
'7	'65	'7	'65	'7	'65	'7	'65	'7	'65
'6	'70	'6	'70	'6	'70	'6	'70	'6	'70
'5	'75	22'5	'75	18'5	'75	14'5	'75	10'5	'75
'4	'80	'4	'80	'4	'80	'4	'80	'4	'80
'3	'85	'3	'85	'3	'85	'3	'85	'3	'85
'2	'90	'2	'90	'2	'90	'2	'90	'2	'90
'1	'95	'1	'95	'1	'95	'1	'95	'1	'95
26'0	2'00	22'0	4'00	18'0	6'00	14'0	8'00	10'0	10'00

TABLE II

Gm. %	Gm.-mol.	α	Mols. + ions.	Gm. %	Gm.-mol.	α	Mols. + ions.
0.1	0.001	.970	.0019	2.65	0.045	.875	.0843
0.15	0.002	.967	.0039	2.7	0.046	.874	.0863
0.2	0.003	.964	.0058	2.75	0.047	.873	.0880
0.25	0.004	.961	.0078	2.8	0.048	.872	.0898
0.3	0.005	.958	.0097	2.85	0.048	.871	.0898
0.35	0.005	.955	.0097	2.9	0.049	.870	.0897
0.4	0.006	.952	.0117	2.95	0.050	.869	.0934
0.45	0.007	.949	.0136	3.00	0.051	.868	.0952
0.5	0.008	.946	.0155	3.05	0.053	.868	.0990
0.55	0.009	.944	.0174	3.10	0.054	.868	.1008
0.6	0.010	.942	.0194	3.15	0.054	.867	.1008
0.65	0.011	.938	.0213	3.20	0.054	.867	.1008
0.7	0.011	.934	.0213	3.25	0.055	.866	.1026
0.75	0.012	.930	.0231	3.3	0.056	.866	.1055
0.8	0.013	.926	.0250	3.35	0.057	.866	.1044
0.85	0.014	.922	.0269	3.4	0.058	.865	.1063
0.9	0.015	.920	.0288	3.45	0.058	.865	.1081
0.95	0.016	.918	.0306	3.5	0.059	.864	.1091
1.00	0.017	.916	.0325	3.55	0.060	.863	.1117
1.05	0.018	.914	.0344	3.6	0.061	.863	.1236
1.1	0.019	.912	.0363	3.65	0.062	.863	.1155
1.15	0.019	.911	.0363	3.7	0.063	.862	.1173
1.2	0.020	.910	.0382	3.75	0.064	.861	.1191
1.25	0.021	.908	.0400	3.8	0.064	.861	.1190
1.3	0.022	.906	.0419	3.85	0.065	.860	.1209
1.35	0.023	.903	.0437	3.9	0.066	.860	.1227
1.4	0.024	.901	.0456	3.95	0.067	.860	.1246
1.45	0.024	.900	.0456	4.00	0.068	.859	.1263
1.5	0.025	.899	.0474	4.05	0.068	.859	.1263
1.55	0.026	.898	.0493	4.10	0.069	.858	.1282
1.6	0.027	.897	.0512	4.15	0.069	.858	.1282
1.65	0.028	.895	.0530	4.2	0.070	.857	.1299
1.7	0.029	.894	.0549	4.25	0.070	.857	.1299
1.75	0.030	.893	.0567	4.3	0.071	.856	.1317
1.8	0.030	.892	.0567	4.35	0.072	.856	.1336
1.85	0.031	.891	.0586	4.4	0.073	.855	.1354
1.9	0.032	.890	.0604	4.45	0.074	.855	.1372
1.95	0.033	.889	.0623	4.5	0.075	.854	.1390
2.00	0.034	.887	.0641	4.55	0.076	.854	.1410
2.05	0.035	.886	.0660	4.6	0.077	.853	.1426
2.10	0.035	.885	.0639	4.65	0.078	.852	.1444
2.15	0.036	.884	.0678	4.7	0.079	.851	.1462
2.2	0.037	.883	.0696	4.75	0.080	.851	.1480
2.25	0.038	.882	.0715	4.8	0.081	.850	.1498
2.3	0.039	.881	.0733	4.85	0.082	.850	.1517
2.35	0.040	.880	.0752	4.9	0.083	.849	.1534
2.4	0.041	.879	.0771	4.95	0.084	.848	.1552
2.45	0.042	.878	.0788	5.00	0.085	.848	.1570
2.5	0.042	.878	.0788	5.05	0.086	.847	.1588
2.55	0.043	.877	.0807	5.1	0.087	.847	.1606
2.6	0.044	.876	.0825	5.15	0.087	.846	.1606

TABLE II—continued

Gm. % ₁₀₀	Gm.-mol.	α	Mols. + ions.	Gm. % ₁₀₀	Gm.-mol.	α	Mols. + ions.
5.2	0.088	.845	.1623	7.8	0.134	.824	.2444
5.25	0.089	.845	.1642	7.85	0.135	.824	.2462
5.3	0.090	.844	.1659	7.9	0.135	.824	.2462
5.35	0.091	.844	.1678	7.95	0.136	.823	.2479
5.4	0.092	.843	.1695	8.00	0.137	.823	.2470
5.45	0.093	.843	.1713	8.05	0.137	.823	.2497
5.5	0.094	.842	.1731	.1	0.138	.822	.2514
5.55	0.095	.842	.1731	.15	0.139	.822	.2532
5.6	0.095	.841	.1748	.2	0.140	.822	.2550
5.65	0.096	.841	.1767	.25	0.141	.822	.2575
5.7	0.097	.841	.1785	.3	0.142	.821	.2585
5.75	0.098	.840	.1803	.35	0.143	.821	.2604
5.8	0.099	.840	.1821	.4	0.143	.821	.2604
5.85	0.100	.839	.1838	.45	0.144	.821	.2622
5.9	0.101	.838	.1856	.5	0.145	.820	.2640
5.95	0.102	.838	.1874	.55	0.146	.820	.2657
6.00	0.103	.837	.1892	.6	0.147	.820	.2675
6.05	0.104	.837	.1910	.65	0.148	.819	.2692
6.10	0.105	.837	.1918	.7	0.149	.819	.2710
6.15	0.105	.837	.1928	.75	0.150	.819	.2728
6.2	0.106	.837	.1947	.8	0.151	.819	.2746
6.25	0.107	.836	.1964	.85	0.151	.819	.2746
6.3	0.107	.836	.1965	.9	0.152	.819	.2764
6.35	0.108	.836	.1982	.95	0.153	.818	.2781
6.4	0.109	.835	.2000	9.00	0.154	.818	.2799
6.45	0.110	.834	.2017	.05	0.155	.818	.2817
6.5	0.111	.834	.2035	.1	0.155	.817	.2815
6.55	0.112	.834	.2054	.15	0.156	.817	.2834
6.6	0.113	.833	.2055	.2	0.157	.816	.2847
6.65	0.114	.832	.2088	.25	0.158	.816	.2869
6.7	0.115	.832	.2106	.3	0.159	.816	.2887
6.75	0.116	.832	.2113	.35	0.160	.815	.2904
6.8	0.116	.831	.2123	.4	0.161	.815	.2920
6.85	0.117	.831	.2142	.45	0.162	.815	.2940
6.9	0.118	.831	.2160	.5	0.162	.815	.2940
6.95	0.119	.831	.2178	.55	0.163	.814	.2956
7.00	0.120	.830	.2200	.6	0.164	.814	.2974
7.05	0.121	.830	.2214	.65	0.165	.813	.2991
7.10	0.122	.830	.2232	.7	0.166	.813	.3009
7.15	0.123	.830	.2250	.75	0.167	.812	.3027
7.2	0.124	.829	.2267	.8	0.167	.812	.3026
7.25	0.124	.829	.2267	.85	0.168	.812	.3044
7.3	0.125	.829	.2286	.9	0.169	.812	.3062
7.35	0.126	.828	.2303	.95	0.170	.811	.3078
7.4	0.127	.827	.2320	10.00	0.171	.811	.3096
7.45	0.128	.826	.2337	10.05	0.171	.811	.3096
7.5	0.129	.826	.2338	.1	0.172	.810	.3113
7.55	0.130	.826	.2383	.15	0.173	.810	.3131
7.6	0.131	.825	.2390	.2	0.174	.809	.3147
7.65	0.132	.825	.2418	.25	0.175	.809	.3165
7.7	0.133	.825	.2436	.3	0.176	.809	.3183
7.75	0.134	.824	.2410	.35	0.177	.808	.3200

TABLE II—continued

Gm. %	Gm.-mol.	α	Mols. + ions.	Gm. %	Gm.-mol.	α	Mols. + ions.
10.4	0.178	.808	.3218	11.25	0.192	.803	.3461
.45	0.178	.808	.3218	.3	0.193	.802	.3477
.5	0.179	.807	.3234	.35	0.194	.802	.3495
.55	0.180	.807	.3256	.4	0.195	.801	.3511
.6	0.181	.807	.3270	.45	0.195	.801	.3521
.65	0.182	.806	.3286	.5	0.197	.801	.3547
.7	0.183	.806	.3304	.55	0.198	.800	.3564
.75	0.184	.806	.3323	.6	0.199	.800	.3582
.8	0.185	.805	.3339	.65	0.199	.800	.3590
.85	0.186	.805	.3357	.7	0.200	.799	.3598
.9	0.186	.805	.3361	.75	0.201	.799	.3615
.95	0.187	.805	.3375	.8	0.202	.798	.3624
11.00	0.188	.804	.3391	.85	0.203	.798	.3649
.05	0.188	.804	.3400	.9	0.203	.798	.3654
.10	0.189	.804	.3409	.95	0.204	.797	.3665
.15	0.190	.804	.3427	12.00	0.205	.797	.3683
.2	0.191	.803	.3443				

TABLE III

Specific Conductivity of decinormal and quinquanormal solutions of Potassium Chloride.

At 16° it is 0.01072 for $\frac{N}{10}$ KCl and 0.002294* for $\frac{N}{50}$ KCl.

17°	1095	0.002345
18°	1119	0.002397
19°	1143	0.002449
20°	1167	0.002501
21°	1191	0.002553
22°	1215	0.002606
23°	1239	0.002659
24°	1264	0.002712
25°	1288	0.002765
26°	1313	0.002819
27°	1337	0.002873

Temperature co-efficients:

NaCl	1	gm. equiv. per litre =	.0238	Correct between 26 and 40° C.
"	0.5	"	= .0241	
"	0.1	"	= .0246	
"	0.01	"	= .0254	
"	0.001	"	= .0253	
Correct between 2 and 34°C.	"	.05	= .02238	(c) + .0000795°C.
	"	.01	= .02255	+ 848 "
	"	.001	= .02269	+ 850 "
	"	.0001	= .02284	+ 740 "
	"	.00005	= .02273	+ 901 "

Given the conductivity at 18°C.,

$$K_t = K_{18} [1 + c(t - 18) + c'(t - 18)^2].$$

* i.e. 229.4×10^{-5}

TABLE IV

RATE OF MIGRATION OF IONS.

		Na	Cl	$\frac{1}{2}\text{CO}_3$
'0005 gm. equiv. per litre	..	43'3	64'8	--
'001	" "	42'9	64'4	69
'002	" "	42'4	63'9	66
'005	" "	41'4	63'0	60
'01	" "	40'5	62'0	55
'02	" "	39'2	60'7	50
'03	" "	38'3	59'9	47
'05	" "	37'0	58'6	43
0'10	" "	35'5	56'5	38

TABLE V

OBACH'S TABLE

a	0	100	200	300	400	500	600	700	800	90
0	'0000	'1111	'2500	'4286	'6667	1'0000	1'500	2'333	4'000	9'00
1	'0010	'1123	'2516	'4306	'6694	1'0040	1'506	2'344	4'025	9'10
2	'0020	'1136	'2531	'4327	'6722	1'0080	1'513	2'356	4'051	9'20
3	'0030	'1148	'2547	'4347	'6750	1'0121	1'519	2'367	4'076	9'31
4	'0040	'1161	'2563	'4368	'6779	1'0161	1'525	2'378	4'102	9'42
5	'0050	'1173	'2579	'4388	'6807	1'0202	1'532	2'390	4'128	9'53
6	'0060	'1186	'2594	'4409	'6835	1'0243	1'538	2'401	4'155	9'64
7	'0070	'1198	'2610	'4430	'6863	1'0284	1'545	2'413	4'181	9'75
8	'0081	'1211	'2626	'4451	'6892	1'0325	1'551	2'425	4'208	9'87
9	'0091	'1223	'2642	'4472	'6921	1'0367	1'558	2'436	4'236	9'99
10	'0101	'1236	'2658	'4493	'6949	1'0408	1'564	2'448	4'263	10'11
11	'0111	'1249	'2674	'4514	'6978	1'0450	1'571	2'460	4'291	10'24
12	'0121	'1261	'2690	'4535	'7007	1'0492	1'577	2'472	4'319	10'36
13	'0132	'1274	'2706	'4556	'7036	1'0534	1'584	2'484	4'348	10'49
14	'0142	'1287	'2723	'4577	'7065	1'0576	1'591	2'497	4'376	10'63
15	'0152	'1299	'2739	'4599	'7094	1'0619	1'597	2'509	4'405	10'76
16	'0163	'1312	'2755	'4620	'7123	1'0661	1'604	2'521	4'435	10'90
17	'0173	'1325	'2771	'4641	'7153	1'0704	1'611	2'534	4'464	11'05
18	'0183	'1337	'2788	'4663	'7182	1'0747	1'618	2'546	4'495	11'20
19	'0194	'1351	'2804	'4684	'7212	1'0790	1'625	2'559	4'525	11'35
20	'0204	'1364	'2820	'4706	'7241	1'0833	1'632	2'571	4'556	11'50
21	'0215	'1377	'2837	'4728	'7271	1'0877	1'639	2'584	4'587	11'66
22	'0225	'1390	'2853	'4749	'7301	1'0921	1'646	2'597	4'618	11'82
23	'0235	'1403	'2870	'4771	'7331	1'0964	1'653	2'610	4'650	11'99
24	'0246	'1416	'2887	'4793	'7361	1'1008	1'660	2'623	4'682	12'16
25	'0256	'1429	'2903	'4815	'7391	1'1053	1'667	2'636	4'714	12'33
26	'0267	'1442	'2920	'4837	'7422	1'1097	1'674	2'650	4'747	12'51
27	'0277	'1455	'2937	'4859	'7452	1'1142	1'681	2'663	4'780	12'70
28	'0288	'1468	'2953	'4871	'7483	1'1186	1'688	2'676	4'814	12'89
29	'0299	'1481	'2970	'4903	'7513	1'1231	1'695	2'690	4'848	13'08

TABLE V—*continued*OBACH'S TABLE—*continued*

a	0	100	200	300	400	500	600	700	800	900
30	·0309	·1494	·2907	·4925	·7544	1·1277	1·703	2·704	4·882	13·29
31	·0320	·1507	·3004	·4948	·7575	1·1322	1·710	2·717	4·917	13·49
32	·0331	·1521	·3021	·4270	·7606	1·1368	1·717	2·731	4·952	13·71
33	·0341	·1534	·3038	·4993	·7637	1·1413	1·725	2·745	4·988	13·93
34	·0352	·1547	·3055	·5015	·7668	1·1459	1·732	2·759	5·024	14·15
35	·0363	·1561	·3072	·5038	·7699	1·1505	1·740	2·774	5·061	14·38
36	·0378	·1574	·3089	·5060	·7731	1·1525	1·747	2·788	5·098	14·63
37	·0384	·1587	·3106	·5083	·7762	1·1598	1·755	2·802	5·135	14·87
38	·0395	·1601	·3123	·5106	·7794	1·1645	1·762	2·817	5·173	15·13
39	·0406	·1614	·3141	·5129	·7825	1·1692	1·770	2·831	5·211	15·39
40	·0417	·1628	·3158	·5152	·7857	1·1739	1·778	2·846	5·250	15·67
41	·0428	·1641	·3175	·5175	·7889	1·1786	1·786	2·861	5·289	15·95
42	·0438	·1655	·3193	·5198	·7921	1·1834	1·793	2·876	5·329	16·24
43	·0449	·1669	·3210	·5221	·7953	1·1882	1·801	2·891	5·369	16·54
44	·0460	·1682	·3228	·5244	·7986	1·1930	1·809	2·906	5·410	16·86
45	·0471	·1696	·3245	·5267	·8018	1·1978	1·817	2·922	5·452	17·18
46	·0482	·1710	·3263	·5291	·8051	1·2026	1·825	2·937	5·494	17·52
47	·0493	·1723	·3280	·5314	·8083	1·2075	1·833	2·953	5·536	17·87
48	·0504	·1737	·3298	·5337	·8116	1·2124	1·841	2·968	5·579	18·23
49	·0515	·1751	·3316	·5361	·8149	1·2173	1·849	2·984	5·623	18·61
50	·0526	·1765	·3333	·5385	·8182	1·2222	1·857	3·000	5·667	19·00
51	·0537	·1779	·3351	·5408	·8215	1·2272	1·865	3·016	5·711	19·41
52	·0549	·1792	·3369	·5432	·8248	1·2321	1·875	3·022	5·757	19·83
53	·0560	·1806	·3387	·5456	·8282	1·2371	1·882	3·049	5·803	20·28
54	·0571	·1820	·3405	·5480	·8315	1·2422	1·890	3·065	5·849	20·74
55	·0582	·1834	·3323	·5504	·8349	1·2472	1·899	3·082	5·897	21·22
56	·0593	·1848	·3441	·5528	·8382	1·2523	1·907	3·098	5·944	21·73
57	·0604	·1862	·3459	·5552	·8416	1·2573	1·915	3·115	5·993	22·26
58	·0616	·1876	·3477	·5576	·8450	1·2624	1·924	3·132	6·042	22·81
59	·0627	·1891	·3495	·5601	·8484	1·2676	1·933	3·149	6·092	23·39
60	·0638	·1905	·3514	·5625	·8519	1·2525	1·941	3·167	6·143	24·00
61	·0650	·1919	·3532	·5649	·8553	1·2779	1·950	3·184	6·194	24·64
62	·0661	·1933	·3550	·5674	·8587	1·2831	1·959	3·202	6·246	25·32
63	·0672	·1947	·3569	·5699	·8622	1·2883	1·966	3·219	6·299	26·03
64	·0684	·1962	·3587	·5723	·8657	1·2936	1·976	3·237	6·353	26·78
65	·0695	·1976	·3605	·5748	·8692	1·2989	1·985	3·255	6·407	27·57
66	·0707	·1990	·3624	·5773	·8727	1·3041	1·994	3·274	6·463	28·41
67	·0718	·2005	·3643	·5798	·8762	1·3095	2·003	3·292	6·519	29·30
68	·0730	·2019	·3661	·5823	·8797	1·3148	2·012	3·310	6·576	30·25
69	·0741	·2034	·3680	·5848	·8832	1·3202	2·021	3·329	6·634	31·26
70	·0753	·2048	·3699	·5873	·8868	1·3256	2·530	3·348	6·692	32·33
71	·0764	·2063	·3717	·5898	·8904	1·3310	2·040	3·637	6·752	33·48
72	·0776	·2077	·3736	·5924	·8939	1·3364	2·049	3·386	6·813	34·71
73	·0787	·2092	·3755	·5949	·8975	1·3419	2·058	3·405	6·874	36·04
74	·0799	·2107	·3774	·5974	·9011	1·3474	2·067	3·425	6·937	37·46
75	·0811	·2121	·3793	·6000	·9048	1·3529	2·077	3·444	7·000	39·00
76	·0823	·2136	·3812	·6026	·9084	1·3585	2·086	3·464	7·065	40·67
77	·0834	·2151	·3831	·6051	·9120	1·3641	2·096	3·484	7·130	42·48
78	·0846	·2165	·3850	·6077	·9157	1·3697	2·106	3·505	7·197	44·45
79	·0858	·2180	·3870	·6103	·9194	1·3753	2·115	3·525	7·264	46·62

TABLE V—*continued*OBACH'S TABLE—*continued*

a	0	100	200	300	400	500	600	700	800	900
80	·0870	·2195	·3889	·6129	·9231	1·3810	2·125	3·545	7·333	49·00
81	·0881	·2210	·3908	·6155	·9268	1·3866	2·135	3·566	7·403	51·63
82	·0893	·2225	·3928	·6181	·9305	1·3923	2·145	3·587	7·475	54·56
83	·0905	·2240	·3947	·6207	·9342	1·3981	2·155	3·608	7·547	57·82
84	·0917	·2255	·3966	·6234	·9380	1·4038	2·165	3·630	7·621	61·50
85	·0929	·2270	·3986	·6260	·9417	1·4096	2·175	3·651	7·696	65·67
86	·0941	·2285	·4006	·6287	·9455	1·4155	2·185	3·673	7·772	70·43
87	·0953	·2300	·4025	·6313	·9493	1·4213	2·195	3·695	7·850	75·92
88	·0965	·2315	·4045	·6340	·9531	1·4272	2·205	3·717	7·929	82·33
89	·0977	·2330	·4065	·6367	·9569	1·4331	2·215	3·739	8·009	89·19
90	·0989	·2346	·4085	·6393	·9608	1·4390	2·226	3·762	8·091	99·00
91	·1001	·2361	·4104	·6420	·9646	1·4450	2·236	3·785	8·174	110·1
92	·1013	·2376	·4124	·6447	·9685	1·4510	2·247	3·808	8·259	124·0
93	·1025	·2392	·4144	·6474	·9724	1·4570	2·257	3·831	8·346	141·9
94	·1038	·2407	·4164	·6502	·9763	1·4631	2·268	3·854	8·434	165·7
95	·1050	·2422	·4184	·6529	·9802	1·4691	2·279	3·878	8·524	199·0
96	·1062	·2438	·4205	·6556	·9841	1·4752	2·289	3·902	8·615	249·0
97	·1074	·2453	·4225	·6584	·9881	1·4814	2·300	3·926	8·709	332·3
98	·1086	·2469	·4245	·6611	·9920	1·4876	2·311	3·950	8·804	499·0
99	·1099	·2484	·4265	·6639	·9966	1·4938	2·322	3·975	8·901	899·0
100	·1111	·2500	·4286	·6667	1·0000	1·5000	2·333	4·000	9·000	

[E.g. to find $\frac{432}{1000 - 432}$, look down the 400 column till 32 of column "a" is reached, the number ·7606 is the one required.]

TABLE VI

Degrees.	Osmotic Concentration.	Pressure in Atmospheres.	Degrees.	Osmotic Concentration.	Pressure in Atmospheres.	Degrees.	Osmotic Concentration.	Pressure in Atmospheres.	Degrees.	Osmotic Concentration.	Pressure in Atmospheres.	Degrees.	Osmotic Concentration.	Pressure in Atmospheres.	Degrees.	Osmotic Concentration.	Pressure in Atmospheres.
.300	.159	3.615	.420	.227	5.06	.540	.291	6.507	.660	.356	7.95	.780	.421	9.39	.900	.486	10.845
.305	.164	3.67	.425	.229	5.12	.545	.294	6.567	.665	.359	8.01	.785	.424	9.45	.905	.489	10.90
.310	.167	3.73	.430	.232	5.18	.550	.297	6.6275	.670	.361	8.07	.790	.427	9.51	.910	.491	10.96
.315	.170	3.79	.435	.235	5.24	.555	.300	6.687	.675	.364	8.13	.795	.429	9.57	.915	.494	11.02
.320	.172	3.85	.440	.237	5.30	.560	.302	6.748	.680	.367	8.19	.800	.432	9.64	.920	.497	11.086
.325	.175	3.91	.445	.240	5.36	.565	.305	6.808	.685	.370	8.25	.805	.435	9.70	.925	.500	11.14
.330	.178	3.97	.450	.243	5.42	.570	.308	6.8685	.690	.372	8.31	.810	.437	9.76	.930	.502	11.20
.335	.181	4.03	.455	.245	5.48	.575	.310	6.928	.695	.375	8.37	.815	.440	9.82	.935	.505	11.26
.340	.183	4.09	.460	.248	5.54	.580	.313	6.989	.700	.378	8.435	.820	.443	9.88	.940	.508	11.32
.345	.186	4.15	.465	.250	5.60	.585	.316	7.049	.705	.381	8.49	.825	.445	9.94	.945	.510	11.38
.350	.189	4.21	.470	.254	5.66	.590	.319	7.1095	.710	.383	8.55	.830	.448	10.001	.950	.513	11.44
.355	.191	4.27	.475	.256	5.72	.595	.321	7.169	.715	.386	8.61	.835	.451	10.06	.955	.516	11.50
.360	.194	4.33	.480	.259	5.78	.600	.324	7.230	.720	.389	8.67	.840	.454	10.12	.960	.519	11.56
.365	.197	4.39	.485	.261	5.84	.605	.327	7.29	.725	.391	8.73	.845	.456	10.18	.965	.520	11.62
.370	.200	4.45	.490	.264	5.90	.610	.329	7.35	.730	.394	8.79	.850	.459	10.24	.970	.524	11.68
.375	.202	4.51	.495	.267	5.96	.615	.332	7.41	.735	.397	8.85	.855	.461	10.30	.975	.526	11.74
.380	.205	4.57	.500	.270	6.025	.620	.335	7.47	.740	.400	8.91	.860	.464	10.36	.980	.529	11.80
.385	.208	4.63	.505	.272	6.085	.625	.337	7.53	.745	.402	8.96	.865	.467	10.42	.985	.532	11.86
.390	.210	4.69	.510	.275	6.1455	.630	.340	7.59	.750	.405	9.03	.870	.470	10.48	.990	.535	11.929
.395	.213	4.75	.515	.278	6.205	.635	.343	7.65	.755	.408	9.09	.875	.472	10.54	.995	.537	11.98
.400	.216	4.82	.520	.281	6.266	.640	.345	7.71	.760	.410	9.15	.880	.475	10.60	1.000	.540	12.05
.405	.218	4.88	.525	.283	6.326	.645	.348	7.77	.765	.413	9.21	.885	.478	10.66	1.005	.543	12.11
.410	.221	4.94	.530	.286	6.3865	.650	.350	7.83	.770	.416	9.27	.890	.481	10.72	1.010	.545	12.17
.415	.224	5.00	.535	.289	6.44	.655	.354	7.89	.775	.418	9.33	.895	.483	10.78	1.015	.548	12.23

1'020	.551	12'29	1'185	.640	14'26	1'350	.729	16'26	1'515	.818	18'25	1'680	.908	20'24	1'845	.997	22'23
1'025	.554	12'35	1'190	.643	14'33	1'355	.732	16'32	1'520	.821	18'31	1'685	.910	20'30	1'850	1'000	22'29
1'030	.556	12'41	1'195	.645	14'39	1'360	.735	16'38	1'525	.824	18'37	1'690	.913	20'36	1'855	1'002	22'35
1'035	.559	12'46	1'200	.648	14'46	1'365	.737	16'44	1'530	.827	18'43	1'695	.916	20'42	1'860	1'005	22'41
1'040	.561	12'53	1'205	.651	14'52	1'370	.740	16'5	1'535	.829	18'49	1'700	.919	20'48.5	1'865	1'008	22'47
1'045	.564	12'59	1'210	.654	14'58	1'375	.743	16'56	1'540	.832	18'55	1'705	.921	20'54	1'870	1'010	22'53
1'050	.567	12'65	1'215	.657	14'64	1'380	.745	16'63	1'545	.835	18'61	1'710	.924	20'60	1'875	1'013	22'59
1'055	.569	12'71	1'220	.659	14'70	1'385	.748	16'69	1'550	.837	18'67	1'715	.927	20'66	1'880	1'016	22'65
1'060	.572	12'77	1'225	.662	14'76	1'390	.751	16'75	1'555	.840	18'73	1'720	.929	20'72	1'885	1'018	22'71
1'065	.575	12'83	1'230	.664	14'82	1'395	.754	16'81	1'560	.843	18'79	1'725	.932	20'78	1'890	1'021	22'77
1'070	.578	12'89	1'235	.667	14'88	1'400	.756	16'87	1'565	.845	18'85	1'730	.935	20'84	1'895	1'024	22'83
1'075	.581	12'95	1'240	.670	14'94	1'405	.759	16'93	1'570	.848	18'91	1'735	.937	20'90	1'900	1'027	22'89
1'080	.583	13'01	1'245	.672	15'00	1'410	.761	16'99	1'575	.851	18'97	1'740	.940	20'96	1'905	1'029	22'95
1'085	.586	13'07	1'250	.675	15'06	1'415	.764	17'05	1'580	.854	19'03	1'745	.943	21'02	1'910	1'032	23'01
1'090	.589	13'13	1'255	.678	15'12	1'420	.767	17'11	1'585	.856	19'09	1'750	.945	21'08	1'915	1'035	23'07
1'095	.591	13'19	1'260	.681	15'18	1'425	.769	17'17	1'590	.859	19'15.9	1'755	.948	21'14	1'920	1'037	23'13
1'100	.594	13'25.5	1'265	.683	15'24	1'430	.772	17'23	1'595	.861	19'21.9	1'760	.950	21'20	1'925	1'040	23'19
1'105	.597	13'31	1'270	.686	15'30	1'435	.775	17'29	1'600	.864	19'28	1'765	.954	21'26	1'930	1'043	23'25
1'110	.600	13'37	1'275	.689	15'36	1'440	.778	17'35	1'605	.867	19'34	1'770	.956	21'32	1'935	1'045	23'31
1'115	.602	13'43	1'280	.691	15'42	1'445	.780	17'41	1'610	.870	19'40	1'775	.959	21'38	1'940	1'048	23'37
1'120	.605	13'49	1'285	.694	15'48	1'450	.783	17'47	1'615	.872	19'46	1'780	.961	21'44	1'945	1'050	23'43
1'125	.608	13'55	1'290	.697	15'54	1'455	.786	17'53	1'620	.875	19'52	1'785	.964	21'50	1'950	1'054	23'49
1'130	.610	13'61	1'295	.700	15'60	1'460	.788	17'59	1'625	.878	19'58	1'790	.967	21'56	1'955	1'056	23'55
1'135	.613	13'67	1'300	.702	15'66.5	1'465	.791	17'65	1'630	.881	19'64	1'795	.970	21'62	1'960	1'059	23'61
1'140	.616	13'73	1'305	.704	15'72	1'470	.793	17'71	1'635	.883	19'70	1'800	.972	21'69	1'965	1'061	23'67
1'145	.618	13'79	1'310	.707	15'78	1'475	.796	17'77	1'640	.886	19'76	1'805	.975	21'75	1'970	1'064	23'73
1'150	.621	13'85	1'315	.710	15'84	1'480	.800	17'83	1'645	.889	19'82	1'810	.978	21'81	1'975	1'067	23'79
1'155	.624	13'91	1'320	.713	15'90	1'485	.802	17'89	1'650	.891	19'88	1'815	.981	21'87	1'980	1'070	23'85
1'160	.627	13'97	1'325	.716	15'96	1'490	.805	17'95	1'655	.894	19'94	1'820	.983	21'93	1'985	1'072	23'91
1'165	.629	14'03	1'330	.718	16'02	1'495	.808	18'01	1'660	.897	20'00	1'825	.986	21'99	1'990	1'075	23'97
1'170	.632	14'09	1'335	.721	16'08	1'500	.810	18'07.5	1'665	.900	20'06	1'830	.989	22'05	1'995	1'078	24'03
1'175	.635	14'15	1'340	.724	16'14	1'505	.813	18'13.5	1'670	.902	20'12	1'835	.991	22'11	2'000	1'081	24'10
1'180	.637	14'21	1'345	.727	16'20	1'510	.816	18'19.	1'675	.905	20'18	1'840	.994	22'17			

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THE following references apply to the authors quoted in the text. As a rule, the title of the Journal and the year are sufficient to identify the reference, so that its title has not been considered necessary.

Some of the references here given have not been definitely quoted in the text, though they have been made use of. On the other hand, many references in the *Biochemische Zeitschrift* and the *Biochemical Journal* have not been inserted, since nearly every one of the papers which have appeared from the very commencement of each of these valuable Journals has been found of use in the studies recorded in this work.

The text-books and other published works which have been made use of appear in the appropriate places.

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