was faint. In 21 cases of general peritonitis, from various causes, the reaction was always present and usually intense, especially where pus was present. It was faint in only one case, after nine hours of a mild inflammation from perforating gastric ulcer. My own experience, though much less extensive, exactly bears this out. In abdominal cases in particular the reaction is a much more trustworthy guide than leucocytosis, for while a large leucocytosis, of more than 20,000, practically always indicates operation, the majority of appendicitis cases have an annoying way of showing a leucocytosis of from 15,000 to 17,000, even with a gangrenous appendix, while some, and those the very worst cases of peritonitis, have no leucocytosis at all. In these, however, the glycogen reaction is always well marked. That the converse holds good the following case will indicate: I was asked some time ago by one of my surgical colleagues to see a case in which the history, symptoms, and signs were so vague that the diagnosis was about equally balanced between perforating gastric ulcer and broncho-pneumonia. A leucocyte count had been made and had given 17,000, but the time and place did not allow a more complete blood examination, and it was decided to operate. Nothing was found in the abdomen, and the patient afterwards went through a broncho-pneumonia. When I had an opportunity of examining the films I found a polymorph percentage of only 68 and a doubtful glycogen reaction. Had the circumstances of the case allowed me to make the complete examination at the time I should have refused to sanction operation. I might multiply instances of the sort, but the point is sufficiently well illustrated by what I have said. A point of some importance is the time after the onset of symptoms at which the reaction appears. Locke found it faint three hours after the perforation of a typhoid ulcer, well marked six hours after. I have found it very intense twelve hours after perforation of a gastric ulcer; well marked fifteen hours after the onset of symptoms in a gangrenous appendix case; and quite recently, well marked an hour and a-half after the onset in an inflamed appendix with peritonitis.

(i) There are numerous other conditions of less importance in this connexion in which the reaction is present, such as late typhoid, advanced phthisis (in both of which it is probably due to secondary infection), severe malaria, acute cystitis, cerebro-spinal meningitis, miliary tuberculosis (sometimes), severe enteritis (slight), gonorrhoeal arthritis, etc.

CONDITIONS IN WHICH IT IS ABSENT.

The conditions in which the reaction is uniformly absent are equally important from the point of view of diagnosis. In addition to those already named—rheumatic fever, chronic rheumatoid arthritis, chorea, rickets, infantile atrophy, congenital heart disease and adult heart disease (unless compensation has failed badly), catarrhal jaundice, uncomplicated tubercle of all kinds, dry and serous pleurisy, bronchitis, whooping-cough, asthma, alcoholism, nervous conditions, early cancer, lead colic, diabetes (except when coma has occurred), and such abdominal conditions as ruptured extrauterine pregnancy, obstructed hernia, intestinal obstruction from bands, etc., unless gangrene or peritonitis has set in, and in stationary abscesses.

The relation of the reaction to leucocytosis has already been hinted at, and may be stated briefly thus—that while many of the causes which produce the reaction also cause leucocytosis, there is no necessary connexion between the two, for in rheumatic fever, for instance, where leucocytosis is the rule, the glycogen reaction is absent, and frequently in the leucocytosis-producing diseases the infection is either so slight or so severe that the leucocytes do not rise, though the glycogen reaction is positive, and in the latter case, of great severity, is always intense. Thus in septicaemia I have seen counts of 7,200, 7,400, 2,100 and so on, with marked glycogen reaction. There is a much closer relation between the reaction and a high percentage of polymorphs (over 75 per cent. in the differential count), in the bacterial infections, and one generally finds the two running parallel, a marked reaction with a high polymorph count. Even this, however, is not invariable, and I have found 66, 68, 72 per cent. of polymorphs in septic cases with marked glycogen reactions. One is therefore entitled to regard it as the most delicate test that we have of constitutional disturbance in the conditions named.

PATHOLOGICAL SIGNIFICANCE.

The actual pathological meaning of the reaction is not very clear. It is almost certainly not a degenerative change, for leucocytes with broken down nucleus do not show it, and all

other evidence goes to show that glycogen is usually found in young and active cells. It is probably associated in some way with the resistance of the organism to poisons, and especially to those which produce positive chemotaxis.

It is unnecessary to repeat in detail the various ways in which the reaction may be used in diagnosis, but it will be obvious that a method which enables us to approach greater certainty of diagnosis in deep-seated pneumonias; to distinguish gonorrhoeal arthritis from ordinary rheumatism, septic or cerebro-spinal meningitis from the tuberculous variety—perhaps, though, on this point observations have not yet been made—cerebral abscess from cerebral tumour, obstructed from strangulated hernia, and which possesses, in addition, the wide range of application already indicated in septic conditions, deserves a more frequent application than has yet been accorded to it. One point which Locke makes is worthy of note, that patients who show it are always "sick," to use the expressive Americanism. Its presence, therefore, in cases which do not admit of a positive diagnosis should always put us on our guard against the possible development of some serious condition.

It must also be clearly understood that, while the method is simplicity itself, it demands in the observer some previous acquaintance with the appearances of blood films, and that it takes some practice before the reaction can be read aright. In its interpretation, also, it necessitates the same precautions as other blood tests, and, indeed, as all laboratory tests. It requires a thorough previous examination of the patient, the exclusion, as far as possible, of complicating conditions, and the endeavour to keep in view a clear issue for diagnosis. If this be done, the method will be found to be of great value.

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OBSERVATIONS ON SOME TESTS FOR ALBUMEN IN URINE.

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IN connexion with what is probably the most commonly employed clinical test for albumen in urine—namely, the application of heat combined with acidulation—my attention has been directed to some facts which I believe are not generally well known, and an explanation of which I have not been able to find in any published work. The great point in this test is the amount of acidulation, and the fallacies which are apt to mislead one in using the test generally arise from inaccurate acidulation. This is especially the case if strong nitric acid is employed, as is still done by many physicians and recommended in many textbooks, diverse directions being given as to the amount of acid to be used.

In connexion with the use of strong nitric acid in performing this heat-coagulation test, Bence-Jones long ago (about 1840) pointed out the fact that, if too little nitric acid be added (2 to 3 drops to 10 c.cm. of albuminous urine), or too much (30 to 40 drops), one may fail altogether to detect the presence of albumen, although it is present in appreciable quantity. On the other hand, if 10 to 12 drops are added, and the upper stratum of the fluid rapidly heated, the test is positive—that is to say, a white turbidity or coagulum is produced. Moreover, when 2 or 3 drops of nitric acid have been used and the test tube boiled with a negative result, the further addition of a certain amount of nitric acid (for example, 6 to 10 drops) leads to coagulation.

These peculiar facts as observed by Bence-Jones seem to have been almost entirely forgotten, for very few, if any, of the present-day textbooks point out this fallacy, and I have not been able to find any published work which gives experimental evidence to explain the above phenomena.

Is the formation of acid-albumen (not coagulable by heat) the reason of the negative result with 2 to 3 drops of nitric acid? There does not seem to be any definite evidence that such is the case, nor any explanation why coagulation is got on a further addition of 6 to 10 drops of nitric acid, since all the textbooks state that solutions of acid-albumen are not coagulable by heat. Again, why should not the use of 10 to

12 drops of nitric acid lead to the formation of acid-albumen also and an absence of coagulation on heating? I have performed a large number of experiments with albuminous urine, and the main results obtained have been similar in all the cases. The following is selected as a typical experiment:

Albuminous urine (0.2 per cent. by Hayward's modified Esbach's albuminometer): Reaction faintly acid (no free acid); specific gravity, 1.010; colour, amber, slightly muddy: 6 test tubes were taken, each containing 10 c.cm. of the filtered urine. Drops of strong nitric acid were added as follows:

A. = 10 c.cm. of urine + 1 drop HNO ₃	
B. = 10 c.cm. " + 2 drops "	
C. = 10 c.cm. " + 10 " "	
D. = 10 c.cm. " + 12 " "	
E. = 10 c.cm. " + 30 " "	
F. = 10 c.cm. " + 40 " "	

After boiling, A., B., E., and F. were quite clear: coagulation was present in C. and D. The addition of another 8 to 10 drops of HNO₃ to A. and B. after boiling, caused turbidity.

This illustrates the phenomena as observed by Bence-Jones. With the larger amounts of nitric acid some turbidity is induced on adding the acid; this may clear up on boiling as in the foregoing experiment, or may not clear up, as often occurs. That acid-albumen had been formed in tubes A. and B. was conclusively proved in this way:—

1. *By Neutralization.*—1 drop of the strong HNO₃ employed was found by experiment to be almost neutralized by 3 drops of 20 per cent. KOH solution. Tubes A. and B. were carefully neutralized first by the addition of 2 to 3 drops and 5 to 6 drops of 20 per cent. KOH respectively, the final part of the neutralization being carried out by 0.1 per cent. KOH solution. At the neutral point down came a cloudy precipitate of acid-albumen, which was soluble in excess of the alkali, but which was again thrown down by neutralizing with weak acid, and again redissolved in excess of the acid.

2. *By Saturation with Neutral Salts, such as Sodium Chloride and Magnesium Sulphate.*—10 c.cm. of albuminous urine were taken in tubes A. and B. as before, and 1 and 2 drops of HNO₃ added respectively: these were then boiled as before. After cooling, both mixtures were saturated with NaCl crystals. There was a copious precipitate of acid-albumen in both tubes. Similar results occurred on saturation with MgSO₄, and on half-saturation with ammonium sulphate. (The urine, before adding the HNO₃, did not give such a precipitate with NaCl, etc.) Now, acid-albumen (like serum-globulin, etc.) is precipitated by saturation with NaCl and MgSO₄, and by half saturation with (NH₄)₂SO₄, while ordinary albumens are soluble in saturated solutions of NaCl and MgSO₄ and half-saturated solutions of (NH₄)₂SO₄. After filtering off the precipitated acid-albumen the filtrate was found to be proteid-free and gave no precipitate when tested with proteid-precipitants (for example, salicyl-sulphonic acid, etc.) showing that all the albumen had been converted into acid-albumen, with the exception, perhaps, of a little in the form of proteose.

Strongly albuminous urine boiled with 1 to 3 drops of HNO₃ without coagulation sometimes becomes gelatinous on standing, so that the tube can be inverted without spilling the contents; this is due to the large amount of acid-albumen present.

I found that similar phenomena occur with the other mineral acids (hydrochloric and sulphuric); they are not peculiar to nitric acid.

It is clear that the first part of Bence-Jones's phenomena (the addition of too little HNO₃) is to be explained by the formation of acid-albumen.

As regards the effects of adding too much nitric acid (30 to 40 drops), the turbidity induced may or may not disappear on boiling (for example, tubes E. and F.). When it does disappear it is due to the destructive influence of the corrosive acid on the proteid (as in the first stage of the xantho-proteid reaction), and not to the formation of acid-albumen. In this case neutralization gives no precipitate, nor when the fluid is boiled after diluting till the HNO₃ present would be in the proportion of 8 to 10 drops in 10 c.cm. does any coagulation occur. (A little proteose may be present.)

The reason why coagulation occurs with 10 to 12 drops of nitric acid is that, although a good deal of acid-albumen is formed, yet acid-albumen does coagulate on heating in the presence of a considerable amount of nitric acid. The great stumbling-block is the ordinary textbook statement that "acid-albumen is not coagulable by heat." This is not strictly correct; it is true only in a weak acid solution. Acid-albumen is rapidly coagulated by heating in certain strengths of the mineral acids; the exact percentage of acid cannot be definitely stated, as it varies with the time and the temperature (for example, acid-albumen will coagulate in a relatively low percentage of mineral acid, even at room temperature, if it is allowed to stand long enough). The change may be regarded as a coagulation by the mineral acid

accelerated by heat, or a heat coagulation accelerated by the presence of a certain amount of mineral acid.

The results obtained by heating after acidulation with nitric acid may fall into one or other of four categories: (1) With faint acidulation, coagulation; (2) with more acid (1 to 3 drops in 10 c.cm.), no coagulation; (3) with 8 to 10 drops, coagulation; (4) with 30 to 40 drops, coagulation or not.

In performing the heat-coagulation test, acidulation with weak acetic acid (2 to 5 per cent.), or a 5 per cent. solution of acid sodium phosphate (the acid salt to which normal urine owes its acidity) is decidedly preferable to acidulation with nitric acid, since with these acidifying agents the danger of forming acid-albumen is reduced to a minimum. The careful production of a suitable degree of acidity followed by the rapid heating of the upper stratum of the fluid is one of the most delicate tests for albumen in urine. The crux of the test lies in the proper amount of acid to be added; the amount needed to give the requisite faint acidulation will vary according to the original reaction of the urine. When using acetic acid in this way the addition of litmus solution as an indicator is necessary. Another way of using acetic acid is to add it, drop by drop, after the urine has been brought to the boiling-point; hardly any time is here given for the formation of acid albumen and the amount of acid used has not to be graduated in the same precise way as when it is added before boiling. On the other hand, a precipitation of "mucin," which might have been seen on acidulating before boiling, will now be indistinguishable from albumen if the acid is added after boiling.

As regards the danger of mistaking urinary "mucin" for albumen when testing is done by acidulation and heat, etc., it is to be remarked that a clear sample of urine which is decidedly acid in reaction can contain little mucin in solution. In a neutral or alkaline urine, however, a very appreciable amount may be dissolved, and here a danger of confusion comes in, and it seems probable that some of the conflicting statistics that have been obtained as to the occurrence of the so-called "physiological or functional albuminuria" of apparently healthy persons (for example, in soldiers and others) are in part at least attributable to urinary "mucin"—either true mucin or nucleo-proteid, or both—being mistaken for albumen. A simple method of distinguishing "mucin" (whether true mucin or nucleo-proteid) is to precipitate it by decided acidulation with acetic or citric acid without heating, for example, by mixing equal volumes of urine and 5 per cent. acetic acid.

Pickering³ recommends an additional procedure in cases of doubt as regards "mucin," namely, boiling a small portion of the precipitate with dilute sulphuric acid, and testing its reducing properties with Fehling's solution. Such a procedure not only takes time, but is inadvisable and is apt to mislead, since it is based on the assumption that the urinary "mucin" is true mucin, whereas it is commonly made up largely or wholly of nucleo-proteid, which of course yields no reducing sugar on boiling with sulphuric acid.

As regards any advantages in the use of dangerous corrosive acids, like nitric, in testing for albumen—as compared with weak acetic or acid salts—I have not been able to find any. Probably force of habit accounts in great measure for the continued use of nitric acid. Of course, it is possible to use faint acidulation with dilute nitric acid before boiling (litmus solution being employed as an indicator), but this has no advantage over the use of weak acetic acid. Strong nitric acid is obviously unsuitable for the purpose. Again, strong nitric acid dropped in after boiling is apt to redissolve proteid if present in small amount.

A saturated solution of acid sodium phosphate may be used like nitric acid in Heller's test; a precipitate of proteose clears up on heating, while albumen does not. With this reagent there is not the liability to redissolving which is seen with nitric acid.

The test for albumen most used in this laboratory for a number of years has been "the salicyl-sulphonic acid test," and an extended experience of its use has abundantly shown its safety, convenience, and delicacy. As recommended by Professor MacWilliam,⁴ the test is done by adding a few drops of a saturated aqueous solution⁵ of salicyl-sulphonic acid to a small amount of urine (20 or 30 minims) in a very small test tube. If no precipitate occurs there is no proteid present; if there is a precipitate the tube is boiled to distinguish albumen, which does not clear up on heating but becomes coagulated and flaky from proteoses (primary), which do clear up to reappear when the fluid cools. In the

absence of precipitation on the addition of the reagent it is not necessary to boil at all, hence the test is a much shorter one than the ordinary acidulation and heat test, a matter of great importance when a large number of urines have to be examined, for example, in hospital practice.

There is no need for care as to the exact amount of the reagent used; no danger of over-acidulation, as with nitric or acetic acids—even large excess of salicyl-sulphonic acid does not redissolve the precipitated proteid. Further, the reagent is non-caustic and exceedingly stable, while it does not stain like picric acid, etc. As regards portability it can be used in the form of crystals, which are much safer than the poisonous and staining picric acid crystals, with their further disadvantage in precipitating certain other bodies besides proteids—for example, many alkaloids, etc. When using the crystals, one should proceed as follows: Half a drachm, or less, of urine is taken in a small test tube—for example, $\frac{1}{2}$ in. diameter, and 3 in. in length—a few crystals of salicyl-sulphonic acid are dropped in, and boiling, if necessary, can easily be done in the absence of a spirit lamp, etc.—over an ordinary lamp or candle.

No substance in the urine, apart from those of the proteid class, has been found to give the reaction⁵, which is an exceedingly delicate one. As a precipitant of proteoses it is greatly superior to nitric and acetic acids, as the latter redissolve the precipitate when too much acid is added.

NOTES AND REFERENCES.

¹ Attention was called to these facts many years ago in this Medical School by Dr. Angus Fraser. ² Some observers deny that acid-albumen is formed. ³ Pickering, *Journal of Physiology*, vol. xiv, p. 374. ⁴ MacWilliam, *BRITISH MEDICAL JOURNAL*, 1891, p. 837. ⁵ To obtain a saturated solution dissolve 25 grams of salicyl-sulphonic acid crystals in 10 c.cm. of water. Roch used a relatively weak solution, and the results got by his method are very inferior in delicacy; Roch did not distinguish between albumen and proteoses. ⁶ MacWilliam, loc. cit., p. 839.

AN INQUIRY INTO THE PRIMARY SEAT OF INFECTION IN 500 CASES OF DEATH FROM TUBERCULOSIS.

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ONE of us having been requested to examine the reports of fatal cases of tuberculosis in the hospitals in Bristol, it was thought that a short account of the analysis might be of some interest.

A question which it is scarcely necessary to remark has much agitated the medical mind of late has been the method of entry of the tubercle bacillus into the system. The statistics of some observers appear to show that in the great majority of cases of tuberculosis invasion takes place by means of the respiratory passages, while others have maintained that their experience shows that entry by means of the alimentary tract is by no means uncommon.

Those whose experience leads them to believe that the abdominal viscera are first invaded naturally lay great stress upon the danger of infected milk, while those who hold that the thorax is almost invariably the primary seat of disease are for the most part inclined to consider that bovine tuberculosis is rarely communicated to man. The whole question is involved in difficulty, and it is needless to remark that it is in the age of childhood that milk is chiefly drunk, and it is at this period of life that primary abdominal tuberculosis is most common; yet it does not follow that this is a proof of infection with bovine tuberculosis.

There are numerous ways by which tubercle bacilli of human origin can enter the alimentary canal. Even in the early months of life such infection is possible. All day long some babies are sucking an india-rubber comforter, and it no sooner drops on to the dirty floor than it is hastily picked up and thrust again into the mouth of the infant. Older children also as they crawl take up every article they can lay hold of and put it into their mouths, to the danger of which their dirt-begrimed cheeks bear witness.

While these risks are real, we have, on the other hand, to remember that the discovery of disease in a certain part of the body does not necessarily imply that the cause of the disease found its way to that spot by the nearest route. There

is what we may call the inborn weakness of certain organs, and the infecting agent may pass through more resistant parts of the body in order to reach these weaker organs.

Recent experimental work seems to show, also, that tubercle bacilli entering by the mouth may reach and cause disease of the lungs, while other parts of the body remain unaffected. However, although we may say that in some cases of disease of the lungs invasion may have taken place by the alimentary canal, so far as we can judge from *post-mortem* evidence it seems to us that infection of the abdomen may, on the other hand, sometimes occur from a small focus in the thorax. Thus we have seen acute tuberculosis limited to the peritoneum when the only caseous focus in the body that could be discovered was a caseous bronchial gland. During an attack of rheumatism in a boy pericarditis had arisen, and the disturbance set up by this inflammation in the thorax appeared to have disseminated tubercle bacilli contained in the caseous bronchial gland, which bacilli had attacked the peritoneum only.

As we have said, the question of the method of invasion is involved in difficulty, but, even if the matter were sufficiently simple to be settled by the evidence afforded by the situation of the oldest foci of disease in the body, we still have the difficulty of deciding in many instances which are the oldest foci. Cases of tuberculosis in children in which the disease is entirely limited to one cavity of the body are considerably in the minority, and the liability to fallacy of personal judgement cannot but influence the conclusions as to which cavity of the body was first attacked. Then, again, there is the difficulty that the pathologist who wrote the *post-mortem* notes may not always have been sufficiently explicit to enable the reader to decide which was probably the primary source of infection. Since, however, one of us has been responsible for fully half of the necropsies, the notes of which have been examined, and has always paid attention to this point, fallacies due to this cause are not likely to be serious. In the following list many cases are classed as doubtfully abdominal or respiratory in origin.

Although there may be intestinal ulcers, and perhaps a few caseous mesenteric glands of apparently recent origin, should there be old pulmonary lesions such as a cavity and perhaps also large masses of caseous bronchial and mediastinal glands, we have generally considered it justifiable to classify the abdominal disease as produced by swallowed sputum infected with tubercle bacilli, and therefore secondary to the disease in the thorax. In other cases, however, when we have considered that there would be the least possibility of difference of opinion, we have placed the case under the heading of "doubtful." For example, when the notes say, "In front of the right bronchus was an abscess cavity with thick walls containing caseous material," and also state that only "two mesenteric glands were caseous and all the others normal," we have classified the case with those in which the disease might have originated either in the abdomen or the thorax, though we are inclined to believe that the thoracic focus was with little doubt the older.

Statistics of 102 Cases of Tuberculosis under the Age of 15 Years.

Apparently definitely abdominal in origin...	12		11.7 per cent.
Definitely thoracic ...	57		55.8 "
Doubtful whether abdominal or thoracic ...	24		23.5 "
Of the remaining cases the primary lesion was possibly in the			
Bones or joints ...	...	...	in 4
The skin ...	...	...	in 1
The tonsil ...	...	...	in 1
No focus found ...	...	...	in 3

It will be noticed that there were 12 abdominal cases to 57 thoracic—that is, the proportion was 1 to 4.7. Examination of the cases under the age of 2 years showed, however, a much higher proportion of abdominal cases. There were 5 apparently primary abdominal cases and 9 apparently primary thoracic in the first two years of life. But if we consider it to be universally true that cases of primary abdominal tuberculosis are most common under the age of 2 years, although it suggests infection by means of milk as a frequent cause of tuberculosis, it does not prove it. The abdominal lymphatic glands are at an early age irritated and weakened by the products of the almost universal dyspeptic disturbances which occur in the first years of life, and this weakness naturally exposes them to infection with tubercle bacilli, whether human or bovine.

To digress from this subject for a moment, it may be remarked that, although cavitation of the lung due to tuberculous disease is decidedly much less common in children than in adults, there were two cavities of considerable size