blown depends on the disruptive charge; and the fragments under such a condition fly out in all directions. The shape of the fragments depends largely on the type of shell—if the shell is segmented the pieces are more or less quadrilateral; if not segmented they have extremely sharp and ragged edges. Such shells, bursting on the ground, naturally become covered with dirt.

Shrapnel.—These shells usually burst in the air with a time-fuse. They are of all sizes and the leaden balls with which they are filled also vary somewhat in size. The penetrating power of such balls is due to the remaining velocity of the shell. The direction of the shrapnel balls will, as a rule, be downwards. In some cases apparently a shell is of a combined high-explosive and shrapnel type.

Bombs and grenades.—In the early part of the war these bombs were often extemporised and consisted of tin boxes filled with an explosive, scrap iron, cobblers' nails, and screws, &c. Now-a-days they are made of iron, the surface of which is marked with grooves, so that on explosion they break up into quadrilateral fragments. Sometimes the force of the explosive will cause a bomb to burst into smaller fragments, so that often they are no bigger than a big match-head. These fragments, although small, have apparently, from their high velocity, a very great penetrating power in the immediate neighbourhood of the explosion, although this is rapidly lost as the distance increases.

Trench mortars.—These are of various shapes, but consist essentially of a very large high-explosive charge with a comparatively thin containing envelope; they therefore burst very often into large ragged fragments as well as minute ones. They are generally timed to burst either on the ground or in the ground.

Bayonet wounds.—These are very seldom met with, being usually fatal on the field. Mention may be made of two cases. In the first the bayonet penetrated the man's back and came out by his umbilicus. No viscus was injured, and the man succumbed to hæmorrhage. The second was the case of a man who was late in answering a challenge and was bayoneted by the sentry. The bayonet entered the left hypochondrium and wounded the stomach near the greater curvature and entered the back wall of the abdomen. Though promptly operated on he died, not of his stomach wound but of retro-peritoneal sepsis.

RELATIVE NUMBER OF DIFFERENT PROJECTILES AND THE PROPORTION RETAINED.

Table II. has been drawn up with the intention of giving some idea of the relative frequency of the different projectiles met with in abdominal wounds; it also shows the relative number retained in the body. Now this table must be taken as

TABLE II.

Bullet.		Shell fragment.		Shrapnel.		Bomb or grenade.	
Out.	Retained.	Out.	Retained.	Out.	Retained.	Out.	Retained.
181	103	24	151	10	52	6	82
284		175		62		88	

only relative for several reasons. In the first place it is often impossible for a man to tell what hit him. In the second place one cannot always be sure from a study of the entrance and exit wounds what was the nature of the projectile; and, again, many do not differentiate between high-explosive shell and shrapnel, the soldier generally including most shells which are not of a large calibre under the name of shrapnel. As a matter of fact, there is very little difference in the nature of the fragments in high-explosive shells, bombs, rifle grenades or trench mortars if one excludes the larger fragments with which we are very little concerned.

Bullets.—Bullets and high-explosive shells furnish a large proportion of abdominal wounds. More bullets pass through the body than other projectiles. The reason for this is obvious. The causes for retention are not so obvious, and I have known bullets fired at a comparatively short distance remain within the body. In other cases the bullet may have passed through the earth and so have had its velocity diminished. Ricochets account for a certain number.

Shells.—These show a high proportion of retention. This is obviously due to the shape of the fragments. Shrapnel

shows a large proportion, and this is no doubt due to the small velocity and round shape.

Bombs and grenades.—The retained fragments are strikingly in excess of those passed out. The large proportion of retained fragments at once suggests the possibility of armour; and, no doubt, armour could be devised which would keep out many fragments. It really is more or less a question of what the soldier is able to carry. One cannot help being struck with the resistive power of an ordinary book, as one has often seen projectiles arrested by such articles when carried by the soldier. The great saving of life produced by the steel helmet makes one hope that something could be produced which would be equally efficacious in protecting the body. Although we could not expect such shields to be supportable and bullet proof, yet the number of passed-out bullets is far out-numbered by the number of retained bullets, shell, and bomb fragments which possess far less penetrative power.

RELATIVE MORTALITY OF THE DIFFERENT PROJECTILES.

TABLE III.

Bullet.		Shell fragment.		Shrapnel.		Bombs or grenades.	
To base.	Died.	To base.	Died.	To base.	Died.	To base.	Died.
50	63	46	68	10	21	30	31

There is nothing very much to be learnt from this table, except that bullets are as dangerous as other projectiles, and that bombs and grenades seem to be the least noxious.

THE IMPORTANCE OF THE DIAGNOSIS OF ENTERIC FEVERS BY AGGLUTININ DETERMINATIONS.

BY E. W. AINLEY WALKER, M.A., D.M. OXON.,
CAPTAIN, O.T.C. UNIV. OXON.; FELLOW AND TUTOR OF UNIVERSITY
COLLEGE, AND LECTURER IN PATHOLOGY IN THE
UNIVERSITY OF OXFORD.

(Report to the Medical Research Committee.)

IN the course of recent criticisms directed to this subject several points of interest have been raised, and some misapprehensions have become evident which it may be of advantage to consider briefly. They are dealt with in the order of their publication.

I.—In a discussion on "The Technique of the Agglutinin Test" Dr. P. N. Pantón¹ strongly dissents from my contention that "the microscopic method entirely fails to give comparable quantitative measures of the titre of a patient's serum on successive occasions." He considers the microscopic technique commonly employed to be fully adequate for this purpose. In defence of this view he expresses certain opinions regarding the constancy of the materials employed, the general accuracy of the microscopic technique, and other matters whose validity ought not to pass unchallenged. Yet merely to deny Dr. Pantón's statements categorically would not assist in illuminating the subject, while to explain in detail *ab initio* the reasons why they are misleading would require not only a repetition, but also a considerable amplification of my previous Note.² For he places one in this dilemma, that he makes statements contradictory of well-established evidence, much of it old and quite familiar, but at the same time he omits to adduce any experimental data in support of his position, such as could be submitted to critical analysis. In some cases he seems actually to be unaware of the factors which militate against accurate quantitative results in the performance of agglutination tests. For example, he still (so far as one can judge) makes the successive dilutions of a serum by adding more and more bacillary suspension, in apparent unconsciousness of the effects of the mass influence of bacteria on the absorption of agglutinins. These effects, however, are such as to render it essential that the serum, whatever its dilution, shall always operate on equal masses of bacteria if accurately quantitative observations are desired. Fortunately many of Dr. Pantón's

arguments have already been admirably dealt with by Glynn.³ It may therefore suffice upon the present occasion to meet his contentions regarding the value of the microscopic method for the purpose under discussion by examining its performance in the only direction in which he has brought forward definite experimental evidence. These observations of his own afford very valuable information. For though he himself interpreted them guardedly as evidence in favour of the exploded theory that in inoculated persons any febrile condition of a certain duration causes, or may cause, disappearance of the inoculation agglutinins, they serve in reality quite a different purpose. They prove to demonstration that the microscopic method as employed by him cannot for accuracy and reliability support any comparison with results obtained by the use of Dreyer's macroscopic technique. Indeed, his figures show that the results obtained by the microscopic method, even as regards the mere detection of agglutinins without considering the question of their measurement, are very much worse than one would have ventured to suggest was probable had these data not been supplied. The results of typhoid agglutination tests are recorded by Dr. Panton for 72 out of 100 consecutive cases in inoculated individuals who had suffered from various febrile conditions. They are tabulated below alongside the results for the first 100 similar cases examined by myself by Dreyer's method.

Cases examined by the microscopic method (Panton).		Cases examined by Dreyer's method (Ainley Walker).	
Typhoid agglutination.	Per cent.	Typhoid agglutination.	Per cent.
"Completely negative."	62.4	Nil, 1 in 25.	0
"Negative or partial."	16.7	"Standard," or trace 1 in 25.	3
"Positive."	20.8	"Standard," 1 in 50 or more.	97

By Dreyer's method every case showed measurable quantities of agglutinin in 1 in 25 dilution of the serum, and 97 per cent. showed "marked agglutination visible to the naked eye" in at least 1 in 50, and frequently in very much higher dilutions. But in the cases examined by Dr. Panton by the microscopic method the test was "completely negative" in nearly two-thirds and "positive" in only about 21 per cent. Nothing could be more convincing as regards the relative delicacy, reliability, and general accuracy of the two methods for the detection of agglutinins. A fuller justification could, indeed, hardly be found for my criticisms of the microscopic method than that here provided by Dr. Panton's own observations.

II.—In a letter to the Editor of THE LANCET Captain R. P. Garrow⁴ makes some interesting comments on my recent "Illustrations of the Diagnostic Value of Agglutinin Determinations."⁵ And one may certainly be permitted to endorse his plea for judgment and caution in the interpretation of the results of agglutination tests, particular regard being had to the methods employed. Probably we are much more nearly in agreement than the incisive character of some of his expressions might suggest. But in charging me with ignoring clinical observation as a means of diagnosis in enteric fevers he misunderstands me. My article was not concerned to discuss the clinical diagnosis of enteric fevers. To do so would be quite beyond my province. The point which it was desired to emphasise is that when and wherever these infections are abroad many mild and atypical cases may occur which are *not capable* of diagnosis by clinical observation alone. Moreover, in every case the differential diagnosis between typhoid, paratyphoid A, and paratyphoid B still rests, and is likely to remain, with the pathologist. The cases charred in my article purposely included a number of such mild or atypical attacks. And the fact that among the mass of cases which receive the provisional diagnosis "pyrexia of uncertain origin" cases of enteric fever may be numerous and important, that they form a grave source of danger, and that the agglutination test (even as commonly applied) affords the *best* criterion for their diagnosis is emphasised by competent authority, as follows (all italics mine):—

Oster and McCrae: *System of Medicine*, Vol. II., p. 178.—"Mild Typhoid Fever.—This is an exceedingly important group, especially as regards prophylaxis, for these mild attacks may not be recognised, and the patients are the means of spreading the disease. The cases

may be divided into two classes, the first including those characterised by a mild and short course, the other those in which despite the short course the onset is severe. In many instances the *mild forms* are taken for malaria, mountain fever, &c., while doubtless they are not uncommonly put down as *febricula*. The recognition of this group comes through one of two ways. They may show characteristic signs. or be recognised only through the routine *Widal reaction*. We are not sufficiently impressed with the importance of this group. Keeping their frequency in mind, the physician will watch more closely and have the *agglutination reaction* tried more frequently in patients with mild fever. For the community the recognition of this group is most important."

If the foregoing statements were valid before the introduction of protective inoculation, much more are they so in relation to the character of enteric infections in inoculated persons, among whom the relative proportion of mild, abortive, and atypical attacks will naturally be higher than among non-inoculated individuals. Thus it appears that in urging the importance of relying on agglutination tests for diagnosis in such cases one is not guilty of ignoring clinical observation, but can claim the strong support of clinical teaching.

That the facts thus indicated were quite fully recognised by experienced observers who have worked upon the epidemiology of enteric infections is illustrated in the subjoined quotations from an article by Grattan and Wood⁶ on paratyphoid fever in India, where the case is stated plainly and with great cogency.

"In the first place, owing to the mildness of the disease in some instances, patients are not diagnosed paratyphoid, but influenza, tonsillitis, pyrexia of uncertain origin, rheumatism or pneumonia, with the result that they are sent out of hospital within a few weeks of going sick, and may return to barracks while still infectious. In the second place, such convalescents are frequently sent with other debilitated men to the hills during the hot weather, and thus the hill stations where men of several corps mix together may serve as distributing centres of the disease. Furthermore, the ambulant case is in reality."

"An epidemic is started from one acute carrier, the nature of whose illness is not recognised owing to its mildness. He infects other men, some of whom are recognised as cases of paratyphoid; others, again, are not diagnosed, and possibly others never report sick at all. These men soon return to barracks and come in contact with other men in soldiers' homes, &c.; and though probably only infectious for a few days manage thus to disseminate the disease."

With regard to the interpretation of agglutination results, Captain Garrow expresses so clearly exactly what ought to be done in carrying out an investigation of this problem that his terms may well be quoted (*italics partly mine*):—

"..... blood culture should be performed at once. Whatever the result of the blood culture, agglutination tests by a strictly quantitative method should be performed and repeated at suitable intervals. The object of this procedure in a case in which an absolute diagnosis by blood culture has been obtained is to furnish *agglutination results controlled by blood culture*. The data so obtained are *essential* as a guide to the interpretation of agglutination results in other cases in which blood culture has failed to give a diagnosis."

But Captain Garrow appears to have overlooked the fact that this fundamental work is precisely what has already been done on a very considerable scale. And there comes a time in the conduct of investigation when the evidence accumulated seems to be sufficient to convince observers that the guide thus obtained may be relied upon, and that the problem may in certain aspects be regarded as solved. Now the evidence obtained by Dreyer⁷ and his co-workers in very long series of cases is that *in every case* bacteriologically proved to be typhoid or paratyphoid B, and also in similar cases of paratyphoid A (since the low level of agglutinin titre found even at the maximum in many cases of this infection was fully recognised), the diagnosis independently arrived at by means of agglutination tests carried out by Dreyer's method was *proved* to be correct. Results of such a character may fairly be termed a *uniformly accurate diagnosis*. They would seem to justify my statement—not in the extravagant interpretation placed on it by Captain Garrow "that agglutination tests alone *invariably* furnish an *absolutely infallible* diagnosis"—but in the form in which it was made, and with the limitations carefully specified in the article, that such tests "..... can be made to yield a uniformly accurate diagnosis in cases examined during active infection." The limitations specified and repeated from a previous article by Dreyer and myself⁸ were that a diagnosis should not be based on a rise of titre of only 100 or 200 per cent. if it is clear that the maximum of that rise falls markedly outside the period sixteenth to twenty-fourth day of the disease. We further stated that so far as experience at present goes, if a regular rise and subsequent fall, even of only 100 or 200 per cent., occurs in the typhoid (or paratyphoid) agglutinin titre of the serum, and its maximum

clearly falls between the sixteenth and twenty-fourth day from the onset of illness, the case is likely to be one of typhoid (or paratyphoid) infection. It would be prudent, therefore, that it should be so regarded under present conditions, when the circumstances are such that it is a prime object to prevent the escape of undetected carriers. For every case that is missed is a potential carrier of infection.

Captain Garrow further takes exception to my use of the graphic method in joining successive points on the charts of agglutinin titre by *curved* lines, which he terms "figments of imagination." Here he is rather led astray by a telling phrase. It is, of course, in one sense never possible to do more than speculate (more or less logically) regarding the course of nature between points of observation. One was therefore careful to speak guardedly of these interpolations as "curved dotted lines to indicate approximately the probable form of the agglutinin curve." But it is surprising that Captain Garrow should express a desire to see the points joined by *straight* lines. For a little more consideration would have shown him that such straight lines would not only equally be figments of imagination, but they would also be deliberately *misleading*. If there is one fact more than another that has been firmly established for years, it is that both the rise and fall in the agglutinin titre of the serum follow *curves*. Why, then, draw straight lines if one wants to illustrate what one thinks is probably occurring between points of observation?

To turn briefly for a moment from the general to the more particular. Captain Garrow very properly draws attention to a bad slip of the pen in a recent article by Professor Dreyer and myself in stating the maximum of a curve lies between the two highest readings, when we should have said on one side or other of the highest reading. But, as he notes, the correct principle is followed in my charts. In reference to Chart 6, in which the T. estimations exhibit a progressive fall, the reason why a maximum is placed between the first two readings is that, as can be seen at once by plotting out on a large scale, a smooth curve through the last five points will not pass through the first. Incidentally, this shows how information is gained from the use of *curved* lines of interpolation. In Chart 10 the reason for the diagnosis "clearly a case of B. infection" is given in the letterpress attached, if read in conjunction with the whole of note 3.

Case 4 of my article receives some special criticism. In publishing this chart attention was drawn to the fact that the case had "simulated a tertian ague." It was selected for publication partly to illustrate the fact (see Osler and McCrae above) that such cases may be *mistaken for malaria*. The clinical data given were a headache, a shivering fit accompanying the first sharp rise of temperature, and two more sharp rises of temperature on the third and fifth day (though without definite chills). The blood was examined for malarial parasites, but none were found, and a *typical typhoid agglutinin reaction was observed*, as shown in the chart. On this evidence Captain Garrow regards the case as one of malaria, but it is quite impossible to assent to such a diagnosis. The patient came from the Mediterranean area, where he had had prolonged fever, associated with severe intestinal disturbance, and he appears to have suffered, at any rate, from paratyphoid A and paratyphoid B infections (mixed or consecutive; *vide* chart). There was no indication of malaria in his history, and he had been three months in England when he was seized with the brief pyrexial attack under discussion and developed an extremely characteristic typhoid agglutinin reaction of moderate grade. Captain Garrow writes in a way that might convey the impression that he supposes every brief tertian periodicity of pyrexia with a chill at the onset to be necessarily of malarial origin. But he is, of course, aware that this is not the case. Repeated chills may occur, with or without marked periodicity, in various stages of enteric fever (to go no further afield) *vide* Osler and McCrae, loc. cit., p. 115, where a chart of enteric fever is given, showing seven successive periodic chills (cp. also Osler and McCrae, p. 105, and Allbutt and Rolleston's "System of Medicine," 1905, vol. i., p. 1108 and p. 1132).

III.—Captain H. Graeme Gibson's letter in THE LANCET of Jan. 13th, 1917 (p. 78), follows quite a different line of criticism. In effect the letter pointedly suggests that Dreyer, Ainley Walker, and A. G. Gibson⁹ have ignored or are ignorant of (it is not clear which is intended) certain

earlier work, which had proved long before the same facts as they imagine themselves to have established more recently. In doing so it, of course, tacitly admits that the facts in question have become established, and on this account its appearance marks an important stage in the gradual crystallisation of opinion regarding the diagnostic value to be attached to systematically repeated and exact agglutinin determinations. But Captain H. G. Gibson is entirely mistaken in his conclusions.

Medical science is deeply indebted in very many directions to the admirable studies carried out by officers of the Royal Army Medical Corps, and it is a matter of particular regret to the present writer that he is compelled—not, be it noted, by any claim or action on the part of authors themselves, but by the misleading charges advanced (however disinterestedly) by Captain H. G. Gibson—to bring under criticism work which constituted at the time a valuable and successful endeavour to advance knowledge in regard to enteric infections, particularly in the domain of epidemiology. This task, which is not undertaken lightly, is rendered personally somewhat less repugnant by the fact that, whatever share may be assigned to his co-workers, any results which may have been attained by the Oxford School of Pathology are pre-eminently due to the scientific insight and experience of my friend and colleague Major Dreyer, now absent on active service.

The statements contained in Captain H. G. Gibson's letter may now be dealt with. Dreyer, Ainley Walker, and A. G. Gibson did not ignore the earlier work referred to.

1. There is no suggestion in any of the authorities whom Captain H. G. Gibson quotes either of the need for accurately quantitative measurement of agglutinin titre, or of a technique and materials by means of which such measurements could be made.

2. Nor is there either the statement, or the evidence to prove it, that reliable diagnostic data can be secured by the systematic repetition of exact agglutinin determinations at appropriate intervals—three to five (or more) successive determinations at intervals of from three to five days.

3. The curves reproduced by Captain H. G. Gibson marked no new departure in pathological investigation. For accurately determined curves of agglutinin titre had been published, at any rate since 1902, by Madsen and Jørgensen, Dreyer and Schroeder, Widal, Dreyer and Ainley Walker, and others, before the appearance of the communications referred to, and were presented in greater detail and with more exactitude.

4. The problem of the diagnosis of enteric fevers in inoculated men by means of agglutination tests became important in India and elsewhere from the period of the re-introduction of typhoid inoculation in the Army in 1905, but it remained unsolved, although the key to its solution had been provided in the work of Madsen and others just mentioned and in the improvements in technique devised by Madsen and by Dreyer.

5. As regards the observations of Grattan and Harvey¹⁰ and Grattan and Wood,⁶ we made reference to them where they were germane to the purpose in terms of which the opening sentence ran as follows:—

"It is in absolute agreement with the important and careful observations carried out by Grattan and Harvey, and Grattan and Wood some years ago in their investigation of paratyphoid fever in India."

6. As concerns the quotations taken from reports on the enteric fever depôt at Naini Tal, they do not represent the views which we maintain. Had we referred to them it could only have been to express our very strong dissent from a number of their conclusions—for example:

Naini Tal Reports.

"In paratyphoid fever the Widal reaction is invariably raised for the *B. typhosus*."

"In all cases of fever in inoculated men the blood should be taken near the beginning and towards the end of the fever. If the two results are the same the case is probably not enteric."

Comment.

It is not invariably raised.

This is not necessarily true, for a marked rise and fall in titre may have occurred between two such distant determinations. We regard three determinations as the normal minimum. And we should consider it quite deplorable to wait until towards the end of the attack before attempting to secure a diagnosis.

In opening his criticism Captain Gibson takes exception to a statement of ours which offered a very moderate presentation of the facts existing at the time when it was written (December, 1914). He states that since 1905 repeated observations of the serum agglutinin content have been

taught at the Royal Army Medical College "and carried out by Army bacteriologists." But the first sentence of his own quotation from the Naini Tal reports throws an interesting light upon the latter claim, and in itself goes far to justify our statement.

Statement by Dreyer, Ainley Walker, and Gibson.

"It is probable that the absence of accurate diagnosis in the past explains the fact that statistical results arrived at in our Army have not always been so convincing as would otherwise have been the case."

Naini Tal Report.

"Some of these errors in diagnosis are due to the fact that the men had been inoculated, and therefore their blood gave a positive Widal reaction, and they were diagnosed enteric fever on the strength of this alone."

Captain Gibson then proceeded to urge that—

"The necessity for using Dreyer's technique is not apparent, and equally reliable results can be, and have been, obtained when using living suspensions of bacilli, 'provided the suspension is standardised.'"

This shows that the writer has failed to understand what is meant by *standardisation of the agglutinability* of a bacterial suspension. The standardisation of the agglutinability of living suspensions is no doubt theoretically possible, but for any practical and routine purpose it is quite out of the question. Indeed, one is almost driven to the hardly credible conclusion that, with Dr. Pantton, Captain Gibson supposes the determination of a given opacity or number of bacilli present per c.c. of the suspension to constitute the "standardisation" of an agglutinable culture.

At the end of the letter a case is stated against Dreyer in error, and with a degree of injustice which might have been avoided had the writer studied more attentively the authorities he quotes. He points out that Grattan and Wood mentioned in 1910 the frequency of *low* titre of serum in paratyphoid A, and would appear to suggest that Dreyer was still ignorant of this fact in 1915, although Grattan, Dreyer, and Wood had been working together. This suggestion is based on Dreyer's statement that "for a considerable time I did not know *how low* is the agglutination given by paratyphoid A in certain cases." But the explanation lies in the meaning of the words "*how low*" and in their context. Grattan and Harvey¹⁰ found in 7 of their 8 cases "*low* dilutions (20 to 40), and always incomplete." Grattan and Wood⁶ mentioned that they *had seen* cases where the agglutination did not reach 1 in 10. Their statement with regard to diagnosis in paratyphoid A is given below, along with the context of Captain Gibson's quotation from Dreyer.

Grattan and Wood⁶ (1910).

"..... a positive agglutination for this organism in a dilution of 1 in 20 is, in our opinion, *very strong evidence* of the nature of the patient's fever."

Dreyer⁷ (1915).

"One had always taken 1 in 25 as the limit below which no positive diagnosis should be made. But in the case of paratyphoid A that is wrong. If one uses standard cultures, and follows the technique laid down for their employment, I am now convinced that an agglutination of 1 in 10 is for all practical purposes *diagnostic* in cases of paratyphoid A."

This (1 in 10) was the *degree of lowness* of titre for positive diagnosis, of which Dreyer (and everyone else) was previously unaware. And no one had ever before suggested that paratyphoid A could be diagnosed with certainty (in non-inoculated and T-inoculated persons) on so low a titre. So far was the fact from being already familiar that Leishman¹¹ was even then unable to accept it, remarking with regard to this conclusion of Dreyer, "Personally, I think he goes too far because of the very low dilutions to which he attaches importance." Dreyer, Grattan, and Wood worked in association for a considerable period, and ample time has passed since Dreyer's statement was made for Grattan and Wood themselves to have drawn attention to any infringement of their priority had such existed. It can constitute no breach of confidence of any kind to state that Lieutenant-Colonel Grattan and Captain Wood were the first officers in the Royal Army Medical Corps to recognise fully the value of Dreyer's method and to make use of it consistently.

References.—1. Pantton: THE LANCET, 1916, ii., 750. 2. Ainley Walker: THE LANCET, 1916, i., 17. 3. Glynn: THE LANCET (Correspondence), 1916, ii., 877. 4. Garrow: THE LANCET (Correspondence), 1916, ii., 1037. 5. Ainley Walker: THE LANCET, 1916, ii., 896. 6. Grattan and Wood: Journal of the R.A.M.C., xvii., 148. 7. Dreyer: Proceedings of the Royal Society of Medicine, 1915, Medical Section, p. 10. 8. Dreyer and Ainley Walker: THE LANCET, 1916, ii., 419. 9. Dreyer, Ainley Walker, and Gibson: THE LANCET, 1915, i., 324. 10. Grattan and Harvey: Journal of the R.A.M.C., xvi., 9. 11. Leishman: Proceedings of the Royal Society of Medicine, 1915, Medical Section, p. 19.

AN EASY AND RAPID METHOD OF DETECTING PROTOZOAL CYSTS IN FÆCES BY MEANS OF WET- STAINED PREPARATIONS.

BY ROBERT DONALDSON, M.A., M.B. EDIN.,
F.R.C.S. EDIN., D.P.H.,

BACTERIOLOGICAL SPECIALIST, WAR HOSPITALS, READING; PATHOLOGIST, ROYAL BERKSHIRE HOSPITAL, READING, ETC.

It is of the highest importance to single out from among those previously infected or exposed to infection the small but dangerous minority who form the little band of *histolytica* carriers. This involves the expenditure of an enormous amount of time on the very necessary routine examination of all suspected fæces in order to detect the presence of protozoal cysts, more especially those of *Entamoeba histolytica*. To fix and stain films from each of the large number of specimens requiring examination is impracticable owing to the amount of time involved.

The usual method of detection is to examine wet preparations, which is done, as a rule, in one of the two following ways. Having determined the existence of cysts by their higher refractivity in a wet film preparation of fæces in normal saline, another similar film is made in which, however, the saline is replaced by Gram's iodine. This method obviously involves the making of two separate films. In the alternative method the first step as above described is omitted, and only a wet Gram's iodine preparation examined. The object in using iodine is to bring out sufficient detail—more particularly the nuclear structures—in order to differentiate between cysts of *Entamoeba coli* and *histolytica* respectively.

These methods are open to two important objections. The first, involving as it does two separate preparations, takes up too much time. In the second place, as the iodine in both methods stains the food debris more or less deeply, there is not sufficient colour contrast between the cyst and the debris surrounding it to enable it to be singled out rapidly and with certainty. Where cysts are very few in number, even an expert at such examinations may miss the occasional cyst on which a diagnosis alone may rest, while those less expert must spend a considerable amount of time carefully scrutinising everything in the film, and even then will probably frequently be in error. The lack of contrast in such preparations where there is so much similarly stained material to distract the eye, necessitates considerable concentration on the part of the observer, involving a greater expenditure of time and labour, and consequently allowing a greater loophole for error.

In order to reduce the amount of time spent on such examinations to a minimum and to render it almost impossible for even a novice to fail to detect the presence of cysts, I have devised the following stain mixtures. After trying various proportions, it has been found that a mixture of equal parts of the two stains used in any of the three following combinations will yield the best results.

A. Five per cent. aqueous solution of KI saturated with iodine to which is added an equal volume of either—

- B. (1) A saturated aqueous solution of rubin S; or
- (2) A saturated aqueous solution of eosin; or
- (3) Stephens's scarlet writing fluid.

(Note.—1. I have found it necessary to specify this particular brand of red ink as some brands are useless for the purpose. 2. Basic fuchsin cannot be used.)

A few loopfuls of one of the above stain combinations are placed on a clean slide, a loopful of fæces taken and rubbed up with the stain to form a fairly smooth suspension, and a clean cover-slip gently lowered over the drop. To get the best possible definition it cannot be emphasised too strongly that the film so made should spread out under the cover-slip by capillarity so as to form the thinnest possible layer—preferably a layer which is no thicker than the diameter of a *coli* cyst. Obviously no gross particles must be left in the film, otherwise the cover-slip will not lie flat. The exact amount of stain to place on the slide will soon be learned after one or two attempts, and will depend on the size of the platinum loop employed and the size of the cover-slip. It is also preferable, although not essential, to mix afresh for each day's use the two elements of the combination stain.