

exuded from the raw surfaces, were united by mattress silkworm-gut sutures. Sites were chosen on the cheek flap before this was fixed to the deep margins of the gap. A threaded needle was passed from the raw surface and out through the nasal orifice where it picked up a small piece of rubber tubing to which was attached a long piece of thread. It was then passed back through the nasal orifice and through the flap, entering this from the skin surface. (Fig. 27.) The second half of the suture was completed by passing each end through the scalp flap and tying them over a piece of drainage tube on its skin surface. (Fig. 28.) The long threads hanging from the nasal orifice served to retrieve the pieces of drainage tube lying on the deep surface of the united flaps when these mattress sutures were cut on the fourth day. Hair grew profusely on this flap, and I therefore sent him to Captain Knox for depilation, the various stages being shown in Plate 3.

It is to be noted that the gap is not filled in, but merely bridged, thus exactly reproducing the normal state. The advantages of this method, aided by depilation, are obvious. It permits a hair-bearing scalp flap to be used to occupy a hairless area of the face. The scar area of the flap is completely hidden by growth of neighbouring hair with resulting absence of disfigurement.

Summary.

Finally, may I be allowed to sum up the points that appear to me to need emphasis?

1. That the result in any given case is largely influenced by the initial treatment adopted.
2. That the whole plan of treatment should be the joint evolution of surgeon and dentist working in concert to attain a common aim.
3. That open-bite splints should invariably be used in the type of case considered.
4. That the method known as "bringing the parts together" should frankly be recognised as unsatisfactory and be abandoned.
5. That skin is an admirable substitute for mucous membrane in that its texture is suitable and its extent unlimited.
6. That radiations may render the plastic surgeon such valuable assistance that facilities for treatment by this method should be provided in the case of any jaw centre or hospital.

ENTAMOEBA HISTOLYTICA INFECTIONS:

THEIR PREVALENCE AMONG BRITISH TROOPS IN
INDIA AND MESOPOTAMIA, WITH SPECIAL
REFERENCE TO THE QUESTION
OF "CLEARING."

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THE following observations are based on the results of an inquiry based on the protozoological examination of the stools of over 2000 men, mostly of the Mesopotamian Field Force, who had been invalided for various ailments to a general hospital stationed in India. A full statistical report with tables will shortly be published in the *Journal of the Royal Army Medical Corps*. We have, however, deemed a summary of our work worthy of separate publication in view of an analysis of our findings pointing to the very marked prevalence of *histolytica* infections among troops not considered to be suffering from any intestinal affection; while certain of our observations appear to us to have an important bearing on the question of the practical value of making a series of protozoological examinations of the stools of men convalescing from amoebic dysentery for the purpose of declaring them "cleared" of their infection.

Scope and General Results of Investigation.

The records on which our observations are based have been obtained from the investigation of the faeces of men whose condition ranged from good general health to one associated with acute and chronic intestinal disturbances. All the stools examined have been those that have followed the

administration of a saline purgative. This routine has been followed because it was found that the examination of material from a constipated motion was unsatisfactory, the percentage of positive findings being considerably greater in a series of liquid or unformed specimens. All stools have been examined both in normal saline and in double Gram iodine solution, at least two specimens from each stool being microscopically examined. In our interpretation of the types of cysts we have followed the teaching of Wenyon. Practically all the *Ent. histolytica* figures are based on our cystic findings, no free form of amoeba having been accepted as *Ent. histolytica* unless it showed ingested red cells.

As regards the prevalence of *histolytica* infection in 1165 cases admitted to the dysentery wards of the hospital we have no intention of stating our findings in any detail. No statistical deductions can be made because the stools examined have come from very different types of cases, both as regards certainty of diagnosis, stage of disease, and previous treatment received, while no features of very special interest have been noted. 10.1 per cent. of these 1165 men were found to be infected with *Ent. histolytica*, and 4.9 per cent. of 3623 examinations carried out on those patients revealed the presence of *Ent. histolytica* cysts. The results of these examinations are here mentioned because they appear to us to act as a control over our differentiation and interpretation of the various types of cysts met with.

It is to be noted that our figures do not show an unusually high percentage of positives for convalescent dysenteries, and are in striking contrast to our findings in the case of men with presumably normal alimentary canals, for the results of the protozoological examination of the stools of 946 men of this latter class have brought to light certain features of considerable interest and importance. This number includes chiefly men who had been recently invalided from Mesopotamia for an affection other than an intestinal one, and who in many instances had suffered from an intestinal disturbance at some more or less remote period.

The incidence of *histolytica* infection among such cases has been especially high, while the general protozoal findings in the stools have been more numerous than the records of Wenyon¹ and of Dobell² show to exist among the troops from the Eastern Mediterranean War Area who have been invalided to England. Only a single examination of each man's faeces has been carried out. This we recognise to be quite inadequate to give any accurate idea of the prevalence of *histolytica* infection among the troops generally, but during the first month of the inquiry the percentage of positive results obtained was so high that we decided that for practical purposes a single examination would be sufficient to supply what, in our opinion, is conclusive evidence of the very large percentage of "healthy" *histolytica* carriers present among the troops who have been in Mesopotamia, and consequently of the futility of attempting to "clear" only those cases of amoebic infection which have suffered from so marked an intestinal disturbance as to result in their reporting sick, and so receiving hospital treatment.

Our results appear to us to reveal features of considerable interest on the three following aspects of the carrier problem.

(a) The incidence of *histolytica* infection among troops invalided from active service in Mesopotamia, apart from cases of acute and convalescent dysentery.

(b) The practical value of attempting to declare convalescent patients, who have suffered from amoebic dysentery, "cleared" of their infection, by the inability to detect the presence of *histolytica* cysts in the stools after a given number of protozoological examinations.

(c) A consideration of some of the factors that enter into any efficient method of "clearing" convalescent dysentery patients—that is, if it be assumed that such "clearing" is of sufficient practical value. These same factors will naturally have to be considered in tests of the therapeutic value of drugs for the "curing" of *histolytica* carriers.

Incidence of *Histolytica* Infection.

The prevalence of *histolytica* infections among the troops formed the main object of our investigation, which was begun because it was found that on examining the stools of the patients in several of the surgical wards a considerably higher percentage of these revealed the presence of *histolytica* cysts than did the cases which were being "cleared" in

the dysentery wards. The results of our inquiry are based on the examinations conducted on 946 men, 351 of whom at the time of their stool examination were in the "non-dysentery" wards of the hospital, while 595 were convalescents stationed in a convalescent dépôt. The percentage of patients in hospital, but not at the time suffering from dysentery, who were found to be *histolytica* carriers was 13.6, while of the men in the convalescent camp waiting to return to their dépôts no less than 17.8 per cent. were found to be infected with *Ent. histolytica*.

These figures are all the more startling when it is considered that they are based on the results of a single examination. Dobell,² in his elaborate statistical deductions from the tabulated results of the protozoological examinations in England, comes to the conclusion that the chances against detecting a *histolytica* infection by any one examination of the average infected case are nearer 2:1 than 3:2; that is to say, from a solitary examination the chances are against the examiner finding the infection in the average infected case. In order not to over-estimate our positive findings let us take the lower figure—viz., 3:2—and we find that the probable number of *histolytica* carriers among the "non-dysenteric" hospital patients is 34 per cent., while the percentage among the men in the convalescent dépôt will be 44.5. In other words, at the very least one-third of the men in the ordinary wards of the hospital, and of the men who have been ill but are now considered fit for duty and ready to return to their dépôts, are harbouring the cysts of what is considered the pathogenic type of entamoeba.

Some interesting points for discussion present themselves when a comparison is made between the number of *histolytica* carriers with a previous history of dysentery or diarrhoea and those with no such history. These comparative figures for the 351 hospital patients are set forth in Table I.:

TABLE I.—Classification of *Histolytica* Carriers among 351 Hospital Patients Based on Presence or Absence of a History of Dysentery.

	Number.	Carriers.
Previous history of dysentery or diarrhoea... ..	218	41 = 18.8%
No previous history	133	7 = 5.2%

The disparity between the percentage of carriers in the two classes is very striking. Among those with a previous history of diarrhoea or dysentery there were 18.8 per cent. of carriers, while among those who gave no such history the number was only 5.2 per cent. The obvious conclusion to be drawn from this at first sight would appear to be that the number of potential carriers who have never shown signs of any intestinal disturbance is less than a third of those who have had a bowel affection; that is, that the "healthy" *histolytica* carrier is a relatively negligible quantity compared with the chronic or convalescent carrier. But a similar analysis of the results obtained from the men living under the conditions of life existing in the convalescent dépôt shows that this disparity between the number of carriers in the two classes of cases is not so marked as the above figures tend to show.

In Table II. are given the corresponding figures for the dépôt cases. Here there exists a third class of case to be considered—viz., the men who had recently been treated for dysentery, the majority of whom had only lately obtained their discharge from hospital, after having been "cleared" by three or more consecutive negative protozoological examinations. The investigation of this class of case has revealed many points of interest, and as it might obviously tend to give a wrong idea as to the relative incidence of carriers among those who give a history of more remote clinical manifestations, and those who give no history of dysentery or diarrhoea, we have considered them separately in Table II.

TABLE II.—Classification of *Histolytica* Carriers among 595 Convalescents, Based on Presence or Absence of a History of Dysentery.

	Number.	Carriers.
Class A.—No dysentery or diarrhoea	154	19 = 12.3%
Class B.—Remote history of dysentery or diarrhoea	246	49 = 19.9%
Class C.—Recently discharged from hospital after dysentery	195	38 = 19.4%

The difference in the percentage of positives between Class B and Class C is, after all, almost negligible, while the

difference between the percentages of Class A and B is still quite considerable—viz., between 12.3 per cent. and 19.9 per cent.—although the disparity is not so marked as in the case of the non-dysenteric patients in hospital. The analysis of the figures both for the hospital patients and the men in the convalescent dépôt therefore leads to the conclusion that the number of carriers is greater among those who have shown symptoms of an intestinal infection than among those who have been unaware of their protozoal infection.

Although these figures appear to show that the great majority of carriers have suffered from some form of intestinal disturbance, yet we must remember that in many instances it does not assume a dysenteric character. On a further consideration of the 90 *histolytica* carriers among the hospital patients and convalescents who gave a past history of dysentery or diarrhoea (but not including those carriers who had just been discharged from hospital, after a typical dysenteric attack) it is found that one-third had suffered from simple diarrhoea, another third had had recurrent diarrhoeic attacks, in addition to acute dysentery, while 47 per cent. had had intestinal symptoms of so trivial a nature that they had never been admitted to hospital for treatment.

Value of "Clearing" Patients.

This wide prevalence of *histolytica* infection among Mesopotamian troops—if our deductions from those "single examination" findings be admissible—naturally raises the question of the practical value of "clearing" patients convalescent from amoebic dysentery. Our observations suggest that the declaring of such cases "cleared" of their intestinal infection by the mere inability to detect the presence of *histolytica* cysts in the stools after a given number of protozoological examinations is of very questionable utility, for it is quite reasonable to conclude that as high a percentage of infection is present among the "healthy" troops as we have found among the present series of hospital patients and men who have just convalesced from other ailments than an intestinal one.

It is the consensus of opinion confirmed by Dobell's recent statistical studies that with proper precautions, the majority of the heavy infections or "gross" human carriers are detected by a series of three or five examinations, and owing to the amount of time and labour involved the segregation of any but such "gross" carriers would be an impossible and futile task. But in view of the fact that we have shown that light and intermittent infections are present in at least one-third of the troops, it is a moot point whether the isolation of a few gross carriers is going to be of much avail from the point of view of prophylaxis.

Nor have we any reliable evidence as to the average duration of infectivity of cases of amoebic dysentery or of *histolytica* carriers in general. Our analysis of the protozoological examinations of those 946 men who give no history of dysentery, as well as of those who have suffered from a recent or more remote attack, does not admit of any satisfactory answer to this question. An attempt, however, has been made to ascertain from the protocols the average period that has elapsed since the initial dysenteric attack, as well as the number of subsequent relapses. On considering our records for the 87 *histolytica* carriers among the 443 convalescents who give a previous history of dysentery it has been possible to obtain accurate dates of the initial attack in 77 instances. An analysis of these cases shows that an average period of 8.5 months elapsed from the onset of dysenteric symptoms up to the date of the stool examination. The figures for the two series of carriers—viz., those who had just convalesced from an attack and those who gave a history of dysentery more or less remote—were also considered separately, and it was found that there was practically no difference in the time that had elapsed since the first onset of symptoms, the figures for the two series being 8.7 months and 8.4 months respectively. The close agreement between these two latter figures naturally suggests that the recent dysenteric attack in those men who had just convalesced was of the nature of a relapse.

An attempt had been made to note the frequency of relapses in the history elicited from the men, but the records do not lend themselves to a statistical analysis, because the usual history given was not so much as to the occurrence of definite dysenteric attacks, as of constant "looseness of the bowels," "diarrhoea on and off," and so on. Hence, owing

to the ind-finite nature and scrappy account obtained of those relapses, we have not thought such an analysis worthy of record.

Therapeutic Methods of Treating Carriers.

Further, the present therapeutic measures which are recognised as efficient in the treatment of acute amœbic dysentery have not proved of similar service in the elimination of the entamoeba and its cysts from the intestine.

It is now recognised that hypodermic courses of emetine are not sufficient to rid carriers of their infection. This is borne out by an analysis of some of our figures. A comparison was made between the number of emetine-treated cases among the *histolytica* carriers who gave a history of dysentery but had not recently suffered from the disease and among the emetine-treated dysentery convalescents. Out of the 49 carriers with a more or less remote dysenteric history it was possible to obtain details in 44 cases, and of these only 7 had had emetine at some time or another; while of the 38 patients recently discharged for and "cleared" from dysentery, a record was obtained in 35 cases, and all except 5 had been on emetine treatment of varying amounts. As these two sets of carriers formed 19.9 per cent. and 19.4 per cent. of the total number of men examined in their respective classes, it does not appear as if emetine had had much effect in diminishing the persistence of those cystic infections. Of course, it must be admitted that in many instances emetine appears to have been administered in a somewhat casual fashion, frequently in a 4-6 grain course at a time, and very few of the cases appear to have had a continuous course of 12 grains given 1 grain daily for 12 consecutive days, nor is there any record of oral administration of ipecacuanha or emetine. Still, a certain number of those men who have had 10 to 12 grains of emetine hypodermically, 20 grains in some instances, occurs in this batch of carriers, so that our deductions are in agreement with the consensus of opinion that the hypodermic administration of emetine alone, although immediately alleviating the dysenteric symptoms, tends only in a small degree to reduce the persistence of *histolytica* infections.

Treatment with emetine bismuth iodide is still on its trial, as is also the combined oral and hypodermic administration of emetine, for, in our opinion, in recently published records of the effects of various modes of treatment certain important factors have been altogether ignored, which, according to our observations, must be considered in any test of the therapeutic value of drugs for the "curing" of *histolytica* carriers.

Number of Protozoological Examinations.

Our methods of declaring cases "cleared" of their *histolytica* infection are distinctly tentative. With regard to the number of stool examinations requisite for efficient "clearing," Dobell concludes that three consecutive negative examinations are quite inadequate and has worked out from the statistical point of view that only a negligible percentage of latent infections will be missed if six examinations are carried out, the first being made three or four days after the end of the treatment, the second about the tenth day, and the four remaining examinations being made a week later when the infection, if still present, will probably have returned to normal. But Dobell himself has shown that of 100 cases, say, infected with *Entamoeba histolytica*, which are discovered after five examinations of the faeces, 93.3 of these will be detected at the end of three examinations. It becomes a question for discussion from the point of view of prophylaxis whether the additional 6 per cent. of positives found by an additional two examinations per case is worth the time and labour involved.

Although the statement that not more than one-half to two-thirds of all infected cases will have been discovered in a series which has been examined three times per case is in all probability mathematically correct, yet from a practical standpoint it gives an erroneous impression of the position. In this series of 100 infections, cited by Dobell, the first examination reveals 55.5 per cent., the second 23.7 per cent., and the third 11.1 per cent., while the fourth and fifth examinations give only 3.7 per cent. and 3 per cent. respectively. Now assuming that 3 per cent. additional infections are detected at each successive examination—and this is much higher than practical experience or statistical deduction would suggest—then if only half the infections are discovered by three examinations, 17

additional examinations will be required in order to detect all the carriers. Hence from Dobell's own figures and on considering how universal *histolytica* infections appear to be, at least in the Mesopotamian Field Force, three examinations seem sufficient for the detection of all cases of heavy infection.

It must be mentioned in reference to the question of the usefulness of protozoological examinations for the "clearing" of *histolytica* infections that there are marked divergences of opinion among authorities as to the morphological differentiation of the species of entamoebæ from one another. The non-pathogenicity of the eight-nucleate cyst—known as that of *Entamoeba coli*—has not yet been definitely established, nor has its relation to *Entamoeba histolytica* been conclusively defined. Knowles and Cole,³ in a recent paper on an enumerative study of the cysts of the intestinal amœbæ, consider that the eight-nucleate cyst of *Entamoeba coli* is the adult and non-pathogenic stage of an entamoeba which, under certain circumstances, becomes pathogenic, and takes on certain morphological forms, both in the free state as the *Entamoeba histolytica* and in the cystic stage as the mononucleate and tetranucleate cysts. Moreover, Gauduchau⁴ concludes that all entamoebæ should be treated in the same way from both a therapeutic and prophylactic standpoint, for he considers that there is only a difference of degree between all entamoebæ, the differences being dependent more on environmental conditions than on intrinsic differences in the amœbæ themselves. Thus there appears to be no consensus of opinion as to what morphological features can be accepted as absolute criteria for the differentiation of pathogenic and non-pathogenic types of entamoebæ.

Factors in Formulation of "Clearing" Scheme.

All the evidence points to the futility of attempting to declare cases of amœbic dysentery free from *histolytica* infection. If, however, it be assumed that such "clearing" is of practical value, certain factors which have come to our notice appear to be of some importance in the formulating of any scheme of dysentery "clearing." In published records dealing with the duration of infectivity, as well as with the therapeutic value of emetine or other drug, one finds that after the patients have undergone a course of treatment with the drug the stools are examined for *histolytica* cysts. After three or more consecutive negative results these examinations are discontinued; the patients are discharged from hospital, and no evidence is cited as to the after-history of the intestinal affection under less favourable conditions than those of hospital life. The patients are thus declared "cleared" under conditions in which the possibilities of intestinal irritation and catarrh are reduced to a minimum. Such intestinal irritation may result from a coarser or less suitable diet, from excessive physical exertion and undue fatigue, and from the lack of hospital discipline in regard to both food and drink.

The nearest approach to such a condition in patients in the non-dysentery wards of the hospital has been attained by administering a mild saline purge. The number of positive results as regards cystic findings in certain controlled batches of specimens has in this way been so considerably increased, as compared with the results obtained even on careful and thorough examination of formed stools, that we now recommend the routine administration of a saline in all cases previous to the protozoological examination of stools of convalescent dysentery patients. Of course, this increase of positive findings may be due to the softer condition of the faeces leading to a more equal distribution of the cysts, as contrasted with the condition in a hard constipated stool, although it is quite as probable that the flushing of the mucous membrane of the intestinal wall and the consequent flooding out of the cysts is an equally important factor.

Effect of Diet and Conditions of Life.

In the case of the men living in the convalescent dépôt conditions somewhat akin to those above described are present and are a faint replica of the conditions on active service, or at least of those experienced at their dépôt. The food is of a much coarser quality than that obtained in hospital; considerable physical exertion is frequently undergone through fatigues, duties, &c., while the privilege of free access to the canteen and coffee-shop is in sharp contrast to the discipline of the hospital régime.

The effect of those factors in leading to a return of intestinal irritation, mild though it be, and to a consequent reappearance of cysts in the stools has been demonstrated

in various ways. A higher percentage of carriers has been found among the men in the convalescent dépôt (17·8 per cent.) than among the non-dysentery patients in hospital (13·6 per cent.). That this difference is not due to the inclusion among the former of a certain proportion of cases who had just convalesced from a recent attack of dysentery is shown by the fact that there is practically the same incidence of persistent infection among the class of case which gave a more or less remote history of dysentery or diarrhoea (19·9 per cent.) as among those recent dysentery convalescents (19·4 per cent.). (See Table II.) The larger percentage of carriers in the convalescent dépôt is found to come from the series of cases which gave no history of any intestinal ailment, 12·3 per cent. being found among them, as compared with 5·2 per cent. in the similar class of case among the hospital patients. In all probability it is those irritative factors which are absent in the hospital régime which have tended to produce this increased number of carriers.

Further, it has been noticed that a distinctly higher percentage of *histolytica* carriers is obtained if the single-stool examination of any case is postponed until the man has been living for at least one week under the conditions of life that prevail in the convalescent dépôt. An attempt has been made to confirm this statistically. Out of the 374 examinations of all classes of cases whose date of discharge to the convalescent dépôt has been obtainable, we find that 15·2 per cent. of carriers have been found among those examined within one week of discharge, while 18·8 per cent. have been detected among those whose stool examination was postponed until they had been resident for over one week at least in the convalescent dépôt. It must be admitted that the percentage difference is not very great, while we find that the larger the number of examinations made the nearer do the two percentages approach each other. This we ascribe to the presence of other factors which are both incidental and indefinite. For example, we have occasionally been struck by the number of positives obtained in certain batches of specimens, and on inquiry we have found that those results have been obtained from stools associated with more or less diarrhoea and accompanied by considerable abdominal pain, following on the consumption of some special article of diet obtained in the dining hall.

A Series of Cases Examined after Discharge to Convalescent Dépôt.

The best illustration, however, of the effects of those conditions conducive to the production of intestinal catarrh is to be found on a perusal of the records of a series of cases of dysentery in which it has been possible to obtain complete reports of the clinical condition, laboratory findings, and drugs administered previous to admission to this hospital, as well as during their period of residence in the hospital itself; while on the discharge of these men to the convalescent dépôt arrangements were made for the examination of their faeces every second day after they had been living for at least one week under the new conditions. These tabulated records we hope to publish in full. The number of cases thus recorded is small, as it was only towards the close of the present inquiry that we became impressed with the number of persistent infections discovered among men discharged to the convalescent dépôt after they had been considered "cleared" on the results of considerably more stool examinations than had previously been thought necessary.

Of the 20 cases which have been followed up, 15 showed the presence of *Ent. histolytica* previous to admission to this hospital, while this amœba or its cyst was found in the others during their residence in hospital. Practically all were convalescent when they first came under observation. Their average period of residence was 9 weeks, the shortest being 4, the longest being 16 weeks. All had received hypodermic courses of emetine of 6 or 12 grains, while four had, in addition, been treated with emetine bismuth iodide, although only one case received as much as 30 grains owing to a further supply of the drug not being at the time obtainable. However, the testing of the therapeutic value of any drug or mode of treatment was not at this time our object. All we were trying to investigate was the relative effect of two different types of diet and conditions of life on the cystic findings in the stools.

The average number of negative protozoological examinations after emetine treatment and before the patient

was discharged from hospital was five. At the time of discharge their general condition of health appeared satisfactory. A consideration of the results of the examinations carried out while the men were in the convalescent dépôt show that 10 were found to have relapsed on the very first examination, while other 5 showed the presence of *Ent. histolytica* cysts on either the second or the third examination. In only 3 out of those 20 cases was there no evidence of any *histolytica* infection after seven consecutive stool examinations. No more, we think, need be said with regard to the futility of any scheme for the "clearing" of dysentery patients where the protozoological examinations of the stools are entirely confined to the period during which the patients are resident in hospital.

We are at present engaged on further investigations into the value of certain drugs and modes of treatment which have been recommended for the curing of *histolytica* carriers. We are subjecting these cases to the "clearing" tests while they are living under the conditions of life in the convalescent dépôt, for we are convinced of the very questionable utility of estimating the therapeutic value of any mode of treatment while the patients are living under a purely hospital régime.

Conclusions.

1. The general protozoal findings in the stools of men invalided from active service in Mesopotamia, apart from cases of acute and convalescent dysentery, are more numerous than the records of Wenyon and of Dobell show to exist among the troops from the Eastern Mediterranean War Area who have been invalided to England.

2. The incidence of *histolytica* infection among the Mesopotamian troops is especially high; 13·6 per cent. of 351 "non-dysentery" patients in a general hospital and 17·8 per cent. of 595 men stationed in a convalescent dépôt were found on a single stool examination to be harbouring the cysts of *Entamœba histolytica*. On applying Dobell's appropriate "figure for correction" to those results obtained on the "single examination" method, we find that at least 33 per cent. of the troops who have been in Mesopotamia are "healthy" or "unhealthy" *histolytica* carriers.

3. The figures for both hospital patients and men of the convalescent dépôt lead to the conclusion that the number of carriers is greater among those who have shown symptoms of intestinal disturbance than among those who have been unaware of their protozoal infection.

4. Of 90 *histolytica* carriers giving a past history of dysentery or diarrhoea, one-third had suffered from simple diarrhoea only, another third had had recurrent diarrhoeic attacks in addition to acute dysentery, while 47 per cent. had had intestinal symptoms of so trivial a nature that they had not been admitted to hospital for treatment.

5. The segregation of any but the "gross" human carrier is an impossible and futile task; while, in view of the fact that light and intermittent infections appear to be present in at least one-third of the troops, it is a moot point whether the isolation of a few gross carriers is going to be of much avail from the point of view of prophylaxis.

6. A comparison of the small number of emetine-treated cases among the *histolytica* carriers who gave a history of dysentery but had not recently suffered from the disease, with the number of the recent dysentery convalescents practically all of whom had received courses of emetine treatment, goes to show that this drug, although alleviating the dysenteric symptoms, tends only in a small degree to lessen *histolytica* cystic infections.

7. This wide prevalence of infection due to *Entamœba histolytica*, coupled with the fact of the proved ineffectiveness of our present therapeutic methods for the destruction of cysts, throws considerable doubt on the utility of attempting to "clear" by a series of protozoological examinations of the stools, only those cases of amœbic infection which have suffered from so marked an intestinal disturbance as to have resulted in their receiving hospital treatment.

8. Various data have been cited showing that factors leading to the production of intestinal irritation, mild though it be, are also associated with the reappearance of *histolytica* cysts in the stools. Such factors are present under the conditions that prevail in the convalescent dépôt, and doubtless exist to a much greater extent during active service in the field, in sharp contrast to the conditions of a hospital régime.

These observations point to a possible fallacy which will have to be considered in any investigation into the duration of the infectivity of *histolytica* carriers, as well as on the therapeutic efficacy of any drug for the "curing" of those cystic carriers, if the protozoological examinations be confined solely to the period during which the patients are living under hospital conditions.

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THE WASSERMANN REACTION:

A RAPID METHOD OF PERFORMING IT WITH SMALL QUANTITIES OF SERUM.

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UNDER the recently introduced scheme of the Local Government Board there has probably been a great increase in the number of those who have to perform or of those who propose to superintend or carry out the Wassermann reaction. It would appear, then, to be opportune to describe a rapid method of carrying out this test with the use of small quantities of serum, such as can readily be obtained by any practitioner from a finger or the ear without undertaking the minor operation of venipuncture. The method here described is one I have taught and made use of for several years with very satisfactory results. In the present communication, therefore, I merely give a detailed description of the technique of the method I have employed and recommend, without entering into any consideration of the theory or rationale of the reaction or attempting to appraise its value.

Apparatus Required.

This consists of:—

1. A Wright's pipette, preferably throttled, graduated in the following manner: A unit-volume is marked with a grease pencil $1/2$ to $3/4$ inch from the point, and above this marks are made indicating 4, 5, 10, and 100 unit-volumes respectively. Alternatively, a pipette, the unit-volume of which is a definite measured quantity—e.g., $1/20$ c.c.—or a dropping pipette.
2. A number of quill tubes about 2 inches in length and about $1/8$ inch in diameter. These can readily be made from glass tubing.
3. A suitable water-bath which can automatically be kept at a temperature of 38° to 40° C., provided with racks to hold the above tubes. The opsonic incubator as modified by Emery is the one actually used.
4. A number of small test-tubes and watch-glasses in which to place and dilute the various reagents.
5. Glass ampoules of 1 c.c. to 2 c.c. capacity to hold the various sera in which they are inactivated.

Reagents Required.

The reagents required are as follows:—

1. *Antigen*.—Originally this consisted of a watery or alcoholic extract of a syphilitic foetal liver, but it has been found that several simpler substances, more readily available, can be used in the preparation of this reagent.

In common with the majority of serologists I employ an extract of normal heart muscle—human, sheep's, or guinea-pig's—fortified with cholesterin and known as "Sachs' antigen."

This is prepared by grinding heart muscle in a mortar with clean fine sand and extracting with absolute alcohol in the proportion of 10 c.c. of absolute alcohol to 1 gm. of heart muscle for three or four days. To 4 parts of this extract add 5 parts of a 1 per cent. alcoholic solution of cholesterin.

2. *Complement*.—This is contained in normal guinea-pig's blood serum and is obtained by bleeding the animal from the ear, by tapping the ventricle through the chest wall with a hypodermic syringe, or by cutting the animal's throat, according to the amount of complement required. As the complement is an extremely unstable substance it should not be obtained until all the other reagents are in readiness for the test, and it should be kept ice-packed throughout the various manipulations in performing the reaction.

3. *Washed sheep's corpuscles*.—The blood is received from the sheep in a sterile glass jar and at once defibrinated by shaking with glass beads. The corpuscles are then poured into a centrifuge tube, thoroughly washed three times in physiological saline solution, the corpuscles being thrown down by centrifuging. For use a 20 per cent. by volume suspension of the corpuscles in saline solution is employed. It is preferable to use the suspension fresh, but it will keep good for about 48 hours if kept in the ice-chest.

4. *Hæmolytic serum*.—This is the blood serum of a rabbit which has received at suitable intervals several intravenous injections of fresh washed sheep's corpuscles. The serum is "inactivated" by heating in a water-bath to 56° C. for half an hour before use. By this means the complement contained in the serum is destroyed. This reagent may conveniently be purchased, reliable hæmolytic sera being placed on the market.

5. Normal "inactivated" human serum, diluted 1 in 2.

6. A known syphilitic "inactivated" human serum, diluted 1 in 2.

7. *The patient's serum or cerebro-spinal fluid*.—This serum is "inactivated" and diluted with an equal bulk of normal saline solution so as to make a dilution of 1 in 2. In inactivating the serum care must be taken not to exceed 56° C. or the serum is liable to become coagulated, and so rendered useless. If the cerebro-spinal fluid is to be tested it should be free from blood or cellular elements (which may be removed by centrifuging if necessary) and it must not be heated.

8. Sterilised 0.85 per cent. saline solution.

Standardisation of the Reagents.

Before proceeding to carry out the test it is necessary to standardise the reagents employed—namely, the hæmolytic serum, the complement, and the antigen. It is best to standardise them in this order.

To standardise the hæmolytic serum make dilutions with 0.85 per cent. saline solution of 1 in 300, 1 in 600, 1 in 900, 1 in 1200, 1 in 1500, 1 in 1800, 1 in 2100, and so on. By means of the calibrated pipette put four unit-measurements of each dilution into a series of small tubes, and to each add one unit-measurement of undiluted complement (so that this may be present in excess), and one unit-measurement of 20 per cent. washed sheep's corpuscles, and make up to a total of 11 unit-measurements by adding five unit-measurements of normal saline. The tubes should then be incubated in the water-bath from 10 to 15 minutes. Then note the end-point of hæmolysis. Thus, if the 1 in 1500 dilution shows complete, and the 1 in 1800 absent or incomplete, hæmolysis, then 1 in 1500 is the end-point. For use three times the strength of this end-point is employed—thus, if 1 in 1500 is the end-point a dilution of 1 in 500 would be employed in the test.

To standardise the complement, dilutions of fresh unheated guinea-pig's serum are made with 0.85 per cent. saline of 1 in 2, 1 in 4, 1 in 6, 1 in 8, 1 in 10, 1 in 12, and so on. Place four unit-measurements of the standardised hæmolytic serum, suitably diluted, together with one unit-measurement of 20 per cent. washed sheep's corpuscles, and five unit-measurements of 0.85 per cent. saline solution into each of several small tubes. One unit-measurement of the various dilutions of complement is then added to the series of tubes. Incubate at 38° to 40° C. for 10 to 15 minutes, and then observe the lowest dilution of complement in which complete hæmolysis occurs. For use in the test double the strength of this hæmolytic end-point is employed. Thus if the 1 in 6 is the lowest dilution of complement to give complete hæmolysis a dilution of 1 in 3 is employed.

To standardise the antigen, make dilutions with 0.85 per cent. saline solution of 1 in 5, 1 in 10, 1 in 15, 1 in 20, and so on. On dilution the clear solution will become slightly milky and opaque. Four unit-measurements of each dilution, plus four unit-measurements of standardised hæmolytic serum, suitably diluted, plus one unit volume of 20 per cent. washed sheep's corpuscles, plus one unit-measurement of complement, standardised and suitably diluted, plus one unit-measurement of saline solution, are placed in a series of tubes and incubated in the water-bath from 10 to 15 minutes. The highest dilution in which hæmolysis is complete—i.e., in which there is no fixation of complement—is taken as the end-point and half this strength is used in the test. Thus if the end-point is 1 in 10 use a dilution of 1 in 20 for the test. Now test this dilution of antigen against a known negative (i.e., non-syphilitic) serum and against a known positive (i.e., syphilitic) serum to make certain that it does not fix with the former and that it does fix with the latter serum.

The hæmolytic serum and antigen only need restandardising from time to time, and this does not need doing every time the test is performed, thus saving much time. The complement, on the other hand, being expressly unstable as well as very variable in quantity, needs to be freshly standardised on each occasion on which the reaction is carried out.