STUDIES ON INTESTINAL AMOEBIASIS

WITH SPECIAL REFERENCE TO THE PATHOGENESIS AND CHRONICITY OF THE INFESTION.

THESIS SUBMITTED TO THE UNIVERSITY OF GLASGOW FOR THE DEGREE OF DOCTOR OF MEDICINE.

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with special references to the pathogenesis and chronicity of the infection

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STUDIES ON INTESTINAL AMOEBIASIS

INTRODUCTION:

In 1945-46 the writer, serving in the Royal Navy as pathologist to the Combined Services Hospital at Trincomalee, in Ceylon, found that at least half of the pathological work and a large proportion of the clinical work of the hospital were, of necessity, concerned with the diagnosis and treatment of amoebiasis. Though rarely fatal, and seldom conspicuous in its immediate effects, amoebiasis was pre-eminent, among the many infections encountered in that area, as a cause of loss of working-hours and chronic ill-health in Service personnel.

The present thesis comprises a series of studies begun in Ceylon in 1945 and developed during the tenure of a research post at Liverpool School of Tropical Medicine in 1947-48. Some of the results have already been published in various journals, or demonstrated at meetings of the Royal Society of Tropical Medicine and Hygiene. Reference is made in the text to these publications.

Analysis of the literature on amoebiasis, from 1875 when Lösch first recognised the causal organism, to the present day, reveals that our knowledge of the disease has been derived from three main sources of investigation: protozoological studies *in vitro*; observations on human patients; and experimental infections in animals. As the result of this constant three-fold approach to the problem, we have today a reasonably complete knowledge of the parasite and the infection, together with rational techniques for the prevention and treatment of amoebiasis. Fully applied, existing knowledge is sufficient to deal effectively with most of the problems arising from the disease. Nevertheless, a few gaps still exist, and one such gap appears to the writer to be of major practical as well as theoretical importance.

Amoebiasis in man is, notoriously, a chronic disease, often characterised by dysenteric relapses and prolonged ill-health. The causes of this chronicity include such obvious factors as the persistence of resistant forms of *E. histolytica* in the bowel, the

nutritional state of the host, inadequate treatment, and re-infection. In the tropics, these factors are of considerable importance but, in the United Kingdom, they can be reduced to the minimum; yet amoebiasis remains a chronic infection. It is clear that further factors should be defined in explanation of the chronicity.

Two factors relevant to this problem emerged from the preliminary studies in Ceylon: firstly, the fact that amoebic dysentery, as well as bacillary dysentery, often subsided to some extent when sulphonamides were given (as a routine in field stations) to Service personnel reporting sick with diarrhoea; and secondly, the fact that the exudate, in cases of mild or early amoebic dysentery which usually responded easily to treatment, was almost devoid of leucocytes or macrophages; whereas, in severe or late cases which relapsed readily, the exudate was invariably purulent. These two facts suggested that added bacterial infection, associated with the purulent exudate, might account for dysenteric relapses, and might be a major factor in the chronicity of the disease; they also support the work of Hargreaves (1946), who showed that penicillin and sulphonamides were beneficial adjuvants to specific anti-amoebic treatment in relapsing cases.

It is obvious that the problem of the chronicity of amoebiasis is closely linked with that of therapy. Emetine and its derivatives, and the iso- and oxy-quinolenes, are all of proved value in the treatment of the disease, but that they are of limited value is shown by the residue of cases for whom these drugs are of limited avail (Adams 1945, Hargreaves 1946, Stewart, O'Meara and Kershaw 1948) and by the occurrence of relapses even among patients receiving expert treatment.

The studies presented in this thesis have therefore been focussed on the factors involved in the aggravation and chronicity of intestinal amoebiasis, with special reference to the role of bacteria. Following the classical trend mentioned above, the author has endeavoured to employ a three-way approach, and the thesis is arranged in three main sections of experimental infections, clinical

observations and protozoological studies, embodying the research done at Liverpool School of Tropical Medicine; the contributory studies, made in Ceylon and elsewhere, are included in the Appendix. Technical methods have been separated as far as possible from the text of the thesis into a fourth section. The various results are discussed and summarised in the appropriate sections, and a general summary of the conclusions afforded by the work as a whole is made at the end.

SECTION I

EXPERIMENTAL AMOEBIASIS.

1. General review.

2. The pathogenesis of an experimental amoebic infection in the rat.

3. The infectivity of E.histolytica outside the bowel.

Experimental Amoebiasis.

1. General review.

The pathology of the early stages of intestinal amoebiasis has been elucidated largely from experimental infections in animals. Human amoebiasis is invariably a disease of slow and uncertain onset, and early lesions are rarely seen; even when they are seen, as in post-mortem examination of accident cases, the course of the infection is unknown. A study of the natural history of the disease requires a suitable animal infection in which the successive stages, and some of the factors governing them, can be inspected and altered at will.

The historical background of experimental infections dates from Lösch (1875) who showed that dogs could be infected by E.histolytica. Cats were successfully infected by Kartulis in 1891, and kittens by Kruse and Pasquale in 1894. Those earlier studies were mainly directed towards fulfilling Koch's postulates and establishing a specific amoeba as a cause of dysentery; Kartulis, however, commented upon the probability of bacteria participating in the infection. Then the classical experiments of Walker and Sellards (1913) showed that man was also susceptible to experimental infection and the primary aetiological problem in "amoebic" dysentery was solved. Thereafter, experimental infections were used to study the pathogenesis and therapy of amoebiasis. Most workers used kittens, since a high percentage of successful infections could be induced in these susceptible animals. The main difficulties were that they could not easily be handled in large groups and that the infection was often excessively acute and rapidly fatal. Rats were infected by Lynch (1915), and monkeys by Dobell (1931) and Hegner et al. (1932). Baetjer and Sellards (1914) and Chatton (1917) claimed to have succeeded in infecting guinea-pigs but most authorities agree that this animal and the rabbit are refractory to the infection (Manson-Bahr 1943).

In the induction of these infections, various methods were employed. The natural method of feeding cysts gave variable results in the hands of most workers and greater success attended the rectal inoculation of cysts or cultures of E.histolytica, or of dysenteric faeces, or the intracaecal inoculation of a culture (Frye and Meleney, 1933). The use of these different methods threw light on certain aspects of the pathogenesis of the infection. Thus Sellards

and Leiva (1923) showed that the initial lesions occurred in the lower part of the colon, irrespectively of whether the infected material was introduced into the caecum or rectum; when the colon was occluded by a ligature, however, lesions developed at the site of stasis, immediately above the ligature. Faust (1932) found that the caecum showed the first lesions when dogs were experimentally infected by feeding them with cysts. Wagner (1935) and Bieling (1935), using cats and dogs, showed that after oral or rectal administration of infected material, the earliest lesions developed at two sites, the caecum or rectum. It became clear that, in the majority of animals, as in man, amoebic lesions occurred typically in the large intestine and were maximal at sites of stasis. This is the only common feature of experimental infections, however: among different animal species, there is wide variation in the course of the infection and, as Manson-Bahr (1943) has indicated, the interpretation of experimental findings is limited by this fact. Thus kittens develop an acute and usually fatal dysenteric infection while dogs acquire a relatively mild, chronic infection with the passage of cysts in the faeces, (Faust 1932). A similar chronic disease, not unlike human amoebiasis, has been described in the monkey but an exact study of the evolution of the lesions is difficult since natural infections with E.histolytica are common in this animal (Dobell 1931, Hegner et al. 1932, Craig 1934).

2. The pathogenesis of an experimental amoebic infection in the rat.

The experimental observations described below suggest that, in the artificially-infected young rat, the course of the disease is intermediate between the fulminating dysentery of the kitten and the protracted, mild disease caused by E.histolytica in the dog. In young rats, the disease has an acute, dysenteric onset, easily recognisable, but its course is limited by various degrees of spontaneous remission and fatalities are unusual. In a few instances, chronic lesions are established and occasionally cysts can be detected in the faeces after 14-28 days.

In the past E.histolytica infections in rats have been studied by several workers with somewhat conflicting results. Natural infections were noted by Lynch (1915) and Atchley (1936) in wild rats but not in laboratory rats. Lynch, Kessel (1923), Chiang (1925), Tsuchiya (1939) and Böe (1939) succeeded in infecting laboratory rats but failed to prove that tissue invasion had occurred, though Lynch and Kessel found ulceration in a few animals.

The present studies were based upon an experimental technique described by Jones (1946) in which invasion of the caecal wall by E.histolytica could clearly be demonstrated in histological sections. In a proportion of animals, this tissue invasion was sufficiently massive to produce gross ulceration, visible to the naked eye. Infection was secured in 67-90 per cent. of animals and there was no evidence that even a fraction of this percentage coincided with a natural infection. Not infrequently, a species of protozoa corresponding to Endolimax muris was present; this organism may possibly be enzootic in some varieties of rats (Chiang 1925), but there was no evidence that it invaded the tissues. A characteristic species of Trichomonas was regularly present and was occasionally associated with mild, superficial inflammation and frothy caecal contents.

With this technique, rats can be infected in numbers sufficient for statistical analysis of some of the factors governing the induction and course of the experimental disease. There is reason to believe that the factors investigated have some bearing on the problems of amoebiasis in man.

Design of the experiments.

The experimental methods described below were designed to study the following aspects of the pathogenesis of amoebiasis.

(a) The relationship between the infectivity and virulence of E.histolytica.

(b) Histological studies of the development, course and healing of the lesions.

(c) The influence of indigenous and added bacteria.

(d) Chemotherapy.

Methods.

1. Cultivation of E.histolytica.

Cultures and inocula of E.histolytica were prepared as described in Section IV (Technical Methods). Rats were inoculated intra-caecally with 0.2 c. cm. of a suspension of E.histolytica, containing about 300,000 trophozoites. This inoculum always included one or more strains of bacteria present in the original amoebic culture from which the suspension was prepared (see page 47).

2. Operative technique.

Four-weeks-old rats (20-35 gm.) were arranged in control and test groups. At least seven rats, evenly matched in weight were assigned to each group. Under ether anaesthesia, the caecum was mobilised through a small laparotomy wound. The inoculum, kept at 37°C, was injected into the caecum and the abdomen closed.

"Blank" operations showed that the operative technique was attended by a negligible mortality (less than 1%) and that it did not affect the animals adversely.

3. Technique used in the Study of added bacteria.

Two sets of experiments were devised to test the effect upon the amoebic infection of certain added bacteria.

(a) 24-hour broth cultures of bacteria were added to suspensions of E.histolytica and inoculated into rats intracaecally. The inocula were adjusted by dilution so that control and test rats received like numbers of E.histolytica and the same volume of inoculum. The bacterial component of the final inoculum amounted to about 10 million cells, equal to approximately 0.1 c.cm. of nutrient-broth culture of Bact.coli.

(b) In other experiments, rats were fed orally by catheter with broth cultures of bacteria, before or after the establishment of the amoebic infection. The individual dose was 1 c.cm. of 24-hour nutrient-broth culture, representing approximately 1000 million coliform bacteria.

As controls, separate groups of animals were given like numbers of heat-killed or living bacteria, with or without the amoebic inoculum respectively.

The intestinal flora of control and infected rats were studied by plating caecal contents taken post-mortem, as described in Section IV.

4. Chemotherapy.

In certain experiments, rats were dosed with the following substances during the 48 hours preceding the inoculation of E.histolytica, or during the first six days thereafter:

Emetine hydrochloride	0.62-10.0 mgm./Kilo/day.
Phthalyl sulphathiazole	1000 mgm./Kilo/day.
Sulphaguanidine	200 " " "
Sulphamezathine	200 " " "
Streptomycin	30 " " "
Penicillin	100-1000 Oxford units/20 gm. rat/day.

5. Assessment of results.

After periods ranging from 1-14 days, according to the nature of the experiment, the rats were killed by chloroform and examined. The caecum was removed, cleared of faeces and inspected. Smears and sections were taken for microscopy. When bacteriological examinations were being conducted, post mortems were performed under aseptic conditions, with instruments sterilised afresh between each examination.

Two quantitative criteria of infection were employed:

(a) The number of animals showing the presence of E.histolytica in smears made from the caecum was noted.

(b) The average degree of infection (A.D.I.) of each group was calculated as the arithmetic mean of the symbols (shown in Table 1) for each animal in the group.

The significance of the differences in A.D.I. between control and test groups was assessed by Fisher's t test (Fisher and Yates, 1943), using the following formula, in which x and y are the A.D.I's of two groups, containing numbers of rats m and n respectively:

$$t = \frac{x - y}{\sqrt{\frac{\sigma^2 x}{m} + \frac{\sigma^2 y}{n}}}$$

σx and σy are the standard deviations of x and y.

This assessment was found to be of value in assessing the significance of a reduction in A.D.I. (i.e. a therapeutic effect). It gave no index of the aggravation of infection produced by certain bacteria, where the change in the appearance of the caecum was essentially qualitative (see page 12).

RESULTS

Infectivity and virulence of E.histolytica.

The term infectivity denotes the ability of the parasite to infect a susceptible host and may be measured by the percentage of animals successfully infected in each group; the virulence is indicated by the severity of the lesions produced and may be measured by the A.D.I. When the percentage of animals infected in each group was plotted against the group A.D.I., a linear graph was obtained (figure 1). This suggests that the infectivity of a strain is directly related to its virulence for the tissues of the host (Stewart and Jones, 1948). The infectivity was independent of the number of amoebae in the inoculum (figure 2).

Passage experiments, conducted by infecting fresh groups of rats with trophozoites cultured from heavily infected rats, failed to endow the amoeba with increased infectivity. Since bacteria from the original lesions were necessarily transferred along with the amoeba, any alteration in virulence could not be assessed. Marked enhancement of the virulence of E.histolytica was obtained by serial passage of the infecting strain through media containing liver infusion.

Tissue invasion.

The mucosa of the caecum showed evidence of invasion of E.histolytica within 24 hrs. after the intracaecal inoculation. This process began with minute erosions of the columnar epithelium in any part of the villus, a process which Craig (1927) and Westphal (1938) have attributed to cytolytic or proteolytic ferments secreted by the amoeba. Thereafter, two types of early lesion were observed:

(a) invasion of the villi and crypts by amoebae, with some necrosis of the adjacent mucosa; at a later stage, the mucosa at the edge of this nidus became heaped-up and formed a follicular ulcer, full of mucus and amoebae. Beyond the ulcer, the mucosa remained healthy. Lesions of this type conformed to the picture of aseptic necrosis described by Westphal and appeared to develop slowly (photograph 1).

(b) a relatively widespread cellular infiltration by polymorphonuclear leucocytes of the tissue around the original breach in the mucosa. The bowel wall showed congestion and focal lymphoid hyperplasia. Amoebae were often scanty. Lesions of this type were especially prevalent when virulent bacteria were added to the inoculum, and probably represented a rapid invasion of tissue originally opened by the amoeba (photograph 3).

Between these two types of lesions various intermediate changes were observed, and the subsequent changes showed features of both. Mucus secretion occurred around the lesion (photograph 4) and the surrounding crypts were often filled with mucus containing numerous amoebae. In some animals, the infection remained superficial but in others the tips of the villi were destroyed and amoebae gained entrance to the submucosa where they could be seen in tissue spaces, and very occasionally, in small blood-vessels. Extension of the invasive process led to wider undermining and desquamation of the mucosa, accompanied by interstitial oedema of all the layers of the bowel wall and round-cell infiltration. In the later stages (3-7 days) the bowel was grossly ulcerated and thickened, with a copious

exudation of mucus into the lumen and local peritonitis which often caused adhesion to the adjacent ileum or to the parietal peritoneum (photograph 5). Amoebae were found chiefly at the bases of the ulcers or in the loose exudate, sometimes forming a solid mass; isolated amoebae were seen in the sub-mucosa and, rarely, outside the muscularis mucosae.

Healing followed the usual mechanism of repair after acute inflammation: the ulcer base became filled with a mound of cellular granulations while epithelial regeneration began at the edges and covered the deficiency. There was comparatively little fibrosis or residual thickening (photograph 6). This process could begin at any time after the initial amoebic invasion but usually it seemed to assert itself after 7 days. In some cases, the healing process was retarded and the infection persisted for a few weeks; it is possible that this was partly attributable to a heavy secondary (bacterial) infection, since tissue invasion was often aggravated by the addition of virulent bacteria, as described below.

When tissue invasion occurred, the animals lost weight and occasionally death occurred after 3-4 days from severe amoebic infection. Most fatalities, however, occurred during the first 24 hours and were associated with a coliform bacteraemia. The operative technique probably permitted some leakage of the inoculum from the caecum and, in the first 24 hours, coliform bacteria could often be cultured from the peritoneal cavity. During this period, a transient bacteraemia was common and a small proportion of the animals may have succumbed to this. When virulent bacteria were added to the inoculum, the death rate increased and the appropriate organism could be recovered from the heart blood and spleen. (See below).

No amoebae were observed in liver sections taken at various stages of the infection, but minor abnormalities were observed in the liver in a few animals; these changes consisted of congestion, peri-portal infiltration by round cells and focal necrosis. Since similar histological appearances were obtained when bacteria were injected intraperitoneally, it is probable that the liver changes in the experimental infection in the rat were attributable to bacteraemia.

Evaluation of the role played by bacteria.

Cultures of E.histolytica trophozoites could not be grown free from bacteria and for this reason the inoculum always contained one or more species of bacteria - usually Bact. coli, Clostridia and enterocci, derived from the host yielding the particular strain of amoeba. These, together with similar bacteria already present in the rat's intestine, may well have played a role in every infection and, in any event, they were inseparable from the experimental conditions.

Rats successfully infected in the usual way showed very few abnormalities in the bacterial flora of the caecum. The composition of this flora in the normal rat is shown in table 2, together with a summary of isolations made from infected rats. The only significant change was the predominance of non-lactose fermenters in a proportion (16 per cent.) of the infected animals. Organisms of this type were present in about 21 per cent. of normal rats. When such animals became infected with E.histolytica, it is possible that pathogenic non-lactose fermenters acted as secondary invaders in the amoebic lesions and thus became more prevalent in cultures made from the infected bowel.

Coliform Organisms:

Variable results were obtained when bacteria of this group were added to the inoculum or administered by mouth to infected rats. Certain strains exhibited a qualitative effect in that established amoebic lesions were rendered more severe. Sections in these cases showed severe ulceration and thickening of the bowel (see photograph 5) with a considerable exudation of muco-pus. This effect did not raise the A.D.I. significantly, since the change affected only those animals in which amoebic infection was already established, and the A.D.I. afforded no measurement of qualitative changes in the ulceration in individual animals (table 3).

Aggravation of the lesions was most pronounced with Bact.coli and paracolon. Correlated with this, the identical strains of paracolon could be recovered from the caecum at autopsy of such animals.

The paracolon strains used in these experiments belonged to groups I and II (Sevitt, 1945). Although other paracolon strains were not

uncommon in the intestinal flora of our rats, no group I or II strains were isolated except where a culture of these organisms had been administered to the rats. Hence it could reasonably be assumed that the organisms recovered were the same as those previously administered. With one strain (group II), an additional check was afforded by preparing an anti-serum (rabbit). The organism under examination was agglutinated by this serum and absorbed the agglutinins for the type organism. When paracolon bacteria were fed to uninfected rats, they remained present in the bowel for several days but caused no damage beyond slight hyperaemia.

Attempts at recovery were unsuccessful with Shigella flexner, an organism which failed appreciably to aggravate the infection. Recovery of Bact. coli was not feasible, since strains with identical reactions were already present in the rat's intestine. One added strain, however, proved able to identify itself by the spontaneous acqusition of unusual haemolytic powers during its sojourn in the rat's tissues. Bact. aerogenes also was occasionally present in the intestinal flora of rats, but the strains used failed to gain further prevalence when introduced artificially.

When coliform bacteria were injected intracaecally, there was usually an increase in the death rate of the rats, depending upon the lethal virulence of the organisms used. This effect was diminished when the bacteria were fed orally at varying intervals after the amoebic inoculum had been injected intracaecally.

Passage experiments, conducted with a paracolon strain, lowered the killing dose but did not endow the organism with added virulence for the colon. Similarly, a strain recovered from heavily infected lesions did not show any increase in local virulence.

Streptococcus faecalis:

When glucose-broth cultures of S.faecalis were added to the inoculum of E.histolytica, the infectivity of the amoeba was significantly reduced or even annulled (table 4). A similar, though less marked, trend was observed when plain- or glucose-broth cultures of S.faecalis were fed by mouth to rats already infected with E.histolytica; the A.D.I. was consistently reduced, but the reduction was only rarely

significant - presumably because lesions were already established in a proportion of the rats (table 4). From in vitro studies (page 52), it appeared that this effect was due to organic acid metabolites of S.faecalis in media containing glucose or related fermentable carbohydrates. Clostridia.

No quantitative or qualitative effect on the infection was observed after the addition of haemolytic strains of Cl.welchii to the inoculum of E.histolytica.

Chemotherapy in the experimental infection.

In theory the use of emetine to control the amoeba and penicillin, streptomycin or sulphonamides to control the bacteria, should provide a measurement of the contribution of each of the dual elements to the complex infection. Emetine can be shown to possess little or no bactericidal or bacteriostatic action in vitro, and penicillin, streptomycin or sulphonamides have no direct action on the propagation or survival of E.histolytica (Section III).

As would be expected, emetine exhibited a marked therapeutic action when given orally to infected rats in doses ranging from 1.25-10 mgm./Kilo/day (Table 5). Less regularly, some therapeutic action was obtained with doses of 0.62 mgm./Kilo. (Table 7). At doses higher than 1.25 mgm./Kilo., emetine was found to be toxic on continued administration, and it appeared that complete eradication of E.histolytica was seldom obtained by doses within the therapeutic range (Table 5). Emetine was found to possess little or no prophylactic activity, and its therapeutic action was most marked when relatively large doses were given during the first 24 hours of the infection, when the process of tissue-invasion by E.histolytica was maximal.

Penicillin and streptomycin both showed marked therapeutic activity at any stage of the infection (Table 6). With sulphonamides, therapeutic activity was less marked, and more variable (Table 6). Penicillin and sulphonamides, however, possessed marked prophylactic activity.

When penicillin and emetine were administered together, each in sub-optimal doses, a striking additive effect was obtained (Table 7). The lesions healed rapidly, and the parasite was more effectively cleared from the tissues. Some enhancement of therapeutic and prophylactic activity was also observed when penicillin and sulphonamide were given together (Table 6), comparable perhaps to the synergic effect demonstrable <u>in vitro</u> against coliform organisms (Stewart 1947 a).

These results show that the activities of <u>E.histolytica</u> and of pathogenic bacteria in the complex infection can be controlled separately by the appropriate chemotherapeutic agents. The remarkable degree of therapeutic activity shown by penicillin and streptomycin proves that bacteria fill a major role in the pathogenesis of the lesions; for ideal therapy, the anti-parasitic agent (emetine) must be used together with an anti-bacterial agent (Table 7).

Examinations of the bacterial flora of the caecal contents were made in rats belonging to the group which had been given 4 doses of penicillin and phthalyl sulphathiazole in the 48 hours preceding inoculation with <u>E.histolytica</u> (Table 6). A random sample of six rats, taken from the group at the time of inoculation, showed no definite changes in the relative proportions of the various intestinal organisms, when compared with controls belonging to the same batch; a significant numerical decrease in the intestinal organisms was only obtained after more prolonged dosage (2 weeks). Since penicillin and phthalyl sulphathiazole both exhibited prophylactic activity in this experiment, it is possible that a concentration of drug can be established in the wall of the caecum, sufficient in residue to antagonise the ingress of bacteria during the period of tissue invasion by the amoeba. Similar results were obtained in bacteriological examinations of the intestinal flora of rats receiving penicillin and sulphonamide therapeutically. It must be concluded, therefore, that these anti-bacterial agents exercise their effect in the experimental infection, not by acting on organisms in the lumen of the bowel, but by inhibiting these same organisms once they have gained access to the tissues of the caecum, either during the first 24 hours of tissue-invasion by the amoeba, or thereafter in the established lesions.

DISCUSSION

In the interpretation of results obtained from experimental infections in the rat, a reservation must be made regarding the artificial nature of such an infection, especially in the mode of induction. Nevertheless, the experimental infection is comparable to human amoebiasis in the histological appearance of the lesions, the influence of bacteria, and the varying degrees of therapeutic response to emetine and other anti-amoebic drugs (Jones, 1947). The essential differences are the absence of liver lesions and the rarity of a protracted, chronic disease in the rat.

The results show that young laboratory rats are susceptible, under experimental conditions, to active infection by human strains of E.histolytica. This infection is a rapid but essentially local tissue invasion in the caecum, with comparatively few systemic manifestations. In the majority of young rats, the infection is self-limiting after a typically acute course of about 7 days. Older rats are normally refractory to tissue-invasion by E.histolytica. This fact, together with the rapidity with which younger animals acquire an immunity, can be linked to the observation of Chiang (1925) that rats may act as carriers of an amoeba indistinguishable from E.histolytica, apparently with minimal tissue invasion. In some of the young rats used in our experiments, E.histolytica cysts were found in the faeces after the acute infection had subsided, and it is possible in a proportion of animals that a carrier state may become established.

In any one group of rats, the number of animals infected is governed primarily by the invasive power of the particular strain of amoeba. In this respect, cysts isolated from human carriers are not necessarily less invasive than trophozoites from cases of amoebic dysentery. A similar finding was reported by Meleney and Frye (1936) from a study of experimental infections in kittens and by Faust (1932) in dogs.

Where the invasive power of the amoeba is high, the infection tends to be severe; the severity of the infection, however, is dependent upon several factors, of which the virulence of the amoeba is only one and probably not the most important factor. When the activities of the amoeba are arrested by Emetine, the lesions may heal: but this response is variable, not necessarily complete, and can be replaced by the exhibition of penicillin or streptomycin; it is evident that other factors are concerned in the maintenance of the infection.

The bacterial flora of the intestine is a major factor in the pathogenesis of the amoebic lesions. This factor probably takes effect as soon as the amoeba establishes a breach in the mucosa, since penicillin and sulphonamide, exhibited in prophylaxis, will prevent the development of the lesions. In a straightforward infection, the lesions rapidly assume the appearance of acute inflammation and, from the fact that the inflammatory response extends far beyond the site of amoebic invasion, it is obvious that much of the tissue damage must be attributed to bacteria (photograph 5). Depending upon the pathogenicity of the bacteria present, this factor probably operates in every infection. When virulent bacteria enter the bowel during the period of tissue-invasion, they are capable of aggravating the lesions and provoke a massive inflammatory reaction with purulent exudation and local peritonitis. In some instances, virulent bacteria are already present in the rat's intestine and it is likely that certain paracolon strains are especially active in this respect (Table 2). Coliform organisms isolated from human dysenteric patients are also capable of aggravating the experimental infection when fed to rats and it may be assumed that the bacteria introduced with the inoculum of amoebae are also active in a similar fashion. When paracolon bacteria are fed to infected rats, the identical organisms can be recovered from the exudate in the diseased caecum.

Penicillin and streptomycin exert a vigorous therapeutic effect on the infection by a purely anti-bacterial action; thus deprived of the auxiliary tissue-invasion of bacteria, the amoebae causes minimal tissue destruction and, more often than not, fails to establish itself further. A maximal therapeutic effect is obtained by giving emetine

and penicillin together, but the action of the anti-bacterial agents, given alone, is so striking as to warrant the conclusion that bacteria play a major rôle at all stages in the infection.

The idea that bacteria are involved in the pathogenesis of amoebiasis was probably first suggested by Kartulis (1891) from the histological appearance of the lesions in man. Since then, the problem has been studied mainly in experimental infections in kittens. Thus Sellards and Leiva (1923) showed that kittens infected with E.histolytica developed a "secondary septicaemia", presumably as a result of bacterial invasion of the amoebic lesions. Reviewing experimental and clinical data, Wenyon (1926) considered that a virulent bacterial flora was necessary for the establishment of the intestinal lesions in the infection. Hiyeda (1930) went further, and contended that bacteria caused a colitis, after which E.histolytica was able to invade the bowel wall. Frye and Meleney (1933) showed that changes in the bacterial flora of cultures of E.histolytica altered its infectivity, while Spector (1935) reported that streptococci and pneumococci aggravated the lesions. Deschiens (1938) demonstrated conclusively that certain bacteria (S.typhi, S.paratyphi B and Bact.coli) or their products increased both the pathogenicity and infectivity of E.histolytica. Nauss and Rappaport (1940) reported that rectal injections of certain fluorescent bacteria of the Pseudomonas group increased the invasiveness of E.histolytica; from the fact that croton oil had a similar effect, they deduced that bacteria caused an irritant or toxic colitis, rendering the mucosa susceptible to invasion by E.histolytica.

From these and other observations, it is now generally accepted that bacteria are involved in the pathogenesis of amoebic infection in the bowel, but there is still some doubt over the identity of the bacteria concerned, and the nature of their action. The experimental results presented above show that in young rats, as in kittens, bacteria are vitally concerned in the pathogenesis of experimental amoebiasis, and that the infection is inevitably a complex in which E.histolytica and

certain intestinal bacteria play complementary rôles. Bacteria of the paracolon group and certain strains of Bact.coli are actually or potentially virulent in the infection, and aggravate the lesions originally established by the amoeba. Enterococci are non-virulent and certain strains (Streptococcus faecalis) produce, under suitable conditions, metabolites toxic to E.histolytica; this lowers the infectivity of the amoeba and may partially suppress an established infection. Other intestinal organisms, belonging to the groups Proteus, Bact. morgani or aerogenes, Shigella flexneri or Clostridia are relatively inert.

Irrespective of their virulence, bacteria do not invade the tissues of the colon freely until E.histolytica establishes a breach in the mucosa, and at this early stage the action of emetine is maximal; but the prophylactic efficacy of penicillin and sulphonamide shows that bacteria are participating even during this early tissue-invasion, while the striking therapeutic action of these compounds at later stages serves to emphasise that much of the tissue damage is attributable to bacteria. When virulent coliform organisms are present, the inflammatory reaction produced by them may indeed dominate the final picture. In view of the differing influences shown, for example, by paracolon bacteria and S.faecalis, it is possible that variations in host-susceptibility and in the course of the infection may be explained to some extent by differences in the bacterial flora, and in the immune-reaction to coliform organisms, of different host-species.

3. The infectivity of E.histolytica outside the bowel.

(a) Subcutaneous tissues and peritoneum.

Suspensions of trophozoites of E.histolytica were injected intra-peritoneally into young rats (20-35 gm.), the inoculum being adjusted by trial and error until a sub-lethal dose was found. This dose depended largely upon the bacteria present in the culture complex, and with strain CWE (table 19) the dose was 0.2-0.5 c.cm. of centrifuged deposit. A mild peritoneal reaction usually followed the injection (24-48 hours) and small amounts of exudate, obtained with a pipette at laparotomy, were examined microscopically for the presence of E.histolytica, but with negative results.

In further experiments, hyaluronidase (prepared from bull testis by Dr. R. H. Townshend) was injected subcutaneously along with the amoebic inoculum; a hyaluronidase-producing strain of <u>Cl. welchii</u> was also tested. These additions failed to facilitate tissue-invasion by <u>E. histolytica</u>. It will be recalled that <u>Cl. welchii</u> was also inert in this respect in the bowel (page 14).

Further experiments were performed by injecting washed cysts, with or without coliform bacteria, into the peritoneal cavity of young rats. In two instances, highly concentrated suspensions, supplying inocula of approximately 500,000 cysts, were used but the results were negative in these as in the other experiments, in that tissue invasion by E.histolytica could not be demonstrated in fresh preparations and histological sections made 2-10 days after inoculation.

The inocula used in the above experiments were also injected subcutaneously into rats, mice and guinea-pigs. Again, no evidence of tissue-invasion by E.histolytica was obtained.

(b) Liver.

The production of experimental liver abscess was attempted by the following methods.

(i) Injection of 0.5 c.cm. of a suspension of trophozoites of E.histolytica, prepared from a culture-complex, directly into the liver tissue. In two kittens and two rabbits, this resulted in a fatal peritonitis in 1-4 days, with an area of necrosis at the point of inoculation in the liver, in which E.histolytica could not be identified. Further experiments with rats were equally unsuccessful.

(ii) Injection of suspensions of trophozoites or washed cysts of E.histolytica into the mesenteric vein of one kitten (trophozoites), three rabbits (cysts and trophozoites) and a number of rats and guinea-pigs. Again the results were unsuccessful. When trophozoites were injected, some animals survived the operation while others succumbed to a fatal bacteraemia or pyaemia. When cysts were injected, the animals survived. In no case was E.histolytica identified, either in fresh preparations made from pyaemic areas in the liver, or in histological sections.

The only conclusion that can be drawn from these experiments is that experimental demonstration of the extra-intestinal pathogenicity of E.histolytica is very difficult to establish. This finding serves perhaps to emphasise the limitations of a purely experimental approach to a problem such as amoebiasis, for abscess of the liver and other tissues in human infections with E.histolytica are by no means uncommon,

whereas no tissue invasion followed the injection in animals of the pathogen into sites presumably favourable for its development.

SUMMARY OF SECTION I.

Under experimental conditions, young laboratory rats are susceptible to active infection of the caecum by human strains of *E.histolytica.*

The experimental infection follows an acute course for seven days, after which a variable degree of spontaneous remission occurs.

The onset of the experimental infection is governed by the invasiveness of the particular strain of *E.histolytica,* and can be to a large extent controlled by emetine.

After the amoeba has established a breach in the mucosa of the caecum, the course of the infection and the character of the lesions are determined largely by the activities of certain intestinal bacteria; the introduction of virulent strains of *Bact.coli* and *paracolon* aggravates the lesions.

Given in prophylaxis, penicillin and sulphonamides counteract early bacterial invasion, and mitigate the infection; for the same reason, penicillin, streptomycin or, to a lesser extent, sulphonamides exert a therapeutic effect on the established infection. The most complete therapeutic effect is obtained by the combined use of emetine and penicillin.

Under experimental conditions, *E.histolytica* fails to exhibit pathogenicity outside the bowel.

SECTION II

CLINICAL STUDIES.

The chronicity of Amoebiasis.

(1) The rôle of bacteria in intestinal amoebiasis in man.

(2) Post-dysenteric colitis.

The chronicity of Amoebiasis.

1. The rôle of bacteria in intestinal amoebiasis in man.

Observations on the incidence of amoebiasis in Ceylon, the carrier rate of E.histolytica cysts among asymptomatic Europeans, and the clinical course of the disease are detailed in two papers included in the appendix (Stewart 1947b, Stewart, O'Meara and Kershaw 1948). These papers emphasise the fact that amoebiasis was the pre-eminent cause of protracted illness and loss of working-hours among Service personnel in Ceylon. A later survey, made in Liverpool, shows that a considerable proportion of the illness observed in Service personnel returning from the tropics to the United Kingdom, is attributable to amoebiasis or post-dysenteric colitis (page 37).

In the second paper (Stewart, O'Meara and Kershaw 1948), reference is made to some of the factors involved in the chronicity of amoebiasis, so distressing a feature of infections contracted in South-east Asia (Lamb and Royston, 1945, Adams 1945). Among such factors must be included the effect of malaria and other privations of the tropical campaigns (Payne 1945); reinfection; delay in diagnosis; and inadequate treatment (Hargreaves 1945, Adams 1945). Even when such factors are minimised or excluded, however, as among patients treated for relapses in hospitals in the United Kingdom, chronicity remains a major problem in amoebiasis; and these factors do not explain the marked variation in individual susceptibility to amoebic infection, whereby one individual becomes an asymptomatic cyst-passer while another develops amoebic dysentery.

It is obvious that the presence of E.histolytica in the tissues does not, in itself, account for the occurrence of dysenteric relapses; and it has already been shown (Section I) that the severity of an experimental amoebic infection is independent, within wide limits, of the number of amoebae introduced into the bowel.

The survey of unselected European cases, made in Ceylon, suggested to the author that the most obvious and most constant factor which could be associated with a high relapse rate was the severity of the original attack of dysentery. This, in turn, was sometimes associated with changes in the bacterial flora of the faeces and it

seemed possible, as Westphal suggested in 1938, that the onset of dysentery was influenced by bacteria rather than by spontaneous changes in the virulence of the amoeba.

There is a considerable volume of experimental and clinical evidence in support of this hypothesis, though few workers have been as explicit in their views as Westphal. Experimental evidence, already reviewed in Section I, suggests that, in several animal species, virulent bacteria are necessary for the development of lesions in the colon. Clinical evidence has been derived from the fact that cases of amoebic dysentery may benefit from treatment with anti-bacterial agents such as sulphonamides (Bloom 1944) and penicillin (Hargreaves 1945) in addition to the usual anti-amoebic drugs. The nature and extent of the element of bacterial infection awaits definition, however, for there is no exact record in the literature of the particular bacteria concerned in the complex infection. The Medical Department of the British War Office, recognising the concurrence of amoebic and bacillary dysenteries in certain theatres of war, recommended the use of sulphonamides for all cases of acute diarrhoea occurring in the field, where accurate diagnosis was impossible. Horster (1942), a German medical officer, had a similar experience, and contended that amoebic infections were activated by bacillary dysentery. In a bacteriological survey already reported (Stewart 1947c), the author failed to incriminate any common bacterial pathogen in a group of patients with severe relapsing amoebic dysentery; the studies made in Ceylon (Stewart, O'Meara and Kershaw, 1948) showed that the occurrence of dysentery in amoebiasis was not necessarily dependent upon a coincident Shigella infection. The experimental studies of Section I, however, suggest that the rôle played by bacteria may be variable in character, depending upon the virulence of bacteria already present in the bowel rather than upon extraneous pathogens. This question is investigated in human patients in the present section.

Method of investigation.

The clinical material consisted of 101 male Service cases of proven intestinal amoebiasis, comprising 35 early dysenteric cases and 66 late relapsing cases. Symptomless carriers were excluded. A group of 120 medical and surgical cases with no intestinal disorders served as controls. The following investigations were carried out.

Pathological Investigation.

The pathological investigation included sigmoidoscopies, leucocyte counts, and, in some cases, blood cultures, serological tests, and biopsies from the colon or rectum. All the cases showed E.histolytica in the faeces during the period of investigation. Bacteriological cultures were made from the faeces in all the cases and in the controls as described in Section IV.

The bacterial results described below were drawn from aerobic cultures. In some preliminary experiments, anaerobic cultures were also made. By this means, fusiform bacteria and gram-positive rods (Clostridia) were preserved. The greater part of the flora, however, was still accounted for by coliform organisms and gram-positive cocci, which are facultatively anaerobic and occurred in approximately the same proportions as in the aerobic cultures. Obligate anaerobes never attained predominance in cultures made from normal or pathological faeces in the series investigated. The present study was therefore confined to aerobic cultures.

Results.

General Findings

The 101 cases all showed the clinical features which are familiar in intestinal amoebiasis - acute or intermittent diarrhoea, abdominal pain and tenderness. The only common manifestations of constitutional upset were varying degrees of lassitude and loss of weight. Even in severe cases pyrexia was slight or absent, and high leucocyte counts did not occur, although dysenteric cases usually showed a mild leucocytosis, of the order of 12,000 cells per c.mm., as described by Manson-Bahr and Willoughby (1928). In some instances a leucocytosis was absent in severe relapsing cases, in spite of the presence of frank pus in the stools.

Sigmoidoscopies were performed upon most of the cases before treatment. The early dysenteric cases showed shallow ulcers with red vascular bases; occasionally yellowish necrotic foci were seen. The intervening mucosa was intact and showed little change beyond hyperaemia. In late cases, the ulcers were deep and full of muco-pus; the intervening mucosa was oedematous and intensely congested. Biopsies taken from this type of case showed that there was a wide-spread inflammatory infiltration of the mucosa. In some cases, known in the Liverpool School of Tropical Medicine as 'post-dysenteric colitis', the entire mucosa was replaced by pyogenic granulations, which often obscured the original amoebic infection (Stewart, 1947c). Post-dysenteric inflammation of this type could develop suddenly, in a few weeks, or gradually over a period of months; the amoeba could persist or disappear during its development, and anti-amoebic treatment _per se_ produced little or no response. Whatever the exact mechanism, it was obvious that other factors in addition to _E.histolytica_ were concerned in the aetiology of this type of colitis.

The exudates from the diseased colon in early dysenteric cases showed vegetative forms of _E.histolytica_, with some loose mucus and blood derived from the ulcers. Microscopically, a few leucocytes and macrophages were present, but there was no 'pavement' of cells and faecal matter was freely admixed. Exudates of this type gave an acid reaction (pH 5), or, if faecal material was present, a weakly acid or neutral reaction. A strongly acid reaction was usually associated with an overgrowth of _Bact. aerogenes._ In late cases, frank pus appeared in the faeces and innumerable leucocytes could be seen microscopically; with the appearance of pus, the reaction of the exudate became neutral or alkaline, and, as will be seen later, this was associated with an increase in non-lactose-fermenting bacteria. By analogy with bacillary dysentery (where the fresh exudate is invariably alkaline) it seemed likely that the alkaline reaction was derived from the non-lactose-fermenters (or the associated tissue reaction), and not vice versa.

Blood cultures were taken from 10 severe cases. The

results were negative. Manipulations, such as sigmoidoscopy, did not cause a bacteraemia.

Bacteriological Findings.

When wet and stained smears of faeces were examined, the bacterial flora, in normal and in dysenteric specimens alike, were found to be abundant and varied. Gram-negative rods were usually predominant, varying greatly in size and in staining properties. Fusiform bacilli, yeast-like cells, spirilla, gram-positive rods and cocci were all present.

In contrast to this, cultures made from faeces were comparatively simple to interpret. The relative distributions of organisms in the cases and controls are shown in Table 8 and the differences are analysed in Table 9.

All the controls showed numerous colonies of Bact. coli. and in a majority (95 per cent.) this organism was predominant. Enterococci were present in 61 per cent., but were rarely predominant. In 27 per cent. of the normal specimens, a few colonies of non-lactose-fermenters or Bact. aerogenes were present, and in isolated instances (2.5 per cent.) the non-lactose-fermenters outnumbered Bact. coli.

The 101 cases of intestinal amoebiasis showed the following changes:

1. Early dysenteric cases (usually in their first known attack of dysentery) showed a significant difference from the controls in the increased incidence of Bact. aerogenes. In many cases this increase amounted to a complete overgrowth by Bact. aerogenes of all the other bacteria present, and where this occurred no final opinion could be reached regarding the relative distribution of the other organisms. Stained smears taken from the plates and direct smears made from the faeces showed that enterococci were usually abundant.

2. Late relapsing cases, with chronic lesions in various stages of activity, showed an increased number of non-lactose-fermenters. In broad terms, this indicated that the bacterial flora was more mixed in these cases than in the controls, but statistical analysis showed that the only organism of which the prevalence was significantly increased

was paracolon (Table 9).

3. Both groups showed a significant increase in the presence of enterococci; quantitatively, the proportion of enterococci was greater in liquid than in solid stools.

4. Coincident infections with Shigella or Salmonella organisms were absent in this series. In this connection it is relevant to mention that, in a corresponding series studied in the tropics (Ceylon), four out of 100 cases showed simultaneously the presence of Shigella (three flexneri, one sonne) along with E.histolytica (Stewart, O'Meara and Kershaw, 1948). In acute cases seen in the tropics, the overgrowth of Bact.aerogenes referred to above was usually very marked.

Serological Reactions.

From 30 cases of amoebiasis, the patient's serum was mixed with formolized and alcoholised suspensions of Bact.coli isolated from his own faeces. Five cases showed 'O' type agglutination to a titre of 1:50. One of the five gave 'H' agglutination to a titre of 1:125. The remainder gave negative results.

Control experiments were performed by testing pooled or individual sera from 10 groups of 10 healthy individuals against strains of Bact. coli isolated from normal stools and from stools containing E.histolytica. This investigation showed that positive reactions could occur at titres of 1:25 and occasionally of 1:50 ('O' type agglutination), though not higher. Furthermore, some strains of Bact. coli isolated from dysenteric stools were agglutinated by normal sera, though not by the patient's own serum. This contradiction showed that the possibility of accidental agglutinations and cross-reactions excluded any pathological significance in the serological reactions of the patients with amoebiasis.

Paracolon bacteria and the other gram-negative bacteria isolated were also tested against sera from patients and controls. One case showed titres of 1:50 ('O') and 1:200 ('H') against his own paracolon organism. Proteus, Bact. morgani. Bact. aerogenes and B.alcaligenes were not agglutinated.

Of the series investigated, therefore, only two showed agglutination titres of importance. It can be concluded that cases of amoebiasis do not commonly develop agglutinins against the predominant members of the intestinal flora, although a reservation must be made concerning severe cases of post-dysenteric colitis (see table 15), where positive reactions are relatively more common.

Notes on Individual Bacteria.

Bact.paracolon.

Twenty-four organisms of this group were isolated from cases of amoebiasis in the present series. The reactions of these organisms are shown in tables 27 and 28. With three exceptions, the strains belonged to the biochemical groups A and D of Dudgeon and Pulvertaft (1927) corresponding to groups I and II of Sevitt (1945). The usual IMViC pattern among groups I and II bacteria was (++−−); when the methyl-red reaction was positive, the Vosges-Proskauer was negative, and vice-versa. Groups I and II bacteria were virulent to rats, mice and guinea-pigs, and it has been shown in Section I that certain strains aggravated an experimental amoebic infection in rats.

All the strains were examined serologically for cross-reactions with Shigella or Salmonella anti-sera, and for agglutination by the patient's own sera. One group II strain (Ne) was agglutinated to titre (1:250) by Flexner Y anti-serum, and to some extent by V, W, X and Z anti-sera. Another strain (K) was agglutinated by standard S.paratyphi (A) '0' anti-serum (1:400). '0' type anti-serum, prepared in a rabbit against strain K, agglutinated S.paratyphi (A) to titre 1:200. Absorption of this anti-serum by S.paratyphi (A) removed the agglutinins for paracolon (K), and absorption of standard S.paratyphi (A) anti-serum by paracolon (K) lowered the titre from 1:800 to 1:200. It appeared, therefore that paracolon (K) possessed somatic antigens identical with Salmonella I-II. A third strain (paracolon (N)) showed cross-agglutination with anti-sera for S.paratyphi (A) and S.senftenberg.

It is obvious that certain paracolon bacilli, notably of group II, are closely related to the Salmonella in biochemical and serological reactions. This relationship was investigated further with

strains K, N and T. Twenty colonies of each strain were lifted from agar plates, and the reactions tested through ten serial subcultures. Strain K produced two spontaneous variants which were indole-negative; two other variants fermented lactose in 24 hours and ten in 1-4 days, the remainder being true non-lactose-fermenters in the primary culture, though subsequent sub-cultures gave a number of late-lactose-fermenters. All variants of strain K were agglutinated by S.paratyphi (A) 'O' anti-serum as before, but none reacted with polyvalent or non-specific 'H' anti-sera. Strain T produced no variants under the conditions described, but strain N behaved similarly to strain K.

The production of variants in vivo was attempted by injecting broth cultures of each of the three strains intra-peritoneally into groups of three mice, and re-isolating them from the heart-blood 24-48 hours later. After each re-isolation, the biochemical and serological reactions of the organisms were tested. Ten original cultures of strain K were examined through ten serial passages, but no indole-negative variants were recovered, nor were there any significant changes in serological properties. With strains N and T, three original cultures were followed through ten passages, again with negative results.

Similar experiments, in vivo and in vitro, were conducted with a strain of S.paratyphi (A) to see if indole-positive variants resembling paracolon organisms could be identified. The results were negative.

These results suggest that certain strains of paracolon bacilli readily produce variants, labile in biochemical reactions, which may make them resemble, on the one hand, lactose-fermenting coliforms or, on the other hand, members of the Salmonella group; serologically, however, the organisms tested were relatively stable. With group II strains, this serological stability is of considerable practical importance as it enables accurate differentiation to be made between indole-negative variants and Salmonella organisms in the non-specific phase.

Sevitt (1945) showed that 75 per cent. of his group I strains were serologically identical, and that many of them contained minor antigens common to the Shigellae. The occurrence and probable importance of paracolon bacilli with Shigella and Salmonella antigens has also been

studied by Felsenfeld and Young (1945). A note of caution, however, was sounded in this subject by Stamp and Stone (1943) who showed that certain strains of lactose and non-lactose-fermenting coliform bacilli possess an α antigen, distinct from the 'O' and 'H' antigens. They found that standard Flexner diagnostic sera contained agglutinins against α strains, and that normal sera from one out of four batches of rabbits agglutinated their α strains in titres 1:25-1:200. Furthermore, the α antigen behaved comparably to the Vi antigen of S.typhi, in that it inhibited 'O' agglutination. It is obvious that the several fallacies arising from the presence of this antigen must be borne in mind when the serological characters of coliform organisms are analysed.

Enterococci.

In the present studies, heat-resistant gram-positive cocci isolated selectively on the azide media were classified as enterococci. The majority were lanceolate diplococci which fermented glucose, mannitol and saccharose, and grew at pH 9.6 in 6.5 per cent. NaCl. No β-haemolysis occurred in horse-blood agar. It is probable that most of these organisms were Streptococcus faecalis. Dible (1921) has shown that, normally, enterococci are of low virulence. Strains isolated from dysenteric cases and controls were equally non-virulent to rats, mice and guinea-pigs by the oral or intraperitoneal routes. Cultures made from different levels in the rat's intestine showed that the organism was more prevalent in the small than in the large intestine. When diarrhoea was provoked by the use of saline purgatives, enterococci became relatively more numerous in the stools. It is probable therefore that prevalence of S.faecalis in dysenteric stools was a physiological consequence of intestinal hurry.

In a few instances, Staphylococci were observed in cultures, but there was no significant difference in their occurrence in the control and in the dysenteric groups.

Discussion.

The results show that the bacterial flora of the colon may exhibit three abnormal deviations in intestinal amoebiasis: the predominance of Bact. aerogenes in the early dysenteric stage; the increased frequency with which paracolon organisms may be isolated in the

later stages of the relapsing disease; and the relative increase in the proportion of enterococci in diarrhoeic stools at any stage. Severe relapsing infections are associated with a pyogenic inflammatory response in the colon, and pus appears in the stools. In spite of these local changes, which are often extensive, there are comparatively few signs of systemic reaction, as evidenced by the comparative fitness of the patient and by the absence of fever or high leucocytosis, bacteraemia or agglutinins in the serum.

The appearance of numerous enterococci in the faeces appears to be a physiological phenomenon, due to the rapid passage of the contents of the small intestine in states of diarrhoea. Investigations described in Sections I and III show that certain strains of enterococci of the S.faecalis group inhibit E.histolytica in vitro and in vivo; in vitro this effect depends upon the production of one or more toxic metabolites from media containing glucose or related carbohydrates. Human strains of S.faecalis, isolated from dysenteric cases in the present series, show similar activity. It is therefore possible that the prevalence of such strains in the active stages of amoebiasis may be associated with a process of natural remission.

The increase in Bact. aerogenes in early acute cases is not easy to explain. This organism may be present in normal human faeces, the frequency of its occurrence varying in different communities from 13 per cent. (Kempny, 1946) to 40 per cent. (Mollari et al., 1939). In the controls of the present series its incidence was 8.3 per cent., but as Bardsley (1934) has shown, it can be isolated with greater frequency by the use of enrichment techniques. It is clear, however, that, in spite of the considerable normal variation in its incidence, Bact. aerogenes does not normally attain predominance in the faeces. Some increase may occur after saline purgation, but the present studies suggest that predominance is associated with the acid reaction of the exudate in amoebic dysentery. The organism is not observed in the acute stage of bacillary dysentery, where the exudate is invariably alkaline, and it is less regularly isolated in late cases of amoebiasis or in post-dysenteric colitis where pus appears. When

cultures were fed to stock rats not carrying the organism, Bact. aerogenes fails to gain prevalence in the intestine - whether by excretion, destruction or modification of its characters is not clear. Cultures fed to young rats experimentally infected with E. histolytica fail to aggravate the infection (page 13). The balance of evidence, therefore, is that the increase in Bact. aerogenes in acute amoebic dysentery is an associated phenomenon, and that it does not necessarily represent an invasion by this particular organism of the amoebic lesions.

Paracolon bacteria have been investigated for pathogenicity by several workers. Glynn et al. (1917) isolated organisms described as 'indole-positive paratyphoid bacilli' from enteritis convalescents during the 1914-18 war, but found no evidence of pathogenicity. Since then, however, evidence has been found that organisms corresponding to those described by Glynn et al. may assume a pathogenic rôle in certain bowel disorders. Dudgeon and Pulvertaft (1927) showed that slow-lactose-fermenting coliforms could often be isolated in pure culture from cases of acute diarrhoea; they classified such organisms, and showed that the majority of the presumptively pathogenic strains were biochemically and serologically uniform. Stuart et al. (1943) considered that paracolon was intermediate in biochemical and antigenic structure between normal Bact. coli and the Salmonella group, and that it might act as a pathogen in conditions of mild enteritis. This finding may be correlated with the fact, familiar to most bacteriologists, that paracolon bacteria appear in the faeces in large numbers during the convalescent stages of acute Salmonella or Shigella infections. Sevitt (1945) reclassified the paracolon group and showed that certain members had an increased incidence among infants with infectious enteritis; many of his strains showed similarities in antigenic structure to the dysentery bacilli. Such strains produced experimentally an enterocolitis in kittens, in which the specific organism could be recovered from the faeces. Ferguson and Wheeler (1946) isolated two paracolon strains antigenically related to Shigella paradysenteriae, and Barnes and Cherry (1946) found paracolon in the stools of 12 out of 17 cases in an outbreak of gastro-enteritis in a United States naval hospital. Thus there is evidence in the literature

that paracolon is associated with certain conditions causing diarrhoea. An attempt was made to investigate further its rôle in amoebiasis by feeding or injecting specific paracolon bacteria, isolated from human cases, to young rats experimentally infected with E.histolytica (page 12). This resulted in an increase in the severity of the lesions, and identical strains could be recovered from the infected bowel and, in some instances, from the blood or peritoneum. When fed to control rats, paracolon provoked hyperaemia in the ileum and caecum and could in some instances be recovered from these sites. Injected intraperitoneally, these strains, in smaller doses, induced a fatal bacteraemia but showed no specific tissue-fixation. Thus, under experimental conditions, paracolon behaves as a facultative pathogen which profoundly influences the course and severity of lesions initiated by E.histolytica. In this sense, Koch's postulates are fulfilled by recovery of the identical strains.

It may be concluded, therefore, that paracolon bacteria are potentially pathogenic when the intestine is diseased by amoebic infection, and that their presence, natural or otherwise, in a proportion of individuals introduces an added element of bacterial infection in amoebic lesions. Since the strains isolated from such cases are not always biochemically or serologically identical, the precise limitations of the pathogenicity of the organism cannot as yet be defined.

Other non-lactose-fermenters isolated from cases of amoebiasis include Bact. morgani, B.faecalis alcaligenes and Proteus. The pathogenicity of these organisms has been repeatedly investigated (Morgan and Ledingham, 1909; Trawinsky and György, 1918; Bengtson, 1919), and Wilson (1929), reviewing their position, found no convincing evidence of their pathogenicity in conditions of enteritis. In the present series there was no significant difference in the incidence of these organisms between cases of amoebiasis and controls. This finding in itself does not mean that the organisms could not assume a pathogenic rôle, but it does suggest that their rôle was inconspicuous.

In many of the severe relapsing cases, the aerobic bacterial flora contained only Bact. coli and enterococci. Strains of Bact.coli isolated from such cases proved highly virulent when fed to rats experimentally infected with E.histolytica (page 19). These strains showed no biochemical or serological uniformity, though in most instances they formed a powerful β-haemolysin active against horse red-cells.

In the interpretation of these findings, the absence of recognized pathogens of the Shigella group is of importance. Such organisms may be found among cases diagnosed in India (Acton, 1933; Marriott, 1945), though their presence is not necessary for the initiation of amoebic dysentery (Stewart, O'Meara and Kershaw, 1948). If unidentified anaerobes be excluded, the element of bacterial infection in the present series of cases must therefore reside in the various organisms described above. This means that added or 'secondary' bacterial infection in amoebiasis depends upon the capacity of organisms already present in the bowel to invade lesions established in the first instance by E.histolytica. The severity and course of the resulting disease depends upon the potential pathogenicity of indigenous strains of Bact.coli and upon the presence, natural or otherwise, of facultative pathogens such as paracolon.

Summary of Section II. (1).

Cases of intestinal amoebiasis in the chronic relapsing stage show pyogenic inflammatory changes in the mucosa of the colon. This pathological process is essentially a local one; a systemic reaction is uncommon.

In the course of the disease alterations occur in the relative distribution of organisms in the faeces.

The early dysenteric stages are characterized by a tendency towards overgrowth of the other organisms by Bacterium aerogenes, a process to which no pathological importance is ascribed.

Enterococci are prevalent in diarrhoeic specimens at any stage. This probably results from the rapid passage of the contents of the small intestine. The majority of enterococci are non-virulent, and the metabolites produced by certain strains are toxic to Entamoeba histolytica.

In chronic relapsing cases the incidence of paracolon bacteria is increased; evidence is adduced to show that these organisms assume a pathogenic rôle in a proportion of such cases.

It is concluded that the inflammatory reaction in the colon in severe relapsing cases is largely attributable to added bacterial infection dependent upon the virulence of the indigenous bacteria and upon the occasional presence of potential pathogens, such as paracolon. This inflammatory reaction is a major factor in the chronicity of active amoebiasis.

(2) **POST-DYSENTERIC COLITIS.**

For many years it has been recognised that a primary attack of dysentery may be followed by a chronic disorder in the function, and sometimes in the structure, of the colon. In amoebiasis, this disorder is the rule rather than the exception, and may manifest itself as periodic dysenteric attacks, or simply as chronic diarrhoea. Acute *Shigella* infections, in a much smaller proportion of cases, may be followed by a condition of chronic dysentery. The diagnosis of one or other of these well-known conditions, however, always depends upon the recovery of the causal organism from the stools or from lesions in the colon, and it is usually inferred that the principal aetiological factor in the persistence of the "colitis" is the continued presence of the organism.

The data presented in this chapter are derived from a general survey of the cases seen in Ceylon and Liverpool in 1945-48. Briefly, this survey revealed that, in a proportion of cases, disorder of the colon persisted long after the removal of the dysenteric organism. This disorder, which might be called post-dysenteric colitis (Hurst 1943), appeared to be of two main types, ulcerative and non-ulcerative, which could be clearly differentiated for purposes of prognosis, treatment and eligibility for pension.

The clinical material from which the present survey was made consisted of 228 cases of dysentery and diarrhoea observed in Ceylon (Combined Services Hospital, Trincomalee) and 246 cases observed in the Tr investigation ~~have already been~~ are described in Section IV.

(a) Cases seen in Ceylon. (Tables 10-11).

The principal known causes of acute diarrhoea and dysentery in the Trincomalee area of Ceylon are shown in Table 10. As might be expected

The principal known causes of acute diarrhoea and dysentery in the Trincomalee area of Ceylon are shown in Table 10. As might be expected the majority of the cases were accounted for by intestinal amoebiasis (50%) and bacillary dysentery (37%). For various reasons, it was impossib to follow up many of these cases, and the analysis of relapses and recurrences of diarrhoea among cases of intestinal amoebiasis and bacillary dysentery were drawn from a smaller group (table 11).

In bacillary dysentery, adequately treated with sulphonamides, the relapse rate was negligible. _Shigella sonne_ displayed its well-known tendency to linger in the bowel beyond the period of treatment, but produced no further disease. Even in the absence of treatment, acute bacillary dysentery was noted to be self-limiting in a few cases admited to hospital on the 3rd or 4th day of illness. The findings therefore, in agreement with those of other investigators (Fairbrother 1944, Scadding 1945), suggest that chronic bacillary dysentery of the type described by Manson-Bahr (1943) after the 1914-1918 war must be extremely uncommon nowadays.

In contrast, there was a high relapse rate among cases of active intestinal amoebiasis. This relapse rate was directly dependent upon the severity and duration of the infection, but a recurrence of diarrhoea was by no means uncommon among early and relatively mild cases (Stewart, O'Meara and Kershaw 1948). A proportion of the later relapses (21%) was accounted for by the persistence of _E.histolytica_ and it was obvious that no further conclusions could be drawn until repeated examinations had failed to reveal the parasite. It was assumed that stool examinations on 12 successive days together with sigmoidoscopic scrapings and flotation technique sufficed to identify the parasite in about 90% of cases (Faust 1939, Kershaw 1946, Kershaw, O'Meara and Stewart 1948). With these criteria fulfilled, it is evident from table II that diarrhoea persisted in the absence of _E.histolytica_ in 23 out of 29 relapses (79%). These cases provide the main source of post-dysenteric colitis.

(b) _Cases seen at the Tropical Diseases Centre, Smithdown Road Hospital, Liverpool._

In the Liverpool area, a number of cases of acute, or chronic diarrhoea occurring in Service or Merchant Navy personnel returning from the tropics were referred to the Tropical Diseases Centre, attached to the Liverpool School of Tropical Medicine. The admissions for 18 months in 1947-48 supported the evidence contained in the Ceylon data, in that

the majority of the cases were accounted for by amoebiasis (table 12). The incidence of bacillary dysentery was negligible and cases of chronic bacillary infection of the type described by Manson-Bahr (1943) and Rogers (1944) were again absent. The Liverpool hospital admissions, however, contained a group not definitely represented among the earlier cases seen in Ceylon: 49 cases, which we have labelled post-dysenteric colitis, in which diarrhoea persisted in the absence of any recognised intestinal pathogen.

There were few diagnostic features in the symptoms of post-dysenteric colitis (table 13). The condition could only be diagnosed when there was a proved history of dysentery, a persistence of diarrhoea and an absence of the original infecting organism. As in most other forms of colitis, the main complaint was diarrhoea, which varied in severity and in frequency: in 62% of cases, it was continuous and in 32% intermittent. 30% of the cases had no complaint other than diarrhoea. The remainder gave histories, or showed signs of lassitude, loss of weight, and abdominal pain, sometimes dyspeptic in character but more often "colicky".

Sigmoidoscopic and stool examinations showed that the cases could be divided into two main types (table 14). In one type (56%) the mucosa of the colon was normal in appearance and the stools, though loose, contained no exudate or a scanty mucoid exudate. In these cases the diarrhoea was usually intermittent, and seldom severe; in some cases a few loose or precipitate stools were passed in the morning only. In other cases, neurotic or anxiety traits were noted and these, together with the absence of organic change in the colon, suggested that the condition was to a large extent functional (case a.). In most cases, there was a slow natural improvement, without treatment. Four cases, rather more refractory, seemed to benefit from treatment with antispasmodics (hyoscine hydrobromide gr $\frac{1}{200}$ 6-hourly) and phenobarbitone.

The second but more important type of case (44%) showed inflammatory or ulcerative changes in the colon, with blood and pus in the faeces (table 15). These cases were seriously and sometimes intractably ill. It is possible that they required further subdivision into two

types, represented by cases b and c, and defined by the aetiological and therapeutic factors detailed below.

Documentary records showed that each of these 49 cases had a previous history of dysentery and had suffered from repeated attacks of diarrhoea prior to admission to hospital. The majority (57%) had suffered from amoebic dysentery, 24% from bacillary dysentery and 19% from a dual infection. Acton (1933) and Silverman and Leslie (1945) have attributed the patency and severity of amoebic dysentery in tropical areas to concomitant infection with Shigella organisms. On the other hand, Stewart, O'Meara and Kershaw (1948) have shown that the occurrence of the dysenteric state in amoebiasis is not necessarily dependent upon a coincident bacillary infection, and that mixed infections are not necessarily more severe or more prone to relapse than "unmixed" amoebic infections. It is worth recalling that the exudate in a bacillary infection is alkaline, whereas that in amoebic dysentery is acid (Stewart, 1948) and it may be that the tissue reaction provoked by the activity of one organism is unfavourable for the simultaneous activity of the other. These findings, together with the absence of chronicity among the Shigella infections in the present series, suggest that the establishment of the post-dysenteric state does not depend upon a previous mixed infection or upon the persistence of a Shigella organism. A proportion (24%) of our cases, however, had histories of one or more attacks of bacillary dysentery, with no past or present evidence of amoebiasis, and it is possible that in such cases some additional factor may lead to the development of a colitis after the elimination of the specific Shigella organism.

The additional factor is one which may be present in cases of amoebiasis as well as bacillary dysentery. In previous communications, Stewart (1947c, 1948) has shown that in severe and relapsing cases of amoebiasis there are changes in the relative distribution of the various organisms in the bacterial flora of the colon, and that there is a significant increase in the presence of paracolon organisms; this group of organisms has often been associated with outbreaks of diarrhoea and "non-specific enteritis", and it is possible that the persistence of diarrhoea in some post-dysenteric cases may be related to the presence of such potential pathogens in the intestine (table 15).

At this stage the differences between the two types of cases must be re-emphasised (see table V). One type developed a mild, intermittent diarrhoea with little or no inflammatory change in the colon and, at most, a scanty mucoid exudate in the faeces while the second type developed an ulcerative colitis with severe and persistent diarrhoea and a purulent exudate. It is in the second or "ulcerative" type (table 14) that the element of added bacterial infection, non-specific in type and bearing some relationship to the factor already described in cases of relapsing amoebiasis, might play a part. The number of cases in the present series, however, is too small for a statistical estimate of the significance of this factor, and it must also be recognised that changes in the intestinal flora per se do not necessarily constitute a pathogenic influence upon the colon (Stewart 1948; Stewart, Jones and Rogers, 1948). In some cases, it was possible to demonstrate serum agglutinins against coliform and paracolon organisms isolated from the faeces (table 15). Blood cultures, taken before and after sigmoidoscopy were negative in ten cases. Leucocyte counts varied from normal to 24,000 cells per c.mm. In other words, there was no definite evidence of a characteristic systemic response to the infection just as there was no specific pathogen which could be incriminated. It would seem, nevertheless (table 15), that some improvement might be expected in such cases from the use of penicillin and sulphonamides; the bacteria concerned, including coliform gram-negative organism, can be inhibited by high concentrations of these agents in vitro (Stewart 1947a) and in experimental amoebiasis in vivo (Stewart and Jones 1948), while penicillin has been shown to retain bacteriostatic potency in the colon (O'Connor 1947). Some improvement was obtained by giving penicillin (1 mega unit) as a retention enema, but the best results were obtained when penicillin was given intramuscularly; large doses were required (e.g. 500,000 units 6-hourly) to provide the requisite blood concentrations for the inhibition or partial inhibition of coliform organisms (table 16). The effect of penicillin upon coliform bacteria, the synergic action of penicillin and sulphonamides, and the production by certain bacteria of anti-penicillin

factors, have already been described (Stewart 1945, 1947a). If penicillin and sulphonamide fail, some improvement may follow the use of a 2½% retention enema of quinoxyl or chiniofon, which have anti-bacterial properties and produce a decrease in the number of coliform organisms in the faeces.

In the series described, there were four cases, showing pronounced inflammatory changes in the colon, in whom anti-bacterial treatment was of no avail, notwithstanding the use of large doses of soluble and insoluble sulphonamides, penicillin and quinoxyl. These cases were virtually intractable. One case showed unexplained remissions, but the others became gradually worse. The mucosa of the colon was evenly oedematous, congested and fragile, like velvet plush in appearance. Large quantities of fresh blood were passed rectally. The patients grew weak, lost weight and developed a hypochromic anaemia. No skin lesions, glossitis or hypoproteinaemia were observed. Leucocyte counts were within normal limits. Skin tests and serum agglutination reactions with intestinal coliform were negative, as were blood cultures. No definite benefit was seen after treatment with supplementary vitamins, high-protein diet, haematinics and blood-transfusion. On the hypothesis that the condition might be maintained by a sensitisation mechanism, two cases were treated with anti-histamine drugs, but without improvement.

It is obvious that such cases resembled the condition of idiopathic ulcerative colitis, in their clinical course and intractability: the only difference was that they arose as direct sequelae to amoebic dysentery, the transition from one condition to the other being well-exemplified by case c.

In the cases described above, the symptoms were sufficiently marked to necessitate admission to hospital, for investigation if not for treatment. It is probable that milder forms of post-dysenteric colitis are very common indeed among personnel returning from active service in certain areas of the tropics. The majority of these cases may be expected to show the slow natural improvement of type I but it is important that the more serious inflammatory conditions of type II should be recognised at the earliest possible stage.

Illustrative Cases.

Case A. Post-dysenteric colitis: type I.

History: Bacillary and amoebic dysentery in India (1941). Recurrent diarrhoea since then, treated in Army Hospitals with courses of emetine and sulphaguanidine.

Condition on admission. Slightly under-weight, but general conditio[n] good. Anxious disposition. 5-10 bowel movements per day. Stools unformed, but no blood or mucus. 12 specimens negative for E.histolytica and 3 negative for bacterial pathogens.

Sigmoidoscopy: mucosa congested. Granular areas at recto-sigmoid junction. Scraping - epithelial cells onl[y].

Treatment: Hyoscine hydrobromide (gr. 1/200 6-hourly) and phenobarbitone (gr. ½ t.d.s.) for 6 days. No change. Without treatment, patient's condition began to improve 3 months later — 3 bowel movements per day, gained weight, felt better.

Case B. Post-dysenteric colitis: type II(a).

History: Amoebic dysentery in Egypt (1942), treated with emetine and quinoxyl. Three subsequent relapses, simila[r]ly treated. Intermittent diarrhoea (1945-46. In 1947 diarrhoea became severe and continuous.

Condition on admission. Pale and fatigued. Temperature 99-100°F. 5-6 bowel movements per day. Stools consisting largely of blood and pus. 12 stools negative for E.histolytica. Culture gave a predominance of group I paracolon bacteria. Serum agglutinated this organism to 1:50 ("O") and 1:200 ("H"). Leucocyte count 16,540 (75% polynuclear).

Sigmoidoscopy: congested mucosa in rectum and lower sigmoid colon, with extensive, irregular ulcers, most marked at recto-sigmoid junction. Scraping - negative for E.histolytica. Biopsy of edge of ulcer: mucosa largely replaced by polynuclear cells and lymphocytes.

Treatment: Rest in bed. Bland, non-residue diet (high protein, high calorie). Penicillin retention enemata daily for 10 days (1 mega unit in 100-400 cc. saline). No improvement.

6-day course of penicillin (500,000 units 4-hourly for 4 days and 6-hourly for 2 days, intramuscularly) and sulphathiazole (gm.76). Diarrhoea stopped and exudate diminished 1 week later. Sigmoidoscopy showed congested mucosa with superficial healing of ulcers. Paracolon bacteria disappeared from stools after treatment.

After 1 month's leave, patient returned symptom-free. Sigmoidoscopy: complete healing of lesions. No relapse during succeeding year.

Case C. Post-dysenteric colitis: type II(b).

History: Amoebic dysentery (India, 1943). Several relapses, all treated with emetine, quinoxyl and sulphonamides.

Admitted to hospital with amoebic dysentery relapse in 1947. Treated with emetine (gr.5), auremetine (gr.30), stovarsol (gr.120) and quinoxyl retention enemata (10). Diarrhoea persisted after treatment, and a week later E.histolytica trophozoites re-appeared in stools. Sigmoidoscopy - oedematous, friable mucosa from anal margin upwards; extensive, irregular ulcers.

Treated with succinyl sulphathiazole (gm 60) and quinoxyl retention enemata (10 days), E.histolytica disappeared, but stools became frankly purulent. Further treatment with sulphathiazole, penicillin, supplementary vitamins, etc. gave no improvement, but a spontaneous remission occurred after 3 months. This was maintained for 6 months, after which a mild relapse occurred. Faeces consistently negative for E.histolytica and bacterial pathogens. Leucocyte count within normal limits. No serum agglutinins for intestinal coliforms.

Summary

Amoebic dysentery or, less commonly, bacillary dysentery may be followed by chronic colitis, supervening after the clearance of the original infecting organism.

Thus defined, the condition of post-dysenteric colitis occurs in three forms, differing in prognosis and management, and possibly in aetiology:

Type I: non-ulcerative; large functional elements; slow, natural improvement.

Type IIa: ulcerative; element of non-specific added bacterial infection; therapeutic response to anti-bacterial treatment.

Type IIb: ulcerative; no response to anti-bacterial treatment; possibly akin to idiopathic ulcerative colitis.

SECTION III

PROTOZOOLOGICAL STUDIES IN VITRO.

1. The cultivation of *Entamoeba histolytica*.
2. *Streptococcus faecalis* and *Entamoeba histolytica*.
3. The action of emetine upon *Entamoeba histolytica*.

1. The cultivation of *Entamoeba histolytica.*

Entamoeba histolytica was first grown under artificial conditions by Boeck and Drbohlav (1925), using a medium composed of coagulated egg with a liquid overlay of Locke's solution and serum. Meanwhile, the studies of Dobell (1926 et seq.), and Yorke and Adams (1926) had furnished many facts about the morphology and life-cycle of the organism. The use of reliable culture media enabled Dobell to pursue these studies further and describe the morphological changes accompanying the growth of *E. histolytica* in remarkable detail. The physiology of the organism is less well understood and there are still two fundamental problems, with regard to its behaviour in culture, which are as yet unanswered:

(a) The dependence of *E. histolytica* upon bacteria.

(b) The exact growth requirements.

These two problems overlap to some extent, for it is probable that bacteria furnish, directly or indirectly, factors necessary for the propagation of *E. histolytica.*

A detailed protozoological study of these problems would be a very considerable task and would, in any case, be irrelevant to the subject of the present thesis. Nevertheless, problem (a) has considerable bearing upon the pathogenicity of E. histolytica, while problem (b) cannot be overlooked if one is trying to maintain reliable cultures for tests *in vitro* and *in vivo.*

The experiments described in the present section fall, therefore, into two classes: firstly, a number of experiments made in the attempt to meet the two fundamental problems, stated above; and, secondly, a coordinated series of tests *in vitro*, designed to investigate two specific problems, arising from the main subject matter of the thesis: the production by *Streptococcus faecalis* of metabolites toxic to *E. histolytica*: and the mechanism of action of emetine upon *E. histolytica.*

(a) The dependence of *Entamoeba histolytica* upon bacteria.

The production of a bacteria-free culture of E. histolytica is a matter of profound scientific importance, as a fundamental contribution to protozoological technique, or for the further study of the growth requirements

and pathogenicity of the organism. Unfortunately, in spite of repeated attempts by various workers, this has not yet been accomplished. The intimacy of the dependence of E. histolytica upon bacteria has been stressed by Dobell (1926, 1947) and Chinn et al. (1942); The latter have claimed that bacteria are necessary not only for the growth and propagation of E. histolytica, but also for the processes of excystation and encystation.

It is less clear whether this dependence, so manifest under conditions of artificial growth, is equally important under natural conditions. In the encysted state, E. histolytica can survive for a week or more at refrigerator temperature in sterile medium. In a liver abscess, trophozoites can multiply and invade the tissue under conditions which are, apparently, bacteriologically sterile (Jordan and Burrows, 1941); though Cleveland and Sanders (1930) have claimed that under experimental conditions, the induction of liver abscess in kittens requires the addition to the amoebic inoculum of bacteria capable of damaging the liver, and that tissue invasion by E. histolytica ceases when the abscess becomes bacteriologically sterile. This important claim has not yet been confirmed (see page 20).

Theoretically, bacteria may be necessary for the growth and propagation of E. histolytica, in culture media, in three ways:

(i) By producing a vital chemical substance.

(ii) By creating physical conditions (e.g. anaerobiosis) necessary for the life of the trophozoite.

(iii) By the intact bacterial cell being a necessary food substance for the amoeba.

These possibilities should be amenable to investigation in the laboratory by a series of very simple experiments; but, in practice, there is one over-riding difficulty: if cysts, sterilised of bacteria by treatment with 0.2% HCl (see page 70) are transferred to sterile medium, excystation may occur but, after this, Clostridia can be isolated from the medium. Under ordinary circumstances, growth and propagation of the metacystic amoebae occur only when certain aerobic bacteria are added to the culture. This again emphasises the intimacy of the association between E. histolytica and bacteria, for it would appear that viable Clostridia can be liberated from the cyst when it opens, or from the cell-substance of the amoeba; under anaerobic conditions,

Clostridium welchii will support further growth of the amoeba, but if the cultures are incubated aerobically, the addition of an aerobic organism is necessary.

Bold attempts to produce bacteria-free cultures were made by Rees and his colleagues (1939, 1941), using a scrupulous micro-isolation technique by which washed cysts could apparently be lifted from microscopic preparations into sterile medium. Cysts isolated in this way failed to propagate, and the initiation of a culture of amoebae required the addition of certain species of bacteria. In his various papers, Rees makes no mention of the emergence of Clostridium welchii, described above - a finding which surely represents a major obstacle to the successful utilisation of his technique.

More recently, Jacobs (1947) reported that he had established a bacteria-free culture of E. histolytica by repeated subcultures of trophozoites through media containing penicillin. After some 40 subcultures, E. histolytica was apparently able to maintain a low rate of growth in bacteriologically-sterile medium, devoid of penicillin. Unfortunately, Jacob's paper supplies few details of the criteria by which bacteriological sterility was assessed. In the writer's hands these experiments could not be confirmed: growth of E. histolytica could be maintained, up to a point, in media in which bacteriostasis and "one-loop sterility" had been obtained by the use of penicillin (1000 units per c.cm.); but the addition of penicillinase to the medium permitted the growth of coliform bacteria. Two cultures became completely sterile, but the growth of E. histolytica ceased.

Further experiments, on the lines described by Jacobs, have been carried out by Shaffer et al. (1948). In a preliminary paper, Shaffer and Frye claimed that the combined use of heat and antibiotics (penicillin and streptomycin) enabled them to carry out 185 serial transplants of E. histolytica trophozoites "from which bacteria capable of rapid multiplication were not subcultured directly, either aerobically or anaerobically". In a later paper, however, Shaffer, Ryden and Frye (1948) modified this claim by the statement that "up to the present time complete elimination of bacterial cells from the substrate for inoculation with amoebae has not been accomplished". They attach significance to the fact that the multiplication of bacteria can be arrested by penicillin to such an extent that "it is doubtful if the bacterial cells are important in the maintenance of growth of the amoebae".

There are obvious inconsistencies in these experimental data, but a definite advance has been made in that the amoeba has been shown to survive almost indefinitely in the absence of rapidly-multiplying bacteria. Whether the reduced rate of growth of the amoeba is proportional to this reduction in the bacterial component of the culture-complex, or whether the persisting bacteria simply represent an insuperable technical obstacle is still uncertain. In other words, there is still no conclusive evidence as to whether or not the apparently vital role of bacteria in cultures of _E. histolytica_ can be replaced by chemical or physical factors.

The present writer made a number of attempts to produce bacteria-free cultures of _E. histolytica_. The problem was approached as follows:-

1. Cysts of _E. histolytica_, washed and "sterilised" by treatment with 0.2% HCl, were transplanted into sterile media containing bacterial filtrates or artificial growth factors.

2. Cultures of _E. histolytica_ trophozoites with bacteria were treated by serial passages through media containing penicillin or streptomycin, with hypothetical growth factors, until complete bacteriostasis or sterility had been attained.

These experiments, summarised in tables 17 and 18, show that _E. histolytica_ failed to propagate in the absence of living bacteria, though a variable degree of survival of the trophozoites was observed, as already reported, in certain media. The only notable finding was the emergence of _Clostridium welchii_ from "sterilised" cysts along with the hatched amoeba. The experiments described hereafter originated perforce from culture-complexes in which _E. histolytica_ was grown, usually in basic medium, along with various strains of bacteria (table 19).

(b) The general growth requirements of E. histolytica.

Various workers (e.g. Cleveland and Collier, 1930; Adler, 1941) have devised culture media giving rich growth and rapid multiplication of E. histolytica, and such studies have conveyed some information as to the type of media in which growth factors exist. Fundamentally, however, a study of the growth requirements of an organism requires simplification rather than elaboration of culture media. Hence perhaps the most important contributions to this study were those of Dobell and Laidlaw (1926) and Pavlova (1938), who found that E. histolytica could be grown satisfactorily in media consisting of serum, buffered saline and rice starch. No "basic" media simpler than these have yet been devised, and each of the constituents mentioned is indispensable, directly or indirectly, for the continued growth of the organism (table 20).

A series of experiments designed to replace or simplify one or more of the "unknown" factors, present in basic media, is summarised in table 21. It is obvious that a suitable synthetic medium, of known chemical constitution, is not readily available. Similar results have been reported by other investigators (Rees et al., 1941, Snyder and Meleney, 1943). Experiments in vitro are therefore best performed in basic media, in which test substances can be easily distributed and preserved.

Cultures maintained in basic media for long periods (more than 6 months) tend to lose virulence. A number of substances were therefore added to the basic media in an attempt to identify accessory growth or "virulence" factors (table 22 and fig. 3). As a result of these experiments, cultures of E. histolytica used for animal inoculation were given serial passages in liver-infusion medium whenever their virulence became lowered.

2. Streptococcus faecalis and Entamoeba histolytica.

Bacteriological studies, conducted upon cases of relapsing amoebiases (page 31), showed that enterococci of the Streptococcus faecalis group were abnormally prevalent in dysenteric exudates.

In the course of experiments made to investigate the influence of S.faecalis in an amoebic infection in rats, it was observed that the addition of a glucose-broth culture or culture filtrate of S.faecalis to a suspension of E.histolytica trophozoites caused the latter to become significantly less infective (table 4). When added in equal volume to a suspension of E.histolytica, these glucose-broth cultures caused conspicuous morphological changes within 2 hours, followed by death of the amoebae in 4-6 hours. The same result was obtained in vitro with twelve strains of S.faecalis, including two obtained from the National Collection of Type Cultures* (Table 16).

This effect was a specific result of the growth of S.faecalis in peptone media containing glucose (0.5-1%), and was due to a heat-stable substance or substances, formed during the fermentation of glucose in the first 12 hours of growth but present also in 4-day cultures and in culture filtrates. When glucose was fermented by growing cultures of coliform organisms or staphylococci, the effect was not obtained (Table 23).

In view of the comparable effect obtained in vivo (page 13) further experiments were performed to identify the factor formed in glucose-broth cultures of S.faecalis. These experiments showed that washed cells of S.faecalis, or cultures grown in media containing less than 0.5% of fermentable carbohydrate, were without effect on E.histolytica in vitro. The effect was obtained only when the streptococcus was grown in broth or peptone media containing glucose or a related sugar (sucrose, laevulose) fermented by the strain of S.faecalis used in the experiment. Such cultures gave an acid reaction (pH 4.8-5 at 24 hours) and lost their effect on E.histolytica when neutralised by the addition of NaOH. The action on E.histolytica was not due directly the lowered pH, as the amoeba remained viable for 4 hours in saline or peptone

* NCTC Nos.370 and 6549.

adjusted to pH 4.6 by the addition of HCl. It appeared therefore that the toxic factor might be an acid substance but that its activity resided in the anion. It has already been reported (Birch-Hirschfeld, 1937) that lactic acid is toxic to E.histolytica in concentrations of 0.05 M. This finding was confirmed, and it was observed that the action of lactic acid was almost exactly comparable to that of culture-filtrates of S.faecalis: the amoebae became rounded and non-motile; the cytoplasm showed large vacuoles and the nuclear outline was lost; in 1-2 hours, the cell was ghost-like and non-viable on subculture. The toxic concentration (0.05 M) of lactic acid was readily attained in glucose-broth cultures of S.faecalis (Stewart, Jones and Rogers, 1948) but not of coliform organisms, and removal of the lactic acid from such cultures by ether-extraction eliminated the toxic action. An active, water-soluble acid product was recovered from the ether-extract. The action of lactic acid on E.histolytica increased as the pH fell, and lactates, which were inactive at pH 7, showed a similar toxic action on E.histolytica at pH 5. It seemed probable, therefore, that the principal toxic metabolite of S.faecalis was lactic acid; another substance which may enter the reaction in complex media is tyramine (Gale, 1940) which was toxic to E.histolytica in concentrations of 10%; besides lactic acid, several other organic acids were also found to be toxic to E.histolytica (table 24).

The main interest of this finding is that it may be related to a biochemical process occurring naturally in amoebic infections (page 32); it is obvious however that the toxic metabolites of S.faecalis are unlikely to be of use therapeutically, and that there is no anti-biotic factor active in high dilutions.

3. The action of Emetine upon E.histolytica.

Relapses may occur in amoebiasis even after careful treatment. Of the many drugs which have some therapeutic effect on the infection, only one, the alkaloid emetine, can be considered highly-specific, and most cases receive some form of emetine during the history of their infection. It is therefore relevant to consider the action of emetine upon E.histolytica and to investigate any bearing which this action might have on the chronicity of the treated infection.

It is a common experience that the administration of emetine to a patient with amoebic dysentery usually causes abatement of the dysenteric state. The parasite itself is not necessarily cleared from the tissues so quickly, for it may be present in scrapings from the colon during the period of administration of emetine, and for many days thereafter, even when the dysenteric phase has subsided. In clinical practice, most authorities agree that complete eradication of the infecting agent is seldom effected by emetine alone; it is now customary to use emetine injections only to control the dysenteric phase, and to follow this by combined treatment with quinoxyl, emetine bismuth iodide, and other drugs (Manson-Bahr, 1943, Adams, 1945).

Under experimental conditions, E. histolytica is not completely cleared from infected animals by therapeutic doses of emetine (table 5); complete clearance can only be effected by using doses which are profoundly toxic on continued administration. Also, it is a well-known fact that cardiac complications occasionally develop even with therapeutic doses of emetine (1 mg./Kilo./day) in man, and this imposes a further limitation upon the use of the drug in clinical practice.

The nature of the action of Emetine upon E. histolytica in vitro.

The fact that emetine exerts a powerful toxic action upon E. histolytica has long been established (Vedder, 1914, Dobell and Laidlaw, 1926). The mechanism of this action, however, is not easily interpreted. Vedder, having shown that emetine, as the main active alkaloid of ipecacuanha, was toxic to free-living amoebae, concluded that the alkaloid exerted an immediate and direct toxic action upon the protozoal cells. Dobell and Laidlaw added emetine to growing cultures of E. histolytica, and found that the toxic effect was variable in degree and in rapidity. They emphasised that the end-point of toxicity was governed largely by such factors as the reaction of the medium, time of observation, composition of the bacterial flora in the culture-complex, and nature of the culture medium. Thus, in media containing solid material, such as that of Boeck and Drbohlav, the added emetine became unequally distributed between the liquid and solid portions. Using simple liquid media (pH 7.2), and with these variable factors more or less controlled, Dobell (1947) has since

then shown that emetine sterilises growing cultures of E. histolytica at dilutions of 1 in 5 million, in 3-4 days.

The influence of the pH of the medium upon the action of emetine was studied in detail by St. John (1933), who showed that the end-point of its toxicity to cultures of E. histolytica fell to 1 in 100,000 in media of pH 6.4. This worker also reported that the drug acted slowly, over a period of 5 days, and that an early lethal action (in less than 5 hours) could only be obtained at dilutions as low as 1 in 1290. Some of the variable factors in the activity of emetine have therefore been defined, but the mechanism of the action is still far from clear (Dobell, 1947).

In the experiments reported below, emetine hydrochloride was added (a) to cultures of E. histolytica in a basic medium of horse-serum, rice starch and saline buffered with phosphate to pH 7.2, and (b) to suspensions, prepared from such cultures, with a cell density of 150-300 per c.mm. Ten strains of E. histolytica were used, each having been isolated originally from cysts in human faeces. One strain CW was grown with Bact. coli plus Cl. welchii; the other strains included in their bacterial flora non-lactose fermenting coliforms, Ps. pyocyaneus, enterococci and Clostridia (table 19). Acting in the basic medium emetine did not inhibit the growth or respiration of these bacteria, individually or in combination.

At pH 7.2, emetine (in dilutions ranging from 10^{-6} to 10^{-7}) sterilised cultures of E. histolytica in 3-7 days (table 25). A study of growth curves showed that this effect was due to a reduction of the propagation rate (figure 4). No division occurred at dilutions 10^{-5} - 10^{-4}; at higher dilutions, there was a progressive increase in the rate of division and dilutions higher than 10^{-7} showed no difference from control cultures. The addition of cysteine (0.02%) or glutathione (0.01%) did not antagonise the action of emetine.

When emetine was added to suspensions of E. histolytica, there was no change in the morphology of the cells and subculture of washed cells was always positive during the first 4 hours. Thereafter, at dilutions of 10^{-3} or less, emetine produced conspicuous degenerative changes in 80-100% of the cells, clearly shown in fresh or iron-haematoxylin-stained preparations. Bi-nucleate or dividing cells were

absent and, after 48 hours, subculture was negative. With higher dilutions, there was a proportionate decrease in the number of degenerate cells; dividing cells were present and subcultures made after 48 hours were positive, as in the controls. The morphological changes observed were, in order of appearance; loss of motility, vacuolation, coarse granulation and central retraction of cytoplasm, loss of nuclear differentiation and disintegration. These morphological changes were not specific for emetine, since cells comparable in appearance though not in numbers were seen in the control suspensions, especially after 24-48 hours when the death rate rose sharply.

From these observations, it would seem that emetine does not exert an immediate toxic reaction upon E. histolytica in the fashion of a general protoplasmic poison. It does, however, have an "amoebostatic" effect, absolute at a dilution of 10^{-5} but still evident at 10^{-7} with some strains. Cultures growing in the presence of emetine are sterilised more rapidly than controls for the reason that the cell count reaches a lower maximum, and the culture dies sooner. The end-point of survival of such a culture is determined by the ration of propagation-rate to natural death-rate of the cells. The cell envelope remains intact, and motility is preserved in the majority of the cells for 4 hours in the presence of emetine. Surviving cells, transferred from an emetine medium after 24-48 hours, grow normally. Emetine does not produce a definite stage of mitotic arrest, and its action is not antagonised by cysteine or glutathione, which serve as -SH donors. It seems likely, therefore, that emetine acts upon E. histolytica by a highly specific interference, in proportion to its concentration, with some essential cytoplasmic reaction at a phase in the growth of the cell prior to cell division.

Emetine-resistance.

Halawani (1930) claimed that E. histolytica acquired resistance to emetine when cultured for a period of months in media containing sub-lethal concentrations of emetine. Halawani's experiments have been criticised on technical grounds by Dobell (1947) and so far they have not been confirmed. It is clear from the data presented above that confirmation of Halawani's findings would be extremely difficult in view of the number of variable factors influencing the precise concentration at which emetine is inhibitory to the amoeba.

A further possibility is that *E. histolytica* may acquire resistance to emetine *in vivo*, and it has been suggested that such an occurrence might account for the high relapse-rate among cases of treated amoebiasis, returning to the United Kingdom after service in South-East Asia (Adams, 1945). Many of these cases had received numerous injections of emetine (over 100 in some cases seen by the author) and Adams noted that a considerable proportion failed to respond to further treatment.

Six strains of *E. histolytica*, isolated from such cases, were tested by the author; none of them displayed a significant degree of resistance to emetine (table 26). In two cases, from which *E. histolytica* was re-isolated after treatment with emetine, there was no definite evidence of acquisition by the parasite of emetine-resistance (table 27). In one case (S), the parasite was slightly more resistant after treatment, but it will be recalled that the inhibitory concentration of emetine tested against fresh strains of *E. histolytica*, normally varies between 10^{-6} and 10^{-7} (table 18). In view of the considerable technical limitations of the method of testing, it would be unwise to attach significance to variations within this range. In all probability, the same difficulty has been experienced by other workers and, apart from Halawani's report, there is no record of experimental support for the idea that *E. histolytica* can acquire emetine-resistance *in vivo* or *in vitro*. The idea has not been conclusively disproved, however, and a final verdict can only be made when major technical improvements are made in the method of cultivating *E. histolytica*.

From the above, it would seem that the action of emetine upon *E. histolytica* is limited and variable, *in vitro* and *in vivo*. *In vitro*, the action requires the maintenance of an inhibitory concentration of emetine in culture media for 3-7 days; *in vivo*, complete clearance of the organism cannot be obtained regularly with therapeutic doses. To some extent, these discrepancies in the action of emetine can be related to the complexity of natural and experimental amoebic infections, in both of which the pathogenesis is influenced by associated bacteria. In practice, the dual elements in the infection can be met by the simultaneous use of emetine and penicillin or other anti-bacterial agent, in the experimental animal (table 7) and in man (Hargreaves, 1946). As

Deschiens (1938) pointed out, the complexity persists even into the test-tube and compels us to regard any strain of E. histolytica (une souche de l'amibe dysenterique) as a complex of amoebae and the inseparable bacterial flora.

Since there is no proof that the parasite can acquire resistance to emetine, any part which the drug may play in the chronicity of amoebiasis in man can only exist in terms of the variability and incompleteness of its action. This fact is, of course, evident from the start, as amoebiasis would not be a chronic infection if a completely effective drug were available; but the activity of emetine in vitro in such high dilutions, and its apparent specificity, justify detailed investigation of its mechanism of action.

Summary of Section III.

The cultivation of E. histolytica.

The dependence of E. histolytica, grown in culture, upon bacteria is confirmed. In suitable media, trophozoites of E. histolytica remain viable for variable periods in bacteriologically-sterile medium, but growth and propagation occurs only in the presence of living bacterial cells. Spores of Clostridia can apparently remain viable within the cysts of E. histolytica.

Factors favouring the growth of virulence of E. histolytica are present in liver infusion. In its effect on growth, the liver factor is similar to, and may replace, the factor or factors present in serum, a necessary constituent of the basic media described by Dobell (1926) and Pavlova (1938). The liver virulence factor is absent from serum.

A number of vitamin-like substances were tested separately and together in attempts to replace bacteria in cultures of E. histolytica, without success.

Streptococcus faecalis and E. histolytica.

Certain metabolites of S. faecalis are toxic to E. histolytica in vitro. A number of substances appear to be concerned in this reaction, but most of these substances are probably simple breakdown products (e.g. lactic acid) of carbohydrates or proteins present in the media. There is no evidence that the toxic metabolites include a true anti-biotic factor, active in high dilutions.

The action of emetine upon E. histolytica.

In high dilutions (10^{-6}-10^{-7}) emetine reduces the propagation-rate of E. histolytica in cultures. At lower dilutions (10^{-3}) this "amoebostatic" effect is absolute, but there is no evidence that emetine exercises an immediate lethal action upon the undivided cells.

The inhibitory concentration of emetine against different strains of E. histolytica varies between 10^{-6} and 10^{-7}. This

variation may be related to differences in the bacterial flora accompanying these strains.

There is no proof that E.histolytica becomes resistant to the action of emetine, in vitro or in vivo.

Within its therapeutic range, emetine seldom produces complete sterilisation of an amoebic infection in vivo: this limitation of the efficacy of the drug can be explained partly by the incompleteness of its action upon E.histolytica in vitro, and partly by the participation of other factors, notably bacteria, in natural and experimental infections.

GENERAL SUMMARY:

The individual results obtained from the animal experiments of Section I, the clinical observations of Section II and the protozoological studies in vitro of Section III have been discussed and summarised in the appropriate sections. These sections of the thesis, representing a convenient three-way approach and presentation of the subject-matter rather than three separate aspects of amoebiasis,, are by no means independent of each other, and numerous cross-references have already been made between them. It is now intended to abstract the principal findings of the individual sections and of the papers in the appendix into a coherent general summary of the following aspects of amoebiasis:-

(a) Pathogenesis.

(b) Chronicity.

(c) Therapy.

(a) The pathogenesis of amoebiasis.

The induction of the intestinal infection depends primarily upon the invasiveness of E.histolytica, defined by its ability to produce a breach in the mucosa of the colon at a site of stasis. Different strains of E.histolytica vary in invasiveness and a single strain may vary in this property over a period of time. There is no evidence that the invasiveness is enhanced by passage. The virulence of E.histolytica, defined by the severity of the lesions produced, is directly related to its invasiveness, but in a more restricted sense than might be expected: widespread invasion of the colon is followed by destruction of the mucosa and submucosa, and an inflammatory reaction beyond, but this depends to a large extent upon concomitant bacterial invasion. Within wide limits, the severity of the infection is independent of the number of amoebae introduced into the bowel.

The virulence of the amoeba for eligible tissues is probably limited to digestion of the mucosa, with the production of a localised, relatively superficial focus of necrotic tissue. In this way, a

breach is established in the intact mucosa and intestinal bacteria gain access to the submucosa, causing a widespread inflammatory reaction. This may lead to extensive ulceration of the affected regions, or it may subside, presumably on account of an immune reaction by the host. Amoebae can penetrate to the deeper tissues, but the majority persist in the areas of the original foci to form the localised ulcers so characteristic of the disease.

Minute foci of the type described form the earliest recognisable lesions of amoebic infection and, in a large proportion of cases, fail to enlarge significantly; such cases are usually described as asymptomatic cyst-passers, and the infection is for practical purposes latent (Stewart, 1947). In a smaller proportion of cases, these primary foci enlarge; blood, mucus and vegetative amoebae are discharged into the bowel and the infection approaches the dysenteric phase. It is probable that mild attacks of dysentery can occur without much added bacterial infection, for the exudate in such cases contains relatively few pus cells; but severe dysenteric infections, and especially dysenteric relapses, are associated with a purulent exudate, a pronounced inflammatory reaction around the ulcerated areas and evidence of widespread tissue-invasion by virulent coliform bacteria.

(b) The chronicity of amoebiasis.

In man, amoebiasis is essentially a chronic infection. Primarily, this depends upon the persistence of *E. histolytica* in the bowel but, in the absence of added bacterial infection, the amoeba is restricted in its activity and the infection tends to remain latent. The chronicity of amoebiasis must therefore be defined in terms of two distinct stages: firstly, a stage of latent infection, of short duration in the experimentally-infected rat or kitten but more prolonged in the monkey (Dobell, 1931), dog (Faust, 1932), and sometimes indefinitely prolonged in man; and secondly, a stage when the infection becomes patent with an attack of dysentery and frequently a prolonged condition of chronic diarrhoea or dysenteric relapses. Between the two

extremes of a latent, asymptomatic infection and relapsing dysentery, amoebic infection may be associated with a number of symptoms: intermittent diarrhoea, dyspepsia, lower abdominal pain, loss of weight and lassitude.

Where the carrier rate of *E.histolytica* is high, and other forms of enteritis are prevalent, the dysenteric stage of amoebiasis is more common. This has been noted especially in the 1914-18 war and in the campaigns in South-East Asia during the 1939-45 war. Such conditions also favoured the spread of bacillary dysentery, but it has been shown that the initiation of dysentery in amoebiasis does not require a coincident *Shigella* infection. Dysenteric relapses are associated with the presence in the bowel of virulent strains of *Bact.coli* and *paracolon*, which may assume a pathogenic role in an intestine already damaged by amoebic erosion. Other intestinal bacteria, such as *Proteus*, *Bact.morgani*, Clostridia and *B.faecalis alkaligenes* are relatively inert in the infection and, under experimental conditions, *Streptococcus faecalis* may actually mitigate the infection by producing metabolites toxic to *E.histolytica*.

In addition to the activities of intestinal bacteria, it is probable that a number of other factors influence the course of amoebiasis. Lamb and Royston (1945), Payne (1945) and Hargreaves (1945, 1946), have emphasised the importance of diet and nutrition, prolonged exposure to infection, inadequate treatment and systemic diseases, such as malaria, as factors associated with the severity of amoebic dysentery in tropical campaigns. It may be inferred that active amoebiasis is a complex infection, and that the occurrence of dysentery, of relapses and remissions, and the general course of the disease are determined by the balance of power between these various factors.

The sequelae of active amoebiasis are well-represented today among many demobilised soldiers, and in Ministry of Pensions Hospitals: general ill-health, lassitude, loss of weight and dyspepsia. These symptoms may persist long after the eradication of *E.histolytica* by specific treatment and, in a proportion of cases, the condition of post-dysenteric colitis develops. Usually

this residual colitis is associated with temporary irritation of the colon by the scars and granulations of the healing lesions, and perhaps with neurotic traits. But in a small proportion of cases, post-dysenteric colitis is ulcerative in type; this may be due to persistent bacterial infection or, more rarely, it may be akin to idiopathic ulcerative colitis, and equally intractable.

(c) Therapy.

No attempt has been made in the preceding pages to assess the relative values of the numerous drugs at present available in the therapy of amoebiasis. Rational therapy demands, primarily, a recognition of the principal factors concerned in the infection : the amoeba, which can be attacked specifically by emetine or one of its derivatives, and to a lesser extent by drugs of the oxyquinolene series; the pathogenic intestinal bacteria which, in varying degree, are inhibited by penicillin, streptomycin, sulphonamides and perhaps also by certain oxyquinolene drugs; and the general hygiene and nutrition of the patient.

Therapy on these lines will effectively arrest or even cure amoebiasis in the majority of cases, but not without cost, for emetine is not infrequently toxic, within its therapeutic dose; and reference has already been made, in some detail, to the incompleteness of its action. This, together with the complexity and natural chronicity of the active infection, permits the recognition of a minority of cases for whom existing forms of therapy are inadequate.

One problem for future research can therefore be defined : the need for a new compound, primarily amoebicidal in its action but compatible with, and even active as, an anti-bacterial agent. Such a compound should replace emetine and it must therefore be much less toxic and nearly as active. This means that its amoebicidal power, or its ability to check amoebic dysentery, must far exceed that of any of the known oxyquinolene or arsenical drugs.

In conclusion, one further problem might be mentioned and, to the writer, this problem is not less important than the discovery of a new chemotherapeutic agent. Present day knowledge of the parasite and the pathogenesis of the infection is sufficient to enable rational prophylaxis and early treatment to be instituted in many endemic areas. Even the rudimentary studies described in the first paper of the appendix led to the introduction of a hygienic policy with regard to amoebiasis in an endemic region in Ceylon: all native food-handlers and a considerable number of European Service personnel were examined for infection; a number of native restaurants were placed out-of-bounds to Service personnel; the eating of uncooked green vegetables was discouraged or forbidden; and a significant number of cases was diagnosed and treated at an early stage of infection. The importance of amoebiasis as a cause of ill-health and loss of working-hours fully justified such a policy under the emergency of the war; it is equally important that a similar hygienic attitude be maintained today in many parts of the world and that amoebiasis should not be neglected until another emergency brings it into prominence.

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The earlier studies on amoebiasis, included in the papers in the Appendix, were conducted during service in the Royal Navy as pathologist to the Combined Services Hospital, Trincomalee, Ceylon. The paper "The effect of penicillin upon gram-negative bacteria: penicillin-sulphonamide therapy" (also included in the appendix) relates to work done at the City Hospital, Aberdeen. A number of the experiments described in Section I were made in the Biological Laboratories of Imperial Chemical Industries Limited, Manchester, in collaboration with Mr.W.R.Jones and by arrangement with Dr.C.M.Scott and Professor B.G.Maegraith.

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SECTION IV.

TECHNICAL METHODS.

1. Protozoological Methods.
2. Bacteriological Methods.
3. Clinical Methods.

TECHNICAL METHODS

1. Protozoological Methods.

The identification of E.histolytica in faeces:

(1) Direct examination: Smears were made in saline and Lugol's iodine, and examined microscopically for the presence of trophozoites or cysts. The morphological features of E.histolytica are well-known and call for no special description. In the early days of the investigation, in Ceylon, some difficulty was found in distinguishing small cysts (7-10 μ) of E.histolytica from Endolimax nana; the presence of a chromidial bar, best seen in saline preparations, serves to identify the former but it is not uncommon to find that the chromidial bar is absent in fully-matured, quadrinucleate cysts of E.histolytica. If small cysts, without chromidial bars are seen, accurate differentiation of the two organisms can only be made by concentrating the cysts and culturing them, or by staining with iron-haematoxylin. In the author's experience, small races of E.histolytica cysts are seen most often in cases of amoebiasis relapsing after treatment with emetine, and it might be noted that Halawani (1930) reported that emetine-resistant strains of E.histolytica were smaller than usual; but these observations have not been analysed statistically, and Halawani's findings have not been confirmed.

(2) Concentration of cysts: Two methods were employed, the first for diagnostic purposes and the second for preparing washed and sterilised cysts for initiating cultures.

Method a: (modified from Faust, 1939)

A tablespoonful of faeces was emulsified in 100 cc. tap-water and filtered through gauze into a glass urine-jar. This was allowed to stand overnight; the supernatant was removed by siphoning and the sediment re-suspended in a saturated solution of copper sulphate or zinc sulphate (specific gravity >1150). After centrifuging at 2000 revs. for 10 minutes, a loopful of fluid was lifted from the meniscus and examined microscopically. Cysts of E.histolytica concentrated in this way could usually be recognised by their size and by the presence of a chromidial bar.

When further staining was necessary, as when two or more types of cysts were present, the cysts were washed and stained with Lugol's iodine.

Cysts treated in this way with copper sulphate were invariably killed; with zinc sulphate, the cysts were viable on culture, but only after repeated washings. Hence another method was employed for concentrating cysts for the initiation of cultures of E. histolytica.

Method b: (modified from Yorke and Adams, 1946).

A portion of faeces was treated as above but the sediment was suspended in a concentrated solution of cane sugar (specific gravity 1120). This suspension was centrifuged at 2,000 revs. for 10 minutes in a conical centrifuge-tube with an elongated constriction, ¼" in diameter, at the neck. The column of liquid in the constricted part of the tube was removed by a pipette and transferred to a conical centrifuge tube containing 5 c.cm. 0.2% HCl. After 30 minutes, the tube was centrifuged at 2,000 revs. for 10 minutes. The supernatant was removed, the sediment twice washed in sterile water and centrifuged. The sediment from the final washing contained bacteriologically sterile cysts, which were transferred to culture media.

The cultivation of E. histolytica.

Cultures of E. histolytica can be prepared directly from stools containing trophozoites or cysts. But this method, although simple, is uncertain in its results, as the growth of certain bacteria or of organisms such as Blastocystis hominis may interfere with the growth of E. histolytica. Many of the cultures used in the experiments described in this thesis were therefore grown from a concentrated suspension of washed cysts, prepared by method b above. In some cases, the cysts were left unsterile, and allowed to excyst in the presence of the natural bacterial flora (coliforms, enterococci and Clostridia); in more critical experiments, such as those on growth factors, sterile cysts were transferred to media previously inoculated with a strain of Bact. coli or Ps. pyocyanea which had been found to favour the growth of E. histolytica.

It will be recalled (page 48) that spores of Cl.welchii were invariably recovered from culture media after excystation of the amoebae.

Culture of E.histolytica were maintained at 37°C. in a basic medium of the following composition (Pavlova, 1938):

Horse serum	0.5	c.cm.
Rice starch	30	mgm.
0.85% sodium chloride	9	c.cm.

The sodium chloride solution was adjusted to pH 7.2 with K_2HPO_4 and KH_2PO_4 and autoclaved in 6" x 5/8" test-tubes. Sterile rice starch and serum were added immediately before inoculation. The pooled sediments from two or more 48-72 hour cultures, grown in larger volumes (150 c.cm.) of the basic medium, were used to prepare dense suspensions of amoebae for experiments in vitro. Variations of the basic medium, or new media, were devised for certain experiments described in Section III and tables 17, 18 and 21.

Inocula for experiments in vivo were prepared from the pooled sediments of 150 c.cm. cultures in the basic medium, with the addition of liver infusion (1%) or marmite. Rats were infected by injecting 0.2 c.cm. of the sediment into the caecum, the cell-density of the suspension being adjusted to about 300 amoebae per c.cm. To infect a kitten, 1 c.cm. of suspension was injected into the ileum, immediately above the ileo-caecal valve, or 2 c.cm. into the rectum by catheter.

Subcultures. The viability of E.histolytica can only be demonstrated satisfactorily by its power to propagate in fresh medium. If the original medium contains metabolites toxic to the amoeba, or test substances such as emetine (page 54), this power may be opposed by the transference of inhibitory concentrations of these substances to the fresh medium on subculture. When this seemed likely to occur, the inoculum for subculture was washed and centrifuged twice in sterile 0.85% NaCl before being transferred to fresh medium.

Quantitative methods.

The rate of propagation of E. histolytica in culture varied considerably, depending upon the size of the inoculum, strain, bacterial flora and medium ~~(figure)~~. On the average, a 10 c.cm. culture in basic medium lived for 7 days but the number of cells present reached a peak in 2-4 days. Test cultures were therefore examined daily for 5-6 days and then subcultured. In suspensions, the optimum cell-density was found to be 150-200 cells per c.mm. If the density exceeded this, the amoebae usually died in 24 hours or less.

A rough assessment of the extent of propagation in cultures was obtained by grading $\frac{7}{8}$" cover-slip preparations of the sediment according to the following code:

0 : no amoebae in whole preparation
\+ : amoeboid cells present.
1 : 2-10 motile amoebae in whole preparation
2 : 1 amoeba per low-power field ($^{2}/_{3}$" objective, x 10 eyepiece).
3 : 2-10 amoeba per low-power field.
4 : >10 amoeba per low-power field.

A more exact count of the number of amoebae per c.mm. of culture or suspension was made by shaking the tube until the sediment was evenly dispersed and withdrawing a small amount from the middle of the tube with a capillary pipette. The liquid withdrawn was then inserted into an "Improved Neubauer" type haemocytometer, and the number (x) of cells counted in five large squares.

$x \times 20$ = no. of amoebae per c.mm. of culture.

All experiments in vitro were performed in duplicate or triplicate and the counts shown represent the mean figures. The number of tubes in any one experiment was planned to provide a fresh and untouched tube for each subculture, since the validity of a cell-count would be nullified by a previous subculture. Thus, where daily subcultures were required, as in certain experiments on emetine (page 54), a minimum of six tubes was arranged for each dilution and for the controls.

Simple differential counts were made by counting the ratio of "normal" to degenerate" forms among 100 or more cells. Degenerative changes in the amoeba appeared in the following order:-

1. The cell became rounded and non-motile (warm-stage preparation).
2. The cytoplasm showed rarefaction and the formation of large vacuoles.
3. The cytoplasm retracted, forming a granular mass in the centre or at one pole of the cell, leaving a completely clear zone in the remainder of the cell.

   In stages 2 and 3, the cells were often larger than normal, and still viable when transferred to fresh medium.
4. The cell became "ghost-like" with crenated ectoplasms, loss of nuclear outline and loose, granular cytoplasm.
5. The ectoplasm disappeared, leaving a small, round, granular mass which soon disintegrated.

These different stages are fairly well-defined, and in practice it was possible to gain some idea of the speed of action of substances toxic to E. histolytica by making full differential counts upon test and control suspensions at hourly intervals. The results shown in tables 16 and 17, however, were simplified by classifying stages 2-4 as "degenerate".

2. Bacteriological Methods.

Analysis of the full bacterial flora of the faeces: Portions of faeces or sigmoidoscopic scrapings were preserved in glycerol-saline and plated on the following agar-media: horse-blood, with or without 0.1% sodium azide; MacConkey; desoxycholate citrate; bismuth sulphite. The blood and MacConkey plates preserved the main aerobic flora; the azide plates suppressed coliform organisms but preserved gram-positive cocci (Snyder and Lichstein, 1940); the desoxycholate-citrate and bismuth sulphite plates were used as selective media to preserve certain non-lactose-fermenters and pathogens of the Shigella or Salmonella groups. The plates were incubated aerobically at 37°C. for 24-48 hours.

In some preliminary experiments, plates were also incubated anaerobically. By this means, fusiform bacteria and gram-positive rods (Clostridia) were preserved. The greater part of the flora, however, was still accounted for by coliform organisms and gram-positive cocci. Obligate anaerobes never attained predominance in cultures made from normal or pathological faeces in the series investigated. The bacteriological findings described in section II were therefore based upon aerobic cultures.

The proportion of different organisms in the faeces was assessed by a numerical survey of the colonies on the non-selective media. The various organisms isolated were subcultured into broth, peptone and carbohydrate media for biochemical reactions, animal virulence, etc.

The virulence of an organism was assessed as follows:- 6 strains of Bact.coli were isolated from normal faeces; the LD_{50} for these strains was 0.25-1.0 c.cm. 24-hour nutrient broth culture, injected intraperitoneally into white mice (20-25 gm.). A strain with LD_{50} = 0.5 c.cm. was used as a standard, and was occasionally given one passage through mice to maintain its virulence. Organisms under test were given to 3 groups of 2 mice in doses of 0.05, 0.5 and 1.0 c.cm. 24-hour broth culture, and the virulence was assessed as greater than, equal to, or less than standard Bact.coli. Alterations in virulence after passage experiments were assessed by a similar technique, using the original strain of the organism under test as a control against ten-fold

dilutions of the passage strain.

The Shigella organisms described in Section II (b) were identified by the usual biochemical and serological reactions. During the early part of the investigation, in Ceylon, standard anti-sera were obtained from the Central Military Pathological Laboratory, Poona. Subsequently, a supply of standard (M.R.C.) suspensions and sera was obtained and used throughout the remainder of the investigation. All agglutination tests were performed in Dreyer's tubes in water-baths at 37°C. or 52°C.; a tube showing visible agglutination, with clear or slightly turbid supernatant, was taken as the end-titre.

Formolized broth cultures and alcoholized suspensions of coliform organisms, isolated from the faeces, were tested for agglutination against the patients' own sera. Paracolon strains were also tested for cross-agglutination against standard Salmonella and Shigella anti-sera. With three strains, showing cross-agglutination (page 29), further identification of the antigenic components causing the reaction was effected by absorbtion tests, performed by the technique described in Mackie and MacCartney's text-book of Practical Bacteriology.

Anti-sera used in the identification of specific group I and II paracolon bacteria were prepared by injecting formolized or alcoholized suspensions of the organisms intravenously into rabbits. The dose was doubled every 7 days, and the animal was bled when samples of serum showed "O" titres of 1:200 or "H" titres of 1:1200 or higher.

The special techniques, used in the study of the effect of added bacteria in experimental amoebic infections, are described in Section I.

Estimation of penicillin in plasma.

Relatively high doses of penicillin were required to inhibit the coliform organisms which were shown to play a part in the complex infections of relapsing amoebiasis and post-dysenteric colitis (section II). Such doses produced penicillin concentrations greater than 1 unit per c.cm. in the blood, and estimation of these by the standard serial dilution

techniques was found to be unsatisfactory. A modified agar-cup assay method was therefore devised.

A shallow layer (about 3 mm. depth) of plain agar was allowed to cool in a 5" Petri plate. 5% horse-blood agar was then prepared in the usual manner and, to each 100 c.cm., melted at 45°C., 4 c.cm. of 24-hour blood-broth culture of a haemolytic streptococcus (H.S.150) was added. This seeded blood-agar was then poured over the layer of plain agar in the Petri plate to give a total depth of about 6 mm. Test-plates were incubated overnight at 37°C. Satisfactory batches showed complete and even β-type haemolysis of the blood layer, rendering the medium transparent. The remainder of the batch was stored at 4°C. In the assays, cups were made by drilling the agar with a cork-borer. The bases of the cups were sealed with a drop of melted agar. When penicillin solutions were added to these cups and the plates incubated, zones of inhibition appeared as uniform red circles with clear-cut edges. These unhaemolysed zones were measured with calipers to the nearest half-millimetre.

A haemolytic streptococcus was used as the test-organism for the following reasons:-

(a) The edges of the zones of inhibition were absolutely clear cut. This avoided the error of the "halo" which occurred with some other test-organisms.

(b) The <u>streptococcus</u> used was regularly inhibited by 0.2 units of penicillin per c.cm. This moderate degree of sensitivity prevented the occurrence of inconveniently large zones of inhibition, as happened with the Oxford <u>staphylococcus</u>.

(c) A sulphonamide-resistant strain of <u>streptococcus</u> was used, since some of the samples of plasma contained sulphonamide.

(d) There was no failure of haemolysis throughout the period of these tests (4 months).

A standard curve was drawn to represent the zones of inhibition in millimetres produced by equal volumes of solutions of crystalline sodium penicillin, in concentrations ranging from 1 to 20 units per c.cm. (figure 5). These solutions were made up by serial dilution of a stock, concentrated solution which had been assayed against the Oxford <u>Staphylococcus</u> "H" strain.

If the volume and concentrations of the penicillin solutions were assumed to be constant, the assay showed errors in zone readings on two accounts:-

(i) variations in the depth of the agar.

(ii) different batches of media.

(iii) differences in the rate of diffusion of solids through agar.

Error (i) had to be accepted, though it was minimised by using thick layers of medium (Hayes, 1945); error (ii), which was sometimes considerable, was obviated by drawing a fresh curve for each batch of media, taking the mean of three or more readings for each point. The graph in figure 5 represents the mean of ten readings, and the standard error of the mean is shown.

To investigate the third error, small volumes of concentrated penicillin in aqueous solution were added to samples of normal plasma, prepared from oxalated or heparinised blood, and assayed against standard control solutions of penicillin in water. The zones produced by the test specimens did not differ from the mean of the controls by more than twice the standard deviation for any one set of readings. It was therefore assumed that the standard curve (figure 5) could be used for estimating concentrations of penicillin in test specimens of plasma.

This method may appear cumbersome when compared with the usual methods of serial dilution; but in actual practice it was very simple, viz; seeded plates were kept at 4°C., ready for use and usually with a standard curve drawn for the batch; a single drop of the plasma under test was added to one cup on each of three plates. The other cups contained standard penicillin solutions and plaasma with added penicillinase. Equal volumes were obtained by using the same dropping pipette, rinsed between each new solution with boiling water. The whole process occupied only a few minutes and required only 1 c.cm. blood.

3. Clinical Methods:

Sigmoidoscopy: This examination was conducted upon all the cases referred to in section II, before and after, and sometimes during, treatment. Smears for microscopy and bacteriological culture were taken from ulcers, granular areas or patches of mucus with a swab or curette. In some cases (page 25) a small wedge of tissue was removed from the edges of ulcers or granulomatous areas with a curette or diathermy loop. and embedded in paraffin for histological examination.

In dysenteric cases, the preliminary sigmoidoscopy was often performed without special preparation. In test-of-cure examinations, patients were prepared as follows:-

Previous day : low-residue diet.
Saline enema in evening.

Day of examination : Saline enema at 6 a.m., retained for 15-20 minutes. Colon emptied again before examination.

The examination was performed in the knee-elbow position. No morphia was given.

Blood-cultures: Blood was withdrawn from a vein into a dry, autoclaved syringe, and then expelled into heart-infusion glucose-broth, and into an oxalated bottle from which a pour-plate was prepared.

Treatment.

General Measures. Dysenteric patients were treated in bed. The diet was bland, low residue and calculated to give at least 3,000 calories daily. The number of bowel movements daily was charted, and one specimen of faeces was retained each day for inspection and, where necessary, laboratory examination.

Special Treatment. Amoebiasis:

The standard course of treatment used in cases of amoebiasis was as follows:-

Emetine gr.1 intramuscularly, daily until the dysenteric phase subsided or until a total of gr.12 had been given. Thereafter a three-weeks' course of "combined treatment" was given as below; in non-dysenteric cases, the injections of emetine were omitted.

Odd days: Auremetine gr. 1 t.d.s.

or

Emetine bismuth iodide gr 3 at night, preceded by Phenobarbitone gr 1.

Even days: Yatren, Quinoxyl or Chiniofon retention enemata - starting with 100 c.cm. of 2½% solution and increasing the amount daily until 50 c.cm. or more were retained for 6-12 hours each day.

Stovarsol gr 4 t.d.s.

Cases relapsing after the above treatment were usually given E.B.I. and quinoxyl or diodoquin daily for two weeks. In some cases, soluble or insoluble sulphonamides and penicillin were given as for post-dysenteric colitis.

Post-dysenteric colitis:

Non-ulcerative : symptomatic treatment only - rest, bland diet, Dover's powder gr 5 t.d.s. A few cases were treated with Hyoscine hydrobromide (gr 1/200 6-hourly for 4 days) and phenobarbitone gr ½-1 t.d.s

Ulcerative : One or more of the following courses were used:-

(i) Penicillin 500,000 units IM 6-hourly for 4-6 days. Sulphathiazole gm 52 in 6 days.

(ii) Phthalyl sulphathiazole gm 80-100 in 6-10 days.

(iii) Quinoxyl retention enemata (2½%) daily for 10 days.

(iv) Penicillin retention enemata (1 mega unit in 200-500 c.cm. saline, daily for 10 days.

(v) "Benadryl" or "Antistin" 50-150 mgm. t.d.s. for 10 days.

(vi) Blood transfusions, supplementary vitamins, high-protein diets, liver extract.

<u>Test of cure</u> (1-3 months after treatment).

(a) General condition good.

(b) 12 stools negative for *E. histolytica* and exudate.

(c) Sigmoidoscopy: no active lesions.

(d) Not more than 3 bowel movements per day.

TABLE 1.

Degree of amoebic infection in experimentally infected rats

Heavy infection; ulceration; numerous amoebae	5
Inflammation and mucus; numerous amoebae	4
Inflammation; many amoebae	3
Normal; many amoebae	2
Normal; few amoebae	1
Normal; no amoebae	0

The Average Degree of Infection (A.D.I.) for a group of rats was found by assigning one of the above symbols to each animal in the group, and dividing the arithmetical total of the symbols by the number of animals in the group (Jones, 1946).

TABLE 2.

Composition of the intestinal bacterial flora in infected and uninfected rats.

Description of group	No. in group	Intestinal flora					
		Bact.coli	Aerogenes	Enterococci		Non-lactose fermenters*	
				Predominant	Present	Predominant	Present
Uninfected caecum normal	42	41	23	16	26	-	9
Infected with <u>E.histolytica</u> †A.D.I. 3 - 5	37	32	11	21	16	6	8
P						0.02	

*Non-lactose fermenters identified: saccharose-fermenting <u>paracolon</u> bacteria; <u>proteus</u>; <u>Morgan.</u>

†Average Degree of Infection (Table 1.)

The effect of bacteria in rats infected with E.histolytica.

Control groups		Test groups				General results.
No. of rats infected †	A.D.I. ●	Organism	Method and time of dosing	No. of rats infected	A.D.I. ●	
42/63	2.5	Paracolon * group I	Intracaecal 0 hrs.	36/58	2.1	Marked inflammatory reaction in caecum (Plate II). Identical paracolon bacteria recovered from exudate.
47/69	2.5	Paracolon	Oral 1-5 days.	67/83	3.0	
5/11	1.8	" group II	"	5/8	2.8	
		"	"	8/11	2.1	
36/55	2.7	Bact.coli	Oral 1-5 days	45/60	3.2	Marked inflammatory reaction in caecum.
5/11	1.8	Sh.flexnesi	Oral 1-2 days	5/8	2.5	No inflammatory reaction. Organism not recovered from exudate.
14/23	2.6	Aerogenes	Intracaecal 0 hrs.	8/19	1.9	No inflammatory reaction.

* Four strains.

● Average degree of infection (see Table 1).

† The numerator indicates the number of rats showing the presence of E.histolytica; the denominator the number in the group or groups.

TABLE 4.

The effect of Streptococcus faecalis in rats infected with E.histolytica.

Control groups		Test groups				"t" test
No. of rats infected †	A.D.I.	Route of administration	Time (hours) x	No. of rats infected	A.D.I.	P
14/23	2.6	Intracaecal	-2	2/26	0.4	< 0.01
10/18	1.9	Oral	-19	8/20	1.7	> 0.1
		"	+24	8/19	1.5	> 0.1
35/46	2.7	"	+24, 30, 48, 54	20/36	2.3	> 0.1
18/24	3.2	"	+72, 80, 96, 104, 120	12/24	1.9	0.03-0.04

x The time is expressed as hours before (-) or after (+) inoculation with E.histolytica.

† The numerator indicates the number of rats showing the presence of E.histolytica; the denominator the number in the group or groups.

TABLE 5.

Therapeutic experiments with Emetine hydrochloride in rats infected with *E.histolytica*.

DOSE		No. of rats infected †		Average degree of infection			"t" test		Notes
mgm./kilo	Days	Treated	Control	Treated	Control	Difference	t	P	
10	2nd	3/11	10/11	0.2	3.4	3.2	4.5614	$\ll$ 0.01	Toxic on continued administration.
5	2nd	3/9	9/11	0.4	2.6	2.2	3.0137	$<$ 0.01	
2.5	2-3	4/9	12/12	1.0	4.6	3.6	3.6000	$\ll$ 0.01	
2.5	5-6	3/9	8/9	1.1	4.0	2.9	3.6541	$\ll$ 0.01	
1.25	2-3	4/12	5/10	0.3	2.1	1.8	2.4791	0.01-0.02	
1.25	5-6	4/8	8/9	2.0	4.0	2.0	2.1742	0.03	

† The numerator indicates the number of rats showing the presence of *E.histolytica*; the denominator the number in the group or groups.

Table 6.

Prophylactic and therapeutic experiments with penicillin, sulphonamides and streptomycin in rats infected with E.histolytica.

TIME OF DOSAGE	DRUG AND DOSE x	No. of rats infected †		Average degree of infection			"t" test
		Treated	Control	Treated	Control	Difference	P
Prophylactic (-40, -24, -18, -1 hrs. before infection).	Phthalyl-sulphathiazole (1000) Penicillin (1000) Pen/sulpha.	7/21 3/19 2/17	11/17	1.3 0.7 0.4	2.6	1.3 1.9 2.2	0.05 <0.01 ≪0.01
Prophylactic	Sulphamezathine (500)	6/9	8/10	1.6	3.2	1.6	0.07-0.08
Therapeutic 2nd and 3rd day after infection.	Phthalyl-sulphathiazole Penicillin	7/9 2/9 1/11	6/10	3.1 0.9 0.5	2.9	- 2.0 2.4	- 0.02-0.03 <0.01
"	Sulphamezathine (200)	8/13	11/12	2.1	4.3	2.2	<0.01
"	Sulphaguanidine (200)	8/11	13/14	2.3	4.0	1.7	0.03-0.04
" (5th & 6th day)	Penicillin	3/9	8/9	1.0	4.0	3.0	≪0.01
" (2nd & 3rd day)	Streptomycin (30)	3/9	10/11	1.2	4.2	3.0	≪0.01
" (5th & 6th day)	Streptomycin	6/11	10/10	1.7	3.3	1.6	0.07-0.08

x Streptomycin and sulphonamides as mgm./kilo/day
Penicillin as units/20 gm. rat/day.

† The numerator indicates the number of rats showing the presence of E.histolytica; the denominator the number in the group or groups.

Table 7.

TABLE 7.

Therapeutic experiments with emetine and penicillin in rats infected with *E. histolytica*.

TIME OF DOSAGE	DRUG AND DOSE ·x·	No. of rats infected +		Average degree of infection			"t" test
		Treated	Control	Treated	Control	Difference	P
2nd & 3rd days	Emetine 0.62	3/11	6/7	0.4	3.7	3.3	<0.01
	Penicillin 100	6/9		2.1		1.6	-
	- Both	1/8		0.1		3.6	$\ll 0.01$
5th & 6th days	Emetine 0.62	6/9	10/10	2.3	3.3	1.0	-
	Penicillin 100	8/10		2.4		0.9	-
	- Both	2/11		0.2		3.1	<0.01

·x· Emetine as mgm/kilo/day
Penicillin as units/20 gm. rat/day.

+ The numerator indicates the number of rats showing the presence of *E. histolytica*; the denominator the number in the group or groups.

Table 8.

TABLE 8.

Relative distribution of organisms in the faeces of controls, cases of early amoebic dysentery and late relapsing amoebiasis.

Group	No. in group	Proportions	*Bact. coli*	*Bact. aerogenes*	Non-lactose-fermenters	Enterococci
Controls	120	Present Predominant	120 (100%) 114 (95%)	10 (8.3%) Nil	23 (19%) 3	74 (61.6%) 2
Early amoebic dysentery	35	Present Predominant	See text 6 (17%)	29 29 (83%)	See text	
Relapsing amoebiasis	66	Present Predominant	63 (95.4%) 47	8 (12.1%) 2	21 (31.8%) 3	59 (89.4%) 7

Table 9.

TABLE 9

Differences in the distribution of certain organisms in the faeces of controls, cases of early amoebic dysentery andlate relapsing amoebiasis.

<table>
<tr><th>Group</th><th>No. in group</th><th>Bact.aerogenes</th><th>Enterococci</th><th>Paracolon</th><th>Bact.morgani</th><th>Proteus</th><th>B.alcaligenes</th></tr>
<tr><td>Controls</td><td>120</td><td rowspan="2">18</td><td>74</td><td>10</td><td>7</td><td>3</td><td>2</td></tr>
<tr><td>Relapsing amoebiasis</td><td>66</td><td>59</td><td>14</td><td>7</td><td>2</td><td>1</td></tr>
<tr><td>Early amoebic dysentery</td><td>35</td><td>29</td><td colspan="5">See text</td></tr>
<tr><td colspan="2">P</td><td>< 0.01</td><td>< 0.01</td><td>0.02</td><td>0.2 > P > 0.1</td><td></td><td></td></tr>
</table>

TABLE 10.

Intestinal infections in Ceylon[x] (European and Asiatic Hospital admissions).

Amoebiasis	314	50%
Bacillary dysentery	230	37%
Flagellate dysentery	69	11%
Salmonella infections	14	2%

x excluding helminthic infections, typhoid fever, undiagnosed or symptomatic diarrhoea.

TABLE 11.

Relapses and recurrences of diarrhoea in a group of cases of amoebiasis and bacillary dysentery
(Ceylon 1945-46).

Group	No. treated	Recurrences after treatment			
		Within 3 weeks		Within 3 months x	
		Organism	Diarrhoea	Organism	Diarrhoea
Bacillary dysentery	123	1	1	-	
Sh. shiga	12	(1 death)			
Sh. schmitz	6	-	-	-	4 return cases
Sh. flexneri	30	-	-	-	each with
Sh. sonne	26	16	6	6	E. histolytica.
Others	10	-	-	-	
Diagnosed by exudate	39	-	1	-	
Intestinal Amoebiasis	100				
Dysentery	54	2	10	4	17
Chronic diarrhoea	46	-	8	2	12
Dual infection	5	-	-	2	2

x After further courses of treatment.

TABLE 12.

Cases of acute and chronic diarrhoea in Service patients or Merchant seamen returning to England from the Tropics. (Hospital admissions to the Tropical Diseases Centre, Smithdown Road Hospital, Liverpool).

Bacillary dysentery		2
Acute	2	
Chronic	-	
Intestinal amoebiasis		
Dysentery	96	246
Chronic diarrhoea	150	
Other infections	4	
Post-dysenteric colitis	49	

TABLE 15.

The main clinical features of 49 cases of post-dysenteric colitis.

History	Bacillary dysentery	12	Previous diarrhoea	50
	Amoebiasis	28	Intermittent diarrhoea	29
	Both	9	Constipation	-
Symptoms	Abdominal pain	26	Continuous diarrhoea	30
	Dyspepsia	15		
	Lassitude	25		
	Loss of weight	23		
	Good health	15		
Sigmoidoscopic findings			**Faeces**	
Normal		18	Loose. No exudate	18
Granular areas		9	Loose. No exudate, or scanty mucoid exudate	9
Ulcers		18	Blood and pus present	22
"Plush"		4		

TABLE 14.

Classification of 49 cases of post-dysenteric colitis.

No.	Condition of colon	Region of colon most affected.	Other findings.	Type of case (Provisional classification)	
18	Normal of spastic. Mucosa normal. No exudate.	-	General condition normally good. Neurotic tendency present.	Neurosis.	Type I. Functional post-dysenteric colitis.
9	Wall normal or spastic. Granular areas on mucosa, formed of small pitted scars, pin-point depressions and patchy hyperaemia. Mucoid exudate.	Recto-sigmoid junction.	General condition good.	"Irritable colon".	
18	Areas of ulceration. intervening mucosa oedematous and congested. Severe diarrhoea with blood and pus in stools.	Recto-sigmoid junction.	General condition variable. Occasionally leucocytosis and serum agglutinins for coliform organisms.	(a) Added bacterial infection.	Type II. Ulcerative post-dysenteric colitis.
4	Entire mucosa intensely congested and oedematous. Superficial sloughing. Copious exudate of blood and muco-pus.	Entire area visualised by sigmoidoscopy.	General condition poor. Anaemia.	(b) Aetiology unknown.	

CASE NO.	HISTORY	BACTERIAL* FLORA OF FAECES	SERUM AGGLUTINATION TITRE	TREATMENT	RESULT
1	Bacillary dys.	Enterococci	ϕ	Penicillin (enemata) 10^6 units x 10.	Improved±
2	Amoebiasis	Bact.coli Enterococci	- ϕ	Quinoxyl + sulphasuccidine Pen. 8 x 10^6 units IM) Sulphathiazole gm. 52)	No change.
3	Amoebiasis	Bact.coli Enterococci Paracolon	- ϕ -	Penicillin (enemata) 10^6 units x 10	Improved
4	Amoebiasis	Bact.coli Enterococci Bact.morgani	(0) 100 ϕ -	Penicillin 8 x 10^6 IM Sulphasuccidine gm.60	Slow improvement
5	Amoebiasis	Bact.coli Enterococci Paracolon+	- - (0) 100	Penicillin (enemata) 10^6 x 10	Improved
6	Amoebiasis	Bact.coli Enterococci Paracolon+	- ϕ (0) 50 (H) 200	Penicillin (enemata) Pen 8 x 10^6 IM) Sulphathiazole gm.52)	No change Rapid improvement
7	Amoebiasis	Bact.coli Paracolon	- -	Quinoxyl - $2\frac{1}{2}$% retention enemata	Slow improvement
8	Bacillary dys. Amoebiasis	Bact.aerogenes Paracolon	- (0) 50	Quinoxyl $2\frac{1}{2}$% R.E. Penicillin RE 10^6 x 10	No change Improvement
9	Bacillary dys. Amoebiasis	Bact.coli Bact.aerogenes Enterococci	ϕ ϕ ϕ	Penicillin 8 x 10^6 IM) Sulphathiazole gm 52)	Improvement
10	Bacillary dys. Amoebiasis	Bact.coli Paracolon	- -	Penicillin R.E. 10^6 x 10	Improvement
11	Amoebiasis	Bact.coli Enterococci	((0) 50 ((H) 200 ϕ	Penicillin 8 x 10^6 IM) Sulphathiazole gm 52) Sulphasuccidine gm 60	Improvement
12	Amoebiasis	Bact.coli Bact.aerogenes Paracolon Enterococci	(0) 50 - - ϕ	Penicillin 8 x 10^6 IM) Sulphathiazole gm 52) Benadryl	No change No change
13	Amoebiasis	Bact.coli Paracolon	(H) 50 (H) 200	Quinoxyl $2\frac{1}{2}$% RE.	Slow improvement

* excluding _Clostridia_

\+ eliminated after treatment

ϕ not done

\- negative

N.B. ± _Improved_ = lessening of diarrhoea, diminution of exudate, healing of lesions (sigmoidoscopy).

TABLE 15.

TABLE 16.

Plasma concentrations attained with large doses of penicillin.
(expressed as Oxford units per c.cm. plasma at hours after injection).

Intra-muscular injection.

Dose (Oxford units)	Vehicle of injection	No. of observations	Mean plasma concentration. (units per c.c.)			
			½ hr.	1 hr.	3 hr.	6 hr.
100,000	Saline	1	3.2	2.7	1	-
125,000	Beeswax	4		2.4	1	1
200,000	Beeswax	5		1.8	1.2	<0.5
500,000	Saline	2	8.5		2.6	1
750,000	"		11.5			1
1,000,000	"		18	20		1

Intra-muscular drip.

Dose in 24 hr. (Oxford units)	No. of observations	Mean plasma concentrations (units per c.cm.)		
		Maximum	Minimum	Mean
1,000,000	2	3.0	1.0	2.0
2,000,000	2	3.3	1.6	2.5
3,000,000	2	5.0	2.0	2.0
5,000,000	2	5.0	3.4	4.4

TABLE 17.

Summary of experiments made in the attempt to grow bacteria free cultures from washed and sterilised cysts of E.histolytica.

Culture medium	Additions	Incubation	Sub-cultures		Growth of E.histolytica (increase in cell-count)
			Amoebae No. of serial positives	Bacteria	
Basic (page)	-	Aerobic	-	Cl.welchii	-
	-	Anaerobic	5	Cl.welchii	+
Basic + 0.1% Marmite	B.coli	Aerobic	>10	Cl.welchii B.coli	+
	" filtrate	"	-	Cl.welchii	-
Basic + 1% liver infusion	" "	"	-	Cl.welchii	-
Complex x	Penicillin 1000 units per c.cm.	"	-	-	-
Complex + 5% chick embryo extract	"	Aerobic Anaerobic	-	-	-

x Composition of complex medium:

Solid

Sucrose	gm.	1
Cholesterol	mgm.	20
Cholin e	mgm.	10
Adenylic acid	mgm.	20
Folic acid	mgm.	10
Ca.panthothenate	mgm.	10
p-Aminobenzoic acid	mgm.	10
Pyridoxin	mgm.	10
Riboflavin	mgm.	10
Thiamin	mgm.	10
Agar	gm.	30
Phosphate-buffer-saline	to	1 litre

Solids added to 3% agar, melted at 60°C., then sloped in 5 c.c. amounts.
10 c.c. liquid overlay added to each tube.

Liquid overlay

Casein hydrolysate 1%
Phosphate-buffer-saline
Penicillin to give 100 units per c.cm.

TABLE 18.

Summary of experiments made to eliminate bacteria from growing cultures of <u>E.histolytica</u>.

Culture Medium	Additions	Incubation	Subcultures		Growth of E.histolytica (increase in cell-count)
			Motile amoebae present (No. of serial positives)	Bacteria	
Basic	Penicillin (1000 units/c.cm.)	Aerobic	> 10	-	-
Egg	"	"	> 10	Coliforms	-
Liver	"	"	> 10	Coliforms	-
Complex (table 17)	"	"	> 10	Coliforms	-
"	(5000 units/c.cm.)	"	> 10	-	-
"	(1000 units/c.cm.)	Anaerobic	NIL	-	-
"	(5000 units/c.cm.)	"	> 10	-	-
"	Streptomycin (15 mgm %)	(Aerobic (Anaerobic	> 10 NIL	-	-

TABLE 19.

Bacterial flora of culture strains of E.histolytica.

Strain	Bacterial Flora	Notes on preparation
ARE	Bact. aerogenes Streptococcus faecalis Clostridia	Cysts washed. Natural flora.
C	Bact.coli	Bact.coli added to "sterilised" cysts in media containing penicillin (1000 u./c.cm.)
CE	Bact.coli	Washed cysts transferred to media containing penicillin.
WSC	Bact.coli Cl.welchii B.subtilis	Subtilis and Bact.coli added to "sterilised" cysts.
CTER	Bact.coli M.tetragenus S.faecalis Clostridia	Natural flora.
CWE	Bact.coli Cl.welchii Enterococci	Natural flora.
CE	Bact.coli Enterococci	"Sterilised" cysts treated with acriflavine 1:50,000.
CR/SA	Bact.coli Staph.aureus Clostridia	Natural flora.
CW	Bact.coli Cl.welchii	Bact.coli added to "sterilised" cysts.
CPW	Bact.coli Cl.welchii Ps.pyocyanea	Natural flora.

TABLE 20.

Conditions necessary for the growth of E.histolytica in culture media.

Condition	Supplied by	Notes
Protein ? amino acid ? peptone	Serum	Serum replaceable by liver infusion or coagulated egg.
Carbohydrate	Rice starch	Antagonised by certain bacterial ferments.
Fat ?phospholipids	Serum	Cholesterol necessary. (Snyder & Meleney,1943)
Minerals Na+	Saline	
Electrolytes Cl^- " H_2PO_4 " PO_4	Saline Phosphate buffer "	Growth at pH range 5.6-8
Low oxygen tension	Aerobic bacteria	
Unknown growth factors	Bacteria	

TABLE 21.

Summary of experiments designed to replace the primary growth factors for E.histolytica in basic medium.

Primary growth factor	Replaced by	Result
Horse-serum	Human serum Monkey serum Rabbit serum	Full growth.
	Glycine 0.1% Alanine 0.1% Cysteine 0.1% Tryptophane 0.1% Peptone Casein hydrolysate	No growth.
	Egg (coagulated)	Full growth (Boeck & Drhobhlav 192
	Liver infusion 1%	Full growth.
	Agar 2%	No growth.
	Liver infusion + agar	Full growth.
Rice starch	Glucose 0.5% Sucrose 0.5% Mannitol 0.5% Dulcitol 0.5% Dextrin 0.5% Corn starch 0.5%	No growth.
Bacteria	Bact.coli-filtrate of 24 hour broth. Bact.coli -filtrate of basic medium. Bact.coli + Cl.welchii - filtrate of basic medium + Marmite 1%	No growth.

TABLE 22.

Substances tested as accessory growth factors of E.histolytica in culture.

Factor	Effect, compared with controls (basic media)	
	Growth	Virulence to rats
Liver - infusion - proteolysed - extract	Promoted	Augmented
Vitamin B group. 0.1% Aneurin 0.1% Riboflavin 0.1% Pyridoxin 0.1% Ca pantothenate 0.1% Folic acid	No effect	Not investigated
0.1% Marmite	Promoted	No effect
Vitamin C	No effect	Not investigated
Amino-acids. 0.1% glycine 0.1% alanine 0.1% cysteine 0.1% tryptophane 0.1% casein	No effect	Not investigated
0.02% cysteine	- retarded	
Horse-blood 1%	Slightly retarded	No effect

TABLE 23.

Antagonism between E. histolytica and S. faecalis in media containing carbohydrate.

Bacteria added	Carbohydrate in medium	% degenerate cells after 2 hrs.*	Sub-culture at 2 hrs.	Sub-culture at 4 hrs.
S. faecalis	Glucose 0.5%	90%	+	+
	" 1.0%	91%	+	-
"	Laevulose 0.3%	88%	+	+
	1.0%	90%	+	-
"	Sucrose 1.0%	82%	+	+
"	Lactose 1.0%	14%	+	+
"	NIL -	16%	+	+
NIL	Glucose 1.0%	20%	+	+
Bact. coli	Glucose 1.0%	36%	+	+
"	" 0.5%	22%	+	+
Staph. aureus	" 1.0%	26%	+	+
Bact. paracolon	" 1.0%	20%	+	+
"	Laevulose 1.0%	22%	+	+
Bact. aerogenes	Glucose 1.0%	21%	+	+

* Technique of cell counts given in Section IV. (Technical methods).

TABLE 24.

Effect of organic acids on E.histolytica in vitro.

Substance added	Molar concentration	pH (adjusted)	% degenerate cells after 2 hrs.	Sub-cultures at 2 hrs.	Sub-cultures at 4 hrs.
Lactic acid	0.025	4.2	20		+
	0.05	4.0	100	-	
	0.05	7.0	10		+
Calcium lactate	0.05	7.0	10		+
	0.1	7.0	12		+
	0.05	4.2	100	-	
	0.05	5.1	67	-	
Lithium lactate	0.05	7.0	12		+
	0.1	7.0	14		+
	0.05	4.2	98	-	
	0.05	5.0	80	+	
Formic acid	0.05	5.2	90	+	-
Succinic acid	0.05	-	80		
Controls: NIL	-	3.9	14		+
Controls: NIL	-	7.0	12		+

TABLE 25.

Dilutions of emetine effective in sterilising cultures of E.histolytica.

Strain (see table 19)	Dilution of emetine	Days required to sterilise culture
ARE	10^{-6}	4
C	5×10^{-6}	4
CE	3×10^{-6}	3
WSC	10^{-6}	3
CTER	10^{-6}	4
CWE	10^{-7}	5
CE	5×10^{-6}	4
CR/SA	10^{-6}	3
CW	10^{-7}	5
CPW	10^{-6}	5

TABLE 26.

Concentrations of Emetine required to inhibit strains of E.histolytica, isolated from cases receiving repeated courses of treatment with emetine.

Strain	Concentration of emetine	
1	10^{-6}	Cultures sterilised in 3-6 days. (see table for comparison with fresh strains of E.histolytica).
2	5×10^{-6}	
3	3×10^{-6}	
4	10^{-7}	
5	10^{-6}	
6	5×10^{-6}	

TABLE 27.

Concentrations of Emetine required to inhibit two strains of E.histolytica, isolated and re-isolated from two cases before and after treatment with Emetine hydrochloride (gr 6) and Auremetine (gr 30).

Strain	Concentration of emetine		
	Before treatment	After treatment	
W	5×10^{-6}	5×10^{-6}	Cultures sterilised in 3-6 days
S	10^{-7}	5×10^{-6}	

TABLE 27.

Biochemical and serological reactions of 24 strains of <u>Bact.paracolon</u>, isolated from cases of relapsing amoebiasis.

STRAIN	Motility	Biochemical reactions: LITMUS MILK	LACTOSE (48 hr.)	GLUCOSE	MANNITOL	DULCITOL	SACCHAROSE	H_2S	INDOLE	METHYL RED	VOSGES-PROSKAUER	Citrate-utilisation	Serological reactions: Agglutination by patient's serum (titre) 'O'	'H'	Cross-agglutination with standard anti-sera (titre)
K	+	alk.	No fermentation (48 hrs.)	AG in 24 hrs.	AG in 24 hrs.	AG	-	-	+	+	-	-	1:50		s.paratyphi A 'O' 1:400
T	+	alk.				-	-	-	-	-	+	+	1:50	1:200	NIL
N	+	alk.				AG	-		+	+	-		NIL		s.paratyphi A 'O' 1:50
Ne	-					AG	-	+	+	+	-		NIL		Flexner Y 1:250
F	+					-	-	-	-	-	+	-	NIL		NIL
W	+					A	-	-	+	+	-	+	NIL		NIL
J	+					-	-	-	+	+	-	-	NIL		NIL
H	-					AG	-	-	+	+	-	-	1:50	NIL	NIL
Me	-					-	-		-	+	-	-	NIL		NIL
R	+					-	AG		+	+	-	-	NIL		NIL
L	-					-	-		-	+	-	-	NIL		NIL
O	+					-	-		+	+	-		NIL		NIL
B	+					-	-		+	+	-		NIL		NIL
A	+					-	-		-	+	-		NIL		NIL
W						-	-		+				NIL		NIL
Ro	+	alk.				-	-		+	+	-		NIL		NIL
M	+	alk.				-	-		-	+	-		NIL		NIL
J	-	alk.				-	-	-	+	+	-		NIL		NIL
S	+	alk.				-	-		+	+	-		NIL		NIL
Ma	+	alk.				AG	AG		-	+	-		NIL		NIL
I						AG	-	+	+				NIL		NIL
P						-	-						NIL		NIL
CB	+					AG	-		+	+	-		NIL		NIL
Ba						-	-		+	+	-		NIL		NIL

A = acid. Alk = alkaline.
AG = acid and gas.
- = negative reaction.

Key to Groups (Sevitt,1945)

Group	Dulcitol	Saccharose	Serological properties
I	-	-	Uniform
II	+	-	Uniform
III	-	+	Diverse
IV	+	+	Diverse

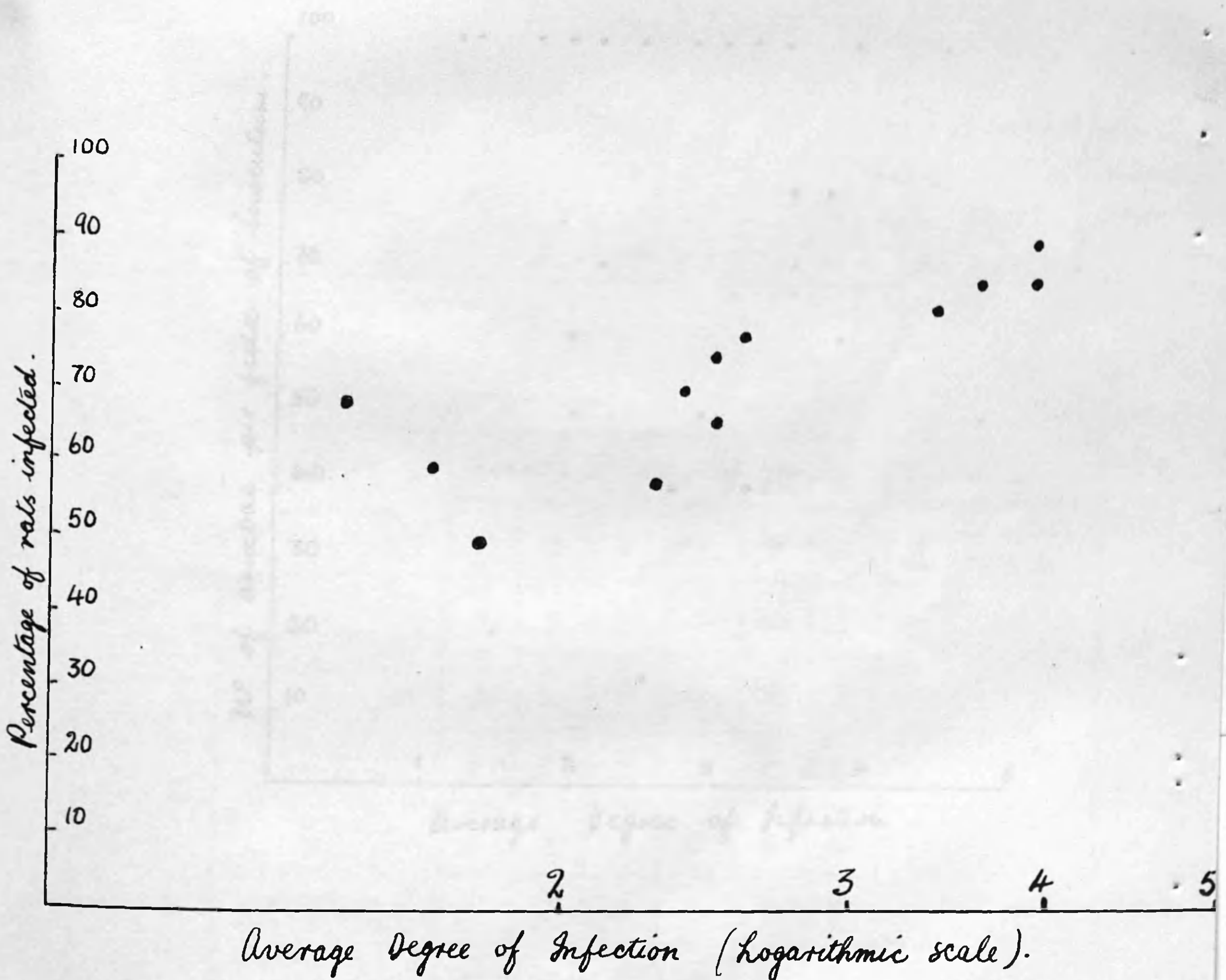
TABLE 28.

Detailed biochemical reactions of six strains of Bact.paracolon.

BIOCHEMICAL REACTIONS	Strain and group of Bact.paracolon: K (II)	H(II)	F(I)	W(IV)	T(I)	J(L)	Salmonella thomson (control)
Fermentations tested over 10 days: Glucose	Monosaccharides rapidly fermented (24-48 hrs.) by all strains.						
Xylose							
Galactose							
Laevulose							
Arabinose							
Lactose	AG(3)	← NIL →					
Maltose	AG(1)→ Neut(4)	AG(1) → Neut(6)	AG(1)	NIL	AG(1)→ Neut(6)	AG (1)	AG(1)
Saccharose	← NIL →			A(2)	← NIL →		
Rhamnose	← AG(1) →			NIL	AG(1)	A(1)	AG(1)
Raffinose	← NIL →						
Trehalose	AG(1) → Neut(6)			NIL	AG(1)→ Neut(6)	AG(1)	
Dextrin	NIL		A(4)	A(4)	NIL	A(3)	NIL
Mannitol	← AG(1) →						
Dulcitol	AG(1)		NIL	A(2)	NIL		AG(1) → Neut(5)
Sorbitol	AG(1)→Neut(4)			← NIL →			AG(1) → Neut(4)
Inositol	← NIL →			A(1) → AG(3)	← NIL →		
Adonitol	← NIL →			AG(1)	← NIL →		AG(1) → Neut(3)
Salicin	← NIL →			AG(1)	A(6)	← NIL →	
Indole (96 hr.)	+	+	-	+	-	+	-
Methyl Red	+	+	-	+	-	+	+
Voges-Proskauer	-	-	+	-	+	-	-
Citrate(growth)	-	-	-	+	+	-	+
H_2S	-	-	-	-	-	-	+

A = acid.
AG(4) = acid and gas in 4 days.
Neut = neutral.

Figure 1. Relationship between infectivity and virulence of E. histolytica in groups of experimentally-infected rats.



Each point represents a group of 8-20 rats.

12 pairs — Correlation coefficient 0.8784. $P \leqslant 0{\cdot}01$.

Figure 2. Degrees of infection produced in the rat by different-sized inocula of E. histolytica.

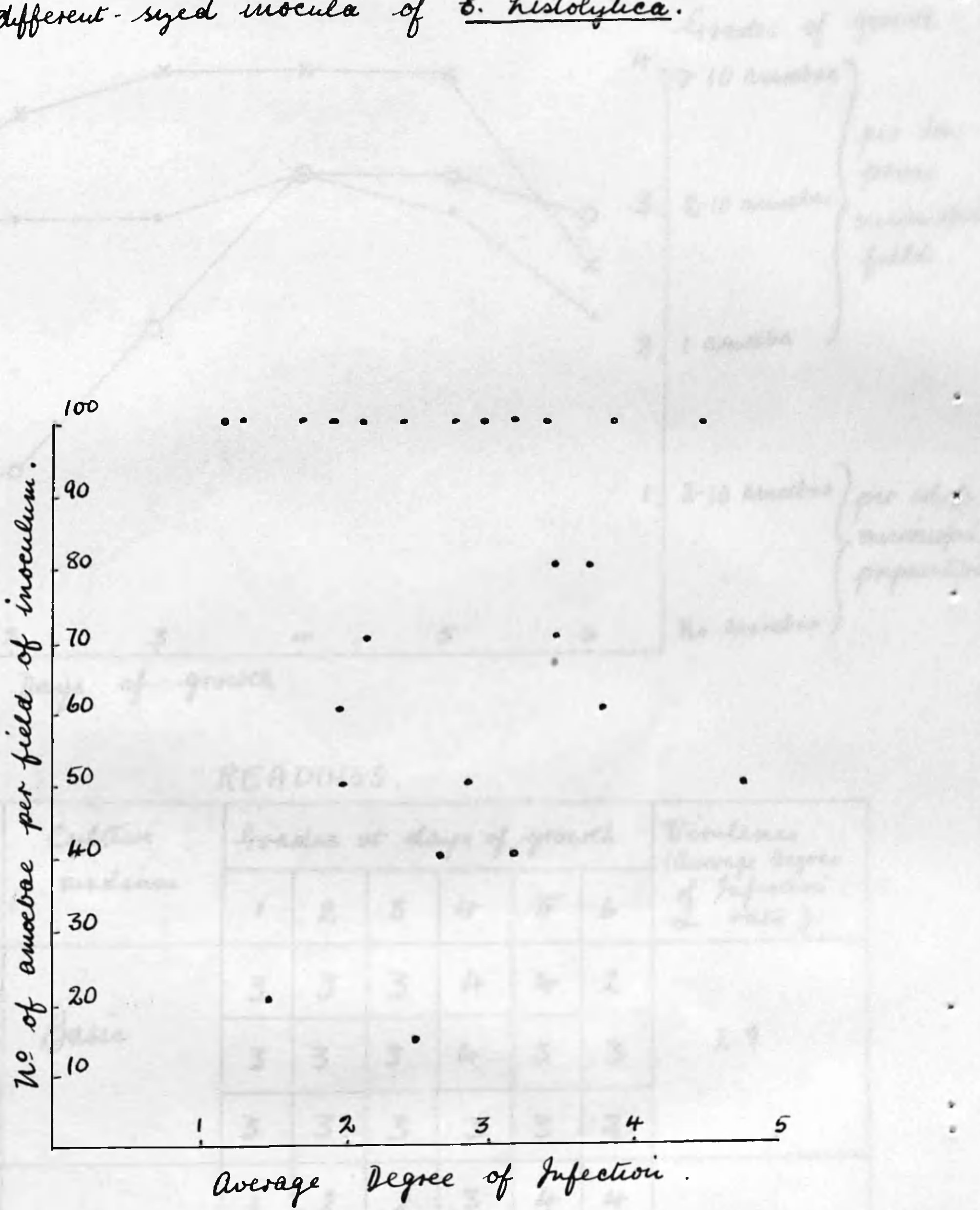
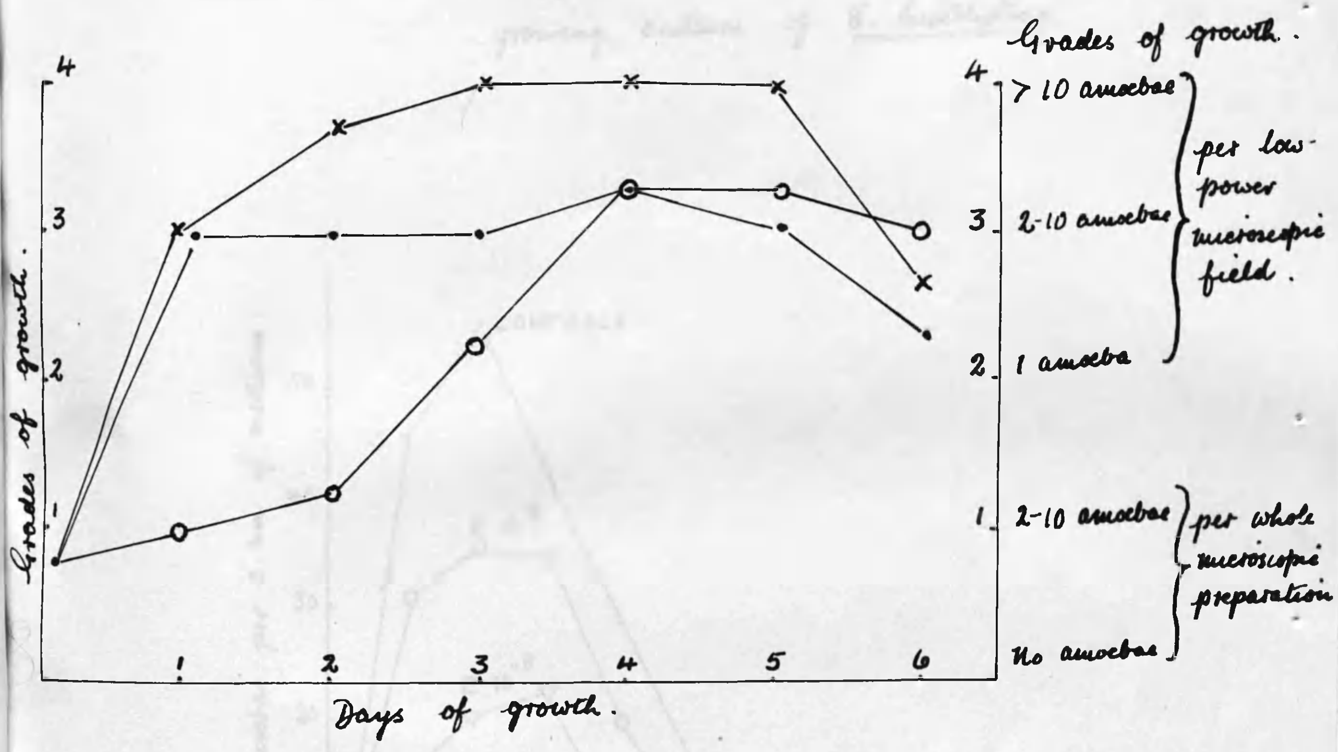


Figure 3: Effect of liver infusion and blood on the growth and virulence of *E. histolytica*.

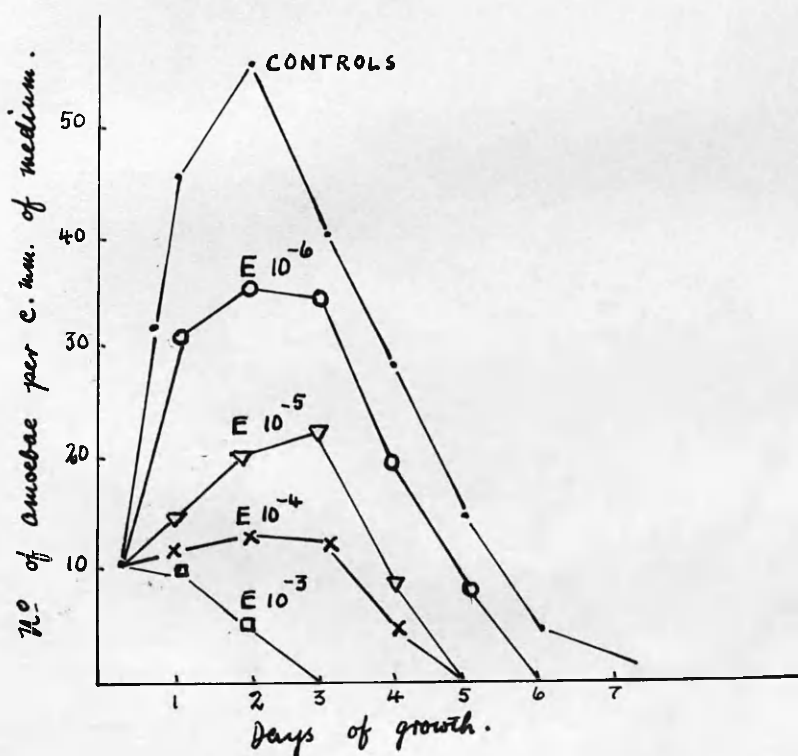


READINGS.

Graph	Culture medium	Grades at days of growth.						Virulence (Average Degree of Infection in rats).
		1	2	3	4	5	6	
•—•	Basic	3	3	3	4	4	2	2·9
		3	3	3	4	3	3	
		3	3	3	3	3	2	
o—o	Basic + 2% horse-blood	1	2	2	3	4	4	0·0
		1	1	3	4	3	3	
		1	1	2	4	4	2	
x—x	Basic + 2% liver infusion.	3	4	4	4	4	3	4·0
		3	4	4	4	4	2	
		3	3	4	4	4	3	

The graph is constructed from the mean values of each set of three readings.

Figure 4. Action of emetine hydrochloride (E) upon a growing culture of E. histolytica.

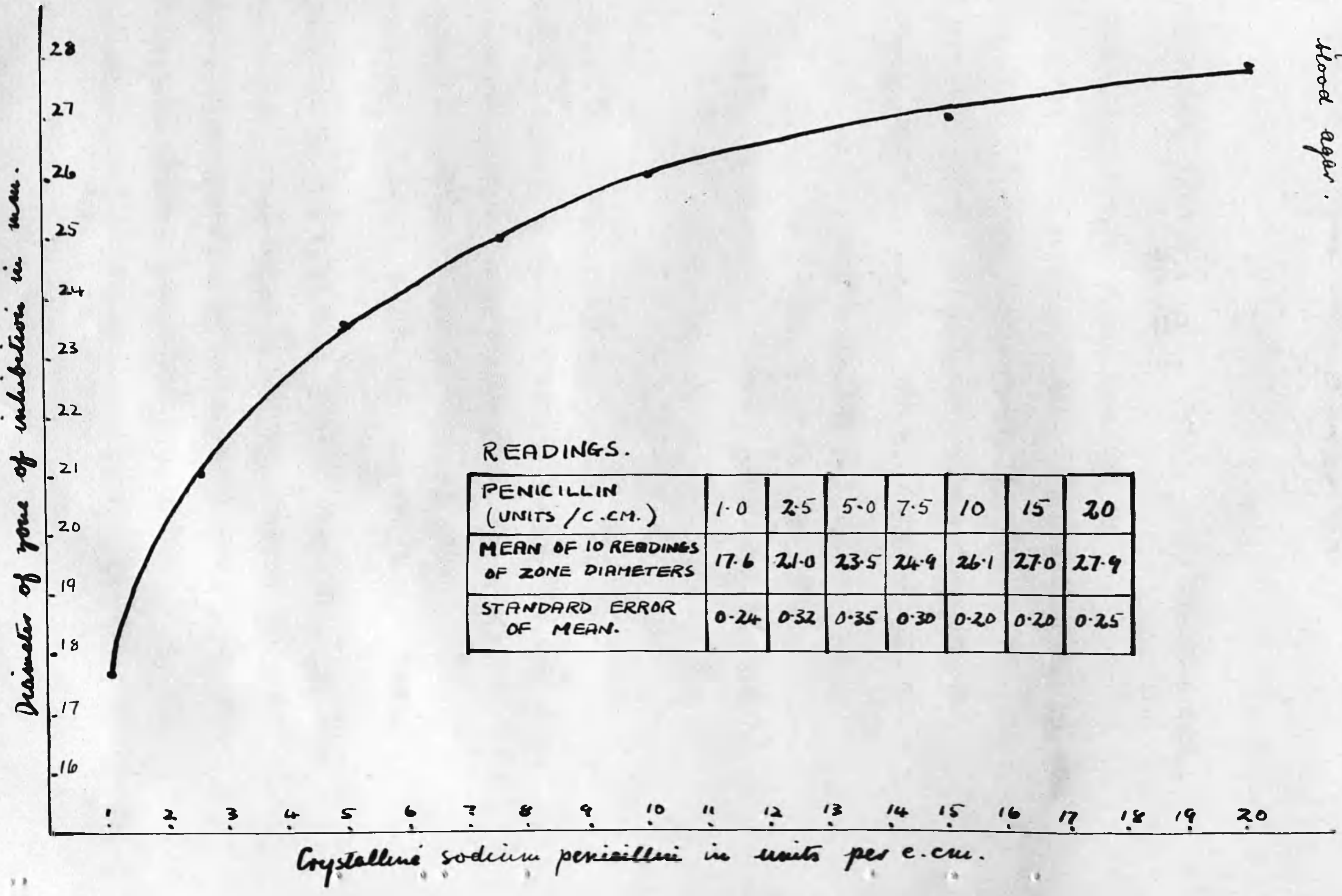


SUBCULTURES AT (DAYS)

		1	2	3	4	5	6	7.
EMETINE	10^{-3}	+	+	−				
	10^{-4}	+	+	+	−			
	10^{-5}	+	+	+	+	−		
	10^{-6}	+	+	+	+	+	−	
	10^{-7}	+	+	+	+	+	+	−
CONTROLS.		+	+	+	+	+	+	+

The graph is constructed from the mean of three readings.

Figure 5. Estimation of penicillin concentrations in plasma — standard curve showing diameters of zones of inhibition of test-organism (Haemolytic streptococcus group A No 150) on blood agar.



PENICILLIN (UNITS / C.CM.)	1·0	2·5	5·0	7·5	10	15	20
MEAN OF 10 READINGS OF ZONE DIAMETERS	17·6	21·0	23·5	24·9	26·1	27·0	27·9
STANDARD ERROR OF MEAN.	0·24	0·32	0·35	0·30	0·20	0·20	0·25

REFERENCES

A Acton, H.W. (1933). Trans.R.Soc.Trop.Med.Hyg., 27, 119.

Adams, A.R.D. (1945). ibid., 38, 237.

Adler, S. and Fouer, A. (1941). Lancet, 240, 243.

Atchley, F.O. (1936). Amer.J.Hyg., 23, 410.

B Baetjer, W.A. and Sellards, A.W. (1914). Johns Hopk.Hosp.Bull., 25, 165.

Bardsley, D.A. (1934). J.Hyg.Camb., 34, 38.

Barnes, L.A. and Cherry, W.B. (1946). Amer.J.Publ.Hlth., 36, 481.

Bengtson, I.A. (1919). J.Infect.Dis., 24, 428.

Bieling, R. (1935). Arch.f.Schiffs-u.Tropenhyg., 39, 49.

Birch-Hirschfeld, L. (1937). Z.Hyg. u. Infekt., 119, 91.

Bloom, H. (1944). Lancet, ii, 558.

Böe, J. (1939). Zbl. Bakt., I., Orig., 143, 393.

Boeck, W.C. and Drbohlav, J. (1925). Amer.J.Hyg., 5, 371.

C Chang, S.L. (1945). J.Infect.Dis., 76, 126.

Chatton, E. (1917). Bull.Soc.Path.Exot., 10, 794.

Chiang, S.F. (1925). Proc.Nat.Acad.Sci., Wash., 11, 239.

―――――― (1925). Nat.Med.J.China, 11, 440.

Chinn, B.D., Jacobs, L, Reardon, L.V. and Rees, C.W., (1942). Amer.J.Trop.Med., 22, 137.

Cleveland, R.D. and Collier, A. (1930). Amer.J.Hyg., 12, 606.

―――――― and Sanders, E.P. (1930). Science, 72, 149.

Coghill, N.F. (1945). (Correspondence) Lancet, 249, 319.

Craig, C.F. (1927). Amer.J.Trop.Med., 7, 225.

―――――― (1934). Amebiasis and Amebic Dysentery. Springfield, Ill. : C.C.Thomas.

D Deschiens, R. (1938). Ann. Inst. Pasteur, 61, 5.

Dible, J.H. (1921). J. Path. Bact., 24, 3.

Dobell, C. (1916). Spec. Rep. Ser. Med. Res. Comm., No. 6.

---------- (1921). M.R.C. Special Report series, 53-59.

---------- (1927). Parasitology, 19, 288.

---------- (1931). Ibid., 23, 1.

---------- (1947). Ann. Soc. Belge. Med. Trop., 27 (Supplement), 201.

---------- and Laidlaw, P.P. (1926). Parasitology, 18, 203.

Dudgeon, L.S. and Pulvertaft, R.J.V. (1927). J. Hyg. Camb., 26, 285.

E

F Fairbrother, R.W. (1946). Brit. Med. J., 2, 489.

Faust, E.C. (1932). Amer. J. Trop. Med., 12, 37.

---------- (1939). J. Parasitol., 25, 241.

Felsenfeld, O. and Young, V.M. (1945). Amer. J. Digest. Dis., 12, 396.

Ferguson, W.W. and Wheeler, W.E. (1946). J. Bact., 51, 107.

Fisher, R.A. and Yates, F. (1943). Statistical tables for biological, agricultural and medical research. Edinburgh: Oliver & Boyd.

Frye, W.W. and Meleney, H.E. (1933). Amer. J. Hyg., 18, 543.

G Gale, E.F. (1940). Biochem. J., 34, 846.

Glynn, E., Berridge, E.M., Foley, V., Price, M. and Robinson, A.L. (1917). Spec. Rep. Ser. Med. Res. Comm., No. 7.

H Halawani, A. (1930). Ann. Trop. Med. Parasitol., 24, 273.

Hargreaves, W.H. (1945). Trans. R. Soc. Trop. Med. Hyg., 38, 244.

---------- (1946). Quart. J. Med., 15, 1.

Hayes, W. (1945). J. Path. Bact., 57, 457.

Hegner, R., Johnson, C.M. and Stabler, R.M. (1932). Amer. J. Hyg., 15, 394.

Hiyeda, K. (1930). Amer. J. Hyg., 12, 401.

Horster, A. (1942). Deutsch. Trop. Zeitschr., 46, 258.

Hurst, A. (1943). Medical Diseases of War. London: Edward Arnold & Co.

I

J Jacobs, L. (1947). Amer.J.Hyg., 46, 172.

Jones, W.R. (1946). Ann.Trop.Med.Parasitol., 40, 130.

---------- (1947). Brit.J.Pharmacol., 2, 217.

Jordan, E.D. and Burrows, W. (1941). Text-book of Bacteriology. Philadelphia: W.B.Saunders Co.

K Kartulis, S. (1891). Zbl.Bakt., I, Orig., 9, 365.

Kempny, J.C. (1946). Rev.Admin.Nac.Agua., Buenos Aires, 10, 15.

Kershaw, W.E. (1946). Brit.med.J., 1, 305.

---------- O'Meara, P.J. and Stewart, G.T. (1948). Trans.R.soc.Trop.Med.Hyg., 41, 441.

Kessel, J.F. (1923). Univ.Calif.Publ.Zool., 20, 409.

Kruse, W. and Pasquale, A. (1894). Z.Hyg.Infektkr., 16, 1.

L Laidlaw, P.P., Dobell, C. and Bishop, A. (1928) Parasitol., 20, 207.

Lamb, W.L. and Royston, G.R. (1945). Lancet, i, 455.

Lösch, F. (1875). Arch.Path.Anat., 65, 196.

Lynch, K.M. (1915). J.Amer.med.Ass., 65, 2232.

M Manson-Bahr, P.H. (1943). Dysenteric Disorders. London: Cassell.

---------- and Willoughby, H.M. (1928) Trans.R.Soc.Trop.Med.Hyg., 22, 125.

Marriott, H.L. (1945). Lancet, i, 679.

Meleney, H.E. and Frye, W.W. (1936). Trans.R.Soc.Trop.Med.Hyg., 29, 369.

Mollari, M., Randall, W.A. and Reedy, R. (1939). J.Trop.Med.Hyg., 42, 34.

Morgan, H. de R., and Ledingham, J.C.G. (1909). Proc.R.Soc.Med., 2, Epidemiol., 133.

N Nauss,R.W. and Rappaport,I. (1940). Amer.J.Trop.Med., 20, 107.

O O'Connor,R.J. (1947). Trans.R.Soc.Trop.Med.Hyg., 41, 78.

P Payne,A.M.M. (1945). Lancet, 2 (Correspondence), 319.

Pavlova,E.A. (1938). Med.Parasitol. and Parasitic Dis., Moscow, I, 224. (In Russian; French summary p.227).

Q

R Rees,C.W. (1939). J.Parasitol., 25, (Suppl.6), 14.

———— Reardon,L.V., Jacobs,L., and Jacobs,F. (1941). Amer.J.Trop.Med., 21, 567.

Rogers,L. (1944). In Rogers and Megaw: "Tropical Medicine". London: Churchill.

S Scadding,J.G. (1945). Lancet, 2, 549.

Sellards,A.W. and Leiva,L. (1923). Phillipines J.Sci., 22, 39;

———— Theiller,M. (1924). Amer.J.Trop.Med., 4, 309.

Sevitt,S. (1945). J.Hyg., Camb., 44, 37.

Shaffer,J.G. and Frye,W.W. (1948). Amer.J.Hyg., 47, 214.

————, Ryden, F.W. and Frye,W.W. (1948). Ibid., 47, 345.

Silverman,D.N. and Leslie,A. (1945). J.Amer.Med.Ass., 129, 187.

Snyder, M.L. and Lichstein,H.C. (1940). J.Infect.Dis., 67, 113.

Snyder,T.L. and Meleney,H.E. (1943). J.Parasitol., 29, 278.

Spector,B.K. (1935). Amer.J.Hyg., 22, 366.

Stamp,Lord and Stone,D.M. (1943). J.Hyg., Camb., 43, 266.

Stewart,G.T. (1945). Lancet, 2, 705.

———— (1947a). J.Hyg., Camb., 45, 282.

———— (1947b). J.R.N.Med.Serv., 33, 6.

———— (1947c). Trans.R.Soc.Trop.Med.Hyg., 41, 75.

———— (1948). Ann.Trop.Med.Parasitol., 42, 198.

————, and Jones,W.R. (1948). Ibid., 42, 33.

————, ————, and Rogers,M.A.T. (1948). Nature, London, 161, 936.

————, O'Meara,P.J. and Kershaw,W.E. (1948). J.R.N. Med.Serv., 34, 2.

St.John,J.H. (1933). Amer.J.Hyg., 18, 414.

Stuart,C.A., Wheeler,K.M., Rustigan,R. and Zimmerman,A. (1943). J.Bact., 45, 101.

T Trawinski,A. and György,P. (1948). Arch.Hyg., Berlin, 87, 277.

Tsuchiya,H. (1939). Amer.J.Trop.Med., 19, 151.

U

V Vedder,E.B. (1914). J.Amer.Med.Assoc., 62, 501.

W Wagner,O. (1935). Arch.f.Schiffs-u.Tropenhyg., 39, 1.

Walker,E.L. and Sellards,A.W. (1913). Philipp.J.Sci., B, 8, 253.

Weil,A.J. (1947). J.Immunol., 55, 363.

Wenyon,C.M. (1926). Protozoology. London: Baillère, Tindall and Cox.

Westphal,A. (1938). Arch.Schiffs-u.Tropenhyg., 42, 343, 441.

Wilson,W.J. (1929). "The Colon group and similar bacteria." In "A system of Bacteriology", 4, 254. London: H.M.S.O.

X

Y Yorke,W. and Adams,A.R.D. (1926). Ann.Trop.Med.Parasitol., 20, 279, 317.

——————— (1927). Ibid., 21, 281.

——————— (1927). Brit.med.J., 2, 486.

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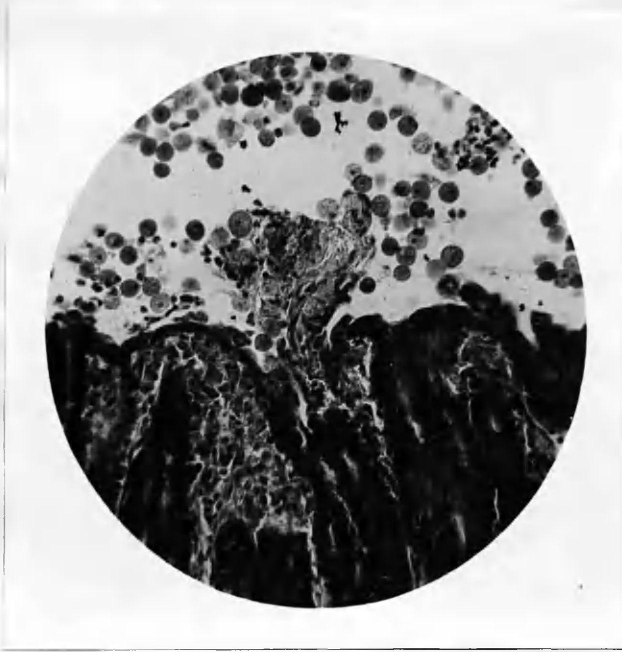
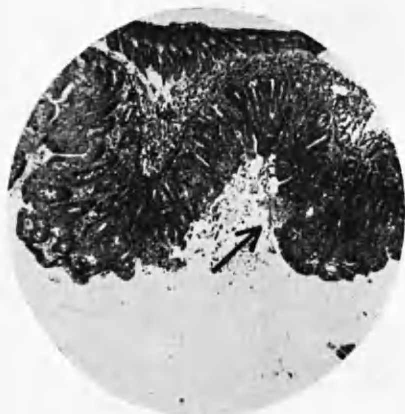
PHOTOGRAPHS.

EXPERIMENTAL AMOEBIASIS. (RAT).

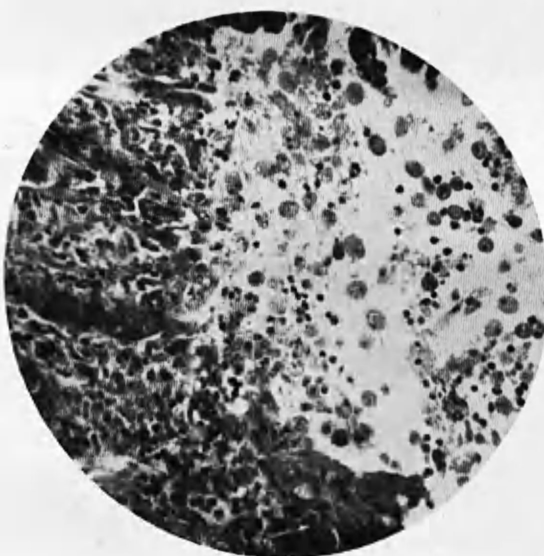


PHOTOGRAPH 1(a): Early invasion of the mucosa of the caecum in a rat experimentally infected with E. histolytica. (× 250 diameters).

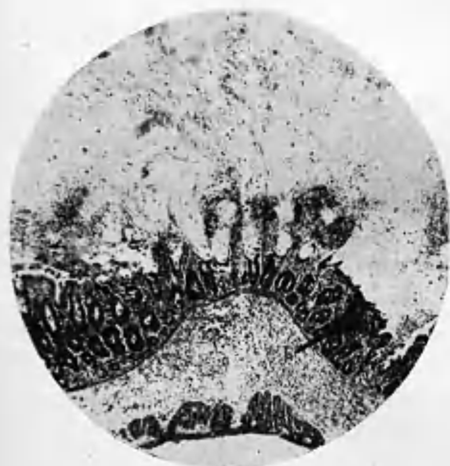
EXPERIMENTAL AMOEBIASIS.
(RAT).



PHOTOGRAPH 1(b). Early destruction of the mucosa by *E. histolytica*. The lesion is localized and there is little or no inflammatory reaction. The adjacent mucosa is undamaged. (× 40.)



PHOTOGRAPH 2. Same as photograph 1. (× 250.)



PHOTOGRAPH 3. Secondary infection of an early amoebic lesion by *paracolon* bacteria. There is a wide-spread inflammatory reaction. (× 40.)

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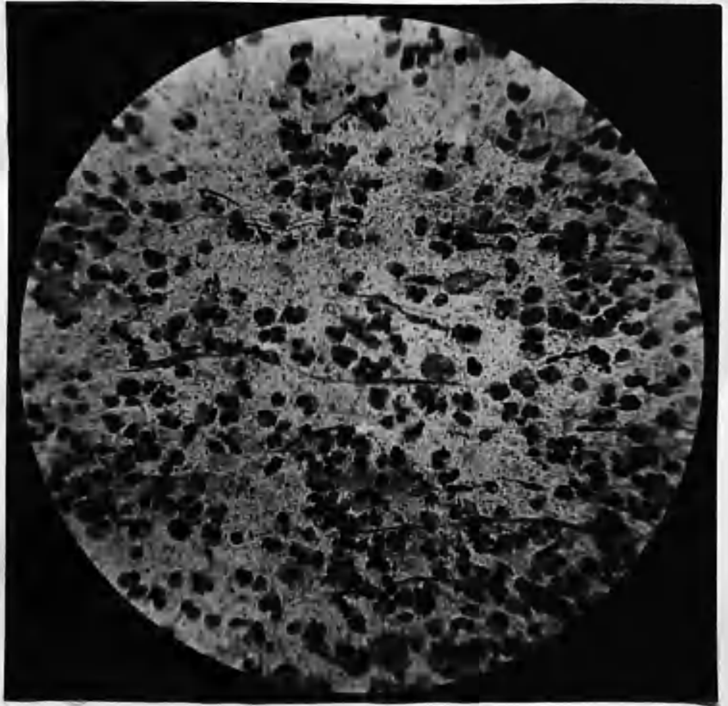
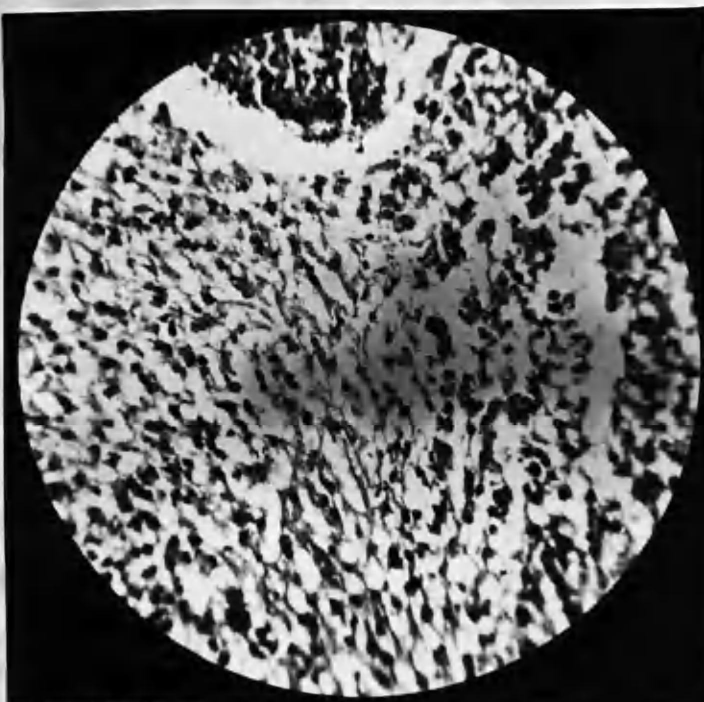
Photograph 6:

Experimental amoebiasis (Rat).

Photograph 7.

Human (post-dysenteric colitis).





Photograph 8.

Human (Post-dysenteric colitis).

Key to Photographs 5, 6 and 7.

Photograph 6: Healing ulcer, in a rat experimentally infected with E. histolytica.

Photograph 7: A smear of the purulent exudate, from a case of post-dysenteric colitis.

Photograph 8: Section of biopsy of the mucosa of the colon, from a case of post-dysenteric colitis. The epithelium is largely replaced by a loose aggregate of inflammatory cells.

Note: All photographs are taken from specimens stained with Haematoxylin and Eosin.