THE CHEMOTHERAPY OF AMEBIASIS

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INTRODUCTION

Agents investigated as potential amebacides have been selected mainly on an empirical basis, and, in fact, the chemical types that have been investigated are not extensive. Early studies were concerned with the isolation of active principles from plants that had some reputation for controlling the dysenteries, *viz.*, ipecacuahana, kurchi alkaloids, ko-sam or ya-tan-tzu and *Chaparro amargosa*. Continuing interest has centered around emetine, the principal alkaloid of ipecac, and attempts have been made to synthesize more stable and effective but less toxic derivatives (Pyman in 1937 (91), Sugasawa and Kabayashi in 1949 (104), Lasslo and Kimura in 1950 (73) and Anastopopulos and Birch in 1950 (4)). Later studies of synthetic compounds resulted in the introduction of the halogenated hydroxyquinolines and the pentavalent arsenicals into the therapy of amebiasis. These were, in part, obvious developments from the therapy of other diseases.

Recent work has been directed toward finding more active compounds within these classes and has resulted in the introduction of vioform, diodoquin, carbarsone and the thioarsenites. Attempts to reduce the toxicity of the arsenicals led to the introduction of the thioarsenites in amebiasis by Anderson *et al.*, in 1947 (10), and trials against *Trypanosoma equiperdum* with oxophenarsine-dimercaptopropanol by Sawyer *et al.*, in 1949 (96).

The antimalarial chloroquine was studied as a substitute for emetine in hepatic amebiasis, but its amebacidal action *in vitro* and *in vivo* in intestinal amebiasis is not significant. Many other series of compounds have been explored *in vitro*, but such studies have been mainly incidental to other interests. Much of this work, which did not lead to the development of clinically effective agents, does not appear in the literature (Goodwin *et al.* (50), Rawson and Hitchcock (92), and Brackett and Bliznick (25)). As an example, the amebacidal tests reported by Anderson and Hansen in 1947 (8) and Anderson, Johnstone and Hansen (14) include but a small part of the many compounds that have been considered.

Historically, the halogenated hydroxyquinolines, beginning with Mühlens' and Menk's (84) introduction of chiniofon (as "yatren"; 26.5–29% I) in 1921, vioform (38–41% I, 11.4–12.2% Cl) by the senior author (H.H.A.) and associates in 1931 (15), and diodoquin (60.5–64% I) by Tenney in 1936 (106), have had considerable use as amebacides. Despite recent reversals of early opinions as to its effectiveness, diodoquin (35) is still used in high dosage and with other agents. David *et al.* (36, 38), in commenting on the toxic effects of

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diodoquin as described by Silverman and Leslie (100), decried the uncontrolled use of oral amebacides. These, like other available agents, are potentially toxic, and David, Phatak and Zener (38) demonstrated blood iodine levels after oral use of diodoquin ranging from 45 to 437 micrograms per hundred ml. Subsequently, Knight and Miller (68) found that vioform was the most readily absorbed of chemically related agents. Iodine blood levels, following vioform medication over a ten-day period, were highest on the seventh day.

Studies on the metabolism of chiniofon, in which radioactive iodine was utilized, have revealed that some absorption of this halogenated hydroxyquinoline may occur. The uptake was small, however, according to Albright, Tabern and Gordon (3) and averaged 12.9% of the dose given. Peak blood level appeared in two hours, the highest urinary level occurred within three hours after ingestion and excretion was complete in 48 hours. Free iodide was split from the molecule, and only 7.4% of the total amount of chiniofon administered was excreted as such. Blood levels of clinical importance could not be attained despite prompt absorption, due to the low percentage absorbed, the rapid urinary excretion, and the breakdown into free iodide and organic rings. Distribution studies of radioiodine after intravenous injection in rabbits by Mann *et al.* (76) have indicated that the liver contains the smallest amount of all tissues.

Emetine has the longest history of use in amebiasis, but its toxicity precludes its recommendation at present. Although Murgatroyd found it difficult to demonstrate emetine in the blood of patients under treatment, in experimental animals it has been possible to show that emetine is present in several viscera other than in the large intestine in larger quantities. Thus, we have evidence as a result of the studies of Parmer and associates (88) that injections of 6 mgm./kg. of emetine hydrochloride in rabbits may be followed by high drug concentrations in the lung, kidney, spleen and liver, and in progressively lower concentrations in heart and lymph nodes, with the lowest concentrations in large intestine and striated muscle. Parmer found no emetine in the brain and also noted that the drug disappears quickly from the muscles and large intestine. Here is an explanation for the effectiveness of emetine in hepatic amebiasis and its lack of specificity in intestinal amebiasis. Since emetine is known to be more toxic to muscular organs, such as the heart, it is believed that, despite the low concentrations detected, the drug interferes with the biochemical function of contractile cells.

Other chemical agents used most extensively are the arsenicals, carbarsone and acetarsone. Marchoux in 1923 (78) introduced acetarsone ("Stovarsol") primarily for use in syphilis. Bender (19) and others have reported frequent reactions, cutaneous and other, to therapeutic doses of acetarsone which have led to its general abandonment as an amebacide in the United States. Thus, among agents in this class, carbarsone introduced by Leake and associates in 1932 (74), has enjoyed the most widespread use and is considered by most authorities as a safe drug in the absence of contraindications to arsenical therapy. Carbarsone, alone or in combination with agents of different chemical type, is satisfactory for the majority of patients. Experience during World War II has revealed that doses

larger than those originally recommended can be used even under field conditions. Hakansson anticipated this conclusion in 1938 (52). Distribution studies (10) have shown that high concentrations of arsenic occur in hepatic tissue and in the intestine following administration of this agent and its trivalent analogues.

Among the locally active agents is "Wia" or "Milibis" (bismuth derivative of p-N-glycolylarsanilic acid), originally studied in amebic infected kittens in Germany by Hauer in 1943 (59). The majority of animals, given doses of 30 mgm. per kg., were freed of amebas or showed a marked reduction in their numbers. Incomplete "cures" were attributed to improper dose schedules and deep-seated ulcerative lesions. Despite only partial success initially, Dennis, Berberian and Hansen (40) have restudied the drug, now known as "Milibis" in the United States, and have initiated its clinical trial. This agent contains approximately 15% arsenic and 42% bismuth. Against the natural, non-pathogenic ameba of Syrian hamsters it was somewhat less active orally than carbarsone. Significant amounts of arsenic could not be demonstrated in the tissues of animals, and only 2% of the orally administered dose is excreted in the urine of man. Because of lack of absorption, this agent may be effective in the lumen of the colon; but, from the initial trials in kittens, it would appear that deep-seated lesions are not likely to be controlled. Berberian (20) cleared 62 of 68 cases. The dose employed was 0.5 gm. thrice daily for seven days. Only 25 of the patients received "Milibis" as the only amebacide. It would appear desirable to demonstrate this drug's usefulness in systemic amebiasis.

Conan (33) has shown that chloroquine will successfully combat hepatic amebiasis, but no consistent effect on intestinal amebiasis has been noted. This finding has also been made by Murgatroyd (85) and has been confirmed by Manson-Bahr (77) and by the authors (H.H.A.) (12). We have been unable to show any amebacidal activity of chloroquine in individuals with intestinal amebiasis.

Chloroquine, despite its minimal direct amebacidal action which precludes its consideration in intestinal amebiasis, is a satisfactory substitute for emetine in hepatic amebiasis. Oral use in man, recommended by Conan in 1948 (33), was designed to fulfill a two-fold purpose: (1) to administer "priming or loading dose" initially to saturate the tissues and rapidly obtain the desired tissue levels which were then maintained over a two- to three-week period; and (2) to exceed the dose levels proposed for antimalarial use, to the point of producing mild toxic symptoms. The author recommended 0.3 gram (of base) orally, thrice daily for the first two days, then a sustaining dose of 0.3 gram daily for 12 to 19 days. Blood levels of approximately 150 micrograms per liter may develop under this regimen. Except for mild gastric upset in two of six patients, toxic symptoms or signs did not develop in other cases.

The use of antibacterial agents, notably the antibiotics, in the chemotherapy of amebiasis has recently received considerable attention. Penicillin by Hargreaves (58), aureomycin by Most (82) and by McVay *et al.* (80), bacitracin by Most (82), neomycin *in vitro* (61), terramycin by Most (83), and a mixture of bacitracin, streptomycin and polymyxin B by the authors, have been investi-

gated in amebiasis as potential agents, either alone or in combination with other therapy. Earlier field reports on the usefulness of sulfaguanidine by Page (87) and others, particularly in military establishments, emphasized alleviation of symptoms in patients with mixed bacillary and amebic infections. Hargreaves (58) has expressed the opinion that the usefulness of antibacterial agents in the therapy of recurrent dysentery depends upon reduction of tissue reaction to the bacterial infection, which then permits amebacides to reach effective concentrations in the previously edematous sites of invasion. Such a result would most likely be obtained in mixed infections by bacteria and amebas.

CURRENT ASPECTS OF THE PROBLEM

The pathologic state in amebiasis that is challenged by the chemical agent is primarily a diffuse invasion of the intestinal mucosa accompanied by tissue lysis and minimal inflammatory reaction. There may also occur a diffuse invasion or abscess formation in distant organs, but the persistence of *Endamoeba histolytica* in the lumen of the large intestine is most common. Cysts, as well as trophozoites, are found in the lumen of the large intestine. Of the available drugs, emetine parenterally administered, acts on the trophozoites in the tissue, and the enteric drugs, chiniofon and diodoquin, act primarily on those in the lumen of the bowel. The halogenated hydroxyquinolines, such as chiniofon, rarely reach levels in the tissues of clinical importance (76), and it is noteworthy that there are no reports of satisfactory response of amebic hepatitis to these drugs. The pentavalent arsenicals (carbarsone and acetarsone) are absorbed and distributed systemically. The trivalent arsenicals recently introduced into amebiasis therapy at the University of California have not yet received extensive clinical trial in patients with massive liver involvement. However, distribution studies (of arsenic) have shown that high concentrations do occur in hepatic tissue and in the intestine, where the amebas also occur. Of the antibiotics thus far tried with some success clinically in amebiasis, only aureomycin is effectively absorbed and may develop systemic levels.

The ideal amebacide is one that can be administered orally, that is effective against the trophozoites and cysts in the lumen of the intestine, that is accumulated in effective concentration in the tissues of the large bowel, and, finally, that is distributed to the liver and other tissues (without damage to them) and there, retaining its active form, reaches therapeutically effective levels against trophozoites. No single, available amebacide meets these criteria. Of the agents currently in use, a breakdown into three classes (irrespective of chemical origin) is possible. First are those agents which act systemically, either on amebas in the bowel or in the tissues elsewhere, presumably by acting directly because they show little or no antibacterial properties on associated bacteria (in the lumen of the bowel) or action on commensal protozoa. In this class are emetine, carbarsone, the thioarsenites and vioform (the only halogenated hydroxyquinoline which may develop effective tissue levels). In the second class are those agents which, in large amounts in the lumen of the bowel, act indirectly on the pathogenic ameba by affecting primarily the intestinal bacterial flora. Among agents

which fall in this class are the antibiotics (with the possible exception of aureomycin), the sulfonamides, and the halogenated hydroxyquinolines, chiniofon and diodoquin. Whenever evidence of tissue invasion by the ameba is not manifest, it is possible, at least theoretically, to clear the lumen of the bowel of *E. histolytica*. However, current data suggest that clearance is temporary only, ranging from a few days to a few weeks or months, after which the bacterial flora returns to its former level and the pathogenic ameba reappears. The third class reaches especially high levels in the liver and are, therefore, effective in amebic hepatitis and abscess, an example being chloroquine. However, this agent does not have appreciable antiamebic action on parasites in the lumen of the large bowel. Thus, amebiasis, like other chronic parasitic diseases, such as syphilis or tuberculosis, because of its differing sites of infection, responds differently to various chemical types, probably on the basis of their differential absorption and distribution. Apparently for this reason, therapy with a succession of different chemical types has been effective when a single agent has not.

In reviewing available methods that have been used for evaluation of antiamebic agents, one of the earliest attempts is found in the study by James in 1913 (63), who observed the morphologic changes which occurred during treatment in the trophozoites found in the stools of human volunteers. Search for a laboratory animal in which the pathology and clinical course would closely resemble that in man revealed that only in the lower primates is the pathologic condition similar to the chronic disease in man. While the ameba may exist in the monkey (or in man) without grossly demonstrable tissue invasion, definite microscopic lesions have been described by Kessel (66) and Johnson (64) in rhesus and ateles monkeys. Histologic material from animals used in drug studies has revealed trophozoites in the gland crypts and lying in and invading tissues (Bond *et al.*, 24). Motile amebas first appear to enter near the mouth of the crypt and then invade the interglandular papillae of the lamina propria. Little tissue reaction resulted from the presence of these parasites, and bacteria were notably absent. Since the natural infection in macaques simulates chronic relapsing amebiasis of man, these animals have been extensively used for the evaluation of new agents. Furthermore, in these naturally infected animals the host response to therapy can be more readily evaluated. Some appreciation of the pharmacologic action of the test agent can be obtained, with respect to potential drug damage to the heart, liver and kidneys. The distribution of the agent and its toxic effects may be more closely parallel in monkeys and man than in the other available animals. Pathogenic protozoa must be distinguished from commensals, and this can only be accomplished by appropriate iron-hematoxylin staining technic.

Numerous attempts have been made to use other types of laboratory animals. Dogs (47, 71 and 107), kittens (32), rabbits, rats (34, 75 and 109) and guinea pigs (28 and 105) have all been employed. With these animals, however, the pathology following experimental infection more closely resembles that seen in acute dysentery which is not as important a therapeutic problem in man. Usually there is an acute fulminating dysentery that may terminate fatally, as in

kittens, or be self-limiting, as in rats (Jones, 65). In the evaluation of the usefulness of these animals, it seems that at best the production of such a fulminating dysentery must be regarded as merely a method of obtaining large numbers of trophozoites in the lumen of the intestine and in the superficial mucosal tissues. Recently Clampit (32) used kittens and found that emetine was relatively ineffective. More recently Coatney *et al.* (105), at the National Institutes of Health, have used amebic infected guinea pigs and have found none of the currently active amebacides effective*. These findings suggest that studies with cats and rodents are not comparable to the experience with even acute disease in man and are certainly not applicable to the minimal invasion of the so-called "carrier" condition. Furthermore, in dogs, diet appears to influence the course of amebiasis far more than it does in man (47 and 71). However, Thompson and Lilligren (107) utilized dogs on controlled diets and obtained some correlation with the therapeutic usefulness of some agents.

Since it has been impossible to devise large-scale mammalian tests in which the human host-parasite relationship can be truly weighed, it becomes important to consider other methods which permit evaluation of amebacidal action. An appropriate test can be carried out with cultures of the pathogenic organism in association with a bacterium or another protozoan. The conditions necessary for standardizing these tests and for distinguishing direct amebacidal action from action primarily against associated bacteria that accompany the amebas in culture have been described by Hansen (54) and Bradin and Hansen (26). Cultures in the presence of anti-amebic drugs have been studied with a view toward elucidating possible mechanism of action of these agents. The major difficulty with regard to such tests is that an agent may be overlooked that is converted to an active form only in the tissues of the infected host. Certain drugs employed in the therapy of parasitic diseases have this characteristic; one of these is carbarsone which is a most useful drug for the treatment of amebiasis and yet has little action *in vitro*. It must be recognized, also, that cultural conditions may alter the response of *E. histolytica* from that exhibited in the tissues of the infected host.

It should be stated further that *in-vitro* tests must be regarded as only preliminary in the evaluation of anti-amebic agents. Laboratory evaluation must include, in addition, successful treatment of infected macaques, complete pharmacologic and toxicologic observations on these and other animals, and finally clinical trials under controlled conditions with adequate follow-up examinations of the stools of treated patients.

It is not the purpose of this review to provide detailed data on the relationship between *in-vitro* tests and the final clinical evaluation of antiamebic agents. It is felt, however, that in view of the necessity for direct specific action of an effective drug, as revealed by considerations of the pathology of the disease,

* According to a manuscript now in press from these workers, "both carbarsone and diodoquin approximately double the survival time of amebic-infected guinea pigs." This observation of Coatney *et al.* agrees with the similar observation based on the use of the same experimental animals and technics, which was reported in 1948. (See reference No. 50.)

and of the exacting requirements of laboratory animal studies, the nature of the results obtained *in-vitro* should be reviewed critically and the requirements met with some precision and uniformity.

THE CULTIVATION OF E. HISTOLYTICA IN RELATION TO APPRAISAL OF AMEBACIDES

The cultivation of *E. histolytica* is a comparatively recent accomplishment, dating only from 1925 when Boeck and Drbohlav (23), at Harvard Medical School, accidentally discovered pathogenic amebas growing in a flagellate culture made from infected stool material. Prior to this, attempts had been made to evaluate amebacidal action by the use of non-pathogenic forms (*e.g.*, Sellards and Leiva, 97) or by direct exposure of amebas to the test agents during microscopic examination. Earlier Vedder, in 1911 (108) in the Philippines, demonstrated the action of emetine *in vitro* against pathogenic ameba. This demonstration of *in-vitro* activity of emetine preceded Rogers' trial of the drug clinically against acute amebiasis and amebic hepatitis in India.

Dobell and Laidlaw in 1926 (45) extended and improved cultivation procedures and were followed by other investigators who introduced numerous changes. It is now known that *E. histolytica* can be maintained in a variety of media. These media were originally of the diphasic type, consisting of a solid slant with a liquid overlay and composed of various combinations of egg, liver extract, albumen or serum. The diphasic media, though still used (*e.g.*, Ishii (62)), present certain disadvantages, such as difficulty of preparation and lack of uniformity of ingredients. Errors may be introduced into drug testing due to variable adsorption of the drug onto the solid slant. This has led to the introduction of liquid medium suitable for continuous cultivation of the ameba and composed of standardized dehydrated ingredients and also of an essentially synthetic medium (55, 56). The latter has permitted the beginning of studies toward elucidation of the nutritional requirements of *E. histolytica*. Such a study may lead to the development of a culture free from the complicating influence of associated micro-organisms and, possibly to the use of anti-metabolites as amebacides.

The problem of possible action of an agent on the associated bacterial flora is most urgently in need of solution. For example, in the study of the action of chloromycetin (Smith *et al.*, 101), Thompson and Lilligren were forced to conclude that the "numbers of motile amebae at 48 hours decreased to an extent attributable to inhibition of the associated bacterial flora." Other workers have confused diminution of bacterial growth (in mixed cultures) with direct amebacidal action. Culture of amebas with *Trypanosoma cruzi* (90) may permit evaluation of direct amebacidal action uninfluenced by the effects of associated bacteria.

While agents that act primarily on the bacteria may have some place in the therapy of amebiasis (when the organisms are confined to the lumen of the bowel), from the point of view of the *in-vitro* tests it is important to recognize how far the observed action against the ameba is conditioned by action on the

bacteria. Agents which kill or adversely affect the bacteria will indirectly kill the amebas, regardless of whether they have any direct amebacidal action or not. It was failure to appreciate the importance of the antibacterial action of the series of compounds studied by Pyman (91) that led both Jones and Goodwin to conclude that "a clinical trial failed to confirm the promise of the *in-vitro* test. It is evident from this careful work of Pyman and his collaborators that an *in-vitro* test alone is not enough to indicate whether or not a compound is likely to be useful in the treatment of amoebiasis" (Goodwin *et al.*, 50). Goodwin has recognized that in the cultures with mixed bacterial flora (used by Pyman) it would be impossible critically to evaluate amebacidal action. Dobell (41) attempted to clarify the confusion such *in-vitro* tests generate by stating that, "The late Dr. Pyman, though he did not make this clear, actually did not use our method in the instance denoted. Indeed when he communicated his findings to me privately before publication, I pointed out to him that the technique of the *in-vitro* tests on which he relied was unallowable. They furnished no sound evidence that the chemical in question possessed any specific toxicity for *E. histolytica*, and consequently I was not surprised to learn later that subsequent clinical trials were unsuccessful."

It should be emphasized that many published results of *in-vitro* tests include amebacidal levels for agents that act primarily by bacteriostasis. This has led to the supposition that tolerance is developed by the amebas to sulfonamides (Work and Work, 110), truly an important phenomenon but unwarranted by the experimental data obtained by Rodaniche and Kirsner (95). In fact, while it may be true that tolerance to conventional amebacides may develop (2), there is as yet no acceptable laboratory evidence that supports this contention (Dobell, 42).

IN-VITRO EVALUATION OF POTENTIAL AMEBACIDES

Biologic aspects of the problem imply knowledge of the pathological state of the amebic infected large bowel and the nutritional factors involved, as well as the influence of associated bacteria. Nicotinic acid deficiency has been reported by Larsh (71) to enhance ulcer formation in the intestines of dogs. Other tissue changes, such as hepatitis and abscess of liver, may occur without associated bacteria. Thus, systemic amebiasis must be recognized as a disease in which a directly active amebacide is required for ultimate control, *e.g.*, beyond the enteral lesion. This fact has been recognized in the therapy of bacillary dysentery in which it has been found that systemically absorbed sulfonamides are most effective.

An improved method for *in-vitro* testing of amebacides (54) has made it possible to recognize whether an agent is acting indirectly by inhibition of associated bacterial growth. The test is based upon recognition of the importance of the intense reducing potentials produced by the associated bacteria in providing an environment suitable for multiplication of the trophozoites. When the dilution of a test agent is found to be "amebacidal" *in vitro*, by the use of the conventional test procedure, a tube containing the identical dilution is set

up concurrently and sealed with petrolatum. This insures that the oxidation-reduction potential established by the associated flora, even though bacterial growth is inhibited, will be preserved. The amebas, then, continue to survive at the apparent "amebacidal" concentration previously determined in the cotton-stoppered tube. The method of sealing cultures with petrolatum to prevent oxidation was adapted from that of Shaffer, Ryden and Frye who used it in developing the clear medium technic (98).

The method has been applied to both biphasic (liquid saline overlay with egg slant) and, more recently, to liquid media (55) utilizing a single bacterial associate, such as organism "t" (C. W. Rees*, National Institutes of Health Bethesda, Maryland). Such mono-bacterial flora, whose growth can be followed and whose contributions to the physical environment are known, serve better than mixed flora. With the latter, varying susceptibility to the test agent by various species or strains of bacteria makes evaluation difficult. This is especially true with antibacterial agents such as the antibiotics. Thus far, the technic has been applied only to detect whether the "apparent amebacidal" activity is dependent on bacteriostasis. With presently available methods, direct amebacidal activity can be inferred only when there is no significant difference in amebacidal levels as determined in cotton-stoppered and petrolatum-sealed tubes.

The current procedure may be briefly summarized: *E. histolytica* was exposed to each agent during 48 hours' incubation at 37° C., and then subcultures were made to determine end-points by the conventional test procedures. The growth of associated organism "t" and the purity of each culture were determined by observation of turbidity and by examination of Gram-stained preparations. Cultures were inspected at 4, 24 and 48 hours, to detect whether inhibition of bacterial growth had occurred, especially during the early half of the growth period. These observations were supported by electrometric measurements (26). Survival of amebas in petrolatum-sealed cultures showed whether the test agent was active primarily on associated bacteria. The "apparent amebacidal" concentration found by the conventional test method in cotton-stoppered tubes was compared with the concentrations tolerated in cultures sealed to reduce the effect of anti-bacterial action. The various antibiotics are compared in Table I.

Interpretation of these results on the basis of *in-vitro* trials alone suggests that possibly antibiotic "S", NA7M10, prodigiosin and neomycin should be subjected to further pharmacological testing. Toxicity tests in various mammalian species should follow, as well as trials in monkeys for effectiveness in tolerated, oral doses against natural amebiasis. Clinical experience with streptomycin metal pectinates, aureomycin and bacitracin, reported by Most (82), confirm this impression since it would appear that enteral amebiasis is controlled only temporarily, *e.g.*, as long as the associated bacterial flora is held static. We have observed similar temporary benefit with subtilin introduced rectally. Given orally to macaques, the combined antibiotics, bacitracin, polymyxin B and streptomycin, cleared 50% of the animals over three months (Hrenoff, 60).

* Isolated by micromanipulation technic, as originally described by this worker in the Am. J. Trop. Med., *22:* 487–492, 1942.

TABLE I

Amebacidal Concentration in Open Cultures and in Cultures Sealed to Reduce Effect of Antibacterial Action

E. histolytica associated with organism *t* exposed during incubation for 48 hours in liver-proteose-peptone medium

ANTIBIOTICS TESTED†	LETHAL LEVEL IN COTTON-STOPPERED TUBES	LETHAL LEVEL IN PETROLATUM-SEALED TUBES
Actidione (Upjohn)	1:10,000,000	1:1,000,000
Aerosporin (Polymyxin B, Burroughs and Wellcome)	1:2,000	1:1,000
Antibiotic "S" (Abbott)	1:100,000 to 1:200,000	1:100,000 to 1:200,000
NA7M10 (Abbott)	1:6,250,000	1:5,000,000
Aureomycin HCl (Lederle, A-377 and C.P. 1141)	1:500,000	1:20,000
Bacitracin (Commercial Solvents)	1:200,000 to 1:260,000	1:1,000 to 1:1,500
Chloromycetin (Parke, Davis & Co.)§	1:100,000‡	1:1,000
Lupulon (West. Reg. Res. Lab., U.S. Dept. of Agric.)	1:20,000‡	1:5,000
Neomycin (Commercial Solvents)	80 units/ml.	80 units/ml.
Penicillin "G", Sodium (Lilly)	1:1,660‡	1:166+
Penicillin "O" (Allyl mercapto-methyl penicillin, Upjohn)	1:100,000‡	1:50,000
Prodigiosin¶	1:100,000**	1:100,000**
Streptomycin (Calcium chloride complex (Merck) & Hydrochloride (Squibb))	1:300 to 1:400	1:100+ to 1:200
Subtilin (West. Reg. Res. Lab., U.S. Dept. of Agric.)	1:500,000 to 1:1,000,000	1:400,000
Terramycin 9M-35 (Chas. Pfizer)	1:100,000	1:5,000
*Tested in combination**		
Streptomycin HCl	1:3,200‡	1:1,600
Bacitracin	1:3,200,000‡	1:16,000
Polymyxin B (Chas. Pfizer)	1:120,000‡	1:40,000
Emetine Hydrochloride (Lilly)	1:100,000 to 1:400,000‖	1:100,000 to 1:400,000

* Modified, Bradin and Hansen (26); Anderson and Anderson (6); and Gould and Hansen* (51).

† Agents were generously supplied by their respective manufacturers.

‡ Lowest concentration tested (amebas killed); + highest concentration tested (amebas alive).

§ Effect of antibacterial action in amebacidal test noted by Thompson (see Smith *et al.* (79, 101)).

¶ Material prepared and supplied by Balamuth who considered the amebacidal level to be 1:200,000 in egg extract medium (17, 18). This antibiotic demonstrated action against *Trypanosoma brucei* infection without concomitant bacterial association (Lack, 69).

‖ Identical levels in parallel cotton-stoppered and sealed cultures.

** Tested in presence of 0.5% ethyl alcohol which may have contributed to the action of this antibiotic.

The high "apparent" activity of actidione would suggest its consideration as an amebacide. This was precluded, however, by the extremely high toxicity of this

agent for mammals, according to Chen (30). NA7M10, available for parenteral use only, killed 4 of 8 animals given effective doses.

Re-examination of accepted amebacides by this modified test procedure revealed interesting comparisons. Emetine hydrochloride, as a standard agent for comparison, was equally effective in cotton-stoppered and petrolatum-sealed tubes (within the range of 1:100,000–1:400,000). Carbarsone and carbarsone-oxide (the trivalent arsenic analog) were likewise equally effective under cotton-stoppered and sealed-tube conditions, at 1:500–1:2,000 and 1:50,000–1:100,000, respectively. The thioarsenites of carbarsone oxide, C. C. No. 914 and 1037, also were equally effective in cotton-stoppered and petrolatum-sealed cultures at 1:10,000–1:50,000 dilutions. The halogenated hydroxyquinolines, notably diodoquin and vioform-soluble, each exhibited some degree of bacteriostasis which might suggest an indirect amebacidal activity, when present in relatively large amounts in the lumen of the bowel. Vioform-soluble was active *in vitro*, however, within the range of 1:10,000–1:50,000, where bacteriostasis was not believed to influence the test. Earlier examinations of various type of agents are summarized in Table II, in which the conventional test procedure was employed.

Three of the chemical types of amebacides which have had clinical appraisal all exhibited direct activity against *E. histolytica*. These are emetine hydrochloride, the pentavalent arsenical, carbarsone, and the trivalent arsenicals, notably the thioarsenites. In contrast, the less certain halogenated hydroxyquinolines, diodoquin and vioform, showed some degree of bacteriostatic action for which the latter was originally introduced into clinical medicine more than 40 years ago.

Thus, it would appear that data obtained in an *in-vitro* test that distinguishes indirect action, together with knowledge of the toxicity and distribution of a proposed amebacide, will provide presumptive evidence of the possible value of a new agent for subsequent trials in macaques and man.

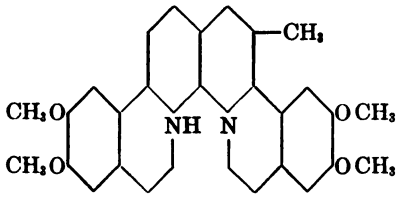
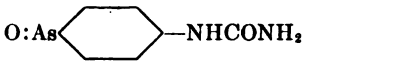
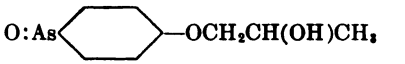
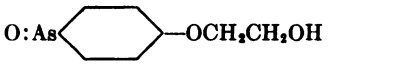
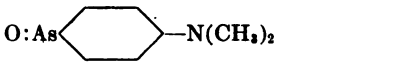
An approach to an understanding of the mode of action of amebacides has been made by comparing the dose-response relationship and morphological changes produced by various agents (9). This, together with data on distribution in the host, may provide an understanding of the variations observed in response to amebacides. The probit of the population surviving at 48 hours, calculated as percentage of the population in the control culture, was graphed against log-dose, as indicated in Figure I. It was found that emetine still caused a 50% kill at $\frac{1}{10}$ its amebacidal level, while vioform-soluble and thioarsenite 914 had no action at $\frac{1}{8}$ their amebacidal levels.

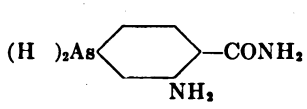
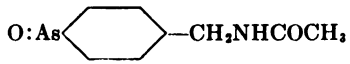
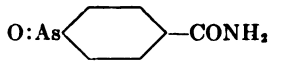
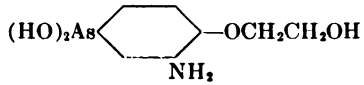
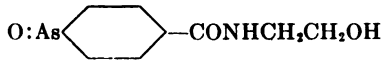
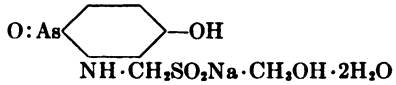
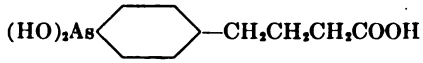
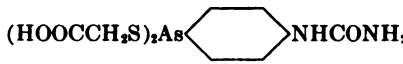
Cultures containing the antibiotics were characterized by survival of a few amebas over at least a ten-fold range of concentrations. These amebas produced high populations in subculture if the effect of the antibacterial agent was reduced by preconditioning the medium with an appropriate flora. Dead cultures were found only when bacterial growth had been completely inhibited, during the first 4 to 10 hours of incubation.

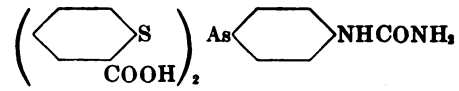
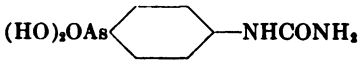
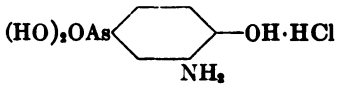
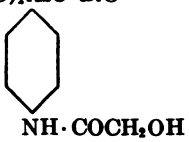
The morphological changes shown by the trophozoites during incubation in

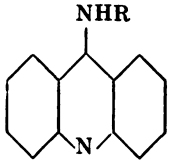
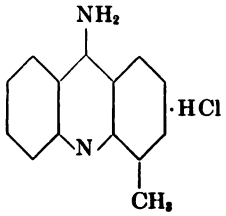
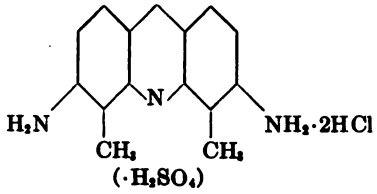
TABLE II

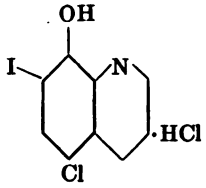
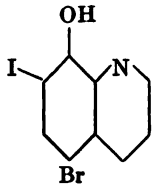
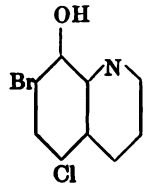
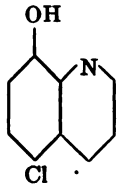
Types of Agents Exhibiting Amebacidal Activity In Vitro[a]

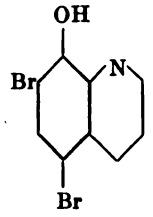
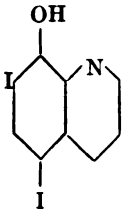
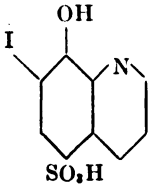
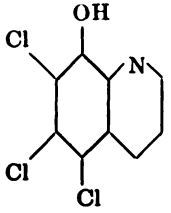
AGENT	FORMULA	SOLVENT REQUIRED[b]	POTENCY RELATIVE TO EMETINE (GIVEN THE VALUE OF 1)
Plant principles			
Emetine		Water	1
Cephaeline isoamyl ether acid phosphate		Water	1–5
Chaparro amargoso		Water	1–6
Yatanocide		Water	<0.1
Arsenicals, trivalent			
p-Arsenosophenylurea ("p-carbamidophenyl arsenoxide")		Propylene glycol	2–5
1-(p-Arsenosophenoxy)-2-propanol		Propylene glycol	10
2-(p-Arsenosophenoxy) ethanol		Propylene glycol	<5
p-Arsenoso-N,N-dimethylaniline		HCl	<5

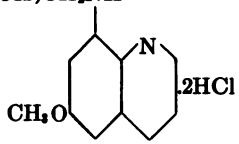
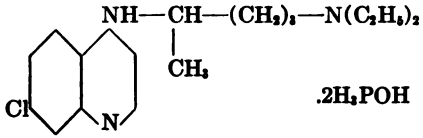
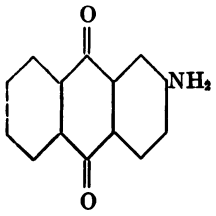
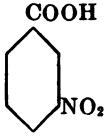
2-Amino-4-arsonosobenzamide (3-amino-4-carbamylbenzenearsonous acid).....		NaOH	5
N-(p-Arsenosobenzyl)acetamide.....		NaOH	2.5
p-Arsenosobenzamide.....		NaOH	2.5
3-Amino-4-(2-hydroxyethoxy)benzenearsonous acid (2-(2-amino-4-arsonosophenoxy) ethanol).....		HCl	1–2.5
p-Arsenoso-N-(2-hydroxyethyl)benzamide .		NaOH	1–2.5
2-Amino-4-arsenosophenol, sodium formaldehydesulfoxylate methanolate dihydrate.....		Water	1
γ-(p-Arsonosophenyl)butyric acid.....		NaOH	1–2
p-[Bis(carboxymethylmercapto)arsino]-phenylurea.....		$NaHCO_3$	1

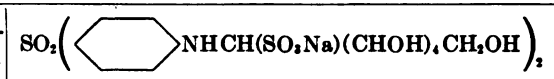
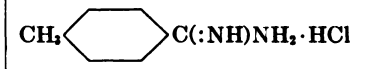
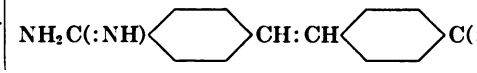
AGENT	FORMULA	SOLVENT REQUIRED[b]	POTENCY RELATIVE TO EMETINE (GIVEN THE VALUE OF 1)
p-[Bis(o-carboxyphenylmercapto)-arsino]-phenylurea		NaOH	1
Arsenicals, pentavalent			
p-Carbamidobenzenearsonic acid (Carbarsone, U.S.P. XIII)		$NaHCO_3$	0.1
3-Amino-4-hydroxybenzenearsonic acid · HCl		Water	>0.1
Bismuth derivative of p-N-glycolyl-arsanilic acid ("Wia" or "Milibis")		NaOH	<0.5
Amines			
N,N′-Bis(2-aminoethyl)-1,3-propanediamine · 4HCl	$NH_2CH_2CH_2NHCH_2CH_2CH_2NHCH_2CH_2NH_2 \cdot 4HCl$	Water	>0.1
N,N′-Tetra-n-butyl-1,6-hexanediamine	$(n\text{-}C_4H_9)_2N(CH_2)_6N(n\text{-}C_4H_9)_2$	Water	>0.1
Cetyl(2-hydroxyethyl)dimethylammonium chloride (n-hexadecyldimethyl(2-hydroxy-ethyl)ammonium chloride)	$n\text{-}C_{16}H_{33}N(CH_2CH_2OH)(CH_3)_2Cl$	HCl	>0.5

N,N′-Diphenethylethylenediamine · 2HCl	$C_6H_5CH_2CH_2NHCH_2CH_2NHCH_2CH_2C_6H_5 \cdot 2HCl$	HCl	0.5
N,N′-Dicuminylethylenediamine	$(CH_3)_2CH$—C$_6$H$_4$—$CH_2NHCH_2CH_2NHCH_2$—C$_6$H$_4$—$CH(CH_3)_2$	Propylene glycol	<0.5
Acridines			
9-Methylaminoacridine·HCl	R = CH_3(·HCl)	Alcohol	1
9-Aminoacridine	R = H	Alcohol	<1
9-Ethylaminoacridine·HCl	R = C_2H_5(·HCl)	Water	1
9-n-Butylaminoacridine·HCl	R = n-C_4H_9(·HCl)	Water	<1
9-Amino-4-methylacridine·HCl (1-methyl-5-aminoacridine·HCl)		Water	1
3,6-Diamino-4,5-dimethylacridine sulfate·2HCl (1,9-dimethylproflavine·2HCl)		Water	5

AGENT	FORMULA	SOLVENT REQUIRED[b]	POTENCY RELATIVE TO EMETINE (GIVEN THE VALUE OF 1)
Hydroxyquinolines			
5-Chloro-7-iodo-8-hydroxyquinoline (iodochlorohydroxyquinoline, N.F., Vioform)·HCl		Water	<1
5-Bromo-7-iodo-8-hydroxyquinoline		NaOH	<0.5
5-Chloro-7-brom-8-hydroxyquinoline		NaOH	<0.5
5-Chloro-8-hydroxyquinoline		Ethylene glycol	<0.5

5,7-Dibromo-8-hydroxyquinoline.....		NaOH	<0.5
5,7-Diido-8-hydroxyquinoline (Diodoquin, N.N.R.).....		NaOH	<0.5
7-Iodo-8-hydroxyquinoline-5-sulfonic acid (Chiniofon, U.S.P.).....		$NaHCO_3$	<0.02
5,6,7-Trichloro-8-oxyquinoline.....		NaOH	<0.5

AGENT	FORMULA	SOLVENT REQUIRED[b]	POTENCY RELATIVE TO EMETINE (GIVEN THE VALUE OF 1)
8-[(3-Diisobutylamino-2-hydroxypropyl)-amino]-6-methoxyquinoline·2HCl.....	$((CH_3)_2CHCH_2)_2NCH_2CH(OH)CH_2NH$  .2HCl	Water	<1
Miscellaneous			
7-Chloro-4(4-diethylamino-1-methylbutyl-amino)quinoline (Chloroquine, N.N.R.)..	 .$2H_3PO_4$	Water	0.05
2-(Guanylmercapto)-N,N,N′,N′-tetraiso-butyl-1,3-propanediamine·3HCl.....	$((CH_3)_2CHCH_2)_2NCH_2CH(SC(:NH)NH_2)\ CH_2N(CH_2CH(CH_3)_2)_2 \cdot 3HCl$	Water	<1
2-Aminoanthraquinone.....		Propylene glycol Water	>0.5 <1
m-Nitrobenzoic acid.....		Water	<0.1

Bis(p-aminophenyl) sulfone N,N′-bis-(sodium dextrosesulfonate) (Promin).....		Water	<0.1
Bis(p-propionylaminophenyl)sulfone (p,p′-sulfonylbispropionanilide).....		Water	<0.1
p-Tol(u)amidine·HCl.....		Water	<0.1
4,4′-Diamidinostilbene (4,4′-stilbenedicarboxamidine; stilbamidine).....		Water	<0.1

[a] Utilizing egg slant medium and liver-proteose-peptone medium (55). All values are relative, and only those agents within the range of activity of emetine are included. The value "1" is arbitrarily assigned to the amebacidal level of emetine active against *E. histolytica* in culture with associated organisms.

[b] Solution is placed in buffered media for test. Concentration of propylene glycol not amebacidal, 2% or less; ethyl alcohol, 5% or less in egg slant medium, and 0.5% or less in liver-proteose-peptone medium.

the presence of a toxic agent were studied by the cover-slip technic of Dobell (41) which provided a means of obtaining trophozoites spread into thin films which were then available for study by phase contrast microscopy of the living organisms, or by fixed stained preparations. Thioarsenite 914 caused marked nuclear degeneration, while only rare trophozoites retained their normal morphology. In the presence of emetine, cytoplasmic aggregates occurred, but the preservation of apparently normal forms was more common (see also Stewart, 102). With the antibiotics, such as aureomycin, the trophozoites did not exhibit

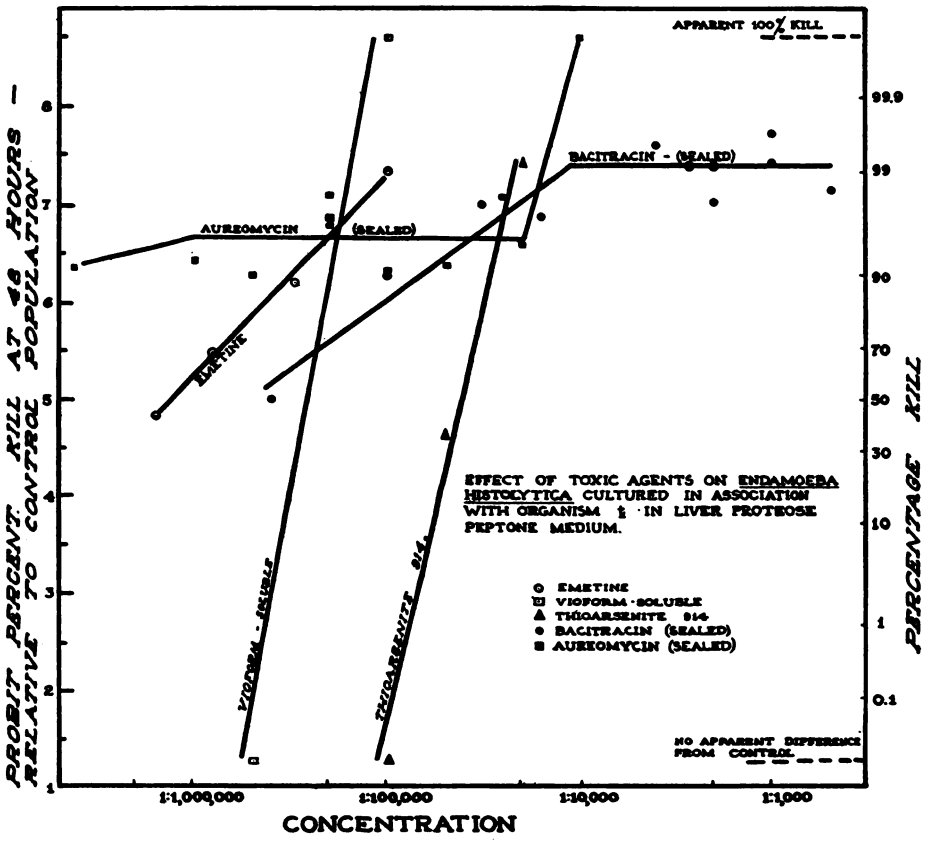


FIG. 1

the characteristics associated with a multiplying culture, and it appeared that the cultures contained only the trophozoites originally introduced with the inoculum. These died during a 48-hour period, at a rate that appeared dependent on the degree of bacterial inhibition in the culture. The most frequent finding in fixed and stained preparations was that of trophozoites showing a fragmented periphery but retaining apparently undisturbed nuclear structure. This change has been reported with subtilin by Anderson *et al.* (16).

Thus, a great variety of chemical types, such as specific plant alkaloids,

arsenicals, hydroxyquinolines, peroxides (57) and antibiotics, together with possible metabolic antagonists, such as 2,6-diaminopurine, are fatal to amebas in culture. The response shown to a radiomimetic agent, such as the triethyleneimide derivative of cyanuric acid, suggests that detailed study may reveal the pattern of drug action of the amebacides. This may contribute to a better understanding of the relationship between chemical structure and biologic activity than now exists.

NATURAL AMEBIASIS IN MACAQUES

Beginning in 1929, shortly after Dobell (43) had shown that emetine, in toxic amounts, would clear macaques of their natural infection with *E. histolytica*, studies with new agents were initiated at the University of California, Division of Pharmacology. After *in-vitro* tests had revealed a level of activity comparable to or greater than that of emetine, toxicity studies in a variety of laboratory animals were undertaken. For acute and chronic toxicity determinations, guinea pigs, rabbits and cats and finally monkeys were employed. Macaques offered certain advantages over rodents, in that it is possible to determine whether or not naturally infected subjects respond differently to the toxic effects of a new drug. Also, since rodents neither vomit nor show the central effects of some drug types, such as the arsenicals, monkeys are preferred. It was subsequently shown by Bertino (21), as well as Lanz and Hamilton (70), that the distribution of some agents, notably the pentavalent arsenicals, is unique for rats, in that nearly 50% of the metal may be retained by the blood cells. These facts argue against reliance upon rodents as a class, and rats in particular, as test animals for the experimental therapy of amebiasis.

Thus far, with the single exception of carbarsone, which appears more active against amebas in rodents and man, it has been possible to develop parallel responses in macaques and in man. This statement applies to emetine, where it has been confirmed that toxic amounts are required for therapeutic effectiveness. Vioform, among the halogenated hydroxyquinolines, was found to be the most effective of the possible iodo, chloro and bromo derivatives (7). More recently, the thio derivatives (C. C. 914 and 1037) of the arsine oxide analog of carbarsone have proved effective in tolerated doses in macaques and man (10).

In monkeys, it is possible to observe changes in the blood, urine, heart, liver and kidneys by clinico-pathologic tests identical with those performed in man. It is also possible to detect blood levels and pathologic changes more readily in the tissues of sacrificed animals. Trials in man can begin with greater confidence with such data, and with less likelihood of producing harm on initial tests with completely new agents. Certainly, the host's response to a new drug is of equal importance to that of the ameba, a factor not always fully appreciated. Table III summarizes the types of agents active in amebiasis in macaques.

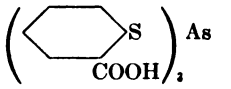
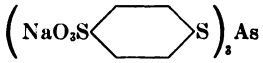
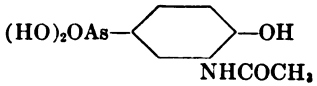
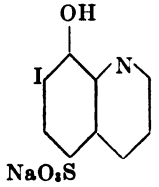
PHARMACOLOGIC STUDIES OF THE THIOARSENITES

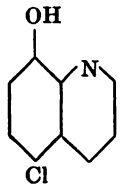
Work with the thioarsenites is presented as an example of the kind of study required for new amebacides (10). They have been found to exert topical effects

TABLE III

Types of Agents Active in Vivo *Against Amebic-Infected Macaques*[a]

AGENT	FORMULA	DAILY DOSE, MGM./KG.	NUMBER OF DAYS GIVEN	FOLLOW-UP EXAMINATION OF STOOLS FOR *E. histolytica*[c]
Plant principles				
Emetine·HCl, subcutaneously	See Table II	10	5	+ (22nd day)[e]
Emetine·HCl, enteric coated tablets		10.5	5	+ (26th day)[e]
Emetine bismuth iodide, orally[b]		50	7	(4/5 cleared)[d]
Cephaeline isoamyl ether acid phosphate, orally[b]		20	10	− (2 months)[e]
Crystalline fraction of chaparro amargoso[b]		25	10	− (3 months)[e]
Yatanocide		5	10	+ (14–21 days)[e]
Antibiotics				
Actidione (Upjohn)		0.1–0.45	10	+ (2/3—8th day)[e] − (1/3—4 months)
Antibiotic "S" (Abbott)		10–40	10	+ (8th day)[e]
Aureomycin HCl (Lederle)		333, 500 and 800	7–10	+ (8th, 21st and 25th day)[e]
Lupulon (W.R.R.L.)		150–250	13	None cleared[e]
NA7M10 (Abbott)		0.8–8.0	2–7	− (2/8 < 30 days)[e]; others not cleared
Polymyxin B		5–20 mgm.	5–7	− (5/10—3 months)[e]; others shorter periods
Bacitracin		1250–5000 units		
Streptomycin (Chas. Pfizer)		62.5– 250 mgm.		
Subtilin (W.R.R.L.)		90	5	None cleared[e]
Terramycin (Chas. Pfizer)		50–200	7–10	− (6/6 cleared over 3 weeks)[e]
Arsenicals, trivalent				
p-Arsenosophenylurea	See Table II	11; 27	30	− (5 months)[e]
3-Amino-4-(2-hydroxyethoxy)benzenearsonous acid intravenously[b]	See Table II	5	5	+ (8th day)[e]
orally[b]		20	10	− (3 months)[e]
γ-(p-Arsonosophenyl)butyric acid, intravenously or intramuscularly[b]	See Table II	3; 5	5; 10	+ (25th day)[e]

2-Amino-4-arsenosophenol, sodium formaldehydesulfoxylate methanolate dihydrate, orally[b]	See Table II	10; 25	7; 10	+ (22nd day)[e] (died at higher dose)
p-[Bis(o-carboxyphenylmercapto)arsino]phenylurea, orally	See Table II	25	7	− (3 months)[g]
Tris(o-carboxyphenylmercapto)arsine (tris(o-carboxyphenyl) trithioarsenite; "arsenious trithiosalicyclic acid"), orally[b]		8; 16	5; 10	(Cleared only with lethal doses)[g]
Tris(p-sulfophenylmercapto)arsine, trisodium salt trisodium tris(p-sulfophenyl) trithioarsenite; "arsenious trithiophenylsulfonic acid," trisodium salt), orally[b]		10; 20	5; 10	(Cleared only with lethal doses)[g]
Arsenicals, pentavalent				
p-Carbamidobenzenearsonic acid (carbarsone, U.S.P.), orally	See Table II	30	23	(3 months)[e] 3/7 cleared
3-Amino-4-(2-hydroxyethoxy)benzenearsonic acid, orally	See Table II	40	10	± (3 months)[e]
3-Acetamido-4-hydroxybenzenearsonic acid (acetarsone, N.N.R.; Stovarsol; N-acetyl-4-hydroxy-m-arsanilic acid), orally		30	12	+ (22nd day)[g]
Halogenated hydroxyquinolines				
5-Chloro-7-iodo-8-hydroxyquinoline (Vioform, N.F.), orally	See Table II	100	10	− (3 months)[g]
Sodium 7-iodo-8-hydroxy-5-quinolinesulfonate (sodium salt of chiniofon, U.S.P.), orally		500	28	(2/4 cleared)[f]

AGENT	FORMULA	DAILY DOSE, MGM./KG.	NUMBER OF DAYS GIVEN	FOLLOW-UP EXAMINATION OF STOOLS FOR *E. histolytica*[a]
5-Chloro-8-hydroxyquinoline, orally.....		300	5	+ (10 days)[g]

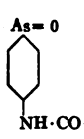
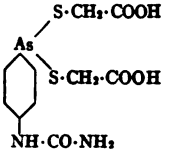
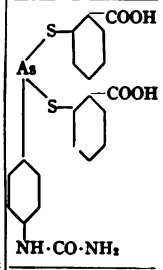
[a] Appraisal technic developed and reported by Anderson and Koch (15); dose based on toxic and tolerated levels in experimental animals.
[b] Untoward or toxic effects noted during therapy.
[c] + = positive; − = negative on examination of iron-hematoxylin stained stool specimens.
[d] Dobell and Bishop (43).
[e] Personal communication, Ethel McNeil *et al.*
[f] Kessel (66).
[g] Anderson *et al.* (6) (10) (15) (60).

on the gastric mucosa similar to but less harmful than those produced by arsine oxides. These are characterized by hyperemia of the gastric mucosa and may be overcome, in part, by the substitution of dithio groups for oxygen attached to trivalent arsenic. Substitution of -SH groups for oxygen detoxifies arsenicals for host's tissue cells. Substitution of methyl or phenyl groups for hydrogen in the

TABLE IV

*Toxic and Tolerated Doses† of Carbarsone Oxide and its Dithio Derivatives**

(Single doses expressed in mgm./kg.)

SPECIES	ROUTE USED	"CARBARSONE OXIDE" 	C. C. NO. 914 	C. C. NO. 1037 
Mice				
LD_{50}'s	I.V.	41.3 ± 1.4‡	42.6 ± 1.9‡	120 ± 6.2
	I.P.	59 ± 6.7	100 ± 9.0	265 ± 12.2
Rats				
LD_{50}'s	I.V.	17	29	70
	I.P.	55 ± 2.1	75 ± 4.7	76 ± 4.1
	I.G.	510 ± 40	1000 ± 39	1220 ± 54
Rabbits				
(lethal range)	I.V.	20–40	100	100–300
(tolerated level)	I.G.	75	300	200–300
Monkeys				
(tolerated level)	Oral (daily)	40	50	50
Man				
(tolerated level)	Oral (daily)	90	600	600

* Results of Anderson *et al.* (10).

† In mgm. per kg. except for man whose total dose is given.

‡ Observations of Dr. K. K. Chen, Lilly Research Laboratories.

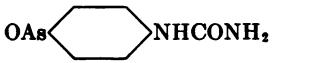
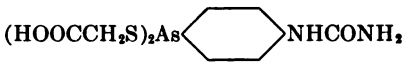
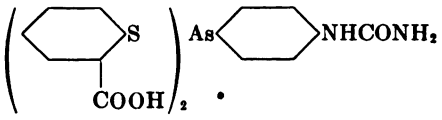
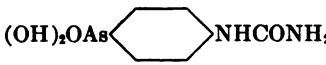
I.P. = Intraperitoneal; I.V. = Intravenous; I.G. = Intragastric.

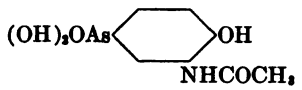
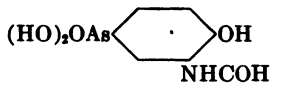
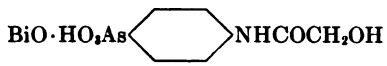
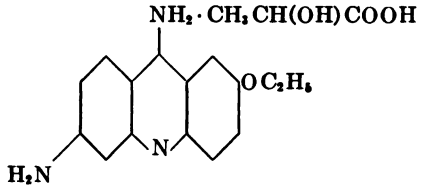
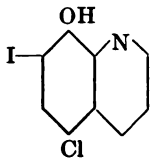
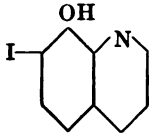
dithio portion of the molecule, plus the addition of carboxyl groups to the thiol radical for solubilizing the compound, results in agents effective orally or rectally in amebiasis, in generally tolerated amounts.

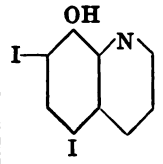
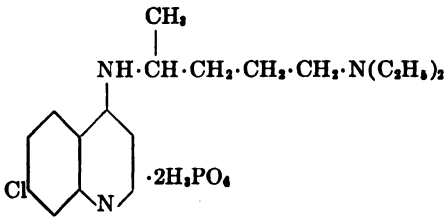
When administered by these routes, slow hydrolysis of the drugs occurs in acid solution, according to Bierwagen (22). Thus, it is conceivable that liberation of small amounts of arsine oxide may continue over a prolonged period.

TABLE V

Types of Amebacides Used with Some Success in Man

AGENT	FORMULA	TOTAL DAILY DOSE (MGM.)	NUMBER OF DAYS GIVEN	ADVANTAGES OR DISADVANTAGES
Plant principles				
Emetine HCl, subcutaneously	See Table II	65	7–10	Clears acute symptoms[b], 16–33% of cases cleared of amebas
Emetine HCl, enteric coated tablets[a], orally		65	10	Clears acute symptoms[c]
Emetine bismuth iodide, tablets, orally		120	10	Clears acute symptoms[d]
Kurchi alkaloids (with bismuth iodide), orally[a]		1300	10	Less effective than emetine[e]
Arsenicals, trivalent				
p-Arsenosophenylurea, enteric coated tablets[a], orally		60–90	10	Effective but irritant to gastro-enteric mucosa[n]
Thioarsenites				
C.C. #914, p-[Bis(carboxymethyl-mercapto)arsino]phenylurea[a], orally		150–300	10–20	90% effective against trophozoites in tissues and cysts, also in lumen of the colon[o]
C.C. #1037, p-[Bis(o-carboxyphenyl-mercapto)arsino]phenylurea[a], orally		150–300	10–20	90% effective against trophozoites in tissues and cysts, also in lumen of the colon[o]; has also been used rectally
Arsenicals, pentavalent				
Carbarsone, p-Carbamidobenzenearsonic acid, orally[a]		750	10–20	90% effective against amebas in lumen of colon or in tissues; has also been used rectally[r]

Acetarsone, 3 - Acetamido - 4 - hydroxy-benzenearsonic acid, orally		750	10	Less effective[f]; arsenic reaction frequent
Treparsol, N-formyl-4-hydroxy-m-arsanilic acid, orally		500	10	Less effective[g]; arsenic reaction frequent
"Wia" ("Milibis") bismuth glycolylarsanilate, orally		1500	8–10 (repeated up to 4 times)	Clinical "cure" in 70–82%[p], ineffective systemic absorption
Acridines				
Rivanol, 6,9-Diamino-2-ethoxyacridine lactate, orally		225	20	Original observations not confirmed[h]
Halogenated quinolines				
Vioform, 5-Chloro-7-iodo-8-hydroxyquinoline, orally[a]		1000	15–30	90% effective against amebas in lumen of colon or in tissues[s]
Chiniofon, 7-Iodo-8-hydroxyquinoline, orally		1,200–1,800	10–20	Less effective[t]; has also been used rectally

AGENT	FORMULA	TOTAL DAILY DOSE (MGM.)	NUMBER OF DAYS GIVEN	ADVANTAGES OR DISADVANTAGES
Diodoquin, 5,7-diiodo-8-hydroxy-quinoline, orally		1,400–2,000	15–20	75% effective in larger doses[j] (33% relapse rate)
Chloroquine ("Aralen"), 7-chloro-4-(4-diethylamino-1-methyl-butylamino) quinoline, orally		900 (first 2–4 days) then 300	14–21	Prompt symptomatic relief of acute hepatitis and abscess[g]
Antibiotics				
Aureomycin, orally		5,000–7,000	3	Temporarily effective against amebas in lumen of colon[m]
Bacitracin, orally		40–160,000 units	10–20	Temporary clearance of 60%; no absorption of drug systemically[m]
Terramycin, orally		2000	10	2 of 3 cleared[i]
Bacitracin + Polymyxin B + Streptomycin[a], orally		1000–2000 (units) 40–80 500–1000	5–10	Under trial[n]; prompt symptomatic relief of acute diarrhea and dysentery, with weight gain (in malnourished)
Miscellaneous				
"Gavano" (cephaeline derivative?)		750	9	Original studies not confirmed in U.S.A.[k]
Bismuth "subcarbonate" and "subnitrate," orally		Massive doses	—	Symptomatic relief only[l]

[a] Appraisal technic utilized by Reed *et al.* (93).
[b] Introduction by Vedder (108).
[c] Shrapnel (99).
[d] Introduced by Manson-Bahr (44).
[e] Introduced by Chopra (1).
[f] Introduced by Marchoux (78).
[g] Introduced by Flandin (49).
[h] Introduced by Peter (89).
[i] Introduced by Mühlens and Menk (84).
[j] Introduced by Tenney (106); Morton (81).
[k] Introduced by Fitzgerald (48).
[l] Introduced by Deeks (39).
[m] McVay *et al.* (80) and Most (quotes Longacre, re: bacitracin) (82).
[n] Anderson and Johnstone (11).
[o] Anderson *et al.* (13).
[p] Introduced by Hauer (59); also E. W. Dennis (personal communication).
[q] Conan and others (33).
[r] Introduced by Reed *et al.* (93).
[s] Introduced by Anderson, Koch *et al.* (15).
[t] Introduced by Most (currently under trial).

This hypothesis is in keeping with the fact that the thioarsenites are tolerated in larger amounts than the arsine oxides, and yet in some individuals nausea, vomiting and diarrhea have occurred. It has been possible to minimize the topical effects on the gastric mucosa by use of appropriate (4 hour) enteric coatings and thus permit continued administration of therapeutically effective doses, namely, 50 to 100 mgm. thrice daily for 10 days in man. Ambulatory patients are more likely to develop side-effects than those who are confined to bed.

Dogs have been used by Chen and Anderson (31) to detect differences in systemic toxicity, and especially in the gastric distress which is not related to total toxicity of the thioarsenites. In man, some lots which were found to be tolerated in appropriate doses by dogs have caused side-effects in about one out of eight ambulatory patients. Occasionally, continued drug use is thus prohibited.

The two thioarsenites (C. C. No. 914 and No. 1037) exhibited a range of activity comparable to that of carbarsone oxide and significantly greater than that of carbarsone, U.S.P. Comparative toxicity studies in mice, rats and rabbits with oral or parenteral administration revealed greater tolerance in acute and chronic dosage. There was less tissue reaction, especially when the thioarsenites were given orally. Data obtained in the several species studied are summarized in Table IV. Monkeys given 25 mgm. per kg. doses by mouth for 30 doses over two months exhibited no impairment of hepatic, renal or cardiac function, and no evidence of damage to the skin, central nervous system or blood. At higher levels, *e.g.*, 50 mgm. per kg., there was, at autopsy, slight fatty infiltration of the liver and, after the dithiocarboxymethyl derivative (C. C. No. 914), mild albuminoid degeneration of the convoluted tubules of the kidneys.

Other pharmacological data, including distribution of arsenic throughout the tissue of rats, rabbits and monkeys, have been obtained. It is of special interest that the distribution of arsenic, given in the trivalent form, differed from that noted after pentavalent arsenic. There were higher arsenic levels in the bile, liver, kidneys, urine and intestinal tract. Appreciable tissue levels, especially in the blood, persisted in rats for at least nine days after the last dose in short-term chronic toxicity tests. These findings, as well as the low levels of arsenic detected in the brain, favored consideration of the trivalent thioarsenites for trial in human amebiasis.

Table V summarizes comparative data on various other amebacides used with some success in man.

THEORETICAL CONSIDERATIONS OF REQUIREMENTS FOR GROWTH AND CONTROL OF E. HISTOLYTICA

Clinical investigations should be more closely integrated with laboratory studies concerned with the essential metabolism of *E. histolytica* in pure culture. Such studies would lead to the development of entirely new and more rational chemotherapeutic agents. Knowledge of the metabolism of the parasitic ameba is a most important passkey for opening the way to effective control.

The fundamental nutritional requirements of the pathogenic ameba have been under investigation in several laboratories since 1944. An essentially synthetic medium and the required physical conditions, notably the optimal pH and reduction potentials, necessary for amebic growth have been suggested. Chang (29) stated that if a strongly reduced system or systems, other than those produced by bacterial metabolism, could be obtained and employed in a medium, it may be found that the preparation of bacteria-free cultures of *E. histolytica* is a relatively easy matter. But until such a reduced system or systems can be devised, we shall have to rely upon associated micro-organisms for the constant maintenance of profound anaerobiasis.

The mode of action of currently available agents which have therapeutic usefulness requires elucidation by appropriately designed laboratory investigations. As a corollary to such studies, it is important to determine the influence of the bacterial flora and the physical-chemical environment of the lumen of the bowel on the pathogenicity of the amebas. Stewart and Jones (103) found that coliform organisms, isolated from patients having ulcerative colitis, aggravated lesions in rats experimentally infected with *E. histolytica*.

The means by which pathogenic amebas lyse host tissue and thus permit invasion in the absence of associated bacteria is a problem of considerable importance in systemic amebiasis. Certainly, when the cytolysin is more fully understood, chronic colitis, abscesses in parts of the body remote to the bowel and many problems incidental to their development may be more clearly understood. It is conceivable that a new approach to the chemotherapy of amebiasis may be developed as a result of greater knowledge of the cytolysin alone.

LABORATORY EVALUATION OF CYSTICIDES

Heteropolar cationic, surface-active agents have been examined by Kessel and Moore (67) for the sterilization of food and water. Halogenated compounds have not been entirely satisfactory because of their partial inactivation by organic nitrogenous materials and by alkaline solutions; high concentrations of iodine and chlorine are also distasteful and may be toxic, thus rendering treated materials unfit for consumption.

From such tests, the effective amounts required to kill 50% of cysts (ED_{50}) were calculated according to the methods of Reed and Muench (94). The results of Kessel and Moore (67), summarized in Table VI, suggest the types most active in dilutions of 1:20,000 or more. Their tests are in agreement with Wright's observations and were confirmed by Fair *et al.* (46).

The relative cysticidal activity *in vitro* of diiodides and triiodides has been reinvestigated by Fair *et al.* (46). For solutions containing 100 p.p.m. of iodide ion, in which 90% or more of the active iodine is present as triiodide, the cysticidal level was found to be 20 to 25 p.p.m. for a 10-minute contact period at 23° C. at pH 5, indicating that triiodide is not as effective as diiodide solution. Monochloramine (NH_2Cl) and dichloramine ($NHCl_2$) solutions were found by Fair *et al.* to show greatest difference in activity at low temperatures (3° C.). Thirty minutes of contact at pH 4 showed dichloramine to be cysticidal at 4.0

TABLE VI

*Cysticidal Activity of Cationic Detergents**

AGENT	CYSTICIDAL ED_{50} (1 PART PER)
n-Hexadecyltrimethylammonium bromide	70,800
n-Hexadecyldimethyl(2-hydroxyethyl)ammonium chloride	87,100
n-Hexadecyldiethyl(2-hydroxyethyl)ammonium chloride	33,900
n-Hexadecyl-di-n-propyl(2-hydroxyethyl)ammonium chloride	20,000
n-Hexadecyl-di-n-butyl(2-hydroxyethyl)ammonium chloride	85,100
"Zephiran" ("Roccal"), industrial grade	20,000
"Zephiran" ("Roccal"), technical crude	40,700
Alkyldimethylbenzylammonium chlorides (C_{14} to C_{18})	41,700
{2-[2-(p-t-Octylphenoxy)ethoxy]ethyl}dimethyl(p-chlorophenyl) ammonium chloride[a]	46,800
{2-[2-(p-t-Octylphenoxy)ethoxy]ethyl}dimethylcinnamylammonium chloride[a]	>20,000
[2-(p-t-Octylphenoxy)ethyl]dimethylbenzylammonium n-undecylate[a]	38,000
1-n-Tetradecylpyridinium chloride	20,900
1-n-Tetradecyl-3-carbamylpyridinium bromide	56,200
1-n-Tetradecyl-4-methylpyridinium chloride	182,000
1-n-Hexadecylpyridinium chloride	74,000
1-n-Hexadecyl-3-carbamylpyridinium bromide	21,600
1-Allyl-4-n-tridecylpyridinium bromide	28,800
1-n-Hexadecyl-1-(2-hydroxyethyl)piperidinium chloride	37,200
1-n-Hexadecyl-1-(2-hydroxyethyl)morpholinium chloride	58,900
1,3-Di-n-octylbenzotriazolium bromide	>42,700

[a] t-Octyl is the group $(CH_3)_3C\cdot CH_2C(CH_3)_2$—.

* Kessel and Moore (67).

TABLE VII

Summary of Current Therapy of Amebiasis

Chronic Amebiasis

Carbarsone, U.S.P. (28.5% As)—0.25 Gm. given B.I.D. or T.I.D. for 20 oral doses. Rest period of 7 to 10 days, then repeat schedule if required.

Vioform, N.F. (40% I, 12% Cl)—0.25 Gm. given T.I.D. or Q.I.D. for 30 oral doses. Rest period as above, and repeat if necessary.

Each of these drugs may be repeated, alone or in combination, over a 4 to 6 weeks' period.

Diodoquin, N.N.R. (64% I), or *Chiniofon*, U.S.P. (29% I), may be substituted for Vioform. Dose to 1.0 Gm. B.I.D. or T.I.D. for 15–20 days.

*Acute Dysenteric Phase**

Emetine HCl, U.S.P., 0.065 Gm., subcutaneously for 7–10 days to control symptoms. Enteric-coated tablets used experimentally. *Interrupt course if untoward reaction develops.* Simultaneous or alternate use of agents listed for chronic amebiasis *is imperative.*

Carbarsone, U.S.P. *or Chiniofon*, U.S.P., *Retention Enemas*, 2.0 Gm. dissolved in 200 cc. of 2% $NaCHO_3$ for five nights, should provide prompt control of acute symptoms.

Amebic Hepatitis or Abscess

Emetine HCl, U.S.P. (of diagnostic aid)—given as above is *not curative.* Agents for chronic amebiasis must be given when hepatic function permits. Aspiration required in localized abscess.

Chloroquine, N.N.R., 0.3 Gm. orally T.I.D. for 2 to 4 days, followed by 0.3 Gm. daily for a total of 14 to 21 days. Currently, this agent is preferred to emetine for control of hepatic (but not enteric) amebiasis.

* Current studies indicate that antibiotics, such as aureomycin or terramycin, given orally may supplant emetine when enteral (superficial) lesions are encountered. Aureomycin, bacitracin, and terramycin have recently been used in limited trials (Most, 82).

p.p.m.; at pH 7, mixed chloramines at 11 p.p.m.; at pH 10, monochloramine at 25 p.p.m.

More recently, Newton and Jones (86) have demonstrated that ozone in 0.7 p.p.m. on five-minute exposures destroyed cysts of *E. histolytica*. Cysticidal action did not appear to be significantly influenced by pH, temperature or organic nitrogen in tests conducted in aqueous solution. Silver has also been studied recently by these authors.

SUMMARY

Drug-refractory, chronic, recurrent amebiasis is a problem of clinical importance in the Western Hemisphere. The usefulness of the currently available drugs has been indicated and their pharmacological characteristics compared with those of more recently developed agents. The requirements for laboratory evaluation of new agents have been indicated with particular reference to the need for distinguishing indirect action of antibacterial chemicals *in vitro* and to the use of the naturally infected macaque as the most suitable laboratory animal in which the pathologic condition compares favorably with that of chronic infection in man. As an example, development of the thioarsenites as chemotherapeutic agents in amebiasis is presented, and an evaluation is made of the place of representative antibiotics in the therapeutic regimen of amebiasis.

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