the bronchial muscle stained rather faintly with the latter). Following this lead, formalin-fixed sections were incubated in the plain alpha naphthol acetate substrate mixture as before, and other sections in the same mixture with 10^{-5} M eserine added. In the latter group of sections, the activity of the alcohol-resistant sites was completely prevented, and the pictures obtained were the exact reverse of those seen after fixation in 95% alcohol.

The specificity of esterases is a rather complicated problem because of multiple overlapping of substrate preferences(3-5) and of sensitivities to various inhibitors(6). The sensitivity to eserine appears to be the most clearcut differential criterion between aliesterases and cholinesterases(7,8). On this basis, the conclusion that alcohol (95% or weaker) destroys the aliesterase hydrolyzing alpha naphthol acetate in dog organs while it spares a cholinesterase hydrolyzing both alpha naphthol acetate and myristoyl choline, is warranted. In other species this need not be the case since the specificity of esterases varies greatly in different species(6).

Summary. In tissues of the dog, 95% alcohol as a fixative selectively destroys aliesterase while it preserves cholinesterase reasonably well.

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Immobilization of *Endamoeba histolytica in vitro* by Antiserum Produced in the Rabbit. (20500)

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The demonstration that syphilitic infection produces antibody which immobilizes *Treponema pallidum*(1) suggested the possibility that amoeba-immobilizing antibody might appear following infection with *Endamoeba histolytica*. The studies reported herein show that the sera of rabbits inoculated repeatedly with suspensions of cultured *E. histolytica* develop the capacity to immobilize trophozoites of this species *in vitro*.

Materials and methods. All glassware was cleaned with dichromate-sulfuric acid mixture prior to sterilization. A single strain* of *E. histolytica* was employed throughout the studies. The active trophozoites required for immobilization tests were obtained from cultures with a flora of mixed bacteria† on Locke-egg-serum medium(2); a modified(3) Locke's salt solution was used. The best and most consistent yield of motile amoebae was obtained after 20-24 hours incubation at 37°C. A measured volume (0.5 ml) of culture taken from the lowest level of liquid phase was transferred by pipette to a 12 x 75 mm test tube, where it was pooled with aliquots from one or more similar cultures. The tube was then stoppered and replaced in the incubator for an additional 3 hours at 37°C; this served to enhance motility of the trophozoites. During

* Strain 200, obtained from the Laboratory of Tropical Diseases, National Microbiological Institute, Bethesda, Md.

† An adventitious combination, the predominating organisms being a Corynebacterium of the xerosis group (sucrose +), an alpha hemolytic streptococcus, and an organism of the achromobacter group.

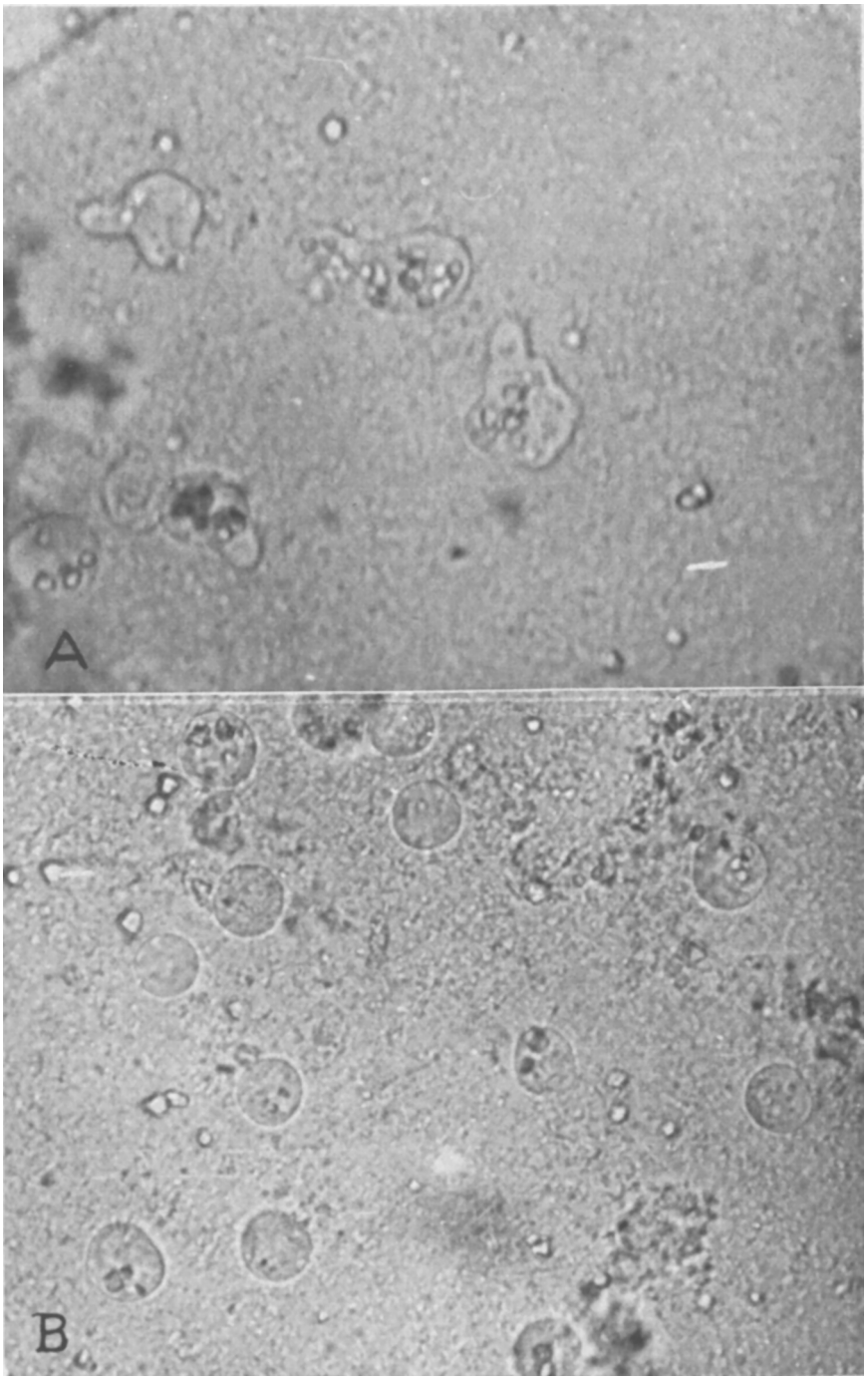

FIG. 1. *Endamoeba histolytica* after 30 min. incubation at 37°C with (A) normal rabbit serum and (B) anti-*E. histolytica* rabbit serum. ×700.

the first half-hour of supplementary incubation the combined cultures were carefully mixed by drawing the material repeatedly into a pipette, and a representative sample was transferred for counting to a Neubauer chamber. Satisfactory culture pools contained

at least 2 x 10^5 amoebae per ml; they were standardized at that count by adding a calculated volume of warm (37°C) Locke's-serum-salt solution(2). In the immunization of rabbits it was desired to avoid if possible any stimulation of antibacterial substances, such as might result from use of the amoeba-bacteria culture as inoculum, so advantage was taken of the fact that *E. histolytica* may be cultured also with *Trypanosoma cruzi*(4). The amoeba-trypanosome inocula were prepared from 48-hour cultures showing heavy growth of amoebae. The organisms were washed 3 times with 0.85% NaCl solution and concentrated by centrifugation for 10 minutes at 500 G. They were then suspended in a volume of NaCl solution such that the desired inoculum would be contained in 0.5 ml. Healthy adult rabbits were injected intravenously 3 times weekly for 3 weeks, receiving the organisms from 2 culture tubes at each injection during the first 2 weeks, and those from 4 tubes each time in the third week. The animals were bled by cardiac puncture 7-10 days after the last injection; the sera were separated aseptically and stored at −20°C. Immediately before testing the required volume of serum was heated for 30 minutes in the water-bath at 56°C, after which serial dilutions were made in Locke's salt solution (3). For the immobilization tests, 0.05 ml of the standardized amoeba suspension was mixed on a glass slide with an equal volume of the serum to be tested. The mixture was then covered with a 22 x 22 mm coverslip, which had been rimmed with petrolatum to minimize drying and maintain partial anaerobiasis. The slide was placed finally on an electric warming stage at 37°C, and examined under the low power of the microscope at intervals from 5 minutes to 4 hours. The number of motile trophozoites among the first 25 observed was recorded. In estimating the percentage of amoebae specifically immobilized, the number (A) of motile trophozoites in the test with antiserum was subtracted from the number (B) found motile in the control with normal serum, and the difference multiplied by 100/B.

Results. Sera from rabbits inoculated with *E. histolytica* plus *T. cruzi* regularly immobilized amoebae of the same strain cultured with

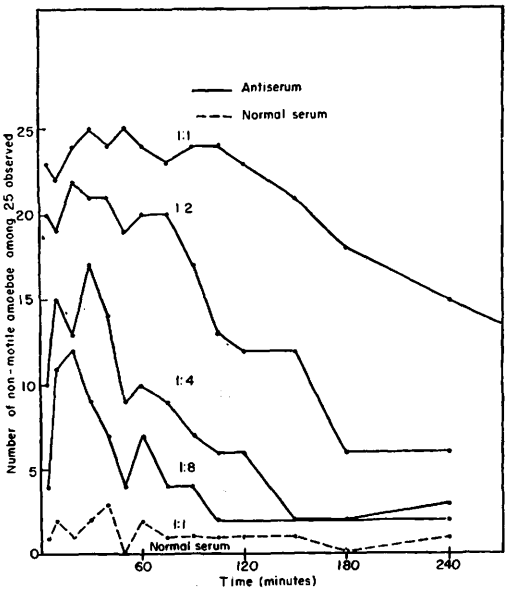

FIG. 2. Immobilization of *E. histolytica* as a function of time and the concentration of rabbit antiserum.

mixed bacteria as commensals. Morphological changes associated with the reaction are illustrated in Fig. 1; when mixed with antiserum, motile trophozoites ceased formation of pseudopodia and assumed a rounded, somewhat contracted form. The results of a representative experiment with serial dilutions of antiserum are given in Fig. 2. The numbers of non-motile amoebae are plotted against the time of incubation in minutes. Included for reference are the numbers of non-motile organisms found concurrently in controls with normal rabbit serum. The number of immobilized trophozoites increased directly with the concentration of antiserum, but varied considerably with the time. Maximal immobilizing effect was observed after 20-30 minutes, the amoebae gradually regaining their activity thereafter. Trophozoites in the controls with normal serum remained actively motile throughout the period of observation. Similar results were obtained with 4 other antisera. The capacity to immobilize *E. histolytica* was not demonstrable in serum from 2 rabbits immunized against *T. cruzi* alone. Evidence of the reproducibility of the immobilization reaction was obtained in repeated titrations of another antiserum; the results appear in Table I. All 7 titrations were per-

TABLE I. Reproducibility of *Endamoeba histolytica* Immobilization Reaction.

Titration No.*	Amoebae immobilized (%) in tests with antiserum (62851-1) diluted in salt sol.				
	1:1	1:2	1:4	1:8	1:16
1	89	71	55	21	3
2	83	74	55	31	17
3	81	67	24	33	10
4	87	63	42	18	8
5	89	64	36	32	16
6	80	69	41	15	13
7	80	65	53	25	15

* Replicate titrations performed during a single day with separate sets of serum dilutions and amoebae from separate cultures.

formed during a single day using separately-prepared sets of serum dilutions and amoebae from separate cultures. The motile amoebae in tests and controls were counted after 30 minutes incubation at 37°C; percentage of specific immobilization was estimated by a method already given herein. Patent discrepancies occurred in individual test mixtures, as in titrations 3 and 5, but the findings otherwise were in good agreement. Two subsequent observations were significant in that they indicated the direction for further standardization, and suggested a practical application of the immobilization reaction. Thus *E. histolytica* cultured with a single bacterial species(5) as commensal could be substituted successfully for the amoebae grown with mixed bacteria. Preliminary tests showed also that the capacity to immobilize amoebae occurs in serum from patients with amoebiasis. In a group of 13 individuals shown to be infected with *E. histolytica* 5 reacted in the immobilization test. The reactive capacity of their sera was relatively low, however, as was that of the rabbit antisera (Fig. 2 and Table I). Serums from a group of 48 normal individuals were without effect upon the amoebae.

Discussion. The immobilization of *E. histolytica* by rabbit antiserum differs in certain essential characteristics from the corresponding reaction between *T. pallidum* and its antibody. In the case of the treponeme the loss of motility appears to be an irreversible one (1). A marked difference in the studies with amoebae was the early recovery of immobilized trophozoites. It appears also that complement (C′), which plays an important role in the reaction with treponemes(1), may not be essential in the immobilization of amoebae. In the present experiments each of the sera had been heated under conditions ordinarily employed for inactivation of labile C′ components, so it could be assumed that the latter at least did not participate in the reaction. Further studies of the nature and kinetics of the amoeba-immobilization phenomenon are clearly indicated. At the same time serious consideration should be given to practical applications of the reaction as a diagnostic aid. Any method which can demonstrate a serologic response to *E. histolytica* may assist in establishing the etiological agent in obscure clinical illnesses. The simplicity and reproducibility of the present technic suggest its early evaluation. Any further studies, moreover, should consider the relationship between complement-fixing antibody in amoebiasis and the capacity to immobilize trophozoites. The demonstration of a distinct immobilizing function, as in the case of the treponematoses, would be especially significant in view of the limited diagnostic usefulness(6) of the complement-fixation test.

Summary. The sera of rabbits inoculated with cultures of *E. histolytica* immobilize the trophozoites of this species *in vitro*. The capacity to immobilize amoebae occurs also in the serum of individuals infected with *E. histolytica*. A technic for demonstrating the immobilization reaction is described, and evidence of its reproducibility is presented.

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