TABLE II.
Neutralizing Antibody Titers of Sera of Guinea Pigs After a Single Injection of Rabies Vaccine.

Group	Serum dil.	1:5*	1:10	1:20	1:40	1:80	1:100	1:1000	1:10,000
34 Days—									
I	Vaccine A		0/6†				6/6	6/6	6/6
II	" A in oil + *M. butyricum*		0/5				1/5	5/5	6/6
III	" B		1/6				4/4	6/6	6/6
IV	" B in oil		0/5				0/6	0/6	6/6
V	" B " " + *M. butyricum*		0/6				0/6	3/6	6/6
VI	" C		0/6				3/5	6/6	4/5
VII	" C in oil		0/5				0/6	2/6	4/5
VIII	" C " " + M. *butyricum*		0/6				0/6	6/6	5/6
71 Days—									
III	Vaccine B		2/7				8/8		
IV	" B in oil		0/8				1/8	8/8	
VI	" C	0/7	4/7	6/7	7/7	7/7	8/8		
VII	" C in oil	1/7	0/8	0/7	1/7	6/7	7/8		

* 0.015 ml of a 1:2.5 dilution of serum + 0.015 ml of a fixed virus-brain suspension diluted 1:300,000. Titer of virus suspension: 10^{-6}: 8/8; 10^{-7}: 5/8; 10^{-8}: 3/8.
† 0 of 6 mice died of rabies.

numerical relationship to the antibody content of the CNS.[5] Deviation from the ratio occurs when antibodies are produced in the CNS itself as a result of infection.[6] Immunization with the aid of water-in-oil emulsion may be applicable to prophylactic immunization of dogs and to the production of "hyperimmune" sera for the treatment of man and lower animals after exposure to rabies infection. Habel[7] found that greater protection against rabies can be obtained in experimental animals with the combination of passive and active immunization than with the latter alone.

Summary. The incorporation of inactivated rabies vaccine into water-in-oil emulsion enhanced the antigenic property of the vaccine as judged by the formation of neutralizing antibodies. Allergic encephalitis did not occur unless mycobacteria were added to the emulsion.

[5] Freund, J., *J. Exp. Med.*, 1930, **51**, 889.
[6] Morgan, I. M., *Am. J. Hygiene*, 1947, **45**, 390.
[7] Habel, K., *Pub. Health Rep.*, 1945, **60**, 545.

16572

Demonstration of Acid Phosphatase in *Endamoeba histolytica.**

G. M. CARRERA AND G. W. CHANGUS.† (Introduced by E. C. Faust.)
From the Department of Tropical Medicine and Public Health and the Department of Pathology and Bacteriology, Tulane University School of Medicine, New Orleans.

Acid phosphatase activity can be demonstrated in *Endamoeba histolytica* using Gomori's histochemical method.[1] The amebae, originally isolated from human cases and grown in Balamuth's liquid media[2] or obtained by rectal aspiration from an experimentally infected dog, consistently give strongly positive histochemical acid phosphatase reaction.

Although the method used is fundamentally the one described by Gomori, slight technical modifications are necessary to make it adap-

* Assisted by a Research Grant-in-aid, National Institute of Health, Bethesda, Md.
† Fellow, American Cancer Society.
[1] Gomori, G., *Arch. Path.*, 1941, **32**, 189.
[2] Balamuth, W., *Am. J. Clin. Path.*, 1946, **16**, 380.

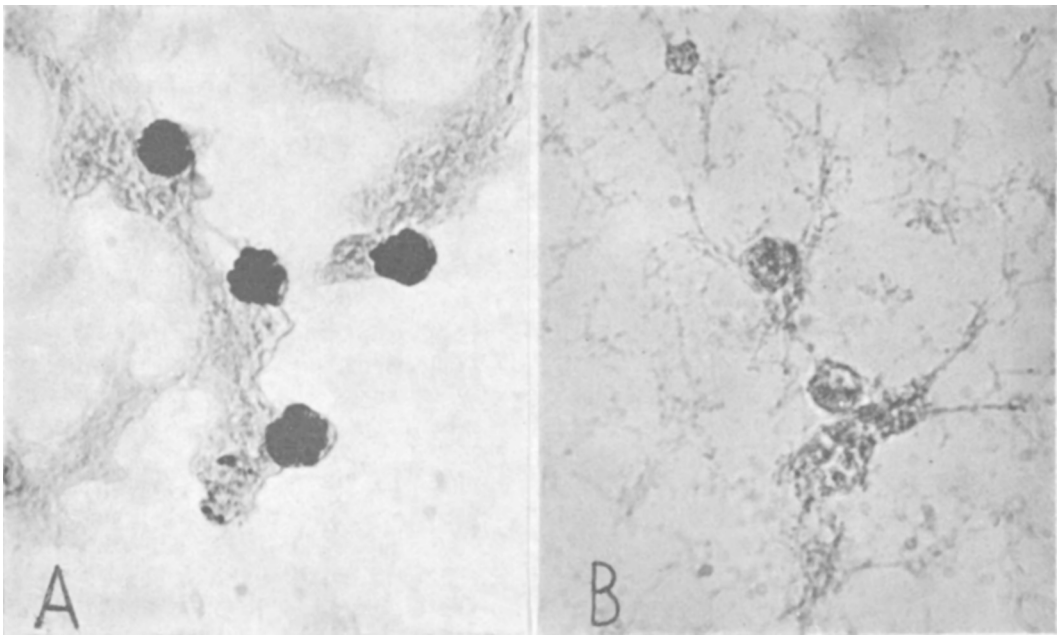

FIG. 1.

A. *E. histolytica* showing positive acid phosphatase reaction. Eosin counterstain. Acid phosphatase is indicated by black deposits in the organism. (Magnification × 510.)

B. Control. *E. histolytica* showing no black deposits. Eosin counterstain. The granularity seen in the background and in the organisms is red in the original side. (Magnification × 510.)

table for amebae. Recently, Rabinovitch, Junqueira and Mendes,[3] and Bayliss, Glick and Siem,[4] have reported modifications of Gomori's method for bone-marrow smears and bacteria and fungi respectively. In dealing with *E. histolytica* care must be taken not to let the smears dry completely before immersing in the fixative, since the limiting membrane of the ameba is very fragile and ruptures upon drying. The smears should be allowed to stand at room temperature until they become "glossy" and are then fixed in chilled acetone for 30 seconds following Gomori's technic, although positive phosphatase reactions have been obtained by the present workers in smears that have been fixed in acetone in the refrigerator for as long as 26 hours. Incubation at room temperature (26-29° C) has been found to be satisfactory, therefore eliminating the need for a water bath. The period required to produce a positive reaction with *E. histolytica* has been found to be at least 45 minutes at room temperature. The amount of precipitate seen in the amebae increases with increased incubation. At 2 hours the cytoplasm is almost completely filled with precipitate, while incubation overnight brings out even greater amounts of precipitate. Control preparations were made in all experiments, either by omitting the glycerophosphate from the buffered reagent or by adding to the control substrate sodium fluoride (M/300), a known acid-phosphatase inhibitor. Since controls were negative, positive results can be interpreted as being due to phosphatase activity. Fig. 1 shows positive reaction (left) and control (right).

Summary. The presence of acid phosphatase in *E. histolytica* has been demonstrated by a modification of Gomori's histochemical method.

[3] Rabinovitch, M., Junqueira, L. C. U., and Mendes, F. T., *Science*, 1948, **107**, 322.

[4] Bayliss, M., Glick, D., and Siem, R. A., *J. Bact.*, 1948, **55**, 307.