TABLE V. Reproducible Infection Rates in Cynomolgus Monkeys by the Oral Route of Virus Inoculation.

No. monkeys	Virus 20% single dose of 5 ml Mahoney virus in 25 ml cream (12%)	Fate	% paralyzed
10	Virus given by stomach	5/10*	50
10	tube 48 hr after	6/10	60
10	fasting	5/10	50
10		7/10	70
10		5/10	50

* 5/10 = 5 monkeys developed paralytic poliomyelitis out of 10 monkeys inoculated.

it difficult to predict the day of infection. In prophylactic as well as therapeutic studies, the day of infection is of considerable importance. Thus virus given orally in one inoculation which produces a consistent infection rate enables one to more nearly predict the time of infection.

Summary. A method of orally infecting cynomolgus monkeys is presented. Consistent and reproducible infections (56%) have been obtained using the Mahoney strain (Type 1) poliomyelitis virus mixed with cream (12% fat) and introducing the virus-cream mixture into the stomach by means of a Fr. No. 16 urethral catheter in a single dose.

1. Kling, C., Levaditi, C., and Lepine, P., *Bull. Acad. de Med. Paris,* 1929, v102, 158.

2. Schultz, E. W., PROC. SOC. EXP. BIOL. AND MED., 1929, v26, 632.

3. Levaditi, C., Kling, C., and Hornus, G., *Compt. rend. Soc. de biol.,* 1933, v112, 43.

4. Vignec, A. J., Paul, J. R., and Trask, J. D., PROC. SOC. EXP. BIOL. AND MED., 1939, v41, 246.

5. Burnet, F. M., *Med. J. Australia,* 1940, v1, 325.

6. Bodian, D., and Howe, H. A., *J. Exp. Med.,* 1945, v81, 255.

7. ———, *Am. J. Hyg.,* 1948, v48, 99.

8. Sabin, A. B., *Am. J. Pub. Health,* 1951, v41, 1215.

9. Bodian, D., *Am. J. Hyg.,* 1953, v56, 78.

Received July 17, 1953. P.S.E.B.M., 1953, v83.

Selective Inactivation of Esterase by Alcohol. (20499)

GEORGE GOMORI AND HÉLIO B. COUTINHO.*

From the Department of Medicine, University of Chicago, Chicago, Ill.

In the course of studies on the distribution of esterases in the organs of the dog, tissues were fixed in 2 different fixatives: a mixture of equal parts of absolute acetone and 95% alcohol, and neutralized 10% formalin, both chilled. Formalin was washed out thoroughly under the tap, and ice cold acetone was used as the first dehydrating agent. Previous experience has shown that failure to wash out the formalin or the use of alcohol for initial dehydration may result in distinct weakening of enzymatic activity. It was soon found that in formalin-fixed tissues the overall intensity of the alpha naphthol acetate reaction(1) was definitely higher and its distribution more uniform than in the acetone-alcohol-fixed ones. The latter often showed a patchy distribution of activity, and the surface layers of the tissues were more intensely positive than the deeper portions. On closer inspection of the slides it became obvious that the activity of some structures remained practically the same, regardless of fixation, while that of other structures such as the hepatic parenchyma, the renal tubules, and the bronchial mucosa became profoundly depressed. Evidently, the acetone-alcohol mixture had a tendency to destroy the enzyme at certain sites.

To determine the nature of the noxious agent, pieces of tissue were fixed in absolute acetone, 90% acetone, absolute alcohol and 95% alcohol. After fixation in acetone, either absolute or 90%, the pictures obtained were almost as good as after formalin. Absolute

* W. K. Kellogg Foundation Fellow from the Department of Histology and Embryology, University of Recife, Brazil.

This work has been aided by grants from the Douglas Smith Foundation for Medical Research, University of Chicago, and from the Pathology Study Section, United States Public Health Service.

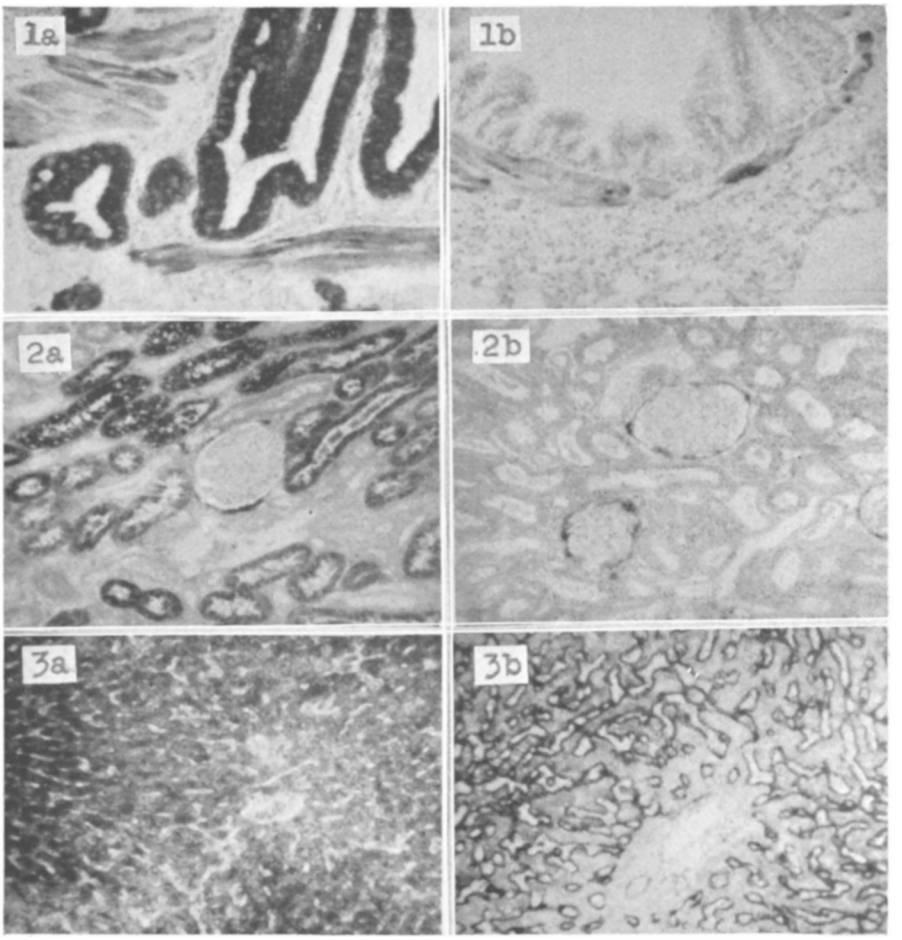

FIG. 1. Lung of the dog. a) Formalin fixation. Bronchial epithelium and smooth muscle stained. b) Alcohol fixation. Epithelium unstained; muscle stained.

FIG. 2. Kidney of the dog. a) Formalin fixation. Renal tubules and Bowman's capsules stained. b) Alcohol fixation. Only Bowman's capsules stained.

FIG. 3. Liver of the dog. a) Formalin fixation. Intense staining of hepatic parenchyma. b) Alcohol fixation. Only the walls of the sinusoids react.

alcohol was distinctly poorer as a fixative and showed some of the effects noted with the acetone-alcohol mixture, while 95% alcohol produced the same effects in an exaggerated fashion. The specific sites mentioned were invariably inactive after fixation in 95% alcohol; on the other hand, other sites such as the walls of the hepatic sinusoids, the mucous neck cells of the stomach, Bowman's capsules in the kidney, etc., lost little if any of their activity (Fig. 1, 2, 3). According to these findings, alcohol, especially in the presence of some water, destroys the enzyme at certain sites.

The question is whether the alcohol-resistant and the alcohol-sensitive localizations the enzyme should be explained by the pr ence of different enzymes or by the same zyme which at certain sites is protected, by unknown mechanism, against the destruct action of alcohol.

The first possibility was considered more likely one, especially when it was disc ered, by comparing slides stained with alpha naphthol acetate method with c responding slides stained with the myrist choline technic(2). The alcohol-resistant calizations of the alpha naphthol method incided remarkably accurately with the str tures revealed by myristoyl choline (altho

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.

1. Gomori, G., *Microscopic Histochemistry,* Chicago, 1952, University of Chicago Press.
2. ———, PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 354.
3. Mendel, B., and Rudney, H., *Biochem. J.,* 1943, v37, 59.
4. Bovet-Nitti, F., *Experientia,* 1947, v3, 283.
5. Whittaker, V. P., *Biochem. J.,* 1949, v44, XLVI.
6. Gomori, G., and Chessick, R. D., *J. Cell. and Comp. Physiol.,* 1953, v41, 51.
7. Easson, L. H., and Stedman, E., *Biochem. J.,* 1937, v31, 1723.
8. Richter, D., and Croft, P. G., *Biochem. J.,* 1942, v36, 746.

Received July 20, 1953. P.S.E.B.M., 1953, v83.

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

BERWIN A. COLE AND JOHN F. KENT. (With technical assistance of Victor A. Lopez.)
From the Tropical Research Medical Laboratory, San Juan, P. R., and the Department of Serology, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D.C.

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.