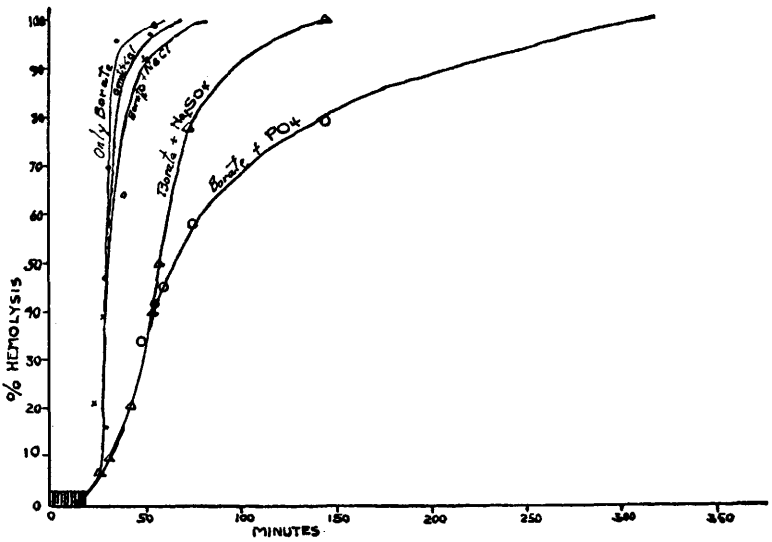

FIG. 3.

As control all suspensions in the various solutions studied containing eosin but unexposed to light were observed for hemolysis. In only one solution was hemolysis noted. That was in the phosphate buffer of pH 5.4. Proper correction in the velocity of hemolysis for the exposed specimen was made. Cell suspensions in the solutions under study containing no eosin were also exposed to light for the same duration as those containing eosin. In no instance was hemolysis observed.

6452

Infection of Kittens with Endamoeba histolytica by Direct Injection of Cultures into the Ileum.

HENRY E. MELENEY AND WILLIAM W. FRYE. (Introduced by E. C. Faust.)

From the Department of Preventive Medicine and Public Health, Vanderbilt University School of Medicine, and the Tennessee State Department of Public Health.

In the course of experiments to test the relative pathogenicity for kittens of a number of strains of *Endamoeba histolytica* in culture, rectal injection proved unsatisfactory because of the small percentage of kittens which became infected. The technique of rectal injection was simply to withhold food on the day of injection, and inject the sediment from a rich culture by means of a rubber-tipped

pipette which was inserted gently through the anus to a distance of about 3 inches. The kitten was then held head downward for a few minutes before being released. In one series the anus was sutured for 24 hours, without producing any better results. A preliminary enema, as used by Kessel[1] was not given because of the desire to avoid even microscopic trauma as far as possible and to avoid early evacuation of the injected material.

Feeding cysts by mouth produced no better results with the one strain with which the method was employed. This method was also impracticable because some strains did not form cysts in culture.

In considering a better route of infection, injection directly into the cecum after laparotomy, as practiced by Rees[2] was not adopted because it was not desirable to pass a needle directly through the wall of the cecum. It was felt that this might create a small lesion in which a few amoebae or bacteria might be left in withdrawing the needle. Faust's method[3] of inoculating dogs directly into the ileum through the anus was not feasible because the colon of the cat is acutely flexed in its upper third, and because there was too much danger of trauma.

It was therefore decided to make the injection directly into the ileum, thus allowing the amoebae to enter the large intestine as they normally would in oral inoculations with cysts. Kittens were anesthetized with ether, the abdomen was opened by an upper mid-line incision, the cecum, which often presented into the wound, was gently pulled outside and the culture injected into the ileum about 5 cm. above the ileocecal valve through a 20 gauge needle. A 5% solution of mercurochrome was applied to the point of injection as the needle was withdrawn to avoid soiling the peritoneum, and the abdomen closed by a continuous silk suture in the muscle and by skin clips. Laparotomy did not shorten the average duration of life of the kittens.

Table I shows a comparison of the results obtained by this method and by rectal injection. With all 5 strains of amoebae the incidence of infection was much higher in the series injected by ileum than in those injected by rectum. The average for all the strains was 54.8% infected by ileum against 21.6% by rectum. The greatest relative increase by ileum was in the strains giving the lowest incidence of infection by rectum. The increased incidence by ileum was obtained despite the fact that, except for the "Ware" strain, the

[1] Kessel, J. F., *Am. J. Hyg.*, 1928, **8**, 311.

[2] Rees, C. W., *Arch. Path.*, 1929, **7**, 1.

[3] Faust, E. C., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 908; *Porto Rico J. Pub. Health and Trop. Med.*, 1931, **6**, 391.

TABLE I.
Inoculation of Kittens with Cultures of *E. histolytica* by Rectum and by Ileum.

Strain of *E. histolytica*	Route of Inoculation	No. Kittens Inoculated	Kittens Developing Amoebic Colitis	
			No.	%
Daffo	Rectum (Not sutured)	20	1	5.0
	Ileum	38	12	31.6
Nelson	Rectum (Not sutured)	36	5	13.9
	Rectum (Sutured)	22	2	9.1
	Ileum	19	15	79.0
Ware	Rectum (Not sutured)	20	3	15.0
	Ileum	20	9	45.0
Cain	Rectum (Not sutured)	30	13	43.3
	Ileum	20	12	60.0
Keen	Rectum (Not sutured)	34	11	32.3
	Ileum	20	13	65.0
Total	Rectum	162	35	21.6
	Ileum	117	61	54.8

amoebae had been in culture a much longer time than they had been when the rectal work was done. Although the average age of the transplants of cultures used in the ileum work happened to be less than the average age of transplants used in the rectal work (we have found that younger transplants produce a higher incidence of infection) this does not invalidate our results, since 4 to 7 day cultures inoculated by ileum produced a considerably higher percentage of infection (45%) than did 1 to 3 day cultures by rectum (31%).

The lesions produced in the kittens injected by ileum did not differ essentially from those produced by rectal injection, except that in the kittens injected by ileum lesions were found more frequently involving the ileo-cecal valve and the cecum adjacent to it. In addition, 3 kittens injected by ileum showed slight lesions in the terminal ileum.

As a result of these observations we believe that injection into the ileum is much more valuable than injection by rectum in testing the pathogenicity for kittens of *E. histolytica* trophozoites in culture. Its only drawback is the necessity for surgical technique, but the uncertainty of giving the amoebae an opportunity to invade the tissues by the rectal route makes the more laborious procedure preferable.