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Effect of Continuous Passage of Endamoeba Histolytica through Experimental Dogs.*

ERNEST CARROLL FAUST AND JOHN CLYDE SWARTZWELDER.

From the Parasitology Laboratory, Department of Tropical Medicine, Tulane University, New Orleans.

Since the development of a satisfactory technic for the cultivation of *Endamoeba histolytica in vitro*[1] it has been shown[2,3] that such cultivation gradually reduces the virulence of this organism and renders it less and less capable of infecting experimental animals. On the other hand, the effect of its successive passages through the experimental host has remained a matter for controversy. Some workers[4, 5, 6] have claimed that the organism becomes less virulent with passage. On the other hand, Baetjer and Sellards[7] state that in 11 direct transfers of an active inoculum through kittens they increased the virulence of the amebæ. Dale and Dobell,[8] after 43 passages through kittens, concluded that evidence of increased virulence was lacking. Cleveland and Sanders[3] believed that the increased incidence of infection was due to an increased virulence in the bacteria accompanying the amebæ. All serious attempts to solve this problem have been carried out thus far on experimental kittens.

The present study is based on the successful passage of a single human strain of *Endamoeba histolytica* through a direct line of 20 dogs and subsidiary infection of 40 additional dogs. The original inoculum, obtained in February, 1934, from a case of amebic dysentery contracted in Louisiana, contained an abundance of active amebæ which were immediately introduced intracecally into 2 young dogs according to the technic previously described.[9] One of the 2 became infected and from it adequate material was obtained to continue direct passage of the strain to the present time. All subse-

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1 Boeck, W. C., and Drbohlav, J., *Am. J. Hyg.*, 1925, **5**, 371.

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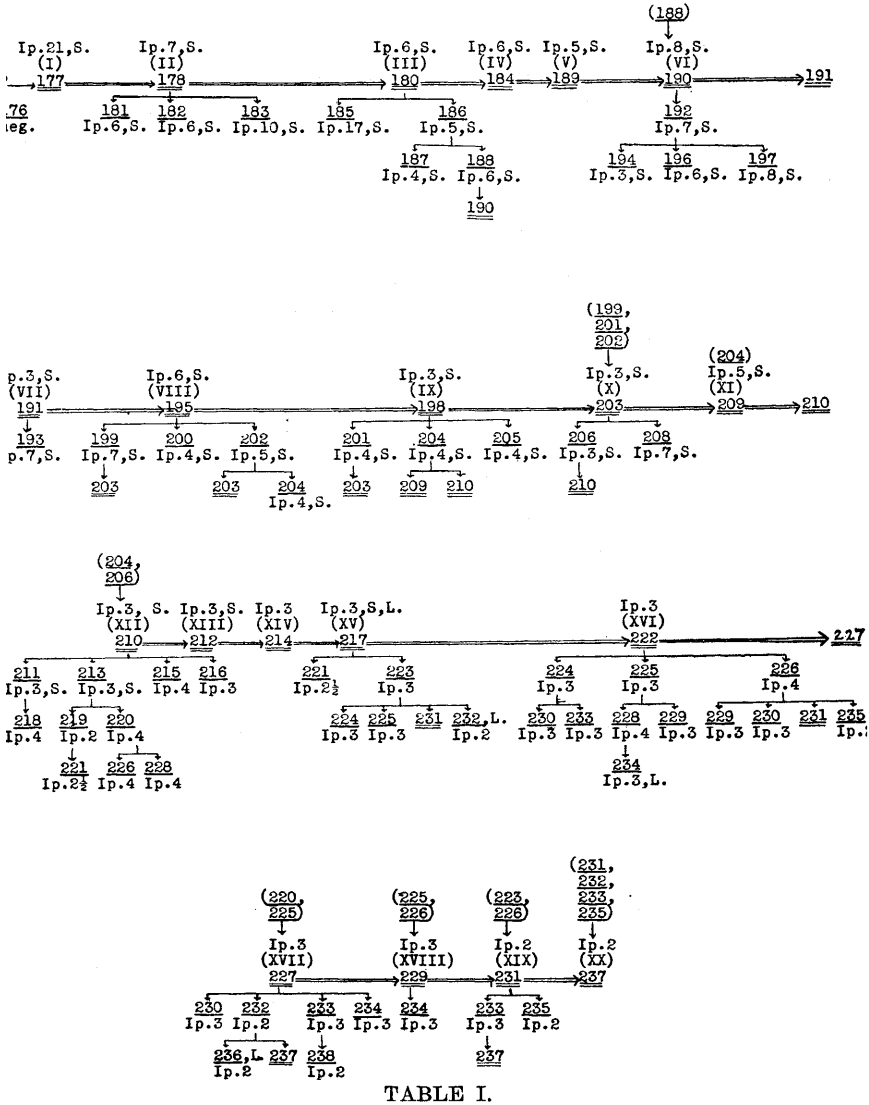

TABLE I.

Showing increased virulence of a human strain of *Endamoeba histolytica* by passage through dogs.

Arabic numerals from 176 to 237 refer to dogs in this series; Roman numerals in parenthesis from (I) to (XX) indicate the particular direct line subpassage from dog to dog.

P, patient from whom the inoculum was originally received.

Ip., incubation period (in days).

S, indicates that the dog was fed tinned salmon before inoculation and during the incubation period.

L, indicates that the dog was fed raw liver following the incubation period.

Series numbers *underlined twice* are in the direct line; those *underlined once* indicate subsidiary lines of subpassage. Arabic numerals in parenthesis above the numbers in the direct series refer to sources of inocula additional to those in the direct line.

quent animals inoculated became infected. All were carefully studied during the course of the infection and all were carefully examined post-mortem. The successive transfers through these animals, utilizing inocula obtained only from living hosts, are diagrammatically represented in Table I. Since all of the inoculated dogs became infected, incidence as a criterion of virulence was *ipso facto* eliminated. Reliance has therefore been placed on other factors, including (1) the length of the incubation period, (2) the degree of pathology induced, (3) the age and size of the animal utilized, and (4) the effect of certain foods as adjuvants in inducing infection.

The incubation or prepatent period is considered terminated[9] as soon as evidence of multiplication of the amebæ and of the earliest host-tissue damage has been obtained by cecoscopic examination. Such evidence may precede clinical amebiasis by several days and in resistant hosts it may be the only evidence of infection ever obtained. But in the present series, except for the first positive animal (No. 177) and one other (No. 185), it never preceded clinical amebiasis more than 48 hours, in 30 % of the cases only 24 hours, and in 60 % was simultaneous with clinical dysentery. During the first 9 months the incubation period was reduced from 7.3 to 3.8 days (average of 10 animals each); later, to 3.25 days, and in the last 10 members of the series, to 2.4 days. Except for the first member of the series (No. 177) all of the first 35 animals were fed tinned salmon during the incubation period.[10] With one exception (No. 217), the later animals had a balanced ration during prepatency. Furthermore, although the early members of the series were all young pups and later in the series larger, more mature and presumably more resistant animals were selected, the incubation period was consistently reduced. On the basis of the incubation period, therefore, the results of amebic infection in our canine series provide consistent evidence supporting the view of Baetjer and Sellards that successive passages of *Endamoeba histolytica* through susceptible hosts enhance the virulence of the inoculum.

The ameba, as originally obtained from the human host, was a medium-sized race. There has never been any tendency toward decrease or increase in the size of the organism. It has apparently propagated entirely by binary fission. Encystation has never been observed in the feces or in the tissues of the infected animals.

Paralleling the reduced incubation period, not only was there an increase in the amount of discharged bloody mucus, but each drop

[10] Faust, E. C., Scott, L. C., and Swartzwelder, J. C., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 540.

of exudate was richer in numbers of amebæ. Examination of sections of representative lesions has demonstrated a remarkable numerical increase of full-sized individual amebæ in each colony, particularly at the base of the glands. As soon as it was discovered that the incubation period was being appreciably shortened, in order to prevent any possible individual host factor from modifying the results, as well as to insure continuity of the strain, material from 2 or more hosts was frequently pooled and used as inocula. Uncomplicated deaths of the experimental hosts occurred most frequently when the incubation period had been reduced to about 3 days (Nos. 211 to 221, after 9 months' passage). Post-mortem examination of all these dogs except No. 216 showed that practically every available site in the entire length of these dogs' large intestines was beset with typical pinpoint or craterous lesions, and in one animal (No. 214) the posterior ileum was comparably involved. The degree of pathogenicity in these intestines was both quantitatively and qualitatively greater than in the early members of the series. In only 2 of these animals was there any evidence of non-amebic ulceration of the intestinal wall. With further reduction in the incubation period, there was less tendency to deep invasion and more superficial confluent ulceration, still without any generalized inflammation of the wall except in isolated cases (Nos. 230 and 237). Clinically these later members of the series have shown more marked early dehydration and exsanguination.

Cleveland and Sanders[3] and more recently Frye and Meleney[11] believe that the bacteria of the large intestine play some part in the development of amebic lesions. The former investigators stress the importance of the increased virulence of the bacteria passed along with the amebæ from host to host, while the latter workers have concluded from their studies that both the incidence of infection and the degree of pathogenicity for the host are to a certain extent dependent on the associated bacteria. Although our dogs have consistently failed to furnish corroborating evidence, we do not deny the possible secondary rôle which bacteria may play in the development of the amebic process. However, at the suggestion of Col. Chas. F. Craig, to rule out bacteria as the primary agents of amebic colitis in our dogs, we have cultured on beef broth in 250 cc. Erlenmeyer flasks the bacteria taken directly from the intestines of our most fulminating cases of amebiasis (pooled from Nos. 224, 226-229, 231-236) and have repeatedly introduced intracecally large amounts of these inocula into 3 negative pups. Neither dysentery

[11] Frye, W. W., and Meleney, H. E., *Am. J. Hyg.*, 1933, **18**, 543.

nor diarrhea has been produced, and at autopsy the wall of the large intestine of each animal was completely free of any inflammatory areas. We conclude, therefore, that the bacteria associated with the amebæ in our inocula have not been responsible for the reduction in the incubation period, for the increased ease with which we have transferred our strain of ameba, or for the increase in the number and degree of severity of the amebic lesions produced.

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Observations on Antigens for Complement Fixation in Amebiasis.*

CHAS. F. CRAIG AND L. C. SCOTT.

From the Department of Tropical Medicine, School of Medicine, Tulane University of Louisiana, New Orleans.

Since the publication of the technique of the complement fixation test for amebiasis devised by the senior author (Craig)[1], in which absolute alcoholic extracts of cultures of *Endamoeba histolytica* were employed as antigens, several modifications of the test have been published and other antigenic extracts have been described.

Menendez[2] and Spector[3] employed simple alcoholic extracts of cultures of *E. histolytica* as antigens with satisfactory results, and Menendez stated that a simple suspension of cultures of *E. histolytica* in formalinized saline also possessed good antigenic properties. Sherwood and Heathman[4] and Heathman[5] used antigens prepared by extracting the dried sediment of cultures of *E. histolytica,* rich in the amebæ, with ether, 96 % alcohol and acetone, after which cholesterin was added in different amounts, and found that such antigens were efficient. Tsuchiya[6] and Weiss and Arnold[7] successfully employed absolute alcohol extracts of cultures of *E. histolytica* in their modifications of this complement fixation test. With all of these antigenic extracts these observers obtained a high percentage of positive results in infections with *E. histolytica* and

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7 Weiss, W., and Arnold, L., *Am. J. Digest. Dis. and Nutrition*, 1934, **1**, 231.