

THE RAT AS A POSSIBLE CARRIER OF THE DYSENTERY
AMOEBIA

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The infection of rats by feeding them human feces containing the cysts of *Endamoeba histolytica* has been reported by (Lynch, 1915) who also claims to have observed this organism in a normal wild rat. The lack of detailed description and figures of the amoeba which he found in his rats leaves its identity uncertain. Later on Brug (1919) through similar experiments confirmed Lynch's observations. Kessel (1923) reported the transmission of various species of parasitic amoebae including *E. histolytica* from the human being to the rat. He at the same time made a study of the amoebae occurring spontaneously in the rat, describing the new species *E. ratti* and reclassifying previously described amoebae of the rat and mouse as *Councilmania muris* and *Councilmania decumani*. None of these investigators utilized the biological test of inoculating kittens with the amoeba in question but based their claims chiefly on morphological grounds.

In the present investigation the experimental infection of young rats that had been raised free from all parasitic amoebae was attempted. Infection having been obtained the morphological study of the organism was supplemented by the inoculation of young kittens in order to determine its transmissibility and pathogenicity. A morphological study of the parasitic amoebae occurring spontaneously in white rats has revealed an endamoeba which if not *E. histolytica*, cannot as yet be differentiated with certainty from it. Another species resembling *Endolimax nana* of the human being has also been found in the laboratory rat. Throughout this study the method previously employed by Dr. Boeck and Dr. Drbohlav (1925)* for the cultivation of *E. histolytica* has been utilized.

Since the laboratory rat is commonly infected with several species of parasitic amoebae it is of primary importance in studying experimental infections to obtain stock which is amoeba-free. Kessel has advocated the employment of purgatives for detecting amoebic infection in rats but this method has certain disadvantages which are overcome in the high-enema method employed by the author in examining rats for the presence of amoebae. In order to obtain a clean stock of young rats free from amoebae, breeding rats are selected which by high enema examination appear to be free from infection. These are placed in sterilized cages, and fed with the exception of pasteurized milk, upon sterilized food. All sawdust, nesting material, etc., which is introduced into the cage is pre-

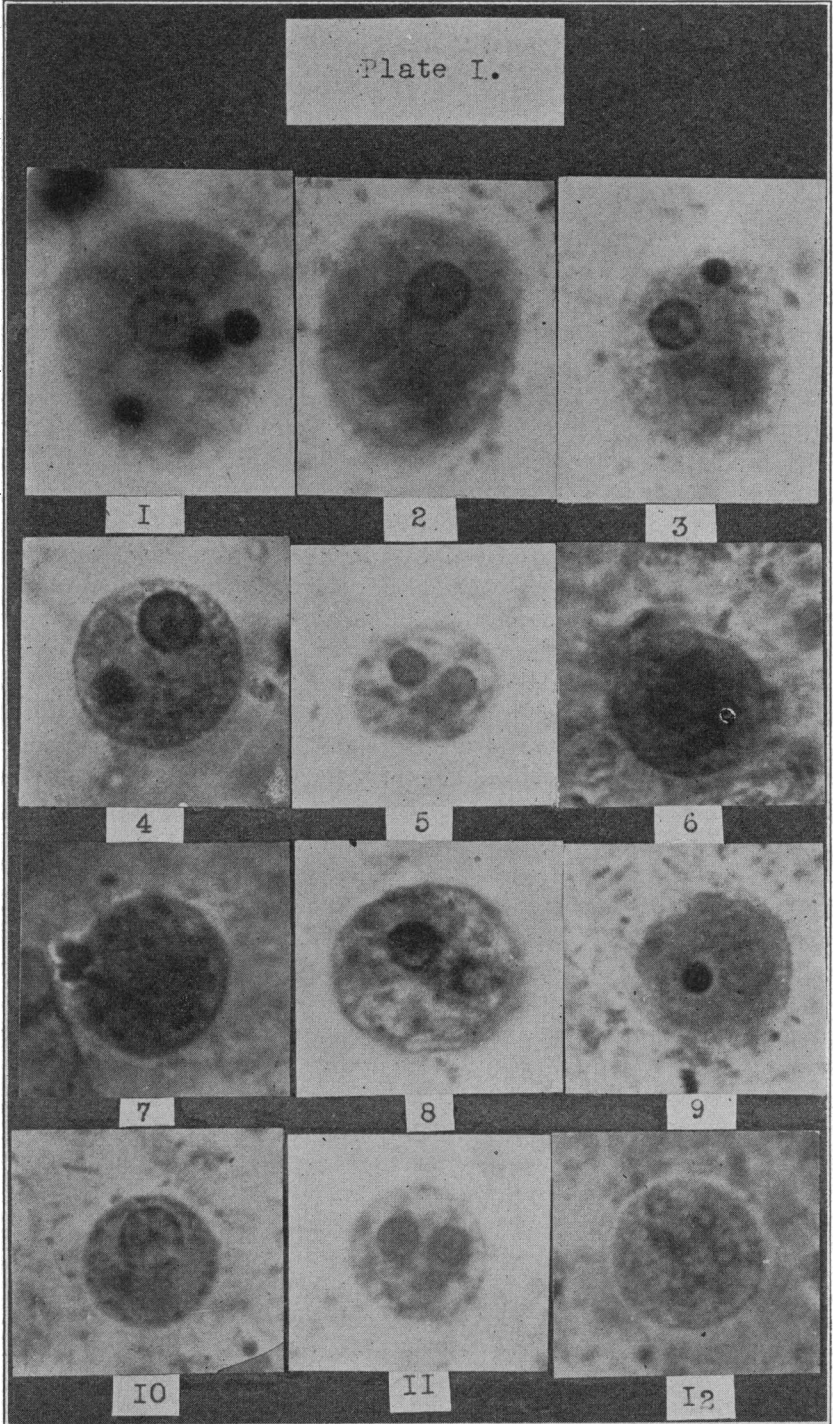


PLATE I

All figures in this plate are photographed from preparations fixed with Schaudinn's solution, and stained with aqueous iron hematoxyline. Magnification 1750 diameters.

Figs. 1 to 6. *E. histolytica* from experimental rats and kittens.

Fig. 1. Original material from kitten used for the first series of experiment. (With red cells.)

Fig. 2. Trophozoite from infected rat.

Fig. 3. Trophozoite from kitten which had been infected with material from experimental rats.

Fig. 4. Uninucleate cyst from infected rat.

Fig. 5. Binucleate cyst from infected rat.

Fig. 6. Quadrinucleate cyst from infected rat.

Figs. 7 to 12. *E. histolytica* (*murina*) found in normal laboratory rats.

Fig. 7. Trophozoite from normal laboratory rats.

Fig. 8. Trophozoite in culture.

Fig. 9. Trophozoite from kitten infected with *E. histolytica* (*murina*). (Showing red blood cell.)

Fig. 10. Uninucleate cyst from normal laboratory rat.

Fig. 11. Binucleate cyst from normal laboratory rat.

Fig. 12. Quadrinucleate cyst from normal laboratory rat.

viously sterilized in the autoclave 20 minutes at 15 pounds pressure. The pregnant mother is isolated under such conditions and the young are removed to another sterilized cage at the age of 3 or 4 weeks. After attaining the age of six weeks each is subjected to at least two high-enema examinations before being used for experimental infection.

Experimental Transmission of E. Histolytica of Human Origin to the Rat.—Two series of experiments were carried out. Material for the inoculation of the first series, derived from a kitten infected with the "E" strain of *E. histolytica* was supplied through the courtesy of Dr. Boeck and Dr. Drbohlav. Two amoeba-free adult rats receiving a rectal injection of this material showed mobile amoeba in seven and ten days, respectively, after the injection. Fecal material obtained by high enema from one of these rats was injected rectally into two young kittens, both of which developed an amoebiasis with lesions of the large intestine. The study of stained section of the affected portions of intestinal wall showed amoebae in the tissues. Fecal material from one of these kittens was injected rectally into two young amoeba-free rats but only one of them became infected.

Material for the second series of experimental infections was furnished by Dr. S., he himself being a healthy carrier of *E. histolytica*, *E. coli* and *Endolimax nana*. The feces was fed on November 13, 15 and 17 to a litter of nine young amoeba-free rats. Neither *E. coli* or *Endolimax nana* became established in any of these rats but they showed instead an infection with an amoeba of the type of *E. histolytica*. In order to test the pathogenicity of this organism fecal material from these rats was injected into a young kitten. This became ill and showing dysentery with amoebae

in the feces on the third day after inoculation, was killed. Amoebae were found in the intestinal mucosa in stained sections. Three months after the infection of the rats, another kitten was injected with fecal material from them but died of pneumonia without developing amoebiasis. Four young amoeba-free rats subsequently placed with the infected ones, became infected through exposure. Periodical observations on the rats infected with these two strains of *E. histolytica* have shown that the infection is at its height at from one to two months after its first appearance and thereafter tends to gradually diminish, but may persist in certain animals for as long as four months.

No macroscopic lesions were found in any of the rats killed during the period of infection and no amoebae were demonstrable in stained sections of the large intestine. Cysts with from one to four nuclei were discharged in the feces of the infected rats.

The results of these experiments indicate that *E. histolytica* is transmissible to the white rat either by rectal injection or by feeding and that the resulting infection continues over a period of several months. The infection is also transmitted from the infected rat to young amoeba-free rats by exposure in the same cage. The pathogenicity of the amoeba employed is demonstrated by transference to kittens in which it produces lesions with amoebae in the affected tissue.

Parasitic Amoebae Found Occurring Spontaneously in the Laboratory White Rat.—Eight nucleated cysts have been described for all species of amoeba thus far reported as occurring naturally in the rat and mouse. In the present investigation one species of endamoeba producing eight nucleated cysts was found in rats. Of greater interest was the occurrence of an endamoeba producing four nucleated cysts and an amoeba closely resembling *Endolimax nana* of the human being. The former since it has not been possible to definitely differentiate it from *E. histolytica* of the human being is designated *E. histolytica* (var. *murina*) and the latter on the ground of biological differences is designated as *Endolimax ratti*.

Endamoeba Histolytica (Murina).—In a rat killed from a lot obtained from another department an endamoeba was found which grew readily on the medium of Boeck and Drbohlav. On further examination, six other rats of this lot were found infected with the same amoeba. The study of this amoeba has revealed so few characters not wholly in agreement with *E. histolytica* of man that only a brief discussion of its morphology will be given at this time. The trophozoites when rounded measure from 12 to 20 microns. Its locomotion is characteristically slug-like as in the case of the human species. In preparations stained with Heidenhain's iron haematoxylin, the nucleus shows the karyosome centrally situated in about 60 per cent of the trophozoites, in the remainder being slightly

eccentric. Except for a less uniform central location of the karyosome, the nucleus agrees in every respect with that of the parasite in human dysentery. The inclusion of large numbers of bacteria in the cytoplasm is another possible difference although it must be taken into account that the trophozoites here studied are obtained directly from the colon of killed animals whereas those of the human being are usually studied from stools.

Cysts in the one, two and four nucleated stages are found in the feces of the rat and measure from 10 to 13 microns. These show chromatoid bodies of various sizes and shapes. In the one nucleated cysts these are identical with those of *E. histolytica* but the typical rod-shaped bars of this material have not been observed in the four nucleated cyst.

E. histolytica (murina) grows readily in the culture media employed by Boeck and Drbohlav and also on a modification of this medium prepared by substituting 100 cc. of beef broth in place of 25 cc. of the Locke Ringer solution in the preparation of the egg slant and omitting the serum in the fluid subsequently added. Encystment has occurred regularly on this medium coincident with concentration through evaporation.

The rectal injection of cultures of this amoeba into young amoeba-free rats produces infection without evidence of disease. The organism is also readily transmitted from infected rats to young normal ones by exposure.

Eight kittens have been injected with *E. histolytica (murina)*. Five of these became infected, two temporarily, complete disappearance of the amoeba following. One of these kittens showed amoebae containing red corpuscles during life but no lesions were found at post mortem and no amoebae were demonstrable in the tissues in stained section. Another showed amoebae with red cells for a time but very few amoeba at post mortem. In one case there were macroscopic lesions with amoebae demonstrable in the tissue in stained sections.

Endolimax Ratti (Spec. Nov.).—Although Kessel reports experimental infection of white rats with *Endolimax nana*, this, as already noted, has failed in the present investigation. In all, fourteen rats were fed feces containing *Endolimax nana* cysts, from three different human cases, but none became infected. An amoeba of this type was, however, found occurring naturally in laboratory rats. The morphology of this organism is identical with that of *End. nana*, but the fact that it parasitizes the rat makes it appear to be at least biologically distinct. The tentative name *Endolimax ratti* is proposed for this amoeba. The mature cysts show four nuclei. The general characters of this organism are illustrated in figures 17 to 22.

Summary.—From the results of the present experiments, it is apparent that *E. histolytica* of human origin may be transmitted to white rats. This may be accomplished either by the rectal inoculation of material containing the trophozoites or by the feeding of material containing its cysts.

Plate II.

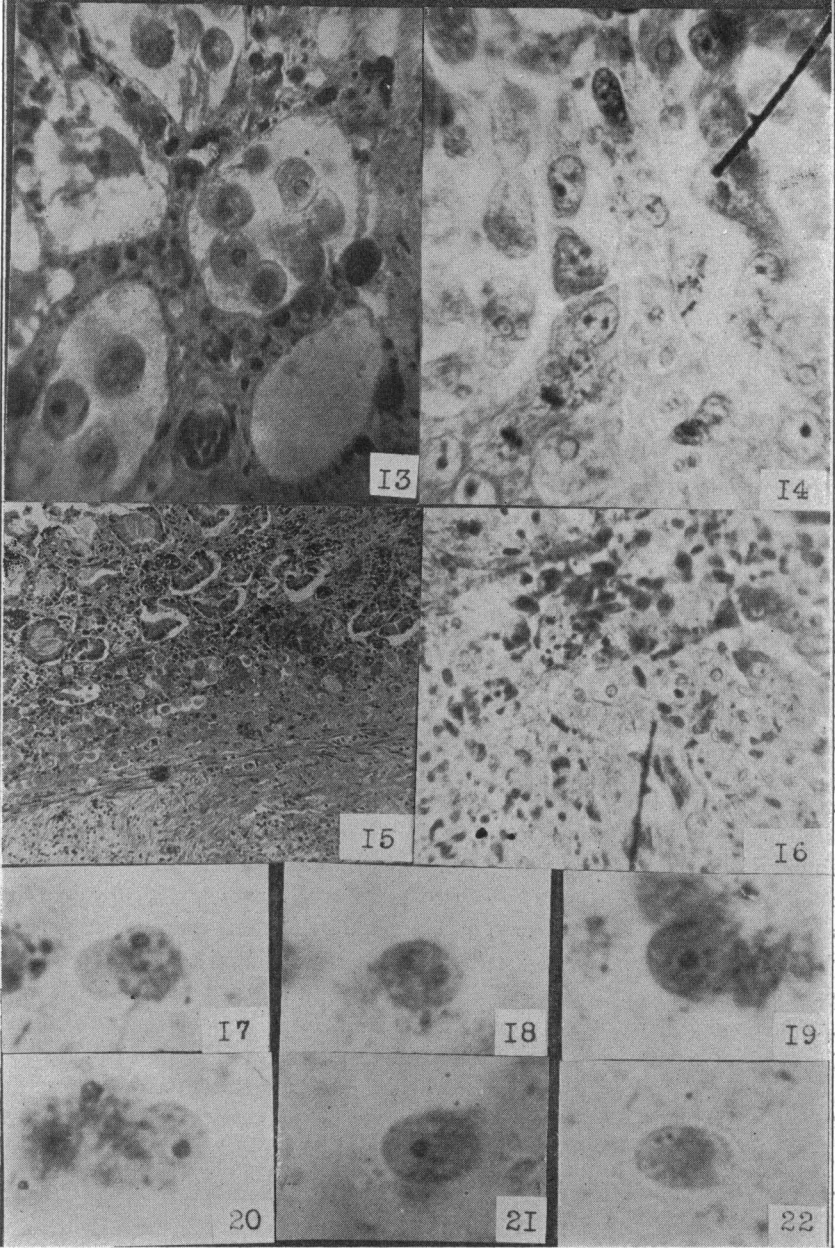


PLATE II

Figures 13 to 16 in this plate are photographed from preparations fixed with sublimate alcohol (with acetic acid) and stained by Mann's methylblue-eosin method. No estimation on magnification.

Figures 17 to 22 in this plate are photographed from preparations fixed with Schaudinn's solution and stained with aqueous iron hematoxylin. Magnification 1750 diameters.

Fig. 13. Section of large intestine of kitten infected with *E. histolytica* (E. strain) from infected rat.

Fig. 14. Section of large intestine of kitten infected with *E. histolytica* (Murina) from normal laboratory rat.

Fig. 15. Section of large intestine of kitten infected with *E. histolytica* (E. strain) from infected rat.

Fig. 16. Section of large intestine of kitten infected with *E. histolytica* (S. strain) from infected rat.

Figures 17 to 22. *Endolimax muris* (sp. nov.) from normal laboratory rats.

Figures 17 to 20. Trophozoites with characteristic karyosome and pseudopodia.

Fig. 21. Precystic trophozoite.

Fig. 22. Four-nucleate cyst.

The pathogenicity of the amoeba found in the experimentally infected rat has been demonstrated by the inoculation of kittens with the production of dysentery and lesions containing amoebae.

The infection of rats with *E. histolytica* of human origin is readily transmitted from rat to rat by association in the same cage. The height of the infection occurs between one and two months after which the organisms tend to gradually diminish in number but may persist for at least four months.

An endamoeba here designated *E. histolytica* (var. *murina*) agreeing very closely in its morphology with *E. histolytica* of human origin is found occurring spontaneously in the laboratory rat. Like the human parasite it is very readily cultivated and is transmissible to kittens for which it appears to be only mildly pathogenic.

Encystment of this organism is readily produced in suitable medium.

An amoeba morphologically similar to *Endolimax nana* but possibly biologically distinct has been found in laboratory rats. This temporarily designated as *Endolimax ratti* requires further investigation to establish it as a distinct species.

The results of this investigation incriminate the rat as a possible carrier of *E. histolytica*, the causal agent of amoebic dysentery.

* See preceding paper in this issue.

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*LINEAR CONNECTIONS OF A SPACE WHICH ARE DETERMINED
BY SIMPLY TRANSITIVE CONTINUOUS GROUPS*

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1. The concept of parallelism introduced by Levi-Civita for general Riemannian manifolds of n dimensions has been extended by many writers to manifolds for which there is not an assigned metric. Weyl¹ used the term *affine connection* to define the relation between elements at different points of a space and used a linear connection in the definition of which the coefficients Γ_{jk}^i are symmetric in j and k . Equivalent definitions have been used by Eddington,² and by Veblen³ and the author⁴ in their papers on the *geometry of paths*. Schouten⁴ has made an analysis of types of linear connection without the restriction that the coefficients be symmetric. It is the purpose of this note to show that the infinitesimal generators of a simply transitive continuous group in n variables serve to define an unsymmetric linear connection for which there exist symmetric tensors g_{ij} , involving $n(n+1)/2$ arbitrary constants, whose first covariant derivatives are zero. Moreover, if one of these tensors is taken for the fundamental tensor of a Riemannian manifold, the group is a transitive group of motions for the manifold.

2. Consider a space of n dimensions of coördinates x^i for $i = 1, \dots, n$. Let $\lambda_{\alpha/}^i$ be the components of n linearly independent contravariant vectors in the space; in this notation a $\lambda_{\alpha/}^i$ given α for $\alpha = 1, \dots, n$ indicates the vector and i for $i = 1, \dots, n$ the component. Since the vectors are linearly independent, their determinant $\Delta = |\lambda_{\alpha/}^i|$ is different from zero. Let Δ_{α}^{α} be the cofactor of $\lambda_{\alpha/}^i$ in Δ divided by Δ ; then

$$\lambda_{\alpha/}^i \Delta_{\alpha}^{\beta} = \epsilon_{\alpha}^{\beta} \quad (2.1)$$

where i is summed from 1 to n , according to the customary convention which will be followed in this paper, and where $\epsilon_{\alpha}^{\beta}$ is 1 for $\alpha = \beta$ and 0 for $\alpha \neq \beta$.

If we denote by $\lambda'_{\alpha/}^i$ the components of the vector $\lambda_{\alpha/}^i$ in a coördinate system x'^i , we have