

Four animals on a balanced diet, carrying Strain A, were utilized for this experiment. In each case the dog had been infected for a week or more, showed active symptoms of dysentery, and was passing trophozoites of *E. histolytica* in the stool. A single portion of 250 gm. of fresh calf's liver was fed to each animal. In each case the bowel movements were markedly reduced within 24 hours and the stools became semi-formed, although considerable blood and mucus was incorporated with the feces. In 3 of the 4 animals cysts were present in the passed stools within one to 7 days and continued intermittently until the death of the animal (a few days to 3 weeks later). In each case proctoscopic examination over the period revealed active lesions, with trophozoites at the site of the lesion. In the one animal in which cysts were not formed *E. histolytica* trophozoites were present on the day following the liver meal but were not recovered subsequently. The animal died a week later of a profound bacteremia.

This feeding test suggests an important rôle which liver substance plays in providing a medium favorable for encystation of *Endamoeba histolytica* in its natural habitat in the bowel. The fraction of the liver responsible for this phenomenon is the subject of a further study. We believe, however, that the dehydration of the large bowel content is intimately associated with the problem of encystment.

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The Panama Strains of Human Strongyloides.

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The studies of Grassi,¹ Perroncito² and particularly those of Leuckhart³ demonstrated that there were 2 different strains of the human Strongyloides, *S. stercoralis*, the one consisting entirely of a parasitic generation and the other having both a parasitic and a

¹ Grassi, G. B., *Rend. Inst. Lomb. sci. e. lett. Rendic.*, Milano, 2 ser., 1879, 12, 228.

² Perroncito, E., *Atti Accad. Lincei*, Ser. 3, 1879-1880, 1, 381.

³ Leuckart, R., *Gesellsch. d. Wissensch. Math. phys. Klasse.* Leipzig, 1882, 34, 85.

free-living generation in its life cycle. Leichtenstern⁴ believed that the organism in warm climates utilized only the indirect development, while those in cooler regions utilized only direct development. This hypothesis, however, has been proved fallacious by the work of Darling,⁵ Sandground,⁶ Nishigori⁷ and others.

Darling's series consisted of cultures from 23 infected persons in Panama, residents of the tropics for many years. Of these cultures 5 proved to be purely indirect in their development, 7 were direct only and 11 were combined strains.

During the summer of 1930 the writer studied 1662 native patients in the Santo Tomas Hospital, Panama, of whom 348 were positive for *Strongyloides stercoralis*. Of this series 276 were each examined 3 or more times. Twenty-nine of these (10.5%) were of clinical importance. From the entire series 27 cases of heavy infection, in which no hookworm infestation was present, were selected for culture. These cultures consisted of active larvae, which had been richly concentrated by the centrifuge method, planted on animal charcoal in sterile Petri dishes, and allowed to develop at room temperature (26-36°C.). Each culture was examined daily for a period of from 3 to 9 weeks. The results of this study indicate that 2 of the infections were purely of the indirect type, 13 were direct only, 7 were of a direct type in which dwarf filariform larvae were present in the freshly passed feces, and 5 were combination of these types. The term *hyperinfective strain* is proposed for the type in which dwarf (unfed) filariform larvae are passed in the feces, since there is considerable evidence that this is the strain which is responsible for hyperinfection of the host.

In 5 cases second cultures were made from the same individuals and in every instance these were similar to the original strains. In the indirect type filariform larvae of the free-living generation had developed as early as the sixth day of culture; by the fifteenth day the strain had completely died out. In the unmodified direct type the rhabditiform larvae of the parasitic generation grew rapidly, became ensheathed between the fourth and sixth day, and continued as active organisms up to 9 weeks, without exsheathing or metamorphosing. In the pure hyperinfective type the feces usually showed both unfed rhabditiform larvae of the parasitic generation

⁴ Leichtenstern, O., *Deutsche Med. Wochenschr.*, 1898, **8**; *Arbeit. aus d. k. Gesundheitsamte*, 1905, **22**, 309.

⁵ Darling, S. T., *J. Exp. Med.*, 1911, **14**, 1.

⁶ Sandground, J. H., *Am. J. Hyg.*, 1926, **6**, 337.

⁷ Nishigori, M., *Taiwan Igakkai Zasshi*, 1928, No. 276. (Japanese text with English abstract.)

and dwarf (unfed) filariform larvae of the same generation. In a few days the former always metamorphosed into the latter, without feeding, and in 6 to 12 days after the culture was started they had died out. In mixed types the indirect and the hyperinfective strains died out between the sixth and the fourteenth day and the active ensheathed rhabditiform larvae of the direct type survived.

This study confirms the work of Darling, Sandground and Nishigori. Furthermore, it substantiates Nishigori's observations on the correlation between filariform larvae in the freshly passed stool and chronic clinical cases in tropical countries. On the basis of the consistent metamorphosis of the larvae without feeding, from the rhabditiform to the filariform stage, frequently before evacuation of the diarrheic stool, there is adequate evidence to designate them as a separate strain, for which the term hyperinfective strain is proposed. These observations indicate that the viability of these respective strains is directly associated with the nourishment which is taken, primarily in the rhabditiform stage of the parasitic generation.

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Effect of Vital Stains on Cultures of Endamoeba Histolytica.

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Because of the more or less elaborate fixation and staining methods used to differentiate the types of Amoeba, the clinician has found it impractical to carry this examination out in routine practice. The vital stains suggested themselves as a simple means by which the differentiated points might be accentuated and recognition made easier in the routine examination. References to vital staining of amoeba in the literature are very few.¹

The technique of Sabine was found impractical because of the nature of the culture media or stool specimen. Aqueous solutions of the dyes were prepared from 1/10 to 2%. A capillary drop of the culture and stain were mixed thoroughly on the slide with an applicator. A cover glass is placed firmly over this, with a blotter or cloth to absorb the excess. If the cover glass is not moderately firm,

¹ Stitt, E. R., *Practical Bacteriology, Blood Work and Parasitology*. P. Blakiston, Son & Co., Philadelphia, 1927, 65.