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.

5259

Effect of Vital Stains on Cultures of Endamoeba Histolytica.

DONOVAN C. BROWNE. (Introduced by C. W. Duval.)

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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.

difficulty may be experienced in focusing the oil emulsion. In 3 to 5 minutes the slides may be examined. The following dyes were used: Neutral red; Trypan blue; Janus green; Brilliant cresol blue; and Nile blue; and the effect noted on the motile forms of the amoeba. Staining reactions on the dying or dead organisms were not consistent.

Neutral red appears non toxic up to 1% solution—the motility being very little affected. The dyes were taken up quickly, and give a very clear differentiation between the endoplasm staining pink and the ectoplasm apparently untouched. Recognition under low power in feces as well as cultures, is very easy, the amoeba appearing as pink refractory bodies. Under oil emersion, the nucleus stands out very distinctly, and by careful focusing as the organism flows along, the chromatin granules appear as a thin line about the periphery, the center appearing more or less clear. As the motility is lost, the nucleus may become larger and the layers of chromatin appear irregular. In some instances the karyosome apparently becomes visible. Unfortunately, at this stage the karyosome seldom maintains its central position, and a definite differential point is lost. I have observed the active amoeba coli with neutral red in only a few instances, but there seems to be a distinct difference in the chromatin arrangement in the nucleus. The band appears much broader and irregular about the circumference and throughout the nucleus dots of chromatin may be irregularly distributed. These observations have not been in sufficient number to draw conclusions.

Brilliant cresol blue in our hands proved unsatisfactory. The active organisms do not take the dye well, and it seems more toxic and the motility is quickly lost.

Janus green stains the endoplasm a pale pink; the ectoplasm remaining unchanged. The nucleus stands out more clearly and because of the paleness of the stain is often more distinct than with the neutral red. This dye is tolerated very well up to 1% solution, but is taken up very slowly, and many organisms remain unstained for long periods. The fact that Janus green stains pink is dependent upon the acidity of the culture media.

Trypan blue, although not as toxic as brilliant cresol blue, is not tolerated well above 0.5% solutions. The staining is unsatisfactory and not consistent. Endoplasm may be a pale blue color, with the ectoplasm unchanged. Nucleus is often well outlined, with the chromatin appearing black, but the contrast with the stained endoplasm does not allow it to stand out as well as with the pink staining.

Nile blue is tolerated well in the weaker solutions up to 0.5%. The endoplasm takes a pale blue color; the ectoplasm appears much paler, with a greenish hue. This latter point was not consistent, and when used in combination with neutral red does not have any particular advantage as a contrast stain.

Neutral red and Janus green are the most satisfactory dyes used, so far. The endoplasm and ectoplasm are more clearly differentiated and the fact that the nucleus with its chromatin arrangement is more visible may have some clinical value.

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Bacteriology of the Liver in Normal Dogs.*

I. M. GAGE. (Introduced by Alton Ochsner.)

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The presence of organisms in the tissue of normal, healthy animals has been a controversy for some time. However, investigators have definitely proved that the tissues of normal, healthy animals harbor organisms. That the occurrence of organisms in normal tissue in the experimental animal has been a source of incorrect observation and deduction in certain experimental problems has recently been shown by Ellis and Dragstedt.[1]

C. B. Hawn,[2] working with Holmes Jackson, first described a spore-bearing bacillus found in the liver of normal dogs. Walbach and Saiki[3] studied this bacillus, using 23 normal, healthy dogs, and found this gram-negative, spore-bearing bacillus in 21 of the 23 dogs. Berg, Zan, and Jobling[4] cultured the liver from 11 normal dogs and found this gram-negative, spore-bearing organism in 100% of their animals. Ellis and Dragstedt,[5] in their investigations on liver autolysis *in vivo,* encountered the same organism in almost 100% of their animals.

We studied the bacteriology of the contents of the stomach, the duodenum, and the liver in a series of normal, healthy dogs and the

* Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

1 Ellis and Dragstedt, *Arch. Surg.*, 1930, **20**, 8.

2 Hawn, C. B., quoted by Wolbach and Saiki.

3 Wolbach and Saiki, *J. Am. Med. Assn.*, 1909, **21**.

4 Berg, Zan and Jobling, PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 433.

5 Ellis and Dragstedt, *Arch. Surg.*, 1930, **20**, 8.