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Filth Flies as Transmitters of *Endamoeba histolytica.*

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During the last 25 years, epidemiological studies[1, 2] have thrown suspicion on flies as possible transmitters of amebiasis. Wenyon and O'Connor,[3] using the eosin viability test, found cysts remained viable as long as 24 hours in the gut of flies. Root[4] showed that cysts apparently remained viable as long as 49 hours after ingestion by flies as judged by the neutral red test.

Due to doubt concerning the absolute reliability of such staining technics as criteria of viability, the present investigation was designed to prove or disprove, by modern culture technics, the viability of cysts or trophozoites of *E. histolytica* washed from the external body surfaces or recovered from or passed through the alimentary tract of flies.

The 5 species of filth flies utilized in these experiments were *Sarcophaga misera, Phormia regina, Cochliomyia macellaria, Lucilia pallescens,* and *Musca domestica.* Cysts used were concentrated by the zinc sulfate technic of Faust, Tobie, *et al.,*[5] and collected by the D.C.F. method of Lane,[6] thoroughly washed and stored in physiological saline at ice-box temperatures. The trophozoites were cultivated on the Frye and Meleney[7] modification of the original Boeck and Drbohlav coagulated egg medium. Cultures were incubated at 37°C and transplanted every 48 hours.

To test the effect of external carriage on the subsequent viability of trophozoites and cysts, 60 flies of each species were contaminated with feces enriched with cultured trophozoites and feces seeded with cyst concentrates. Over a period of 10 minutes, flies were removed from the exposure cage in groups of 3 at 30-second intervals and washed, and the washings inoculated into culture media.

1 Craig, C. F., *The Military Surgeon,* 1917, **40**, 286.

2 Frye, W. W., and Meleney, H. E., *Am. J. Hygiene,* 1932, **16**, 729.

3 Wenyon, C. M., and O'Connor, F. W., *Human Intestinal Protozoa in the Near East* (Monograph), pp. 218, 1917, London and New York.

4 Root, F. M., *Am. J. Hyg.,* 1921, **1**, 131.

5 Faust, E. C., Tobie, J., *et al., Am. J. Trop. Med.,* 1938, **18**, 169.

6 Lane, C., *Trans. Roy. Soc. Trop. Med. and Hyg.,* Part I, 1923, **16**, 274.

7 Frye, W. W., and Meleney, H. E., *Science,* 1939, **89**, 564.

Controls consisted of the cultivation of washings from 3 flies immediately following contamination. Trophozoites were found to be culturable after periods of from ½ minute (for *L. pallescens*) to 1½ minutes *(S. misera* and *P. regina)* of external exposure. Cysts were found to be culturable after periods of from 1 minute *(M. domestica)* to 4 minutes *(S. misera)* of external exposure.

To obtain some indication of the survival of trophozoites and cysts at different levels of the alimentary tract, the following experiments were performed. Groups of from 15 to 20 flies of the designated species were fed singly on cultured trophozoites or on feces seeded with cyst concentrates. Flies fed on trophozoites were killed and examined at 5-minute intervals up through 1½ hours, while those fed on cysts were sacrificed at 30-minute intervals up through 6 hours. Controls consisted of the cultivation of the same trophozoite and cyst material which was fed to the flies.

Trophozoites were found culturable after remaining in the crop for periods of from 15 minutes *(M. domestica* and *L. pallescens)* to 40 minutes *(S. misera),* and after remaining in the gut for periods of from 5 minutes *(M. domestica, L. pallescens,* and *P. regina)* to 30 minutes *(S. misera).* Motile trophozoites were not recovered from the recta of any of the 5 species. Cysts were found to be culturable after remaining in the crop for periods of from 20 minutes *(L. pallescens)* to 210 minutes *(S. misera),* however all species of flies other than *S. misera* gave values below 30-minute maxima. Cysts were found to be culturable after remaining in the gut for periods of from 20 minutes *(L. pallescens)* to 240 minutes *(M. domestica)* and were found to be culturable after remaining in the rectum for periods of from 30 minutes *(P. regina)* to 180 minutes *(S. misera).*

To test the viability of cysts and trophozoites deposited in the vomitus and fecal droplets of flies, the following experiments were performed. Groups of 15 flies of each species were fed separately on cultured trophozoites or feces seeded with cyst concentrates. Vomitus and fecal droplets were cultured immediately after deposition. The cyst experiments have been repeated several times for each species to add significance to the results. To date a total of 375 flies of the 5 species have been tested. Controls consisted of the cultivation of the same trophozoite and cyst material fed to the flies.

It was found that trophozoites recovered from the vomitus were culturable after periods of from 9 minutes *(C. macellaria)* to 17 minutes *(L. pallescens)* of internal carriage. Cysts were culturable from the vomitus after periods of from 28 minutes *(C. macellaria)*

to 64 minutes *(P. regina)* of internal carriage. Cysts were found culturable from fecal droplets for periods of from 137 minutes *(P. regina)* to 218 minutes *(C. macellaria)*.

Conclusions. From the evidence cited above, it seems highly probable that the filth flies studied play no important rôle as transmitters of trophozoites or cysts of *E. histolytica* by external carriage. However, the viability of cysts for periods as long as 3½ hours after initial ingestion and later dejection indicates a potential natural method of transmission.

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Carbohydrates of the Gonadotropic Hormones.

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The presence of carbohydrate in various purified gonadotropic hormone preparations raises a number of questions concerning the significance of the carbohydrate and its relationhip to biological activity. Evans, *et al.*, have studied the carbohydrate of various pituitary[1, 2] as well as pregnant mare serum preparations.[3] McShan and Meyer[4] have recently called attention to the possible significance of carbohydrate in their studies of pituitary follicle stimulating hormone preparations. The nature of the carbohydrate in the gonadotropic hormone of urine of pregnancy has likewise been studied.[5]

The data reported by Evans, *et al.*,[2] indicate that the interstitial cell stimulating hormone of the pituitary contains mannose, while a preparation made from pregnant mares' serum was found to contain galactose. Studies along these lines have been carried out in this laboratory and a comparison of the carbohydrate in various gonadotropic hormones is reported here.

The carbazole method[6] was employed for the identification and

1 Evans, H. M., Fraenkel-Conrat, H., Simpson, M. E., and Li, C. H., *Science*, 1939, **89**, 249.

2 Li, C. H., Simpson, M. E., and Evans, H. M., *Endocrinology*, 1940, **27**, 803.

3 Li, C. H., Evans, H. M., and Wonder, D. H., *J. Gen. Physiol.*, 1940, **23**, 733.

4 McShan, W. H., and Meyer, R. K., *J. Biol. Chem.*, 1940, **135**, 473.

5 Gurin, S., Bachman, C., and Wilson, D. W., *J. Biol. Chem.*, 1940, **133**, 467.

6 Gurin, S., and Hood, D. B., *J. Biol. Chem.*, 1939, **131**, 211.