

the egg and contains a series of very small pore canals which penetrate this layer at right angles, linking up with the larger pore canals in the outer layer. These small canals are very difficult to demonstrate, but they show up distinctly in paraffin sections slightly overstained in hæmatoxylin. This suggests that they contain cytoplasmic filaments originating in the underlying hydropyle cells. The white cuticle of the remainder of the egg is distinctly laminated, and like the inner layer of the hydropyle it also contains pore canals but of a much larger size.

A detailed account of these and other features of the egg of *Locustana* will appear elsewhere.

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<sup>1</sup> Slifer, E. H., *Quart. J. Mic. Sci.*, 8, 437 (1938).

### Control of Intestinal Microflora

IN view of recent work which has directed attention to the importance of the intestinal microflora in nutrition, examinations of the faeces were made on a number of children receiving treatment with streptomycin.

Films showed a fair proportion of Gram-negative bacilli; but in most cases none could be grown. In other cases, streptomycin-resistant strains of *A. aerogenes* were found on culture. In no case could any *B. coli* or Gram-negative bacillus other than *A. aerogenes* be grown.

A strain of smooth *B. coli* was made streptomycin-resistant, and after attempts at inoculation by mouth had failed, rectal inoculation was successful. This has now been carried out on two cases, and in each case the organism appeared in the stools in less than 48 hours; in one case it persisted until treatment was suspended (11 days), and in the other case it is still present at the time of writing (16 days). It is now only just resistant to 10–20 units of streptomycin per ml.

This technique may be useful in assessing the part played by various other species of micro-organisms in nutrition; and also possibly in epidemiological problems.

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### Growth of *Culex molestus* under Sterile Conditions

*Aedes aegyptii* and *Culex pipiens* are the only two mosquitoes which have hitherto been reared from egg to adult under sterile conditions. It is interesting to note that the autogenous *Culex molestus* can also be bred without difficulty on sterile media.

The following media were found suitable for this purpose (all experiments were carried out at a temperature of 28° C.).

(1) Dried or fresh yeast (autoclaved) 1.2 gm. to 15 ml. distilled water to which 25 eggs of *C. molestus* are added under sterile conditions. On this medium the mosquitoes developed from eggs to adult in 10.5–13 days, but the adults were not viable.

(2) Water plus dried yeast plus the following vitamins: thiamin, riboflavin, pantothenic acid, niacin, pyridoxin, folic acid (each 10  $\gamma$  per ml.), biotin 0.14  $\gamma$  per ml. This was the optimum medium, and the development from egg to adult lasted 7.5 days. The mosquitoes were viable. Obviously 1.2 gm. of the best samples of yeast available did not contain sufficient of the above factors to produce viable adults.

(3) Liver extract plus dried yeast (autoclaved) (Trager's medium for *A. aegyptii*). Development from egg to adult lasted 14 days and the adults were viable. Water extract of the same yeast and liver extract allowed development from egg to adult in 20 days; but the adults which emerged were not viable.

(4) Water, glucose (0.1 per cent), a mixture of salts (0.2 per cent), casein hydrolysate (2 per cent) and the above-mentioned vitamin B complex (but with only 7.5  $\gamma$  biotin per ml.) plus ascorbic acid 150  $\gamma$  per ml. Although development on this medium was relatively slow (eggs to viable adults in 25 days), it proved to be the most suitable for studying the nutritional requirements of all stages of *Culex molestus*. Thus in the absence of pantothenic acid, all other factors being present, there was no development beyond the first instar; in the absence of thiamin, riboflavin or pyridoxin, the larvae die in the second instar; in the absence of folic acid the larvae reach the fourth instar but never pupate. In the absence of niacin the whole developmental cycle lasts 42 days and the adults are viable. In the absence of biotin the cycle lasts 36 days, but the adults are not viable. Ascorbic acid is not essential for complete development of viable mosquitoes, but in its absence the cycle is prolonged to 29 days.

It is interesting to note that the viable females raised on sterile media were autogenous and laid eggs in the absence of any kind of nutrition. Full details will be published elsewhere.

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### Mosquitoes and Malaria

WITH reference to the recent article on the Ronald Ross Jubilee<sup>1</sup>, it may be of interest to note that, in a paper read at a meeting of the Royal Scottish Geographical Society in April 1892, Dr. H. Martyn Clark, of Amritsar, remarked "that, as some insects carry the pollen of one plant to the flower of another, so mosquitos [*sic*] transplant the malarial germ to a suitable *nidus* by directly inoculating their victims with it"<sup>2</sup>. He further stated that "it will probably some day be ascertained that the mosquito fulfils a part in their [that is, the germs of malaria] transmission". As Ross met Manson in 1894, and Manson brought forward the mosquito theory in that year, Clark's adumbration seems to be worthy of recall.

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<sup>1</sup> *Nature*, 162, 50 (1948).

<sup>2</sup> Clark, H. M., "Remarks on Malaria and Acclimatisation", *Scot. Geog. Mag.*, 9, 294 (1893).