BIOLOGY

Inclusion of Parental Somatic Cells in Sponge Larvæ

THE common boring sponge *Cliona celata* (a marine siliceous type) is oviparous. This characteristic, exceptional among sponges, makes it particularly useful for embryological investigation.

When the fertilized eggs are ejected from a maternal osculum, each carries 20–35 small amœboid cells clinging to its surface. These contain numerous brownish-yellow cytoplasmic granules, and also retain some of the yellow picric acid stain of Bouin's fixative even after prolonged washing. These two points identify them with a type of small amœbocyte widespread in sections of the adult sponge, and conspicuous in the thin edges of reconstitution cultures¹. They stain deeply if the eggs are immersed in dilute brilliant cresyl blue.

During cleavage, the amœboid cells move into the clefts between the blastomeres, apparently without themselves dividing. In sections of mature larvæ, they are found in the inner mass of amœboid cells and between it and the external flagellated epithelium. Though their cytoplasmic granules have almost disappeared, they are still recognizable by their stubborn retention of picric acid during histological preparation. They can be found in undiminished numbers throughout the 2-day larval life.



Fig. 1. Embryo of *Cliona celata* showing maternal cells on and among the blastomeres

Unfortunately, I have not been able to learn whether these maternal cells persist through metamorphosis, and become incorporated in the new young sponge, or (as seems more likely) merely serve as a food reserve for the larva. In either case, the larva of *Cliona* is a chimera, composed of parental somatic cells mixed with cells formed by cleavage of the zygote.

In 1920, Gatenby² mentioned that maternal cells from the nutrient capsule of the embryo sometimes enter the posterior mass of non-flagellated cells in the embryo of *Grantia compressa*, a calcareous sponge. This suggests that the chimeric structure of the larva may be common throughout the phylum Porifera.

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¹ Warburton, F. E., Canad. J. Zool., 36, 555 (1958).

³ Gatenby, J. B., J. Linn. Soc. Lond., 34, 261 (1920).

Cyclical Infection of Glossina morsitans with Culture Forms of Trypanosoma rhodesiense

The natural rate of salivary gland infection of tsetse flies with trypanosomes of the *brucei* subgroup is very low, being only about 0.25 per cent¹.

One of the main problems associated with the work on trypanosomiases of this sub-group is the difficulty research workers experience in obtaining adequate supplies of metacyclic trypanosomes for experimental purposes. No circumstances are known which will invariably lead to cyclical infection of the natural vector¹.

During 1959-60, one of us (the late R. M. G.) noticed that tsetse flies fed artificially on culture forms of *Trypanosoma rhodesiense* appeared more readily to develop gut infections than flies fed naturally on infected mammalian hosts. In view of the potential importance of this observation, a pilot experiment was planned in which a small number of *Glossina morsitans* were fed on culture forms of *Trypanosoma rhodesiense*, the tsetse flies afterwards being maintained for three weeks on heparinized human blood. One of these flies developed a patent gland infection.

The trypanosome strain used (S.S. 354) had been isolated from a human infection in 1959 and sent from Africa in deep freeze. Weinman² cultures were set up in January 1960 from the blood of mice previously inoculated with the human material. Since this date regular *in vitro* passages have been made, and in this experiment tsetse flies were fed on the 31st and 32nd sub-cultures.

The tsetse flies had hatched from puparia sent from East Africa, by the courtesy of the Director of the East African Trypanosomiasis Research Organization. They were given three feeds of the cultured trypanosomes. For each feed 0.2 ml. of the water of condensation from the culture containing approximately 2,000,000 trypanosomes was added to 10 ml. of fresh, heparinized human blood. This mixture, heated to 37° C., was offered to the tsetse flies through a specially prepared guinea pig skin membrane. Afterwards the same diluent without culture was fed twice-weekly to maintain the flies.

At time-intervals of 19–27 days after the first infective feed the tsetse flies were induced to probe on to a warmed glass slide³, and Giemsa-stained drops of saliva were examined for metacyclic trypanosomes. One smear, from a fly first fed 27 days previously, showed typical metacyclic trypanosomes.

The whole group of experimental tsetse flies were then allowed to probe and to feed on an uninfected guinea pig which showed typical blood forms in peripheral wet blood films on the 9th day after the infective bite. Infection is persisting in this animal at a low level at the end of the fifth week after the infective bite. The trypanosomes are polymorphic.

It is hoped to confirm this finding in further experiments by showing that a significantly high proportion of tsetse flies will develop a salivary gland infection after feeding on trypanosome cultures, and that this can be cyclically transmitted by the arthropod vector.

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¹ Buxton, P. A., The Natural History of Tsetse Flies (Lewis, London, 1955).

² Weinman, D., Ann. N.Y. Acad. Sci., 56, 995 (1953).

³ Burtt, E., Ann. Trop. Med. Parasit., 40, 141 (1946).