

days. In the *maculatus* progeny the average isolation age of 8 thyroid-fed males was 99.1 days, that of 10 control males 189.7 days. The sharp difference in the isolation ages shown by *maculatus* and *variatus* appears to be characteristic of these species (Bellamy—unpublished data). There seems to be little doubt that thyroid feeding results in precocious maturation in both species tested.

Besides being precocious, differentiation of the gonopod in thyroid-fed males is atypical. In the normal male the third, fourth, and fifth anal rays grow out to nearly twice the length of the sixth and seventh and undergo a closely integrated differentiation. In the thyroid-fed male, however, the sixth and seventh rays also participate in the elongation process and the typical relations of the rays are upset. Subsequent differentiation is incomplete and distorted. Description of the details of the process must be postponed for a later publication.

*Summary.* Thyroid-feeding of immature *Platyopocilus maculatus* and *P. variatus* resulted in exophthalmos, decreased growth rate and altered body proportions, in addition to precocious sex maturation as indicated by the early but atypical differentiation of the male gonopod.

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### Cultivation of Various Species of Trypanosomes in the Developing Chick Embryo.

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One of the strains of trypanosomes (*T. rhodesiense*) maintained in this laboratory throughout a period of several years became arsenic-fast for some unknown cause. Cross-transfer to various kinds of mammalian hosts failed to correct this abnormality, hence the idea occurred to us to attempt cultivation of the organism. Since cultivation in artificial media was unsatisfactory, we turned next to the chick embryo as a further possibility.

Early in the work we used the commonly employed window technic but soon found that a simplified method was entirely adequate. Eggs were incubated for 8 or 10 days prior to inoculation. By means of a sterile dissecting needle, two small holes were made through the shell previously cleaned with alcohol. One hole was made into the

air sac and the other immediately over the embryo. The inoculum consisted of rat blood, highly infected with trypanosomes obtained aseptically and diluted with an equal volume of 0.9% NaCl solution. A 26-gauge needle attached to a syringe was then inserted into the allantoic cavity by placing the needle parallel to and immediately under the chorio-allantoic membrane. The volume of inoculum for each egg was usually approximately 0.5 cc. After withdrawing the needle, the holes in the shell were closed by small drops of melted paraffin. A heavy infection in embryo blood was regularly obtained which was the cause of death of the embryos in 4 or 5 days.

Subcultures were made by transferring diluted embryo blood obtained aseptically on the fifth day. By this technic this strain was maintained in developing chick embryos for 8 generations in 41 days. Neither change in virulence nor arsenic-fastness occurred in this strain of trypanosomes during this period of observation.

Since *T. rhodesiense* was so easily cultivated in the chick embryo, it seemed appropriate to attempt cultivation of other species of trypanosomes. On trial it was found that other species, *viz.*, *T. equiperdum*, *T. brucei*, *T. evansi* and *T. hippicum*, were equally readily cultivated for 15 days and maintained their normal virulence for rats. There appeared to be no obvious reason why these strains could not have been cultivated indefinitely had there been any object for such a continuation. *T. lewisi*, on the other hand, was cultivated, but not very satisfactorily, because of its very slow growth.

None of these species of trypanosomes was infective for the hatched chick.

*Conclusions.* The following species of trypanosomes were successfully cultivated in the developing chick embryo: *rhodesiense*, *equiperdum*, *brucei*, *evansi* and *hippicum*. *T. lewisi* was cultivated but not very satisfactorily. No change in virulence of these organisms appeared during the time of cultivation. The strain of *T. rhodesiense* remained arsenic-fast throughout the period of 41 days of cultivation in the chick embryo.