

With Lloyds' reagent we accomplished just the reverse of the ultraviolet light effect, that is, the factor effective *in vitro* is removed, while that effective *in vivo* is left unharmed, perhaps even increased in potency.

Boiling the tissue fibrinogen solution vigorously for a few minutes, and filtering, yielded a clear filtrate which was without effect on blood clotting *in vitro*; but which gave a marked inhibition of clotting when injected intraperitoneally.

We feel justified in concluding that tissue fibrinogen, as we previously prepared it, contains 2 substances, one of which is associated with the turbidity of the solution and acts as a strong blood coagulant only when added directly to the blood. The other can be obtained in a crystal clear solution, is without coagulative activity *in vitro* but acts well in the body. This latter substance is readily inactivated by ultraviolet light, while the former is resistant to such effects.

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#### Delayed Reproduction in *Amblystoma Punctatum*.

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Nine specimens of adult *Amblystoma punctatum*, captured in pools in the Pocono Mountains, near the village of Effort, Pennsylvania, during the breeding season (the first week of April) of 1929, were kept in the laboratory of the Morris Biological Farm of the Wistar Institute until February 14, 1930, when they were placed in a mechanical refrigerator. On May 28 two females and one male were found frozen to death. The others of the lot, one female and 5 males, were placed in an aquarium. On the morning of May 29 numerous spermathecae were found deposited on sphagnum in the aquarium, and during the night of May 29-30 two clutches of eggs were deposited. On the morning of May 31 another clutch, very small, was found, but it was probably deposited on the first night, for the eggs did not appear to differ from the others in degree of development.

The eggs were examined critically on June 7. There proved to be 125 in all. Seventy-six of these were developing normally in various neural groove and early tube stages (Harrison's stages 15 to 19);

36 were abnormal, mostly in an apparently abortive segmentation (possibly parthenogenetic); 11 were apparently infertile. The normal embryos progressed in development according to Harrison's stages approximately as follows: June 10, stage 26; June 11, stage 27; June 12, stage 31; June 13, stage 31+; June 15, stages 32 and 34; June 16, stage 34; June 17, stages 34 and 35; June 18, stages 35 and 36. On June 15, 9 were in the early flexure stage (Harrison's stages 33 and 34); 18 in the non-motile stage (Harrison's stages 33 and 34); the others were in the premitile stage. On June 16 almost all of the embryos were in various flexure stages; but none were in the coil stage. On June 17 several were in the coil stage. On June 18 the embryos were mostly in the S-reaction stage (Harrison's stage 36). On June 19 they were approaching closely to the swimming stage, and on June 20 (the time of writing) some were beginning to swim. The delayed reproduction has, therefore, not prevented normal structural and physiological development.

The parents of these embryos were kept from April, 1929, to February 14, 1930, in a tightly closed wooden box with a bedding of sphagnum but without water excepting as water was dashed over the sphagnum to keep it moist. The box in which they lived was kept in a moist place on the cement floor of a cellar and adjacent to a gutter of running spring water the temperature of which ranged during July and August from 12.4° C. to 14.6° C. During the remainder of the year it ran as low as 9.6° C. The temperature immediately about the specimens was probably a little above that of the water in the gutter. The food was almost exclusively earthworms which were thrown into the box without regard to individual feeding. The temperature of the refrigerator ranged from 33° F. to 36° F. except during defrosting, when it rose considerably, and on one occasion it fell below freezing.

The dimensions of the aquarium in which the eggs were deposited are 14x14x36 inches. It contained a large amount of sphagnum, and was supplied with a jet of compressed air, and a small stream of spring water which on the days concerned ran at a temperature of 11° C. The room temperature was 15.8° C.

Probably an important factor in the excitation of egg laying in this species under laboratory conditions is the presence of a number of males. Blanchard<sup>1</sup> has observed several males of *Amblystoma punctatum* swimming in a whorl around a single female at the time of egg-laying; and one of us has seen the same performance in the native breeding place.

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<sup>1</sup> Blanchard, *Am. Naturalist*, 1930, 64, 154.