

was cut on the dorsal side, and the eye was gently turned ventrally. A crescentic cut was then made through the coats of the eye dorsal to the optic nerve, and the conjunctiva was sutured back to the edge of the orbit. With the dorsal half of the eye destroyed, the fish became very dark in both black and white dishes. In complete darkness an intermediate shade was assumed. When the lower region of the eye was destroyed by the method used on the upper region, fish became lightish to light in a white dish and intermediate in a black dish. Since they lacked the lower half of the eye, they were unable to adapt dark. A very uniform response was obtained in these various experiments. More confirmatory evidence of regional differences in the eye was thus obtained.

The dispersion of pigment and the length of myoids of retinal elements are similar in the upper and lower regions of the eyes of both light and dark fish. Therefore, pigment migration and the elongation of the retinal elements do not account for this mechanism of adaptation.

The lower region of the eye differs from the upper region in having a specialized crescentic ridge and no single cones. The differing response of the two regions of the eye, however, may not be due to these structural differences, but it may be due to the difference in distribution of the optic nerve fibers of the two regions of the eye in the C.N.S. Information has not been secured on the destination of the nerve fibers. Confirmation has thus been secured that the upper region of the eye is associated with the adaptation to light background and the lower portion to the darkening of the body. The responses are due to either the qualitatively different regions of the eye or to different central connections.

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### THE EFFECTS OF ANESTHETICS ON CHROMOSOME BEHAVIOR DURING POLAR BODY FORMATION IN THE EGGS OF THE SNAIL *NASSA*

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One of the first questions that arise regarding the behavior of chromosomes concerns the causes which underlie the difference between mitosis and meiosis. In the case of eggs the factors responsible for the formation of polar bodies present a second though closely related problem. Except for some centrifuging experiments on polar bodies (Conklin, 1917; Clement, 1935), previous work of an experimental nature on either of these problems is almost non-existent (see

reviews by Conklin 1924, Darlington 1937). Recent work on the action of a wide variety of agents on chromosomes (Sharp 1934, Ludford 1936, Nebel 1937) has been confined to somatic mitosis.

The following experiments were intended to help elucidate these related problems. The eggs of two species, *Nassa (Ilyanassa) obsoleta* and *N. trivittata* were used as a check on each other since both are found abundantly, though in different localities, near the laboratory. A slight difference in the size of the eggs is at once apparent, but their development is essentially similar and their reactions to anesthetics have so far been the same.

The eggs of these two snails have proved to be favorable material for an experimental study of polar body formation because the intervals between laying and the emergence of the first polar body and between that event and the emergence of the second polar body are long enough to permit manipulation and are separated by sharply defined points from which time can be measured. Fertilization takes place before laying. The first polar body appears 30 or more minutes after laying, followed 50 or 60 minutes later by the second polar body. The first cleavage occurs about two hours after the second polar body. No attempt was made to maintain a rigid control of temperature. This schedule holds good at approximately 22°. At higher temperatures development is much more rapid.

The recently advanced precocity theory of meiosis (Sharp p. 275) holds that the difference between reduction divisions and ordinary somatic mitosis lies in a difference in the relative times at which the various events in the mitotic cycle occur. According to this concept it might be expected that artificially increasing the time between the two maturation divisions would give the chromosomes an opportunity to divide, with the result that an ordinary somatic mitosis instead of a reduction division would follow. This assumption presupposes of course that the multiplication (or division) of the chromosomes would not be retarded as much as the division of the egg itself. That this is not at all impossible is shown by the fact that a differential effect has been observed (Twitty 1928, Needham 1933) in the case of other developmental processes.

In a preliminary series of tests (Moment 1936), chloral hydrate was found to be the most satisfactory anesthetic and a concentration of 0.005 M in sea water, the minimum concentration which would completely inhibit the formation of the second polar body. Various times for putting the eggs into and removing them from the anesthetic were tried but the best results were obtained by placing the eggs in the chloral hydrate ten minutes after the extrusion of the first polar body and allowing them to remain there until the controls were at the height of the first cleavage; i.e., at the brief climax of the so-called trefoil stage. They were then returned to sea water.

Two different results were obtained. After the eggs had been in sea water again for an hour or longer, either a single second polar body several times the normal size was formed, or else two, or in very rare cases three, 'second' polar bodies emerged simultaneously

from the animal pole of the egg. They were usually but by no means always separated by a small space (Fig. 1). Sometimes one was larger than the other. There was a tendency for any of these polar bodies which were larger than those shown in the accompanying figures to be resorbed back into the body of the egg. The smaller double second polar bodies were completely cut off from the egg.

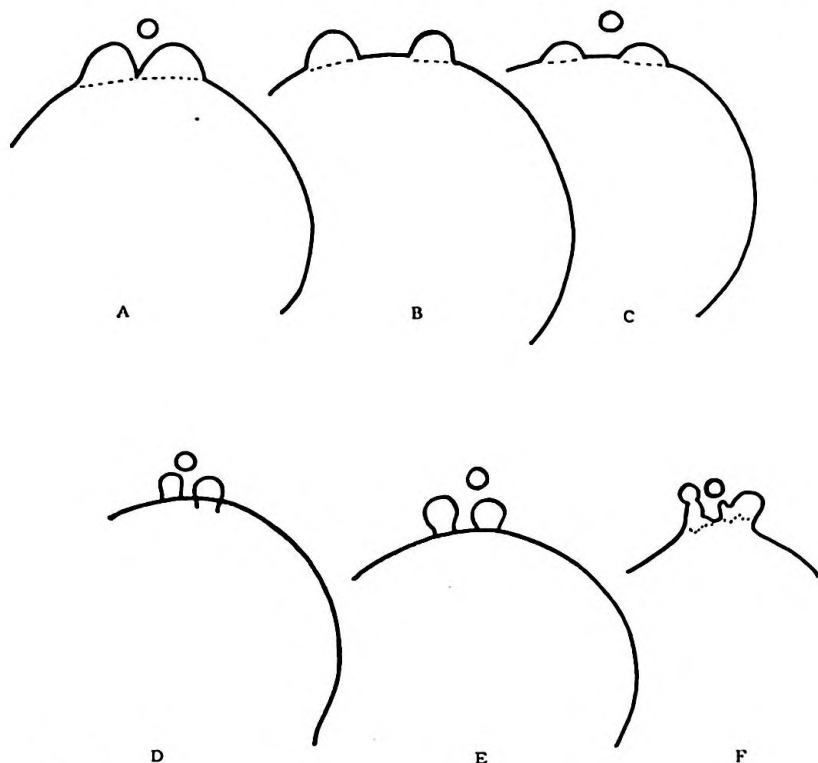


FIG. 1.—Camera lucida drawings of living eggs of *Nassa obsoleta* showing the simultaneous formation of two second polar bodies. Figures *a*, *b*, and *c* represent earlier stages than do figures *d*, *e*, and *f*. The normal first polar body is shown in all except *b* where it had become detached from the egg.

Eggs from different capsules varied greatly in the percentage which reacted in any given way.

The chromosomes were studied after sectioning the eggs and staining with Heidenhain's iron hematoxylin and in whole mounts after staining with Conklin's modification of Delafield's hematoxylin. Much the best results, however, were obtained by fixing in equal parts of saturated aqueous sublimate and absolute alcohol and then using Feulgen's reaction without sectioning. Both the large single and the smaller double second polar bodies contained chromosomes in 100% of the cases. Chromosomes could also be seen in the normal

first polar bodies in those cases in which they remained attached to the egg after fixing and staining. The sperm nucleus could also always be found, although there was a good deal of variability in regard to its position. It was never very close to the chromosomes forming the second polar bodies and was often far removed on another side of the egg.

The anti-polar, or yolk, lobe, recently the object of intensive study (Morgan 1936), showed striking and characteristic differences when the eggs from several capsules were compared. Ordinarily this lobe protrudes during the formation of the second polar body in such a way that the egg as a whole becomes pear shaped. After the treatment with chloral hydrate, however, the lobes were sometimes much shorter and blunter than usual while in other very frequent cases they were exceedingly long and thin, longer than the diameter of the rest of the egg. At the end of this narrow finger-like structure there were usually three to five slightly ameboid processes resembling roots. All the eggs from the same capsule reacted in the same way as far as the anti-polar lobe was concerned. No correlation was observed between the behavior of the anti-polar lobe and of the polar bodies. The meaning of this is uncertain, but that the eggs of a single species should react in two or more ways is not surprising in view of the known diversity of reaction in *Urechis* eggs (Tyler 1932) and in the eggs of *Triton* (Fankhauser 1937).

Individual chromosomes can be seen and counted in the double polar bodies but the counts are as yet inconclusive. A considerable amount of material has been fixed and is in the course of study. Any conclusions would seem premature and further work with chloral hydrate and other agents is planned.

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