

an opalescent solution of the latter (normal rabbit's serum, diluted with 3 volumes of water, steamed for 15 minutes, and centrifuged) with specific precipitative systems (hemocyanin and ovalbumin) and have found that the amounts of precipitated nitrogen were no greater than those produced in mixtures not containing the heated normal serum whether in the zone of equivalence, of excessive antigen, or of excessive antibody. In these respective zones the comparative figures, averages from duplicate analyses, were 0.512 and 0.489; 0.517 and 0.509; 0.402 and 0.408 mg. N in the *Fulgur carica* hemocyanin-system; 0.103 and 0.110; 0.106 and 0.117; 0.172 and 0.181 mg. N in the *Limulus polyphemus*-system. Mixtures of ovalbumin and its antiserum (rabbit) were made only in the zone of equivalence and of excessive antibody. The nitrogens in the precipitates were 0.743 and 0.749; 0.498 and 0.502 mg.

Thus, the prominent cohesive property of specifically combined antibody-globulin is not developed by thermally denatured normal globulin, although, in Shibley's experiments, the  $\zeta$ -potentials of the two forms were identical. This negative evidence is reported because it slightly narrows the field wherein we must search for an answer to the question: What makes combined antibody sticky?

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### Occurrence of Birefringence in the Fertilized Egg of the Sea Urchin.

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In preliminary experiments it was observed that, after fertilization, the eggs of *Strongylocentrotus purpuratus* showed, near the periphery, light and dark phases when rotated between crossed Nicols. The effect, however, was partially masked by the opaque granules of the cytoplasm. In order to eliminate this difficulty the unfertilized ova were centrifuged at approximately 60,000 gravities for 3 minutes, flotation of the eggs being insured by the addition of sufficient M/1 solution of sucrose to the sea water in the tubes. After centrifugation the eggs were transferred to sea water and microscopic preparations made in the usual way. The eggs appeared elliptical in shape, with the nucleus at the centripetal end and the

opaque granules at the opposite pole occupying about  $\frac{1}{3}$  of the egg. The remaining  $\frac{2}{3}$  of the egg was transparent and showed no birefringent effects with polarized light. Within 3 minutes after fertilization, however, when the membrane was forming and afterward, the clear cytoplasm gave evidence of birefringence so definite that the angle between light and dark phases could be measured. In all 35 measurements of the angle were made and these yield a mean value of  $31^\circ$ , with extremes of  $24^\circ$  and  $37^\circ$ , the intermediate values being consistently distributed on a probability curve. Now the optical section of the egg is something less than  $72\mu$ , and since such a minute quantity of polarizing molecules (*e. g.*, sugar) in solution would not be sufficient to rotate the beam of polarized light, it is necessary to assume that the rotation is due to a regular grouping of molecules to form a multimolecular lattice with birefringent properties similar to those of a crystal, a starch grain, a filament of silk, a muscle fibril. Fertilization, therefore, causes an arrangement in regular pattern of certain molecules or micels of the cytoplasm. It is obvious that with crossed Nicols there would be, with an anisotropic crystal, 4 dark and 4 light phases for a complete rotation of the specimen, whereas we have obtained 6. The solution of this problem probably lies in the same direction as that of the peculiar birefringence of the starch grain. The effect which we have obtained may be related to the chemical change reported by Mirsky,<sup>1</sup> who found that the percentage of soluble protein in the egg of the sea urchin diminishes and that of the insoluble protein increases as the result of fertilization. A relation of the effect under consideration to the migration of the nucleus after fertilization is also suggested, since it has been shown that the nucleus is probably pulled into position by contractile structures in the cytoplasm.<sup>2</sup>

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<sup>1</sup> Mirsky, A. E., *Science*, 1936, **84**, 333.

<sup>2</sup> Moore, A. R., *Protoplasma*, 1937, **27**, 544.