conditions visual purple can be formed again in solution from its products of decomposition by light.

For the record we give the essential details for the preparation of visual purple solutions used in these experiments. Freshly caught, summer (1936) frogs (*R. pipiens*) from Alburg, Vermont, were dark adapted over night. Their heads were cut off in the light of a 10 watt ruby lamp, and the eyes removed and cut in half just back of the iris. The parts containing the retinas were left in a 4 per cent. alum solution for 2½ to 3 hours at 25° C., after which the retinas were removed as free of pigment as possible and washed once with 0.04 *M* borate-KCl buffer of pH 9.3 and three times with water by decantation. Finally, the excess water was removed by centrifugation. A 4 per cent. solution of purified bile salts was next added in the proportion of 1 ml of solution to 27 retinas, and the mixture gently stirred for 10 minutes at 30° C. The suspension was then centrifuged at a high speed for 1½ hours at 5° C., and the clear, supernatant liquid removed and stored in the dark at 0° C. For bleaching the solutions, we used a 100 watt lamp at 25 cm distance with a heat-absorbing glass interposed, and a mirror backing the solution. The particular experiment shown in Fig. 1 began with 3 ml of solution to which was added 1 ml of 0.4 *M* phosphate buffer of pH 7.6; the colorless precipitate which formed was allowed to settle and then filtered off, and the filtrate containing the visual purple was divided into samples A and B, each placed in a 5 mm cell for absorption measurements. After bleaching, one drop of 1 *N* NaOH was added to change the pH to 10.5; this caused a colorless precipitate to form, which was allowed to settle to the bottom of the cell before the absorption spectrum of the solution was measured. The pH measurements were made with a glass electrode.

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PROTEIN COAGULATION AS A RESULT OF FERTILIZATION

DURING the past two years I have developed a technique for studying the proteins of the sea-urchin egg and have found that a pronounced change in state of the proteins in the egg takes place soon after fertilization. Experiments on the quantities of soluble protein in unfertilized and fertilized eggs of the sea-urchin (*Arbacia punctulata* at Woods Hole, Mass., and *Strongylocentrotus purpuratus* at Corona del Mar, Calif.) show that about 12 per cent. of the total protein in the cell becomes insoluble as a consequence of fertilization. The protein fraction in which the change occurs has been identified, and some of the properties of this protein have been investigated. The time-course of the change has been followed, beginning at three minutes after insemination and ending two hours later, when the egg is in the two-cell stage. As a result of these experiments, the significance of the change in state of protein for the egg can perhaps be understood.

To investigate the protein contents of the egg the first need is to disrupt the cell by a method that does not involve the use of reagents known to alter proteins. I have found that both fertilized and unfertilized eggs can be disintegrated by freezing in a solid carbon dioxide-ether mixture at −77° C., drying in a vacuum at about −25° C., and then grinding the powder so obtained. Scarcely an intact cell remains after this treatment. The disintegrated cell material is extracted with a large volume of cold 1 M KCl solution at a pH of about 7.3. Under these conditions between 82 and 85 per cent. of the total protein of unfertilized eggs goes into solution, whereas only 69 to 72 per cent. of the protein of fertilized eggs dissolves.

Some of the reasons for the technique developed may be briefly given. Freezing and thawing suffice to disintegrate unfertilized eggs; this procedure, however, kills fertilized eggs, but it does not break them; hence the need for the drying and grinding procedure. The instability of the protein system in the cell also presents difficulties. If eggs are frozen and then allowed to thaw, it is found, after subsequent drying and grinding, that in both unfertilized and fertilized eggs only about 50 per cent. of the total protein is soluble. And if the protein fraction that becomes insoluble during fertilization is isolated from unfertilized eggs and then frozen in the same way as were the eggs from which it was prepared, this protein becomes insoluble, whereas it remains soluble when the intact egg is frozen. Once these properties of the material have been recognized, however, it is not difficult to estimate with accuracy the relative quantities of soluble and insoluble protein in a mass of eggs.

The protein that coagulates during fertilization can be prepared from unfertilized eggs by freezing, drying, extraction with potassium chloride and precipitation by ammonium sulfate, this protein being in the fraction first salted-out. The protein can be freed from many other cell constituents by repeated reprecipitation, but it is not claimed that a single protein has been isolated. In solution at a pH of about 7.0 the protein particles appear to be exceedingly elongated, for the solution possesses high viscosity (a 1.4 per cent. solution is 9.6 times as viscous as water),

and the relative viscosity, measured in an Ostwald viscosimeter, decreases when pressure is applied. Furthermore, the solution shows double refraction of flow.[1]

I have found by investigating protein sulfhydryl and disulfide groups that when coagulation occurs in the cell the change in protein is distinctly different from that caused by the usual protein denaturing agents—heat, acid, etc. When these agents coagulate proteins, changes in their sulfhydryl and disulfide groups are observed;[2] whereas, when coagulation takes place in the cell, no change in these groups is detectable, although a change is observed if the proteins of the egg are treated with acid. In this respect coagulation in the egg resembles the coagulation of myosin as it occurs in the contraction and rigor of muscle.[3] Of all the known ways of coagulating myosin *in vitro* only in dehydration (either by drying or freezing) is loss of solubility *not* accompanied by a change in sulfhydryl groups.[4] In this kind of coagulation it would appear that when the shell of water enveloping a protein particle is removed the outer groups of the particle become firmly attached to the outer groups of other particles. An insoluble mass of protein is formed with far less disturbance of the inner configuration of the molecule than when coagulation is caused by any of the usual denaturing agents.[5] The type of coagulation that takes place in muscle and in the egg is of the type observed in dehydrated myosin. It should be noted that the protein in the egg which coagulates during fertilization readily coagulates if, when isolated, it is dehydrated.

The time-course of protein coagulation after fertilization shows that the protein change is not associated either with elevation of the fertilization membrane or with the cycle of cell-division that follows fertilization. The fertilization membrane is formed within a minute after insemination; coagulation begins about three minutes later and is completed within the next ten minutes. No change in the quantity of coagulated protein is detected during the next two hours, during which time the egg passes through a complete mitotic cycle. Coagulation is, however, associated with another change in structure of the egg, an increase in strength and elasticity. The unfertilized egg is broken by freezing and thawing, whereas the fertilized egg is not broken by this treatment. The increase in strength manifested in this way is not due to presence of the fertilization membrane, for even after it has formed the cell is still fragile. Increased strength of the cell appears within the ten minutes following membrane elevation, during the time of protein coagulation. And the ability of the cell to withstand freezing and thawing remains thereafter (at least for two hours) just as the coagulated state remains. The explanation of this increase in strength and elasticity is that in coagulation the elongated protein particles unite to form a fibrous net-work. This, an early step in development of the egg, may be regarded as a skeleton framework within which differentiation proceeds.

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THE BERGER RHYTHM IN CATS[1]

THE disappearance of the slower potential fluctuations of about 10 per second in the human electroencephalogram when the eyes are opened was one of the most striking observations reported by Berger in his early papers on the electrical activity of the human brain. This phenomenon has been studied in detail by Adrian and Matthews[2] and by Adrian and Yamagiwa.[3] It is so easy to produce in the average subject that it has now become a commonplace. Kornmueller, who has worked extensively on rabbits, did not report any comparable change in animals. Range,[4] working in the same laboratory, however, has recently published a figure which shows disappearance of the slower potentials in the rabbit with painful stimulation.

Ectors[5] reported that modifications similar to those seen in man are produced by sensory stimulation in the rabbit. The frequency of the slower components, which Berger calls alpha waves, is somewhat slower in the rabbit than in the normal adult human, but, as in the human, they diminish in amplitude or disappear when the eyes are illuminated. Adrian[6] has confirmed this observation on monkeys under light anesthesia.

Work which is now in press,[7] in which one of us had a part, shows a similar effect in the cat. Attention, in the cat as in the rabbit and man, abolishes or dimin-

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