true—began with Lamarck as an intuition, became a conviction, and, unsupported by proofs such as we could give to-day, went down in apparent defeat. No one fails so completely as a genius" (p. 185).

Leaving Europe, we now come to chapters on the early American naturalists; Bartram and Michaux, the botanists; Wilson and Audubon, the ornithologists; Say and Lesueur, and that singular character Rafinesque. There is then an account of Goethe and the Romanticists, a quite full account of Darwin and Wallace and a final chapter on Fabre. Agassiz is omitted, "because most of what he stood for in natural history had already been expressed by Cuvier."

It is a charming book, certain to do a great deal of good. There should, however, be a somewhat revised edition. Peattie's emotional impulse, the very intensity of his convictions, sometimes leads him astray. He seems like a man on a splendid horse, riding in hot haste to a certain goal, sometimes careless of what is trampled under foot on the way. Thus (p. 298) "For the East Indies are the isles of five times ten thousand beetles, the country of the gorilla, the archipelago of birds of paradise, each species with an island to itself." Or (p. 292) "It was the 'Brownian Movement,' the spontaneous streaming of protoplasm, as disturbing for human eyes to see as the procession of the suns of space across a telescopic field, and fraught, as much, with human destiny." Or (p. 318) "But Father Gregor, superior of an Austrian monastery, was working his marvelous sweet-pea plot all unknown to the world of science." Some of the statements about Rafinesque are fantastically extravagant: "Amongst all the naturalists who have ever worked on the American continent, Rafinesque is the only one who might clearly be called a titan" (p. 263). "If the rules of priority were strictly and justly applied, Rafinesque would be found to have antedated a large part of the work of Say amongst shells, of his enemy Harlan amongst mammals, in botany of Gray and De Candolle." (p. 266.) A curious point has to do with "the deep roar of the gorilla" (p. 164). This appears to be traditional,[1] but last year I spent considerable time watching the two gorillas (the mountain form, from the region near Lake Kivu) in the zoological gardens at San Diego. They are in robust health, and play together a great deal, running and wrestling. They make a noise by beating the chest, clapping the hands and beating rapidly on an iron door. They seem not to be vocal at all, under ordinary circumstances, but Mr. B. J. Benchley, the superintendent, tells me that they will occasionally utter a sort of scream under stress. They were tested with different kinds of music, and it was found that they were interested in anything of the nature of a drum, but wind or string instruments had no meaning for them. They pay no attention to the gibbon in the next cage, which sits on its perch and cries like a lost soul, just as I heard the gibbons crying in the tree-tops at dawn in Siam.

The illustrations in Peattie's book are of necessity those already familiar elsewhere, but they are well chosen and most excellently reproduced.

It is interesting to think how the historian, after many years, can perceive the significance or interest of events which could not be so understood when they happened. Peattie gives a figure of the Franklinia, a beautiful plant related to the camellia, discovered by Bartram in Georgia in the latter part of the eighteenth century. It still exists in cultivation, but how was Bartram to know that he had found the last wild specimen of a species on the point of extinction, a species which, but for him, would never have been known to man? When Say, in the far west, found the oval striped beetle which we call *Leptinotarsa decemlineata* (Say), he could not tell that eventually it would be known as a major pest of potatoes and related plants, of tremendous importance to mankind. It is for the historian to relate the present to the past, and so far as the naturalists are concerned, work of the type of Peattie's can be extended almost indefinitely.

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SPECIAL ARTICLES

THE REGENERATION OF VISUAL PURPLE IN SOLUTION

IN 1878 Kühne[1] reported that visual purple in solution, after being bleached by light, will regenerate some of its color in the dark. To our knowledge this observation has never been confirmed, though it is commonly known that most investigators concerned with visual purple have vainly tried to repeat it. In fact, many of the published measurements with visual purple depend on the fact that a bleached solution remains constant and does not change in color. We wish to report that not only have we been able to confirm Kühne's observation, but that we have learned to specify some of the conditions controlling the regeneration of visual purple *in vitro,* and have measured its kinetics; and in addition have critically established the

[1] A. Ewald and W. Kühne, *Untersuchungen aus dem physiologischen Institut der Universität Heidelberg,* 1: 267, 1878.

[1] See, for instance, Flower and Lydekker, "Introduction to the Study of Mammals," p. 736.

phenomenon by measuring the absorption spectrum of the newly formed visual purple.

A visual purple solution, freshly prepared from frogs, is pinkish purple. When exposed to strong light, the color changes rapidly to yellow and then very slowly to colorless. If the bleached yellow solution is placed in the dark at 25° C. and examined an hour later, it is found to be distinctly pinkish again, and this color fades rapidly on exposure to light. This procedure may be repeated several times and is what Kühne described.

We have measured the course of regeneration in the solution by following its photometric density at 500 mμ with a highly sensitive photoelectric spectrophotometer which uses so little light that it leaves the visual purple unaffected. Regeneration begins immediately and proceeds rapidly; more than half the increase in density is accomplished in the first 10 minutes, though the density continues to increase for over an hour, reaching asymptotically a concentration about 15 per cent. of the original solution. The curve of density against time resembles the usual kinetics of chemical reactions.

Reformation of visual purple is demonstrable by this means only when the bleaching by light has reduced the visual purple to below 15 per cent. of its original concentration. If the bleached concentration is much higher, the density continues to decrease in the dark. At between 15 to 20 per cent. of the original concentration, the density hardly changes at all in the dark. Evidently after bleaching, two processes take place in the dark: one concerned with forming visual purple from its decomposition products, the other with the further changes of the decomposition products.

The regeneration of visual purple takes place only in relatively fresh solutions made either with bile salts or with digitalin crystals (Eimer and Amend), and in a narrow range of pH. Above pH 8.0 the effect is negligible, while below pH 6.6 the results are uncertain and are complicated by the presence of a decomposition product of visual purple which is yellow at acid pH's[2] and which fades in the dark.[3]

The fact that regeneration takes place only to a slight extent beyond pH 8.0 enables us to measure the absorption spectrum of the freshly formed visual purple. A visual purple solution buffered to pH 7.6 is divided into two equal samples A and B. Both are exposed to the same bright light for 10 minutes, which bleaches them almost completely. To sample A NaOH is added to make its pH 10.5. The two samples are then placed in the dark. Sample B, being at pH 7.6, regenerates noticeably, while sample A, being alkaline, regenerates only slightly if at all. After an hour in the dark, NaOH is added to sample B to make its pH also 10.5. The absorption spectrum of both samples is then measured. Any difference between the two samples represents the regenerated visual purple.

There are two criteria for recognizing visual purple. One is its absorption spectrum,[4] which in the case of frog visual purple is a nearly symmetrical curve with a maximum at about 500 mμ. In Fig. 1 the open

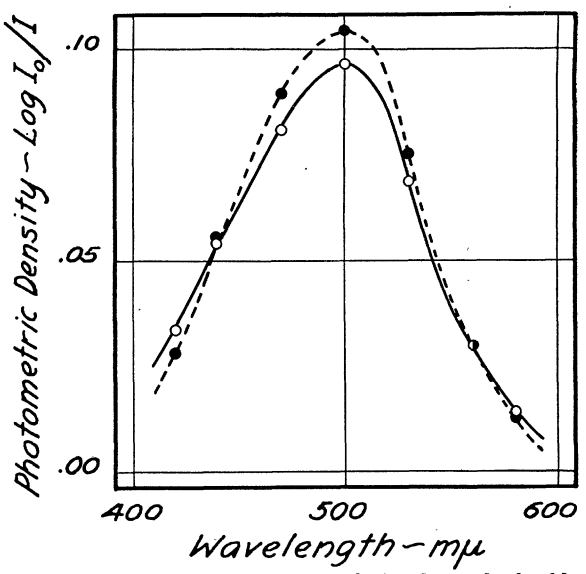

FIG. 1. Absorption spectrum of visual purple freshly formed in solution from its photochemical products.

circles represent the difference between the absorption spectrum of sample A which had remained at pH 10.5 in the dark, and the absorption spectrum of sample B which had been at pH 7.6 in the dark, and in which regeneration had occurred. It is apparent that the difference between the two solutions represents a substance with a nearly symmetrical absorption spectrum whose maximum is at about 500 mμ.

The second criterion for recognizing visual purple is its sensitivity to light. If sample B (now at pH 10.5) is exposed to strong light for 10 minutes, it is rapidly bleached. An indication of the amount of material which has disappeared on bleaching is the difference in absorption spectrum of sample B before and after bleaching. These values are the solid circles in Fig. 1. It is apparent that within the errors of an experiment of this nature, the concentration and the absorption spectrum of the material removed by bleaching are practically identical with the concentration and absorption spectrum of the material freshly formed during the stay of sample B in the dark. There can therefore be no doubt that under the proper

[2] A. M. Chase, *Jour. Gen. Physiol.*, 19: 577, 1936.
[3] Y. Hosoya, *Arch. ges. Physiol.*, 233: 57, 1933.
[4] E. Köttgen and G. Abelsdorff, *Zeit. Psychol. u. Physiol. d. Sinnesorgane*, 12: 161, 1896.

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),