was introduced into the diffusion cell; then the calibration was repeated after the establishment of a steady state of diffusion, with the visual purple still in the cell. With two preparations of visual purple the first calibration gives an average diffusion coefficient of 0.0156, while the second calibration makes it 0.0190 sq. cm. per day. The latter is probably more nearly correct, because the second calibration records the actual state of the disk during the diffusion of the visual purple.

Efforts to eliminate the clogging factor by using alundum disks were not satisfactory because they trapped air easily, and the individual determinations varied widely (from 0.0108 to 0.0177 sq. cm./day) even in a single run. However, the values are of the same order of magnitude as with the glass disks.

To derive the molecular size of visual purple from diffusion data involves the application of Einstein's[2] equation which relates the diffusion coefficient to the radius of the molecule by way of the coefficient of friction and Stokes's law. Such an application is quite reasonable,[3] provided one is interested only in an order of magnitude. Taking the diffusion constant as 0.0148 sq. cm./day, the equations yield 8.04×10^{-7} cm. as the radius of the molecule of visual purple; using the more probable value of 0.0190 sq. cm./day, the radius becomes 6.26×10^{-7} cm. Either of these values shows visual purple to be a large molecule of the size heretofore found only for proteins. Its molecular volume computed from the smaller and more probable radius is 623,000; and if its specific gravity is 1.3 like that of most proteins, its molecular weight is 810,000.

Molecular weights computed from diffusion coefficients are frequently higher, often by a factor of 2 or even 3, than those computed from osmotic pressure or sedimentation data. The real molecular weight of visual purple may therefore be half or even a third of the value here given. For our purposes, this is of minor importance because even so it would still be a very large molecule of the kind found only among the proteins.[4]

This is powerful evidence that visual purple is really a protein, and agrees with Kühne's[5] inability to separate visual purple from the neurokeratin of the rods, and with his demonstration of the high temperature coefficient of its thermal destruction. Adding Wald's contribution[6] that visual purple liberates a carotenoid when it is bleached by light or acted upon by chloroform, it definitely places visual purple among the conjugated proteins—the carotenoid proteins.

[2] A. Einstein, *Z. Elektrochem.*, 14: 235, 1908.
[3] M. L. Anson and J. H. Northrop, *Jour. Gen. Physiol.*, 20: 575, 1937.
[4] T. Svedberg, *Trans. Faraday Soc.*, 26: 740, 1930.
[5] W. Kühne, in *Hermann's Handbuch d. Physiol.*, 3 (1): 235, 1879.
[6] G. Wald, *Jour. Gen. Physiol.*, 19: 351, 1935.

The visual purple solutions were prepared as previously described,[7] and were buffered with borate-KCl to a pH of 9. The glass disks of porosity 4G had a diameter of 30 mm and were about 0.5 mm thick. The inner diffusion cell contained 10 cc of visual purple solution, and the outer cell contained 7 cc of solvent—either digitonin or bile salts solution—also buffered to pH 9. When the disks were to be calibrated after the diffusion had reached equilibrium, NaCl was added at the beginning to both the visual purple and the outside solutions to make a concentration of 2 M; then when the diffusion rate had become constant, we determined the passage of the NaCl into an outer digitonin solution without NaCl. The entire apparatus was immersed in a water bath at 6° C. in a dark cold-room kept at the same temperature. The water bath was carefully mounted on rubber and then on a heavy concrete block to avoid undue vibration. The concentration of visual purple was determined with a photoelectric spectrophotometer, using as a measure the difference in photometric density at 500 mμ between the unbleached and subsequently bleached solution. The pH of 9 is to avoid any regeneration after bleaching. The experiments were made during the past year, and were aided by a grant from the Rockefeller Foundation.

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RATE OF MATURATION OF YOUNG RED CELLS IN CANARIES

THE erythrocytes that are present in the peripheral blood of birds may be separated into three types, all of which are nucleated.

(1) Nucleated basophilic erythrocytes are spheroidal in shape, free from hemoglobin and stain only with basic dyes. The nucleus is large and spheroidal, and the chromatin is coarsely reticulated. Only a fraction of one per cent. of these occur in the peripheral blood of canaries.

(2) Nucleated amphophilic erythrocytes are spheroidal or elongate in shape, contain a small amount of hemoglobin and stain with either acid or basic dyes. The nucleus is large and oval in shape and the chromatin is present in the form of irregularly shaped clumps of various sizes; such nuclei are called polychromaphilic. Usually from one to 6 per cent. of these occur in the peripheral blood of canaries.

(3) Nucleated oxyphilic erythrocytes are spheroidal or elongate in shape, and contain hemoglobiniferous or oxyphilic cytoplasm. The nucleus is small, being

[7] S. Hecht, A. M. Chase, S. Shlaer and C. Haig, SCIENCE, 84: 331, 1936.

only about half as large as the nuclei in (1) and (2), and the chromatin is compact.

When stained by the Giemsa method, the cytoplasm of (1) and (2) is light blue or gray and that of (3) is light yellow or orange; the nucleus of (1) and (2) is a mottled purple, the reticulated chromatin and lumps of chromatin being clearly differentiated; the nucleus of (3) is a more or less uniform or homogeneous purple. Various stages intermediate between (1) and (2) and (2) and (3) occur.

Types (1) and (2) are considered to be "young red cells" and type (3) "old red cells." These young and old red cells are very easily distinguished from one another after being stained by the Giemsa method.

Several investigators have called attention to the fact that when the mature schizonts in the asexual cycle of certain species of malaria parasites of both man and birds undergo segmentation, the merozoites attack young red cells more readily than they do old red cells. The approximate rate of maturation of young red cells after they are liberated into the peripheral blood of the canary can be determined by observing the comparative age of the red cell and of the growing malaria parasite within it. *Plasmodium cathemerium* was used for our determinations. The merozoites of this species enter young red cells and the schizonts into which they develop grow to maturity in 24 hours; then they segment, giving rise to a new litter of usually from 12 to 20 merozoites. Synchronicity in the segmentation of *P. cathemerium* is pronounced; most of the schizonts segment in the early evening hours. At 10 P.M. most of the recently produced merozoites are present in young red cells. A few hours later the trophozoites have become obviously larger and the red cells have changed from stages (1) or (2) in the direction of stage (3). By the end of 21 hours, that is, at 7 P.M. on the succeeding day, only mature schizonts are present, and these are all in mature red cells. The conclusion reached is that the young parasitized red cells become mature within a period of 21 hours. The percentages of merozoites and mature parasites in young and old red cells obtained from a study of three birds are given in Table 1.

TABLE 1

Bird	Time	Merozoites in young cells	Time (21 hours later)	Mature trophozoites in old cells
1	10 P.M.	88.4 per cent.	7 P.M.	100 per cent.
2	"	75.3 " "	"	100 " "
3	"	84.6 " "	"	100 " "

The rate of maturation of these young red cells may have been accelerated or retarded because of the presence of the malaria parasites, hence the following *in vitro* experiments were carried out. Blood was drawn from canaries and kept at room temperature. Counts of young and old red cells were made at the time the blood was drawn and again at the end of 24 hours. The percentages of young cells present are given in Table 2.

TABLE 2

Bird	Young red cells in fresh blood	Young red cells after 24 hours
1	6 per cent.	0.4 per cent.
2	12.3 " "	0.2 " "
3	33.4 " "	0.2 " "

Birds 2 and 3 had been treated with phenylhydrazine in order to bring about an increase in the number of young red cells.

These *in vitro* experiments indicate that in drawn blood at room temperature practically all the young cells became mature within 24 hours. Thus the period required for the maturation of the young red cells in the peripheral blood of canaries appears to be less than 24 hours both in parasitized and in non-parasitized cells.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

EMBRYONIC SERIES IN SNAKES

DATA on Ophidian embryology are incomplete largely because of the difficulties involved in acquiring a satisfactory series of stages. Sacrifice of a gravid female yields an abundance of material of one stage, but to obtain a continuous record of development by this method would be almost impossible for rare or secretive snakes and both laborious and wasteful for common snakes.

To obviate this waste, Professor Peter Okkelberg, of the department of zoology of the University of Michigan, suggested that successive stages in development might presumably be obtained by a series of "Caesarean" operations on a single female. Such operations were begun in the summer of 1935 and proved highly successful.

Nembutal (sodium pentabarbital) is employed as the anesthetic. The strength most generally applicable is 0.5 per cent. in physiological saline solution. About two to three cc of this solution are injected intraperitoneally approximately at the middle of the body. Exact dosage is not important. Small snakes, such as *Diadophis* and *Tropidoclonion*, require proportionately less of the anesthetic (0.1 cc per 10 grams of