We believe it is more likely that testosterone results in an increased production of erythropoietin and that this is the material responsible for the erythropoietic stimulating properties of normal plasma 4 days post-testosterone. Testosterone is known to stimulate kidney growth[9], and since the kidney has been implicated in the production of erythropoietin, perhaps testosterone may initiate erythropoietin production. Indeed, if erythropoietin is the physiological regulator of erythropoiesis, it would not be surprising for any substance exerting an erythropoietic effect to do so by initiating erythropoietin formation. Cobalt is known to act in this manner[10], and we suggest that testosterone also does so, although the fundamental mechanisms by which cobalt and testosterone stimulate elaboration of erythropoietin may be quite different.

WALTER FRIED*
CLIFFORD W. GURNEY

Argonne Cancer Research Hospital†, and the
Department of Medicine,
University of Chicago.
Chicago.

* Postdoctoral Public Health Service Fellow, U.S. National Institute of Arthritis and Metabolic Diseases, *F2–AM–20*, 919–01*A*1.
† Operated by the University of Chicago for the U.S. Atomic Energy Commission.

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Visual Pigments in a Fish exposed to Different Light-Environments

THE poeciliid *Belonesox belizanus* Kner has a mixture of two visual pigments in its retina. One of these is based on vitamin A_1 (λ_{max} = 498 mμ), the other on vitamin A_2 (λ_{max} = 521 mμ)[1,2]. Like the cyprinids *Scardinius*[3] and *Notemigonus*[2] the proportions of these pigments in the retina vary throughout the year, there being more of the vitamin A_2-based pigment in the winter and less in summer[3]. As in *Notemigonus*[4], there is considerable variation of retinal visual pigment composition among individuals of *Belonesox* caught at the same time and in the same place (Bridges, C. D. B., unpublished observations).

This communication describes the results of an experiment in which batches of this species were kept in the laboratory under different conditions of illumination, ranging from complete darkness to an artificial day-length of 12 h. In this way the influence of light on the mean pigment composition of a population and on the variation among its individual members was investigated.

A large number of *Belonesox* was seined in the usual location[2]. Eight of these fish were dark-adapted overnight and eight individual retinal extracts were prepared and analysed (see refs. 2 and 4 for methods). Meanwhile, the remaining fish had been divided into three groups, each group being placed in its own 50-gallon tank (containing 'natural' water, salinity approximately 10 parts per thousand). Two tanks were glass-sided and located in light-tight cubicles each illuminated by a 100-W tungsten filament lamp (illumination at water surface = 80 ft. candles). One lamp was automatically switched to give a 24 h cycle of 6 h light and 18 h darkness, the other to give 12 h light and 12 h darkness. The remaining fibreglass-treated wooden tank was kept in a darkroom. At the end of 1 month extracts from 8 individuals in each group and in a fresh group of fish from the natural habitat were prepared and analysed. Fig. 1 summarizes the analysis of all 40 extracts from these 5 batches.

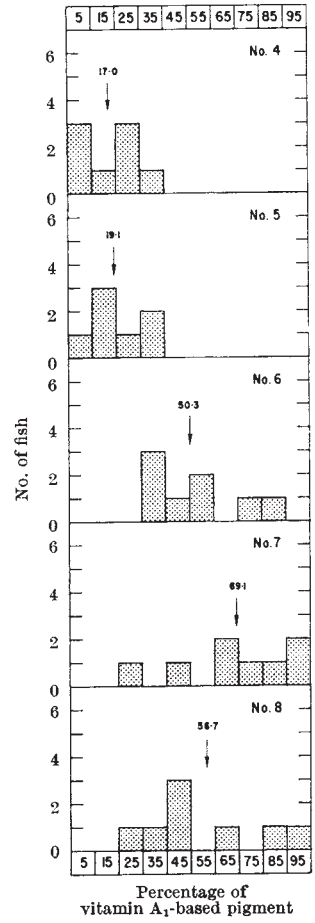

Fig. 1. Visual pigment composition of individual retinal extracts from *Belonesox* kept under different conditions (the figures along the abscissa represent the centre of each interval, that is, 65 includes all fish with vitamin A_1-based pigment percentages between 60 and 70). No. 4 (Feb. 24, 1964), sample of initial batch; No. 5 (Mar. 23, 1964), 1 month darkness; No. 6 (Mar. 24, 1964), 1 month 6-h day; No. 7 (Mar. 25, 1964), 1 month 12-h day; No. 8 (Mar. 31, 1964), sample of a fresh batch of fish from the same habitat as before

One result is immediately obvious: there is no less variation among fish in any one group after they have lived for 1 month under identical conditions, whether of light or darkness; this supports the view implicitly expressed by Bridges[4] that individual variation in paired-pigment species is not attributable to local differences of environment. In actual fact, as may be seen in Fig. 1, in the light tanks a significant increase in individual variation had occurred, paralleling that in the natural habitat over the same period (group 8, Fig. 1). Thus in the '12 h' day group the variance was five times that of the 'dark' group.

Turning now to the mean pigment composition of the various batches examined, it is clear that the fish kept in the light tanks had, on the average, higher proportions of vitamin A_1-based pigment in their retinae than the initial ones or those kept in total darkness. Moreover, this switch from vitamin A_2 pigment to vitamin A_1 pigment had apparently progressed further in the '12 h' fish than in the '6 h' ones. The 'dark' group, on the other hand, was nearly identical in variance and in mean composition with the initial group. At first sight this is surprising, for observations on bulked extracts from *Scardinius*[3] reveal that this species increases its retinal vitamin A_2 pigment in darkness. In the present case, however, the fish were collected at the time of their winter vitamin A_2 maximum (February 1964; ref. 1, Fig. 1) and may have reached their equilibrium positions.

In spite of varying compositions, the average yield of pigment per fish in each group was virtually constant, viz. in terms of ΔD_{max} for a 1 cm optical path it was 0·2235 initially, 0·2242 for the dark fish, 0·2037 for the 6-h fish and 0·2141 for the 12-h fish. This suggests that whatever changes were occurring, they involve the direct conversion of one pigment into the other (cf. ref. 3).

Seasonal changes in the paired-pigment species *Scardinius* have been tentatively ascribed to a kind of 'photoperiodic' effect, where long days would bring about conversion of vitamin A_2- to vitamin A_1-based pigment and short days would reverse the process[3]. The findings recorded here, however, cannot be accounted for in this simple way. Thus, although *Belonesox* has its lowest proportion of vitamin A_2 pigment in summer when the day-length is 13·75 h and the highest in winter when the day-length is 10·5 h, the change observed when winter fish were reduced to an artificial 6 h day was in the 'summer' direction. Although it is difficult to compare laboratory conditions with those of the natural habitat, it is likely that the light-tank fish experienced much higher light exposure, for the tanks were glass-sided and contained filter-clear water. A probable explanation of these preliminary findings is that light-quantity (as determined by light intensity as well as exposure time) is the factor influencing pigment changes in this species.

This work was supported by the U.S. National Science Foundation (grant *GB* 186) and the U.S. National Council to Combat Blindness (grants *G*-268 and *G*-268(*C*2)).

C. D. B. Bridges*

Bascom Palmer Eye Institute,
Department of Ophthalmology,
University of Miami School of Medicine,
Miami, Florida.

* Present address: Vision Research Unit (Medical Research Council), Institute of Ophthalmology, Judd St., London, W.C.1.

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PATHOLOGY

Significance of Electrochemical Phenomena in Intravascular Thrombosis

It appears increasingly certain that vascular interfacial phenomena are critically involved in the development of intravascular thrombosis. However, comments on the role of the vessel wall in the maintenance of vascular homoeostasis are conspicuously absent in discussions of the subject. In a recent article by Macfarlane[1] it was suggested that foreign surfaces activate Factor XII, triggering the cascade of reactions involved in 'physiologic clotting'. A series of experiments in this laboratory, including measurement of transmural ion fluxes[2], absorption and concentration of various important anions and cations by vessel wall cells and fibres[3,4], and measurement of the zeta potential in the electric double layer at the blood-intimal interface as shown by both electro-osmosis[5] and streaming potential[6] experiments, indicate that charge on the vessel wall is important in vascular homoeostasis.

A number of correlated findings emphasize the relationships involved. Among others, Zucker[7] has pointed out that the clot which closes a hole in a cut blood vessel is composed almost entirely of agglutinated blood platelets. We suggest that this finding can be attributed to local electric fields due, at least in part, to the presence of oriented ionic charges on the vessel wall. An additional confirmatory experiment from our laboratory indicates that human and canine erythrocytes, leucocytes, and platelets have a determinable precipitation potential of 0·4 ± 0·1 V on platinum, with respect to the normal hydrogen electrode[8,9] (Figs. 1, 2, and 3). These observations suggest that the initiation of intravascular thrombosis is primarily biophysical.

Katchalsky[10], Bangham[11], and Surgenor[12] have indicated that charged biocolloids act as highly polarizable particles because of their surrounding electrical atmosphere, and that the thrombotic potential of many of the phospholipids and lipoproteins is related to their electrophoretic mobility. A charged surface such as the blood vessel wall will, therefore, interact with these particles. In addition, perpendicular orientation of cells on attachment to the wall is predicted by static electrical theory. This has actually been observed in cinemicroscopic pictures of induced mural thrombus formation produced by small electric fields[13]. Precipitation of blood cells on the platinum electrode in electrochemical precipitation experiments displays the same phenomenon[8,9].

To test this new concept of the mechanism of intravascular thrombosis, solid vascular prostheses were constructed of various metallic elements. These metals exhibited markedly differing rates of thrombosis when used to replace aortic or vena-caval segments, depending primarily on their position in the electro-motive series[14].

We conclude that surface charge of the blood vessel wall, as elucidated by findings among a substantial group of investigators, must be considered a major homoeostatic mechanism, normally preventing abnormal thrombosis. Conversely, it may also be a source of vascular thrombosis in the event of injury, as well as in the case of metabolic

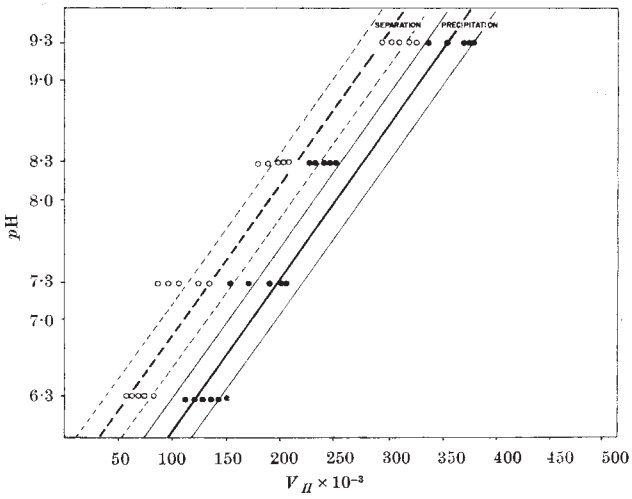

Fig. 1. Human erythrocyte precipitation and separation potentials (V_H) versus pH change. Human blood in ACD-solution

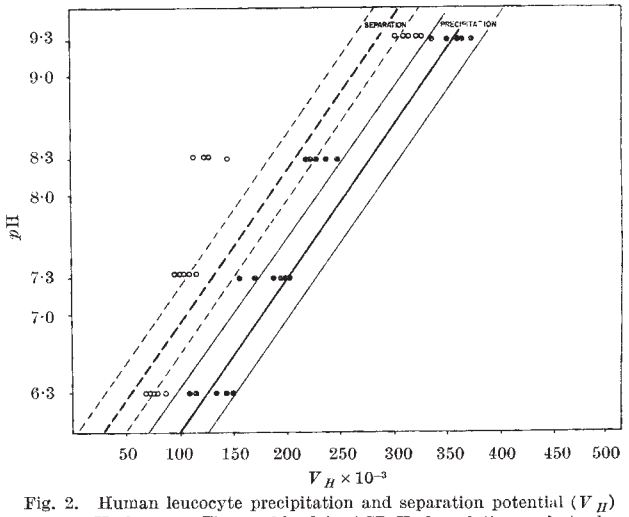

Fig. 2. Human leucocyte precipitation and separation potential (V_H) versus pH change. Human blood in ACD-Krebs; platinum electrode