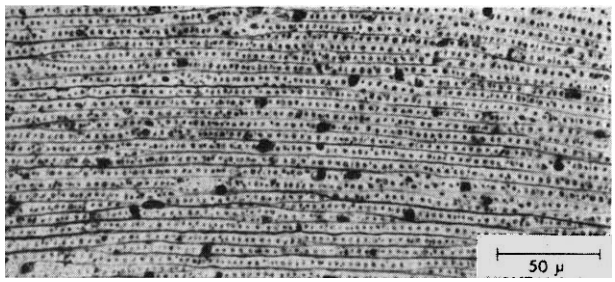

Fig. 5. Tangential section through the molecular layer of the intermediate lobe of the valvula. The transversely cut Purkinje dendrites are separated by bundles of parallel fibres.

distinctly impregnated. In this region bundles of parallel fibres alternate with the rows of Purkinje dendrites (Fig. 5). (The granular cells, the axons of which give rise to the parallel fibres, resemble in every respect those of other teleosts[2,8].)

In the lateral lobes of the valvula the parallel fibres pass directly from the granular layer into the molecular zone, and pass straight to the top of the ridges (Fig. 2). There is no bifurcation. The dendritic trees of the Purkinje cells are oriented perpendicular to the direction of the parallel fibres. It is noteworthy that the cerebellum of birds and mammals shows a transversely oriented folding, whereas the valvula of Mormyrids, judging from the orientation of its parallel fibres and Purkinje dendrites, is folded sagittally. Also remarkable is the fact that the most basal parts of the two cell layers of each valvular ridge contain a row of large cells, the perikarya of which are enveloped by a conspicuous axonal plexus. Two of these elements are visible in Fig. 2. Golgi preparations have shown that the dendrites of these cells extend into the molecular layer but are both thinner and more widely spaced than those of Purkinje cells. The destination of their axons has not yet been determined.

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Received April 21; revised May 23, 1967.

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Monolayer Films of Retinal$_1$ and the Effects of Light on Them

SEVERAL stereoisomers of retinal$_1$ (vitamin A_1 aldehyde) are known and their interconversions can easily be promoted by the action of light[1]. The photoconversion of 11-cis to all-trans retinal$_1$ takes place with the highest efficiency[2,3] and is involved in the first step of the visual process[4]. Studies of vitamin A and its derivatives have suggested that their surface-active properties are closely related to their physiological functions[5,6]. The purpose of the present investigation was to study the properties of monolayer films of all-trans and 11-cis retinal$_1$ at air–water interfaces, and to determine the effect of light on the films.

Vitamin A oil (15×10^5 unit) was obtained from Riken Vitamin Oil Co., Ltd. All-trans and 11-cis retinal$_1$ were prepared by a modification of Wald's method[7] and recrystallized three times from light petroleum ether. The spreading solutions were prepared by dissolving 3 mg of the respective retinal$_1$ in 10 ml. of ethanol. The solutions were spread on a clean surface of water by means of a micrometer syringe. Surface pressures were measured for various film areas at room temperature using the hanging plate method.

To minimize oxidation of retinal$_1$, the measurements for every force-area curve were completed within 20 min of spreading the solution. For the radiation experiments a 500-W iodine lamp, placed 45 cm from the surface, was used. The light passed through a 10-cm thick water cell to avoid heating the films. Surface pressures were measured before and after irradiation, and the area of the film was held constant at $15 \times 14{\cdot}5$ cm^2. All experimental procedures were carried out under a dim red light.

Fig. 1 shows force-area curves of all-trans and 11-cis retinal$_1$. Both isomers give liquid expanded films, that of the 11-cis isomer being more expanded than that of the all-trans. The collapse pressures and limiting areas of each molecule of the isomers are shown in Fig. 1. The 11-cis isomer has a larger limiting area and lower collapse pressure than the all-trans one.

Retinal$_1$ has a bulky lipophilic β-ionone ring attached to a side chain with four conjugated double bonds and terminating in a hydrophilic aldehyde group. The side chain of the all-trans isomer is considered to be flat and straight, while that of the 11-cis one is bent and twisted at the central carbon atom[1]. The relatively straight and flat form of the all-trans isomer allows a closer and more ordered packing on the water surface than with the cis-isomer. The intermolecular forces, which make the film able to withstand the increasing lateral compression, would therefore be stronger than in the other case.

When all-trans and 11-cis films were irradiated, changes were observed in the surface pressures required to keep the film area constant. The changes were maximal after irradiation for 30 sec. In Fig. 2 the changes (percentage increase or decrease) in the surface pressure of films after irradiation for 30 sec are plotted against the initial surface pressure. The surface pressure of the film of all-trans retinal$_1$ increases in the region below 9 dynes/cm, changes little between 9 and 12 dynes/cm and decreases in the region above 12 dynes/cm. The increase of the pressure on irradiation may be explained by supposing that any other isomers formed will tend to expand the film, or

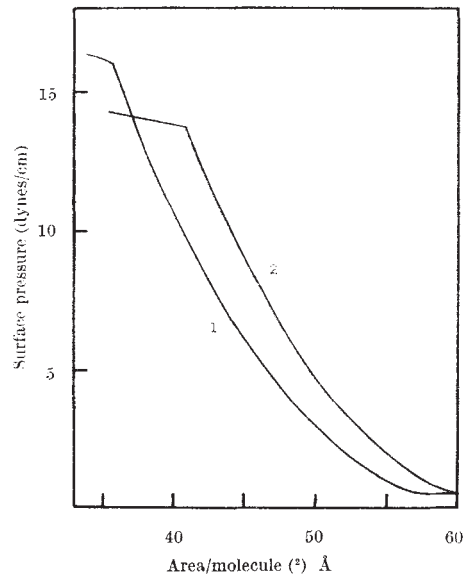

Fig. 1. Force-area curves of all-trans and 11-cis retinal$_1$. Curve 1, all-trans retinal$_1$; curve 2, 11-cis retinal$_1$. The curves show that all-trans and 11-cis retinal$_1$ have collapse pressures of 16·1 and 13·8 dynes respectively, and limiting areas of 35·7 and 41 Å^2/molecule respectively.

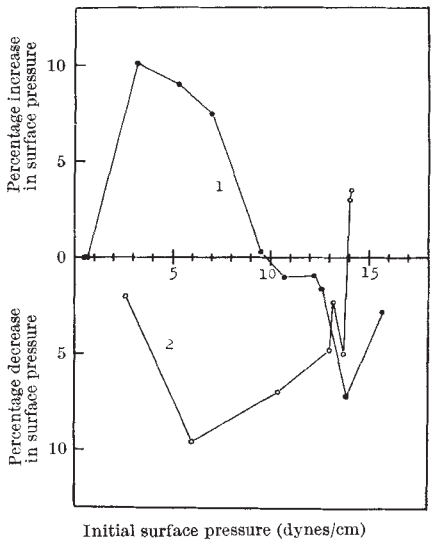

Fig. 2. The changes (percentage increase or decrease) in the surface pressure of films of retinal$_1$ after irradiation for 30 sec. Curve 1, all-trans retinal$_1$; curve 2, 11-cis retinal$_1$.

because the area of the film is kept constant, to increase the surface pressure. The decrease in pressures at regions above our initial pressure of 12 dynes/cm could be caused by the squeezing of some part of the retinal$_1$ out of the film. Conversely, irradiation of the films of 11-cis retinal$_1$ results in decrease of surface pressure. This similarly may be explained by supposing that the 11-cis isomer is photoisomerized to the lower pressure all-trans one. These properties of retinal$_1$ films contrast with those of other vitamin *A* derivatives (our unpublished results).

The change of molecular packing in the film may also be expected to occur in the native physiological membrane when conformational change of the retinal$_1$ molecule occurs. The molecular packing of rhodopsin in rod outer segments is not well known and the orientation of 11-cis retinal$_1$ in the membrane is still more obscure. It is probable, however, that the retinal$_1$ moiety of the rhodopsin molecule exists in the lipid-rich part of the disk-like membrane in the rod outer segment, because retinal$_1$ is itself a member of the lipid family. Photoisomerization of the retinal$_1$ would affect the permeability of the membrane, or at least the conformation of the rhodopsin in that membrane[8–10]. Further experiments are in progress along this line.

We thank all members of the Photobiology Group of Honjo Laboratory of the Department of Biology for criticism and encouragement.

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Received March 21, 1967.

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Pituitary–Adrenal Regulation of Ceruloplasmin

INVESTIGATIONS of the effect of sex hormones on copper metabolism in man and animals have shown a sharp rise in ceruloplasmin after hormone injection[1]; however, the mechanism of this elevation has not previously been discussed. Hormonal control of the synthesis of RNA has been demonstrated (reviewed by Davidson[2]), and other investigations have shown that both testosterone and oestradiol can bind to DNA and stimulate RNA synthesis[3]. Ceruloplasmin is a protein and as such depends on RNA synthesis for its production. Molecules which act as inhibitors or stimulators of RNA synthesis could therefore control ceruloplasmin production.

In this laboratory, studies of the effect of copper metabolism on the rat have shown that hypophysectomy and bilateral adrenalectomy cause a significant rise in concentrations of ceruloplasmin. In contrast, ACTH administered to intact animals caused a significant decrease in the concentration of ceruloplasmin, as did corticosterone given to adrenalectomized rats. Correlating these findings with recent reports on factors regulating RNA, we have attempted to discover a mechanism to explain the influence of hormone on ceruloplasmin.

Hypophysectomized rats (Hormone Assay) were purchased at 30 days of age and raised to maturity on 'Metrecal', 'Purina' laboratory chow, wheat and water. The remainder of the animals were adult Wistar strain rats, fed 'Purina' laboratory chow, wheat and water. Bilateral adrenalectomy was performed by the trans-abdominal approach via midline incision. After surgery, the animals were continued on the normal diet supplemented with 5 per cent dextrose in saline, and experiments were begun two weeks later.

Blood was drawn by cardiac puncture into a heparinized syringe after the animals had been lightly anaesthetized with sodium pentobarbital (2·5 mg/100 g). Plasma ceruloplasmin concentrations were determined by the method of Houchin[4], which measures the oxidase activity of the enzyme. Plasma blanks were used with each determination. Oxidase activity was converted to mg/100 ml. ceruloplasmin by multiplying optical density times 157, the constant we have determined for the rat.

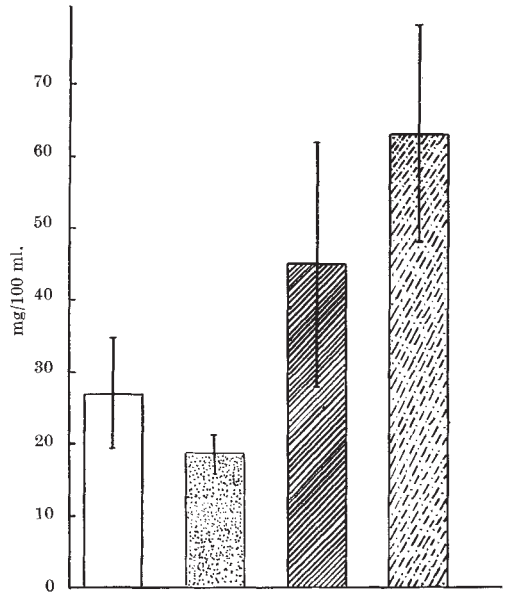

Fig. 1. Concentration of ceruloplasmin for the four groups studied. There were twelve animals in each group. The bar represents the mean and the centre line indicates standard deviation.