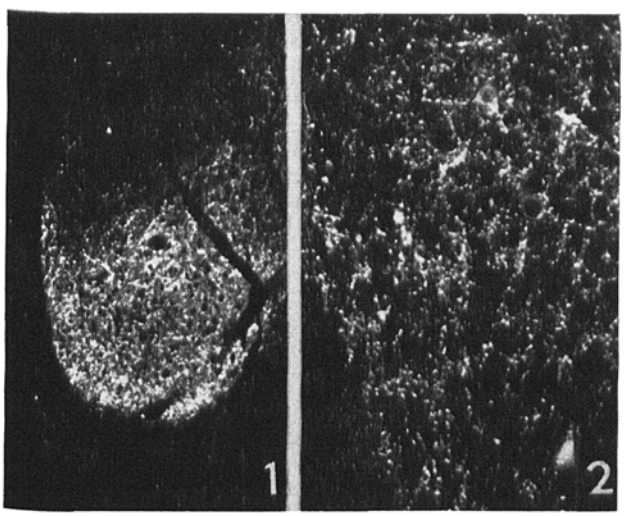
fine and had abundant varicosities, which showed an intense green or yellow fluorescence. Histochemically and pharmacologically, three types of terminals could be distinguished containing very high concentrations of – in all probability – dopamine, noradrenaline and 5-hydroxytryptamine respectively.

Noradrenaline and dopamine terminals. In agreement with the chemical determinations[4], most of the green fluorescent terminals were found to be of the noradrenaline type, which have a wide-spread distribution, i.a. in the entire reticular formation. More or less dense accumulations of such terminals exist in several nuclei of the lower brain stem (e.g. nucleus of Edinger-Westphal, motor nucleus of the trigeminal nerve). The nucleus tractus solitarius and the dorsal motor nucleus of vagus have a very high accumulation of green fluorescent terminals (Figures 1 and 2) but a large proportion of them seem to contain dopamine. Other nuclei of the cranial nerves receive a much sparser innervation (Nucleus n. VII, XII acusticus, and nucleus ambiguus) or none at all (Nucleus n. III, IV, VI and vestibularis).



The cranial part of the nucleus tractus solitarius (1) and the dorsal motor nucleus of the vagus (2) in the rat. A high accumulation of fine, varicose, green fluorescent fibres is present. Fluorescence microphotograph: ×75 and ×190.

5-Hydroxytryptamine terminals. These terminals were more difficult to observe. They are mostly very fine, and their yellow fluorescence disappears rapidly on irradiation with UV-light[3]. In contrast to that of the catecholamine terminals, the fluorescence of the yellow terminals reappears in reserpinized animals after nialamide treatment[3]. Many terminals of this type were found together with catecholamine terminals in the lower brain stem, e.g. in the motor nuclei of the vagus and trigeminal nerves and the nucleus tractus solitarius. A more dense accumulation of terminals, almost exclusively of this type, was detected in an area approximately corresponding to nucleus intercalatus. Some of the nuclei of the cranial nerves, such as nucleus cochlearis, Edinger-Westphal and ambiguus, which receive catecholamine terminals, seem to lack terminals of the 5-hydroxytryptamine type.

Several groups of nerve cells with low concentrations of either a primary catecholamine or 5-hydroxytryptamine in their cell bodies and processes were found in the lower brain stem. Of particular interest is the finding that a large group of nerve cells which seem to contain dopamine is present in the area of substantia nigra. This area, which is connected to the caudate nucleus, has been found to have a fairly high concentration of dopamine[4]. Since nerve terminals containing very high concentrations of dopamine have been identified in the caudate nucleus[6], it seems possible that the nerve cells detected in the substantia nigra area are dopaminergic neurons that send their axons to the caudate nucleus.

Zusammenfassung. Histochemische und pharmakologische Experimente sprechen stark dafür, dass Dopamin, Noradrenalin und 5-Hydroxitryptamin im Hirnstamm der Ratte in drei Typen von Nervenzellen und Endsynapsen gespeichert werden.

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Department of Histology, Karolinska Institutet, Stockholm (Sweden), February 14, 1964.

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[7] This work has been supported by research grants from the United States Public Health Service (NB 02854-04), the Knut and Alice Wallenberg Foundation, and the Swedish Medical Research Council.

The Importance of Sulfur and Iron in the Retina as Determined by Paramagnetic Resonance Studies

Several mechanisms have been suggested for the mode of action of light transfer in the retina[1]. The complete mechanism, however, is still unknown. Intermolecular energy transfer in the retina is considered to be the most probable process for nervous excitation in the rods and cones. It is the purpose of this communication to discuss another possible concept and to give some of the evidence which supports it.

Recently we have reported[2] the presence of sulfur in many biological systems as determined by means of electron paramagnetic resonance (EPR) studies. The importance of sulfur for charge transfer was pointed out.

A sulfur signal (characterized by a delocalization of odd electrons by the sulfur-containing group) was also found to be present in retinae which were obtained from deceased human beings within hours after death occurred. After air drying, the EPR spectrum of samples of the retinal tissue (a few mg) was measured at 295° K as well as at 77° K. Experimental details are given elsewhere[2].

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The EPR spectrum of retina is shown in Figure 1. Since the sulfur signal was relatively large, its part of the spectrum was recorded under different operational conditions as marked. Arrows indicate which parts of the curve belong together. The spectrum from the retinal tissue contains two clearly defined signals, one of which is the sulfur signal near $g = 2$. The iron signal, at about $g = 5$, is already recognizable in the upper curve but more pronounced in the sample measured at 77° K. The iron signal at this g-value is due to a very strong asymmetry of the crystal field surrounding the iron, which is caused by occupying the different iron axis with one of each H_2O and N atom[3].

From these results it might be concluded that sulfur and iron are of importance in the process of photoreception. This is not surprising since it is well known that sulfur and another metal, Ag, are important and necessary for the production of a latent image in photographic emulsions. No picture can be obtained with highly purified gelatine (removal of all sulfur). Therefore, one might be tempted to compare the processes in photographic emulsions[4] with those occurring in the eye. Then, the process of photoreception may be divided into several phases: the initial events characterized by the production, motion, and trapping of photoelectrons within the crystal called retina, and the final event, the mechanism of nervous excitation. Initially, electrons of negative ions, such as sulfur (electron donor) are raised to stationary or quasistationary energy levels in the continuum (conductance band of the crystal) by the passage of incident light. These photoelectrons then migrate freely through the retina until they are trapped in places (sensitivity specks) characterized by localized energy levels below those of the conductance band. The sensitivity specks might be the iron ions and/or other electron acceptors which are probably located at the beginning of the nerves. Thus, an impulse is induced in the nerves and is part of the total image.

In the model outlined above, the presence of iron seems to be important. Light absorption studies done by SCHELER[5] demonstrated that protohemin as well as ferrihemoproteins have a maximum absorption at about 500 mμ (rods?, scotopic), while protoporphyrins have a clearly defined absorption peak at 560 mμ (cones?, photopic). The coincidence of these absorption peaks at 500 and 560 mμ with the ones observed in rods and cones, respectively, may show a possible connection between these retinal structural elements and the proteins (iron).

The mechanism of bleaching can also be described by the proposed model. To investigate this effect the magnetic sweeping field was stopped in the peak of the sulfur signal. The retinal sample then was irradiated with bright white light. As can be seen in Figure 2, the sulfur signal *increases*, reaches a plateau and *decreases* again after turning off the light. CAMPBELL and RUSHTON[6] also measured bleaching (open circles, *decrease*) and subsequent regeneration (black dots, *increase*) of rhodopsin in

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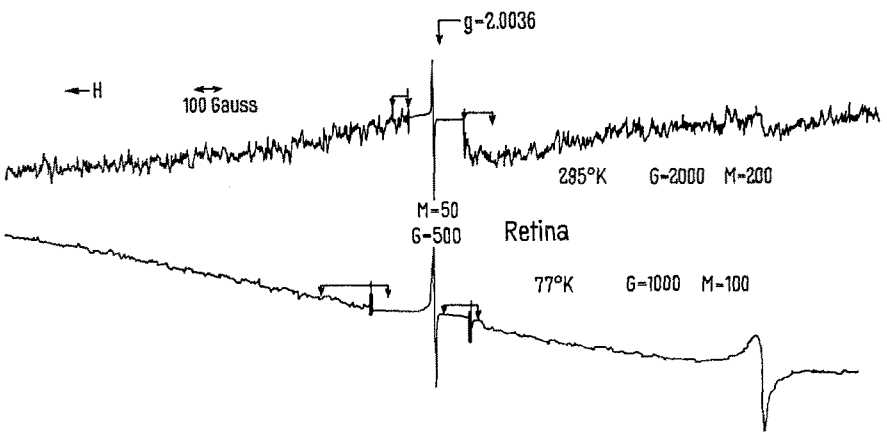

Fig. 1. Electron paramagnetic resonance spectrum of human retina. The curves represent the second derivative of the actual resonance curves.

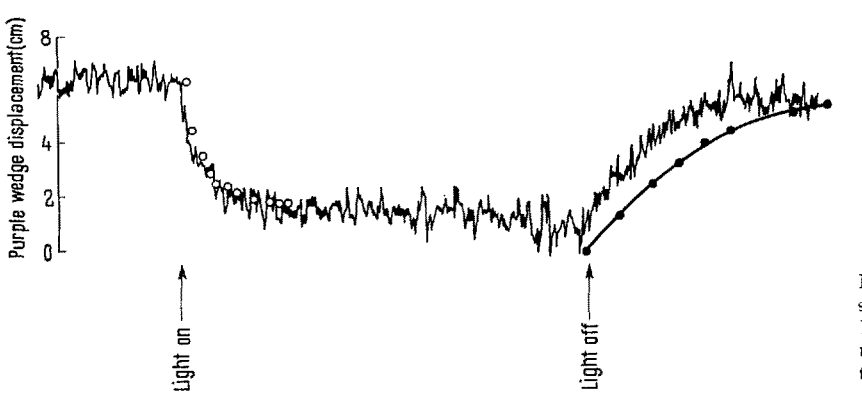

Fig. 2. Time course of bleaching and subsequent regeneration of the sulfur signal. Results of purple wedge displacement measurements[6] (○, ●) are superimposed. Note that both results are actually inverted.

the human eye. Their results, obtained by a method using purple wedge displacements, showed an inverted effect to the EPR results but were, nevertheless, plotted in the same direction. The results of both measurements agree well, except for the opposite direction of the changes. The EPR data suggest that more odd electrons are produced or released from the S^{2-}; that is, the electron donor intensity is reduced. This not only supports the proposed charge transfer hypothesis but is also in contrast to the previously accepted mechanism of bleaching[1]. It was usually explained as hydrolyzing of the visual pigment, rhodopsin, into retinene and opsin. This bleaching process requires the presence of water.

We investigated, therefore, the influence of the relative humidity on the sulfur signal. After equilibrating retinal samples in atmospheres of constant humidity by storage for 3 weeks over saturated salt solutions[7], their peak heights (p_h) were measured. The peak heights of the control values (p_c) were measured at 18% relative humidity.

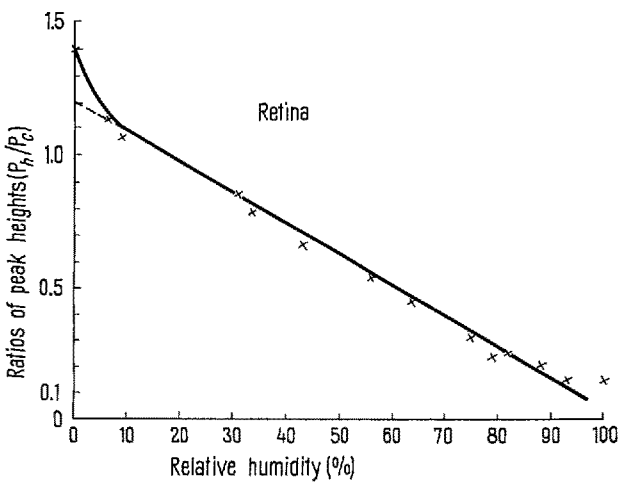

Fig. 3. Influence of relative humidity on the peak height of the sulfur signal.

The ratios of these peak heights as function of the relative humidity are plotted in Figure 3. The greatest signal is obtained from completely dry material, hence, water and light have opposite effects on the sulfur signal. Therefore, it seems doubtful that water is involved in the actual bleaching process (decrease in electron donor intensity). Its influence seems to be on the electron acceptor site (energy sink). Together with oxygen it might be responsible for restoring the higher positive charge of iron, changed by light absorption, according to

$$2Fe^{2+} + {}^1/_2\, O_2 + H_2O \rightarrow 2Fe^{3+} + 2OH^-$$

ALBOUY and FARAGGI[8] and LOHMANN[9] proposed this reaction to explain the fading process occurring in photographic emulsions. The sensitivity of the retina to anoxia[10] might be explained by this reaction as well. The elevation of visual threshold (or % reduction of rhodopsin[11]) might be based on the change in valence of iron under the influence of oxygen and water[12].

Zusammenfassung. Eine neue Theorie über Photorezeption wird vorgeschlagen. Mittels EPR-Untersuchungen wird gezeigt, dass die in der Retina vorkommenden Elemente Schwefel und Eisen eine wichtige Rolle im Photorezeptionsprozess spielen. Für die Nervenerregung scheinen Ladungsleitungen und nicht intermolekulare Energieleitungen verantwortlich zu sein.

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Departments of Physiology and Radiology, University of Arkansas Medical Center, and Southern Research Support Center, Little Rock (Arkansas, USA), February 27, 1964.

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[12] I thank Dr. R. D. HOKE for kindly supplying us with the eye material and L. MARTIN for his technical assistance.

Influence de la température de l'air ambiant sur quelques activités métaboliques d'animaux hétérothermes

On sait bien que la température exerce *in vitro* une action d'importance fondamentale sur toutes les réactions chimiques et sur les activités enzymatiques en particulier[1]. Cependant, dans les organismes vivants et spécialement dans les animaux à sang chaud qui ont été ceux qu'on a le plus amplement étudiés, il y a simultanément et ordonnément d'innombrables réactions enzymatiques dont le parfait équilibre assure la réalisation normale des fonctions vitales. Tout procès enzymatique ne fonctionne donc pas au maximum de ses possibilités, mais de façon à s'intégrer avec les activités à lui liées; pourtant la rapidité et l'intensité de chaque réaction peuvent être stimulées ou ralenties selon les exigences. Cet étonnant equilibre de fonctions se vérifie de façon optima à la température physiologique qui est, en gros, entre 36°–39°C pour les animaux à sang chaud.

Les changements de la température corporelle ont donc une profonde influence sur la vie à travers une action sur les réactions enzymatiques et sur les structures qui conditionnent ces réactions mêmes. On a obtenu des résultats très interessants en étudiant les changements biochimiques d'animaux à sang chaud qui ont été rendus artificiellement hyperthermiques[2] ou hypothermiques[3–5].

Sur la base de ces considérations, il nous a semblé utile d'étudier les effets des changements thermiques *in vivo*

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