ing color development with *p*-dimethylaminobenzaldehyde in HCl · acetone as well as with nitrosonaphthol in nitrous acid.

The ability of iproniazid to block the increased excretion of 5-hydroxyindoleacetic acid induced by banana suggests that oxidative deamination of this precursor of the acid occurs. These increases in excretion of 5-hydroxyindoleacetic acid in monkeys and in the two children, induced by banana feeding, are of the order observed for certain adult patients with carcinoid tumors and could lead to erroneous diagnosis.

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28 October 1957

Response of Single Cells in Monkey Lateral Geniculate Nucleus to Monochromatic Light

The lateral geniculate nucleus of the rhesus monkey (and of man) consists of six layers of cells separated by fiber layers. Three of these layers receive impulses from one eye, three from the other eye. Thus the visual fibers from each eye split three ways in the thalamus.

We have been investigating the functional significance of the lamination of the lateral geniculate nucleus and, in addition, the nature of the responses of color-selective cells at the thalamic level. The problems of human color vision—for example, the number of color systems and their spectral sensitivities—have been widely debated. The question of whether the systems operate independently, as Helmholtz maintained, or in opposing, complementary pairs, as suggested first by Hering, has never been satisfactorily decided. By tapping in on the visual messages as they are transmitted to the cortex, it should be possible to answer some of these questions.

Granit and his coworkers (*1*) have recorded from single ganglion cells in the retinae of numerous subprimate vertebrates. While the results of these experiments are very interesting and important, the applicability of the results to problems of human vision is limited by the fact that the primate visual system differs in many respects from that of other vertebrates: the most commonly studied animal, the cat, is, for instance, nocturnal and color-blind. It would seem more appropriate to study the mechanisms of human vision on an animal, such as the rhesus monkey, whose visual system (including color vision) closely approximates that of man.

Five monkeys have been used in this experiment (*2*). The animal, under barbiturate anesthesia, is held in a stereotaxic instrument. A tungsten microelectrode (*3*) with a tip diameter estimated to be from 1 to 3 μ is lowered by a micrometer drive until a single cell of the lateral geniculate nucleus is isolated. The output of the microelectrode, with reference to an indifferent point on the skull, is fed by way of a cathode follower to a conventional amplifying, recording, and monitoring system.

Each of the monkey's eyes is stimulated by one beam of a dual-beam optical system; a beam of light from each side of a ribbon-filament lamp is passed through a shutter and collimated before it is passed through monochromatic interference filters and neutral-density filters. The beams are then focussed on the eyes. This makes it possible to stimulate either eye with any of a number of different wavelengths of variable intensity and duration. Customarily, we adjust the intensity of the light passed by each monochromatic filter so that equal amounts of physical energy are transmitted by each filter. The number of spikes elicited by each monochromatic light stimulus gives a direct indication of the spectral sensitivity of the element under study. The locations of the cells recorded from are estimated from histological analyses of electrode tracks and of coagulation marks made at the end of each experiment.

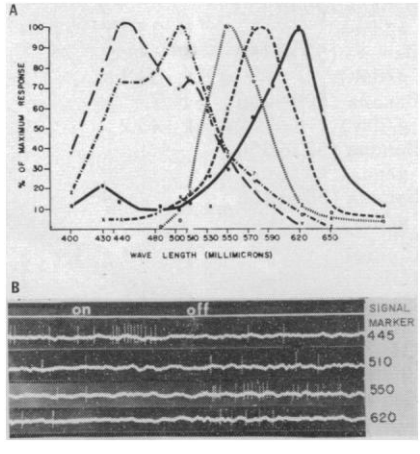

Fig. 1. Responses of single units in the lateral geniculate nucleus. (Top) Response curves for five different types of cells in the dorsal layers. (Bottom) Response of an intermediate-layer cell to four different wavelengths of monochromatic light.

In an earlier experiment (*4*) in which larger electrodes were used, we found that the patterns of responses from the laminae of the lateral geniculate nucleus differed from one another. On-responses were found in the dorsal layers; in the most ventral pair we observed a striking inhibition of spontaneous activity followed by an off-response when the light was turned off. The responses obtained from the two intermediate layers, although more difficult to interpret, appeared to be a combination of the dorsal and ventral types, with on- and off-bursts. Our current single-cell recordings support these earlier findings on different types of responses in the various layers.

Among the cells in the dorsal pair of laminae, we have found some that respond to various narrow ranges of wavelengths in different parts of the spectrum. In our work to date we have found cells with peaks at five different points in the spectrum. In Fig. 1*A* are shown curves of the spectral sensitivity of the different types of elements we have found. These results should be considered preliminary since we have recorded so far from only 66 of the thousands of geniculate cells. The peak at 510 mμ corresponds to the sensitivity of the scotopic system, and presumably it represents mainly a rod connection. The other four presumably correspond to the various color-vision elements. It should be noted that the "blue" cell has a secondary peak at 510 mμ; this is true of all the "blue" cells we have isolated so far and, together with the secondary peak in the blue of the 510 mμ cell, may indicate that "blue" receptors and rods feed into the same neural pathways. In the case of cells with other sensitivities, we have also often found ones with multiple peaks, red and green being a particularly common combination.

Thus there seems to be a complete color-vision system represented in the two dorsal layers of the lateral geniculate nucleus. This is in contradiction to Le Gros Clark's hypothesis (*5*) that each pair of laminae receives impulses from only one of the three classical color receptors, the top layers, for example, containing only "red" cells, the middle "green," and the bottom "blue."

In some ways, the responses recorded from certain cells in the two middle laminae of the lateral geniculate nucleus are even more interesting. Here we have located cells which fire either on- or off-responses, depending on the wavelength of the light. In Fig. 1*B* is a record from one of these cells. It can be seen that the cell gives a large on-response to blue light, little response of any sort to green light, but gives a substantial off-response to greenish-yellow light. We have similarly found "red-on,

green-off" cells. This raises the intriguing possibility that there may well be a second type of color-vision system represented in these layers, one perhaps having to do with such phenomena as afterimages and contrast.

The majority of cells recorded from in this intermediate pair of layers, however, are pure off-cells. We have not had the opportunity to study these layers or the inhibitory ventral layers to the same extent as we have the dorsal layers, but work on them is continuing.

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14 November 1957

Complex Nature of the Step in Immune Hemolysis Involving Third Component of Complement

The immune hemolytic reaction is complex because complement (C′) consists of four recognized components (C'_1, C'_2, C'_3, and C'_4) (*1*). As a result of the elegant studies of Mayer and his coworkers (*2*), the mechanism of immune hemolysis involving guinea pig C′ and sensitized sheep erythrocytes is considered to comprise the following sequence of steps:

$$E + A \rightarrow EA \quad (1)$$

$$EA + C'_1 + C'_4 \xrightarrow{Ca^{++}} EAC'_{1,4} \quad (2)$$

$$EAC'_{1,4} + C'_2 \xrightarrow{Mg^{++}} EAC'_{1,4,2} \quad (3)$$

$$EAC'_{1,4,2} + C'_3 \rightarrow E^* \quad (4)$$

$$E^* \rightarrow \text{inactive product} \quad (4a)$$

$$E^* \rightarrow \text{ghost} + \text{hemoglobin} \quad (5)$$

where EA represents a sensitized cell, $EAC'_{1,4}$ and $EAC'_{1,4,2}$ represent cells in a state resulting from interaction with C'_1 and C'_4, and C'_1, C'_2, and C'_4, respectively, and E* represents an activated cell which lyses in the absence of C′.

In a comparative study of inhibitors in several sera and of the effect of these inhibitors in the step involving C'_3 (reaction 4), it was found that the titration curve for C'_3 depended on the source of C'_3. This titration was carried out by the addition of different volumes of a given serum to a fixed concentration of $EAC'_{1,4,2}$ in the presence of 0.017*M* disodium ethylenediaminetetraacetate (EDTA). Disodium ethylenediaminetetraacetate was added to prevent further reaction of C'_1, C'_2, and C'_4 with the cells (reactions 2 and 3). Typical titrations for C'_3 of human, pig, and guinea pig sera are given in Fig. 1, which shows that pig serum gives the greatest limiting extent of hemolysis, although at low concentrations it is not as effective as guinea pig serum. Human serum shows only a trace of activity in this system. One would expect, irrespective of the concentration of C'_3, that the final extent of hemolysis would depend only on the fraction of cells in the state $EAC'_{1,4,2}$ which require presumably only C'_3 for lysis. Therefore, the titration curves for all sera would be expected to have a common limiting end point.

In an attempt to account for these differences, the role of inhibitors in this step was investigated (*3*). The addition of sheep serum to guinea pig serum produces inhibition at this step, resulting in a decrease both in the initial slope and in the limiting value of the curve. The differences observed at low concentrations in the curves for pig and guinea pig sera (Fig. 1) might therefore be explained by the presence of inhibitors in pig serum (*4, 5*). However, these same inhibitors cannot be invoked to explain the intersection of these curves and the greater extent of hemolysis produced by high concentrations of pig serum.

Pig serum was heated at 56°C for 15 minutes to destroy C'_1 and C'_2 activity and titrated together with normal pig and guinea pig sera. Heating the pig serum had only a slight effect on its reactivity with $EAC'_{1,4,2}$, and the limiting extent of hemolysis obtained remained greater than that obtained with normal guinea pig serum. Evidently C'_1 and C'_2 are not responsible for the increased reactivity of pig serum.

Classical reagents for the titration of the components of C′, namely R_1, R_2, and R_4, which are deficient in C'_1, C'_2, and C'_4, respectively, were prepared from pig serum (*6*) and titrated. The results are shown in Fig. 2. These reagents, which are nonhemolytic when added to equivalent concentrations of sensitized cells, gave final extents of hemolysis greater than that of normal guinea pig serum. This confirms the observation that C'_1 and C'_2 are not involved and indicates that C'_4 is probably not responsible either.

Pig serum was fractionated by column electrophoresis on powdered cellulose. One fraction, which was found among the β-globulins, did not lyse $EAC'_{1,4,2}$ in the presence of EDTA, but it enhanced the activity of guinea pig serum in this system. Pig serum therefore contains a factor which reacts in the presence of EDTA and is responsible for the increased activity of pig C′.

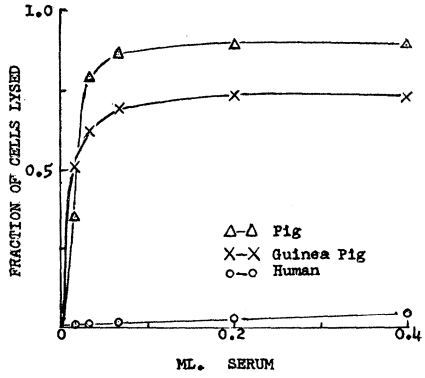

Fig. 1. Lysis of $EAC'_{1,4,2}$ with different sources of C'_3.

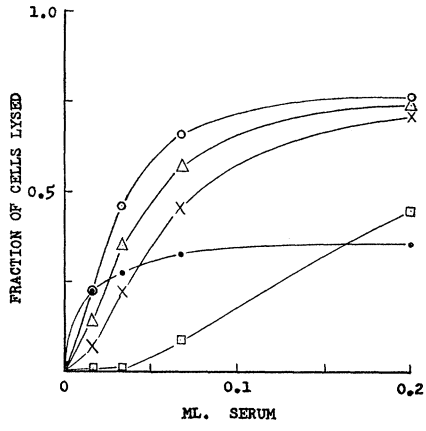

Fig. 2. Lysis of $EAC'_{1,4,2}$ with classical reagents derived from pig serum compared with lysis with guinea pig and pig sera. Open circles, pig serum; triangles, R_4 reagent; crosses, R_1 reagent; squares, R_2 reagent; solid circles, guinea pig serum.

The lysis of $EAC'_{1,4,2}$ appears, therefore, to involve a component, other than C'_3, whose properties do not coincide with those of C'_1, C'_2, or C'_4. This component, necessarily present in guinea pig serum, is not reactive in the presence of 0.017*M* EDTA, while its counterpart in pig serum is active. In this respect it differs from the dual C'_3 factors that Rapp (*7*) found in guinea pig serum, which, when mixed, are active in the presence of EDTA.

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