results cited in Table 1. Thus, it is conceivable that one of the fractions might contain C'_3 in admixture with an inhibitor, while the other fraction might contain an anti-inhibitor. If this were the case, both fractions would be inactive when used alone, but activity would be restored by admixture. Tests with the fractions described in this paper showed that the acid precipitate, after incubation with whole C′ at 37°C for 30 minutes, strongly inhibited the action of the latter on EA, while the methanol precipitate lacked inhibitory action. These results, however, are not relevant to the system under study—that is, the conversion of $EAC'_{1,4,2}$ to E* by C′ in the presence of EDTA.

An appropriate test would involve incubation of whole C′ in EDTA with the fractions alone and in combination followed by tests of these mixtures with $EAC'_{1,4,2}$ as substrate. This was done with an incubation temperature of 37°C and incubation time of 30 minutes, and with a concentration of the acid precipitate which had been found to be strongly inhibitory to whole C′ in the previous test. No anticomplementary action was observed in the modified test with $EAC'_{1,4,2}$ as substrate, and, indeed, in all cases such treatment enhanced the conversion of $EAC'_{1,4,2}$ to E*.

The results of the fractionation experiments permit two alternative interpretations: (i) Each of the fractions contains a different component of complement; these two components act sequentially to transform $EAC'_{1,4,2}$ to E*. (ii) One of the fractions contains a component of complement (presumably C'_3) which converts $EAC'_{1,4,2}$ to E* by two successive reactions, while the other fraction contains a cofactor or activator for this process.

Further experiments are needed to distinguish between these interpretations. With respect to the first interpretation, since either R_1, R_2, or R_4 is able to convert $EAC'_{1,4,2}$ to E*, it would appear that both of the factors involved in this reaction are distinct from C'_1, C'_2, or C'_4. Furthermore, since R_3 does not convert $EAC'_{1,4,2}$ to E*, at least one of these factors is C'_3. Attempts were made to reconstitute R_3 (at a nonlytic level) by addition of the alcohol and acid precipitable fractions, alone and in combination (in the proportions corresponding to the original guinea pig serum). The three mixtures, as well as whole C′, were assayed for their ability to hemolyze sensitized sheep red cells (EA) in the presence of Ca^{++} and Mg^{++}. The results were expressed as the reciprocal of the final dilution required to yield 50-percent lysis, as judged by visual inspection, and are given as follows: R_3 + methanol precipitate, 120; R_3 + acid precipitate, 350; R_3 + mixture, 750; whole C′, 1500.

This experiment indicates that both the methanol precipitate and the acid precipitate contain C'_3, in the sense that they are lytic with R_3. However, the experiment is inconclusive with respect to the possible duality of C'_3, since the titer of the mixture of the two fractions was only moderately higher than the sum of the titers of the fractions alone.

One of the limitations in the use of R_3 arises from the fact that it does not furnish an excess of C'_1, C'_2, and C'_4 and that the purified fractions may contribute one or more of these components, leading to the formation of $EAC'_{1,4,2}$ of different activity in the different mixtures. By contrast, in the experiments described in Table 1, in which the fractions were tested with preformed $EAC'_{1,4,2}$, further formation of $C'_{1,4,2}$ sites on the cells was blocked by the EDTA.

While it is evident that one of the factors is C'_3, it is not clear whether the other one is a second part of C'_3, an activating factor, or a new component of complement. This question requires further study of the nature of R_3. In addition, it is not known whether the two factors act on $EAC'_{1,4,2}$ in a definite sequence. Finally, it will be necessary to reexamine the properties of $EAC'_{1,4,2}$ with the aim of determining whether a population of cells "in the state $EAC'_{1,4,2}$," when prepared as described by Levine, Mayer, and Rapp (*3*), has progressed, at least in part, beyond this stage by interaction with one of the factors. This possibility must be considered since in the preparation of $EAC'_{1,4,2}$ a small proportion of cells is lysed.

In regard to the nature of R_3, it is pertinent that Da Costa Cruz and De Azevedo Penna (*7*), employing conventional C′ reagents, concluded that C'_3 consists of at least two different substances, on the basis of the differential destruction of C'_3 by formaldehyde on the one hand and by sodium hydrosulfite on the other.

There have been quite a few reports presenting evidence of the existence of complement components other than C'_1, C'_2, C'_3, and C'_4 (*8*). Some workers in the field, including the members of this laboratory, have been reluctant to accept the existence of more than four components because of the limitations inherent in the use of serum fractions which are subjected to destructive treatments and which are recombined in order to reconstitute hemolytic activity. The present evidence on a new component or factor of the complement system is not based on the use of reagents prepared by destructive treatment (*9*).

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4. R_1, R_2, R_3, and R_4 are reagents for the detection of the four complement components and are prepared by selective removal or inactivation of either C'_1, or C'_2, or C'_3, or C'_4, respectively, from whole guinea pig serum.
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9. This work was supported by grants from the National Science Foundation and by contract Nonr 248(17) with the Office of Naval Research.
10. It should be noted that C'_3 concentrations are comparable only within a given experiment, since the reactivity of $EAC'_{1,4,2}$, which varies from experiment to experiment, affects the extent of reaction. The reaction mixtures contained 3×10^7 erythrocytes per milliliter and 5.0 ml of the C'_3 dilutions indicated for each experiment in a total volume of 25.0 ml.

24 September 1957

Banana Feeding and Urinary Excretion of 5-Hydroxyindoleacetic Acid

The diagnosis of malignant carcinoid in man can be made by the presence of markedly increased excretion of 5-hydroxyindoleacetic acid (5-HIAA) in the urine. The increased excretion of this acid in the urine of patients with carcinoid tumors is derived from 5-hydroxytryptamine, which is present in very large concentration in the carcinoid tumors (*1*). 5-Hydroxytryptamine is converted to 5-HIAA by the action of aminooxidases (*2*). The range of excretion values for 5-HIAA obtained from carcinoid patients is 21 to 680 mg/day, as compared with 2 to 9 mg/day for adult normal subjects (*3*).

The 5-hydroxyindoles found in the urine of carcinoid patients and of normal man are derived primarily from the metabolism of tryptophan. The excretion in normal man is relatively constant over wide ranges of tryptophan intake. However, addition of very large amounts of tryptophan to the diet of normal man results in increased excretion of 5-HIAA in the urine. A twofold increase in excretion of this acid has been demonstrated following loading with 3.5 g of tryptophan.

To date, the elevated values of

5-HIAA found in the urine of patients with carcinoid tumors have not been observed in other clinical conditions in man. No reports have been found on the influence of dietary constituents other than tryptophan in producing an increase in excretion of 5-HIAA of such a striking nature.

The effect of banana on the excretion of 5-HIAA was accidentally observed. At the conclusion of a study on the excretion of this acid in monkeys maintained on a monkey chow diet, a banana was given on the final day as a reward for satisfactory performance. Surprisingly, a 24-fold increase in the 24-hour excretion of 5-HIAA was found in this final urine sample. This observation led to the studies described in this report.

Twenty-four-hour urine specimens were collected from four rhesus monkeys. The excretion of 5-HIAA was measured by means of the method of Udenfriend, Titus, and Weissbach, which is presumably highly specific for this acid (4). The 5-HIAA excreted during control periods has been compared with that observed following the feeding of banana. Iproniazid (1-isonicotinyl-2-isopropylhydrazide) is capable of blocking the action of monoaminooxidases, both in vivo and in vitro (5). The effect of the administration of iproniazid on the excretion of 5-HIAA was observed in the monkeys during the feeding of a normal monkey chow diet and during the period of addition of banana to the diet. The results are presented in Table 1.

The excretion of the acid during control periods was relatively constant. Feeding of banana produced a prompt rise in the amount of acid excreted. Addition of 50 to 150 grams of banana to the diet resulted in acid excretion of from 5 to 30 times the values obtained for control periods.

The administration of iproniazid (50 mg/kg intraperitoneally twice daily, at 8 A.M. and at 4 P.M.) resulted in a marked reduction in the excretion of the acid. Decreases to 0.1 that observed for the control period occurred by the second day of iproniazid administration. The acid excretion returned to normal values within 48 hours after discontinuation of the iproniazid administration.

Iproniazid was administered to the monkeys following 4 days of banana feeding (150 g of whole banana per day). A prompt decrease in excretion of the acid occurred. This was progressive throughout the 3 days of iproniazid administration. In spite of banana feeding, iproniazid appeared to return the excretion value to control, or low control, values. Three days after discontinuation of both banana and iproniazid, the excretion values had returned to the average for control periods.

A similar effect of banana feeding was observed in one 10-year-old male child with recurrent thrombocytopenic purpura and in one 9-year-old female child with phenylpyruvic oligophrenia. The range of 24-hour urinary excretion values in a child with thrombocytopenic purpura, when maintained on a normal hospital diet, was 1.57 to 2.04 mg for a 3-day period. Feeding 300 g of banana a day resulted in an excretion of 13.3 to 18.0 mg of 5-HIAA per 24 hours.

The child with phenylpyruvic oligophrenia excreted an average of 0.66 ± 0.81 mg/day while she was on a general hospital diet for 6 days. The addition of approximately 300 g of banana daily for the next 3 days resulted in urinary excretion values of 7.2, 5.7, and 4.0 mg/day. The excretion of 5-HIAA in this child when on a low phenylalanine diet for 8 days was 0.93 ± 0.38 mg/day. The addition of approximately 200 g of banana per day to this diet resulted in an average excretion of 5-HIAA of 5.77 ± 2.9 mg/day for a 5-day period. The effect of iproniazid in blocking the increased excretion of 5-HIAA produced by banana feeding which was observed in monkeys was also observed in this child. Large oral doses of iproniazid were required; 5 mg/kg per day for 6 days produced no reduction in the excretion values. However, doses of 25 mg/kg for 2 days reduced the 24-hour excretion value to 0.80 mg/day.

Extraction of fresh banana skin, fresh banana pulp, or whole banana baked at 100°C for 2 hours failed to reveal evidence of nitrosonaphthol reacting materials. However, banana pulp that was autoclaved at pressure of 15 lb/in.^{2} and at 250°F, when fed to two monkeys, produced a striking rise in the amount of 5-HIAA in the urine. The administration of large doses of ascorbic acid, or of neomycin sufficient to produce a relatively sterile intestinal tract, did not influence the excretion of the acid in monkeys on a control monkey chow diet or in monkeys on a diet in which banana was fed.

Urine, obtained from a monkey that had been fed banana, and containing approximately 10 μg of 5-HIAA per milliliter, was extracted by the method of Udenfriend into ether. Volumes containing approximately 12 μg of the acid were placed on No. 1 Whatman paper for descending chromatographic analysis, with 5-HIAA as the reference standard. The solvent consisted of isopropanol, water, and ammonia (200:20:10). Identical spots at the same R_f as the standard 5-HIAA were obtained follow-

Table 1. Effect of banana feeding and administration of 1-isonicotinyl-2-isopropyl hydrazide (IIH) on urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA) in monkeys.

Item	Monkey 510		Monkey 513		Monkey 514		Monkey 405	
	Days	5-HIAA (μg/hr day)	Days	5-HIAA (μg/hr day)	Days	5-HIAA (μg/hr day)	Days	5-HIAA (μg/hr day)
Control	5	1.6 ± 1.0	6	10.4 ± 5.9	6	17.5 ± 2.9	6	10.8 ± 4.7
Banana (50 to 150 g/day)					1	81.8	1	138.9
Banana (50 to 150 g/day)	1	19.0	1	31.8	1	32.3	1	32.0
Banana (50 to 150 g/day)	1	17.1	1	52.4	1	78.3	1	97.5
Banana (50 to 150 g/day)	1	24.1	1	90.7	1	79.8	1	52.4
Banana (50 to 150 g/day)	1	43.2	1	77.6	1	87.1	1	105.8
Banana (50 to 150 g/day)					1	76.9	1	72.5
Banana (50 to 150 g/day)					1	33.1	1	35.6
Control	3	8.1 ± 1.4	3	19.0 ± 11.3	3	19.4 ± 5.1	3	16.9 ± 5.1
IIH	1	7.6	1	5.8	1	10.0		
IIH	1	1.0	1	1.0	1	3.7		
IIH	1	1.2	1	1.9	1	2.1		
IIH					1	4.0		
Control	1	4.0	1	3.3	1	10.9		
Control	1	3.1	1	17.3	1	17.0		
Banana (150 g/day)	4	25.8 ± 11.8	4	63.1 ± 26.2	7	67.1 ± 23.8	7	76.4 ± 39.6
Banana + IIH	1	14.3	1	28.5	1	32.2	1	7.7
Banana + IIH	1	14.1	1	22.1	1	7.2	1	1.0
Banana + IIH	1	3.2	1	4.3	1	4.9	1	1.1
Control	1	3.8	1	8.8	1	1.4	1	7.2
Control	1	4.7	1	3.1	1	45.2	1	21.7
Control	1	11.3	1	13.4	1	14.8	1	12.0
Control					1	9.3		

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.

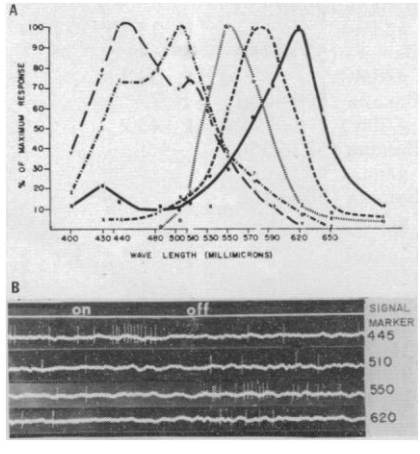

Fig. 1. Responses of single units in the lateral geniculate nucleus. (Top) Response curves for five different types of cells in the dorsal layers. (Bottom) Response of an intermediate-layer cell to four different wavelengths of monochromatic light.

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,