the untreated enzyme does (*2*). In the presence of trypsin and glucose, Type II hexokinase splits into three separate active enzyme forms, none of which migrates with a mobility equal to that of untreated Type II (*2*). The significance of the seven bands observed by Eaton *et al.* thus remains to be established. The possibility that these multiple bands are produced in the course of preparing the hemolyzates and during electrophoresis remains to be excluded (*5*).

A possible association between Type II hexokinase and fetal hemoglobin was suggested by the chance observation that the red cells of a dysmature (*8*) infant studied on the first day of life demonstrated only hemoglobin A and Types I and III hexokinase on starch-gel electrophoresis. The erythrocytes of this newborn dysmature infant thus lacked both the high concentration of fetal hemoglobin and the Type II hexokinase normally present.

We were fortunate in obtaining blood samples from a well-studied family that includes one subject known to be homozygous for the hereditary persistence of fetal hemoglobin (*9*). Results of starch-gel electrophoresis indicate that Type II hexokinase is present in the red cells of the members of this family who have the trait for persistent fetal hemoglobin (Table 1). Three unrelated adult patients with the persistent fetal hemoglobin trait were also studied and found to have Type II hexokinase in their erythrocytes. This trait thus appears to be associated with the presence of Type II hexokinase in adult erythrocytes. Heretofore it has been thought that, apart from the hemoglobin components, the red cells of the patient homozygous for persistent fetal hemoglobin are comparable to those of the normal adult and are, with respect to enzyme composition, unlike the erythrocytes of the newborn (*9*). These data suggest that hexokinase activity, which is a probable rate-limiting step in red-cell glycolysis (*10*), may be mediated by distinct proteins in the erythrocytes of the fetus and the adult. Furthermore, they strongly indicate that the regulation of synthesis of Type II hexokinase is in some way related to that of the γ-chain of hemoglobin.

The K_m for glucose of Type I hexokinase from human erythrocytes when separated by DEAE-cellulose chromatography is of the order of $5.3 \times 10^{-5}M$, and the K_m for glucose of Type II is $1.4 \times 10^{-4}M$. The substrate specificity and kinetic characteristics of the isoenzymes of red-cell hexokinase may prove to be significant with regard to glucose homeostasis, for, as noted by Krebs (*11*), the obligatory glucose utilization of the erythron is of great quantitative significance in the fasting state.

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Amino Acids and the Spikes from the Retinal Ganglion Cells

Abstract. *The effects of amino acids or mixtures of amino acids on the spike discharges from the optic ganglion cells were studied. Mixtures of amino acids have different effects than single amino acids. Proper composition of excitatory and inhibitory amino acids can enhance only the light-induced spikes without giving rise to any spontaneous discharges. It is implied that amino acids may play an important role in the regulation of the general sensitivity of cells in the central nervous system.*

The iontophoretic injection of amino acids has given us valuable information on the action of amino acids in the central nervous system. These results have shown that the amino acids could be classified into three types: (i) excitatory amino acids such as glutamic acid which gave rise to spike discharges when they were iontophoretically injected; (ii) depressing amino acids such as γ-aminobutyric acid (GABA) which inhibited (spontaneous) spike discharges and antagonized the action of excitatory amino acids; and (iii) those amino acids such as glutamine which exhibited no apparent effect (*1, 2*).

This report describes preliminary results of experiments in which amino acids or mixtures of amino acids were applied to a vertebrate retina. In the vertebrate retina, the discharge pattern of the ganglion cell is the result of the mutual antagonism between the central and peripheral portion of the receptive field (*3*). The "on" mechanism is thought to be related to an excitatory process and the "off" mechanism to an inhibitory process. Intracellular recording from the ganglion cell of the bullfrog retina also showed that there was a complex interaction of excitatory and inhibitory postsynaptic potentials (*4, 5*). Study of the effects of amino acids on the retinal ganglion cell may help to clarify the role of the amino acids in the central nervous system.

The eye of the bullfrog, *Rana catesbiana*, was dissected and the frontal half removed together with the lens. Test solutions were applied to the vitreous side of the posterior half of the eye by means of a pipette positioned close to the recording site. In the bullfrog the vitreous humor could easily be removed and the solutions could be introduced directly onto the retinal surface. Test solutions were applied in the order of 0.01 ml per trial.

Commercially obtained amino acids were dissolved in the frog Ringer solution, the *p*H of which was adjusted to 7 by HCl or by NaOH. Spikes were recorded extracellularly by glass pipettes filled with saturated NaCl solution. In the vertebrate retina only the ganglion cells are known to produce spikes (*5, 6*) and, therefore, it

can safely be postulated that the spikes obtained in the present experiments were from the ganglion cells. After amplification and filtering, each spike triggered a pulse which was fed into the z-axis of an oscilloscope. The flash used was of diffused white light (duration about 100 msec given once in every 3 seconds) and its intensity was controlled by a 4 log neutral wedge (Kodak, annular). The wedge was mechanically coupled to a potentiometer, the output of which controlled the horizontal level of the oscilloscope beam. The spikes appeared as dots on a plane whose ordinate was the log relative intensity and the abscissa was time. This allowed us to display on a single frame discharge patterns to flashes of different intensities.

The results obtained with single amino acids were what one could expect from the results of iontophoretic experiments, namely, that the effects of amino acids could be classified into three typical groups, excitatory, depressing, and those without any apparent effect. Both excitatory and depressing amino acids were effective when applied at concentrations of $10^{-4}M$. Considering the methods of application, this is in good agreement with the effective concentrations postulated in the iontophoretic methods (*2*) and with the results obtained by the intravenous injection of GABA (*7*). Excitatory amino acids irreversibly abolished spikes at concentrations of about $10^{-3}M$, while in the case of depressing amino acids, recovery was always observed in 10 to 20 minutes, even with concentrations of amino acids as high as $10^{-2}M$. This might have been owing to enzymatic removal of the amino acid.

However, more interesting results were obtained when mixtures of excitatory and depressing amino acids were applied. Mixtures of suitable composition could markedly increase the number of spikes obtained in response to a flash without giving rise to any spontaneous firing. The composition of a mixture found at present to be most effective is shown in Table 1, together with a preliminary estimate of the corresponding amino acids in the water-soluble fraction of the frog retina.

Figure 1A shows typical results obtained by application of the amino acid mixture shown in Table 1 (the units shown in Fig. 1, A–D, were "on-off" type, and the unit in E was "off" type). In Fig. 1, column 1 is the control, 2 is the record obtained immediately after application of the mixture, and 3 is the recovery observed 15 minutes later. A marked increase in the number of light-induced spikes was seen after the application of the mixture, and no spontaneous discharge was seen. The effect would be more drastic if the spikes were previously depressed by application of a depressing amino acid. Records B to E (Fig. 1) show the effects obtained after removal of one amino acid out of the mixture of four. The mixture without aspartic acid (excitatory amino acid) slightly reduced the number of spikes (Fig. 1B).

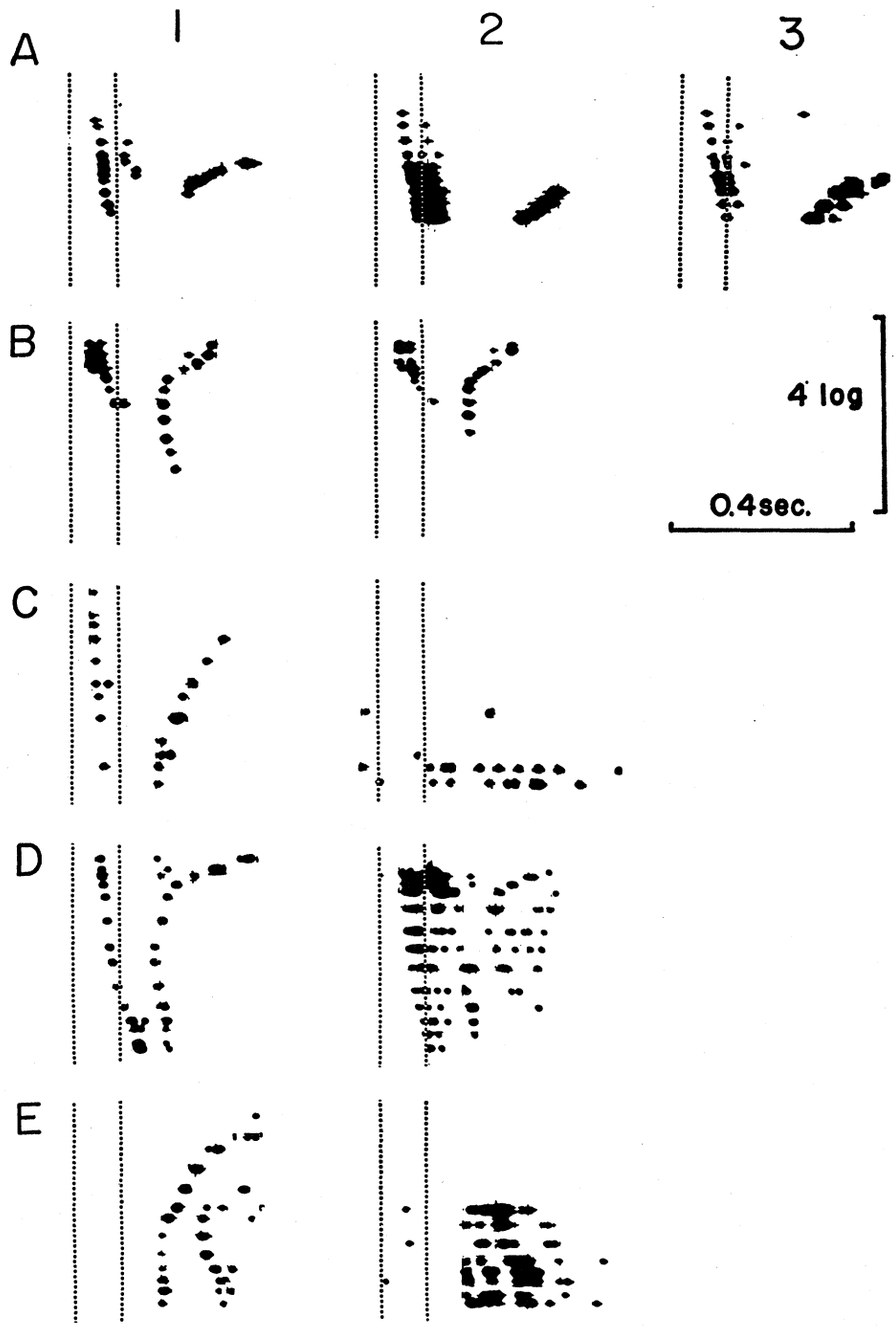

Fig. 1. Effects of a mixture of amino acids (composition shown in Table 1) on the spikes from the frog optic ganglion cell. Column 1 is control, column 2 is response obtained immediately after application of the mixture, and column 3 is recovery observed 15 minutes later. (A) Effect of the mixture. (B–E) Effect of mixtures without aspartic acid, glutamic acid, alanine, and glycine, respectively. In (C) recovery was observed, while in (D) and (E) units were destroyed following a burst of spontaneous discharge. (A) to (D) were "on-off" units, while (E) was an "off" unit. Pairs of dotted vertical lines show the approximate duration of the flash. A shift of the horizontal line upward indicates an increase in the intensity of the flash.

Table 1. Composition of amino acids mixture.

Amino acid	Amount (μg/ml)	Water-soluble amino acids in frog retina (*11*) (μg/g)
L-glutamic acid (excitatory)	30	69.3
L-aspartic acid (excitatory)	20	15.7
L-alanine (depressing)	20	98.4
Glycine (depressing)	10	40.8

Application of the mixture without glutamic acid (excitatory amino acid) resulted in a transient increase in the number of spikes, followed by a period of depression (Fig. 1C). Mixtures lacking either glycine or alanine (both depressing amino acids, Fig. 1, D and E) gave rise to a transient increase in the number of light-induced spikes, followed by a burst of spontaneous discharge. In either case, units irreversibly stopped firing.

These results suggest that there is a balance of action between excitatory and depressing amino acids. Withdrawal of one excitatory amino acid shifts the balance more to the depressing side, and withdrawal of one depressing amino acid shifts the balance more to the excitatory side. The composition of the mixture of four amino acids shown in Table 1 was obtained after various combinations of amino acids had been tried. Mixtures of the same ratio as in Table 1 but of higher amino acid concentration depressed the spike discharge in response to a flash, while mixtures of lower amino acid concentration had weaker spike-enhancing action. The composition may not be the optimum one, and a better ratio may be found by further experimentation.

The mixture having the same composition as the water-soluble amino acid fraction in the bullfrog retina (as shown in Table 1) slightly enhanced spike discharges. Various combinations of one excitatory and one depressing amino acid were also tried. Although some of them had effects similar to that of the mixture of four amino acids, results were not always consistent.

The electroretinogram simultaneously recorded from the spike-recording electrode (through a low pass filter) was not affected by the application of either group of amino acids. Amino acids thus applied were acting mainly on the retinal ganglion cells (see Tomita, *5*). Apparently the spike discharge pattern is not only the function of stimulus parameters and of the state of adaptation but also of the amino acid composition in the retinal layer.

Several authors have suggested that some amino acids, such as glutamic acid or GABA, could be transmitters in the central nervous system (*2, 8, 9*). The amino acid(s) applied to the retina in the present experiment are probably not acting as transmitters. First, the total amount of amino acids in the mixture is 80 μg/ml, while in the frog retina the amount of free glutamic acid, aspartic acid, alanine, and glycine totals about 225 μg/g. It seems more reasonable to assume that the effects observed were due to changes in the amino acid composition, rather than to the action of amino acids as transmitters. Second, there is no specificity in the type of amino acids in the mixture. Glycine or alanine could be replaced by GABA.

The results of this experiment imply that the excitability of the cells could be controlled by changing the amino acid composition in the external media (see McLennan, *10*). In this connection it is interesting to note that the excitatory amino acids, such as glutamic acid, could easily be converted into depressing amino acids, such as GABA, by decarboxylation. The control of excitability of a number of cells through amino acid metabolism appears as important as control of cells through synaptic inputs.

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Plastic Films on Plants as Antitranspirants

Abstract. *Plastic-film-type antitranspirants are more permeable to water vapor than to carbon dioxide. Photosynthesis in treated plants is reduced to the same or greater extent than transpiration, except under conditions of stress, when photosynthesis in treated plants is greater than in controls. This exception is attributed to reduction of stress, as the selective permeability of the films to gases would tend to produce the reverse effect.*

Wax and plastic-based emulsions were long ago suggested as antitranspirants (*1, 2*). The emulsions are usually sprayed on plants in order to form a film on the surface of the leaves that will be more permeable to carbon dioxide and oxygen than to water vapor. Such a film would decrease the transpiration : photosynthesis ratio, thus reducing the irrigation requirement and alleviating the effects of water stress under dry conditions.

It is widely accepted that certain plastic materials, such as polyethylene, possess the desired selective-permeability characteristics (*1*). Permeabilities of plastic films are, however, very difficult to determine; the methods used and the units in which the results are expressed often cannot be compared. The high permeability of polyethylene to carbon dioxide has often been compared in the literature to its low permeability to water vapor (*3*), but such comparisons can be misleading. Polyethylene is in fact four to five times more permeable to water vapor than to CO_2 (Table 1), but, in comparison with other plastic films, its permeability is low to water vapor and high to CO_2.

Gas permeabilities of plastic films have been compared in terms of the same units; Table 1 summarizes typi-

Table 1. Relative permeabilities of plastic films to water vapor and carbon dioxide. Approximate ranges calculated (from *4, 5*) on the basis of volume-diffusion rates.

Film	$P_{H20} : P_{CO2}$
Poly(vinylidene chloride)	48–3500
Rubber hydrochloride	145–1044
Poly(vinyl chloride)	170–255
Polystyrene	7.7–38.5
Polyethylene (density, 0.954)	4.2
Polyethylene (density, 0.922)	4.45
Polypropylene	6.8–7.6
Natural rubber	22.6
Silicone rubber	3.5–17.7