pounds that are excreted in the bile as glucuronide conjugates are deconjugated by bacterial β-glucuronidase and further modified by intestinal bacteria in the large bowel (*5*, *8*). Since the intestinal microflora is changed by diet, these changes might alter the biological activity, toxicity, excretion, and resorption of many endogenous and exogenous compounds, such as carcinogen metabolites, acid and neutral sterols, ammonia and select amines, major products of urea and protein degradation, and tryptophan metabolites. Diet may also control the secretory and functional ability of the liver to yield potentially harmful metabolites that are subsequently split and released by gut microflora. Since the microflora is more active in populations on a mixed Western high meat diet, these reactions, including the release of carcinogens or other toxic components, would be more likely to occur in the gut of these populations on a mixed Western diet.

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Synaptic Transmission between Photoreceptors and Horizontal Cells in the Turtle Retina

Abstract. *Low calcium, high magnesium, and cobalt hyperpolarize the horizontal cell membrane and suppress the response to light, but only partially affect the response of receptor cells. These observations are consistent with the interpretation that a depolarizing transmitter is released by photoreceptors in darkness. The hyperpolarizing response to light of the horizontal cells would then result from a reduction in the amount of transmitter released.*

Vertebrate photoreceptors do not generate nerve impulses, but respond to light with a graded increase in transmembrane potential (hyperpolarization). The mechanism by which receptor hyperpolarization affects second order neurons and causes a hyperpolarization in the luminosity-type horizontal cell is not completely known. It has been suggested that the hyperpolarization of the receptor membrane by light reduces the release of a depolarizing transmitter which, according to this view, would flow continuously from the receptor pedicle (*1*). If this explanation is correct one would expect that reducing the release of transmitter by the action of blocking agents would result, not only in a decrease of the response to light, but also in a hyperpolarization of the horizontal cell membrane.

Divalent cations play a fundamental role in the process of transmitter release from presynaptic terminals both of the neuromuscular junction and of spinal cord neurons (*2*). Calcium must be present in the extracellular fluid for the transmitter to be released and its action is antagonized by an excess of magnesium or by the presence of other divalent cations. On the assumption that the principle for such an action is extensible to retinal synapses, we studied the effects of varying concentrations of calcium, magnesium, and cobalt on the intracellularly recorded responses of photoreceptors and horizontal cells in the perfused retina of turtle (*Pseudemys scripta elegans*). The effect of magnesium on horizontal cell activity in the skate retina has been reported by Dowling and Ripps (*3*).

The receptors of the turtle retina are predominantly cones (*4*) and their synaptic organization has been studied in detail by Lasansky (*5*).

The turtle eye was removed and cut along the medial lateral axis. After the vitreous chamber was drained, the eyecup was mounted in a chamber where an oxygenated and buffered Ringer solution continuously flowed over the vitreous side at 4 to 5 cm^3/min. The ionic composition of the Ringer solution used was similar to that of the cerebrospinal fluid (*6*). The *p*H was adjusted to 7.7 ± 0.2 with appropriate amounts of sodium bicarbonate. The room temperature was kept around 20°C. Test solutions containing magnesium and cobalt in different proportions were not compensated for changes in osmolarity when changes did not exceed 5 meq. Calcium-free solutions were prepared by omitting calcium chloride and adding 1 m*M* ethylenediaminetetraacetic acid (EDTA).

Intracellular recordings were made with glass micropipettes filled with 4*M* potassium acetate. With microelectrodes of low tip potential there was no need to correct the results for changes of

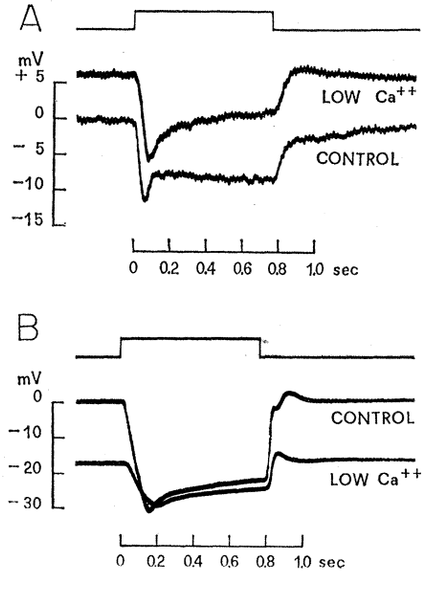

Fig. 1. Effects of low calcium on the activity recorded from (A) a cone and (B) a horizontal cell. Responses to light were obtained during perfusion with normal Ringer solution (control) and after the retina was perfused for 15 minutes with a calcium-free solution containing 1 m*M* EDTA (low Ca^{2+}). The light intensity used was attenuated 1.8 log units with respect to the maximum available energy; the area illuminated was 1500 μm in diameter. The raised bars at the top indicate durations of illumination. The zero level of membrane potential is arbitrary and indicates the level of membrane potential in darkness.

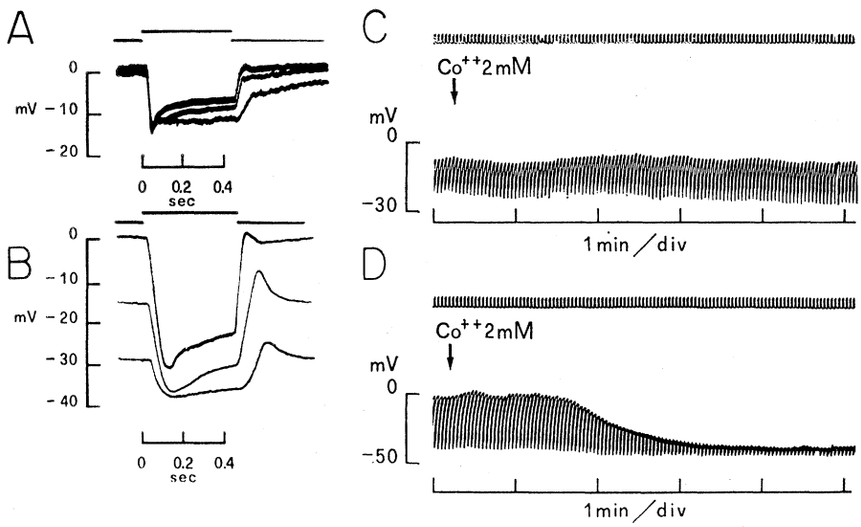

Fig. 2. (A and B) Effects of high magnesium on the activity recorded from (A) a cone and (B) a horizontal cell. The records at the top were obtained during perfusion with control Ringer solution. The two lower records were obtained during perfusion with a solution containing 10 m*M* magnesium chloride. The raised bars at the top indicate periods of illumination. The stimulus parameters are the same as reported in the legend of Fig. 1. (C and D) Effects of cobalt on the activity recorded from (C) a cone and (D) a horizontal cell. At the time indicated by the arrows we began to substitute a solution containing 2 m*M* cobalt chloride for the control Ringer solution. The traces at the top indicate light periods. The stimulus parameters are the same as for Fig. 1.

liquid junction potentials due to substitution of the bathing fluids. The retina was stimulated by white light from a quartz iodine lamp. The reduced image of a circular diaphragm was focused on the retina with a diameter that could be varied between 100 and 2000 μm. The light intensity was attenuated by neutral density filters. The total irradiance of the unattenuated light of wavelength between 4000 and 8000 Å was approximately 8×10^3 μw/cm^2. Receptor and horizontal cell responses were identified by the criteria given by Baylor and Fuortes (*7*).

The effect of calcium deprivation was studied on the intracellular responses of both cones and horizontal cells. Results of a representative experiment are shown in Fig. 1. The records were obtained in normal Ringer solution and during perfusion of the retina with a calcium-free solution containing 1 m*M* EDTA. Although calcium deprivation produced an appreciable depolarization of the cone membrane and altered the shape of the hyperpolarizing potential, the amplitude of the response to light was not reduced (Fig. 1A). In the majority of cases the amplitude of the response to light was increased. By contrast, the horizontal cell membrane hyperpolarized and the response to light decreased (Fig. 1B). The changes observed in the photoreceptor response in low calcium were likely a consequence of a direct action on the membrane processes, but could not be the cause for the effects observed on the horizontal cell (membrane hyperpolarization and decrease of response).

The effects of a high external magnesium concentration were studied in separate experiments. Typical results are illustrated in Fig. 2. Records were obtained from a cone (Fig. 2A) and from a horizontal cell (Fig. 2B). In both cases the record at the top was taken in normal Ringer solution and the two lower records were obtained during perfusion with a solution containing 10 m*M* magnesium (five times higher than the normal concentration). After the test solution was substituted for the control Ringer solution, the membrane of the horizontal cell gradually hyperpolarized and the response to light decreased (Fig. 2B). The amplitude of the cone response was not affected by the presence of high external magnesium, as shown in Fig. 2A. The shape of the receptor potential, however, appeared modified when the light responses of horizontal cells were suppressed by high magnesium. These alterations of the intracellular responses of cones are very similar to those observed in retinas treated with aspartate and glutamate (*8*).

Hyperpolarizations of the horizontal cell membrane associated with decrease of the response to light were also obtained by applying small amounts of cobalt chloride to the retina. The effects produced with this cation, which is not present in the natural medium, resembled those described for high magnesium. These results are illustrated in Fig. 2, C and D, showing the intracellular responses from a cone and a horizontal cell during perfusion of the retina with a solution containing 2 m*M* cobalt chloride. Shortly after the test solution was substituted for the control solution, the horizontal cell membrane (Fig. 2D) hyperpolarized and the response to light rapidly decreased. The photoreceptor, however, could produce a response to light in conditions in which the horizontal cell was unresponsive. The effects produced by low calcium or high magnesium and cobalt were reversed when the retina was again perfused with the normal control solution.

When an excess of calcium was added to the retina to counteract the blocking action of magnesium, the horizontal cell activity was not restored. Control experiments, however, showed that in those conditions the response of photoreceptors was suppressed [see (*9*)].

These results show that with low calcium, high magnesium, and cobalt responses to light from horizontal cells decrease and the cell membrane hyperpolarizes. However, responses from photoreceptors, although altered, are not diminished. The alterations observed in the cone responses could be due in part to a direct action exerted on the membrane excitability and in part to the inactivation of the horizontal cells. In fact, horizontal cells have been shown to affect the cone response by a feedback mechanism (*10*).

It seems likely that presynaptic signals from photoreceptors cannot be transmitted to horizontal cells because low external calcium, an excess of magnesium, and the presence of cobalt all prevent the release of transmitter. Accordingly, the data we have reported can be best explained by assuming that cones release a depolarizing transmitter continuously in darkness; by hyperpolarizing the cone membrane, light reduces the amount of transmitter released, and consequently the horizontal cell membrane hyperpolarizes.

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Inhibition of Cellular Mediated Immunity in Marihuana Smokers

Abstract. *The cellular mediated immunity of 51 young chronic marihuana smokers, as evaluated by the lymphocyte response in vitro to allogeneic cells and to phytohemagglutinin, was significantly decreased and similar to that of patients in whom impairment of T (thymus derived) cell immunity is known to occur. This inhibition of blastogenesis might be related to an impairment of DNA synthesis.*

It has been previously reported (*1*) that delta-9-tetrahydrocannabinol (Δ^9-THC), a psychoactive substance of *Cannabis*, when administered to rodents alters their cellular mediated immune responsiveness, and it was suggested that similar changes might also occur in man. In our study the mixed lymphocyte culture (MLC) and phytohemagglutinin (PHA) responsiveness of 51 marihuana smokers, 16 to 35 years old (median age 22), were studied. Only subjects who had used *Cannabis* products (at the exclusion of other drugs) at least once a week (average four times a week) for at least 1 year (average 4 years) were selected for this investigation.

Eighty-one healthy volunteers, 20 to 72 years of age (median age 44) were used as controls. Purified lymphocyte suspensions were prepared from fresh samples of venous blood by the Ficoll-Isopaque density gradient method (*2*). A microculture system was used for screening of cellular responsiveness (*3*). For the MLC test, 1×10^5 responding cells were incubated, per well, with 2×10^5 stimulating cells pooled from a panel of ten donors, phenotypically different [allogeneic cells in which 25 different HL-A specificities were represented (*4*)].

For the PHA test, 2×10^5 responding cells were incubated per well with 1 μg of purified PHA. The medium used was RPMI 1640 with penicillin, streptomycin, and glutamine, to which 25 percent autologous serum was added.

Results are summarized in Table 2 and compared with data obtained in 60 patients with cancer, 20 patients with uremia, and 24 renal allograft recipients with iatrogenically induced immunosuppression. The mean values registered in the group of marihuana users were significantly lower than those of the normal, but much older, control group. Since an inverse correlation exists between cellular immunity, as reflected by in vitro lymphocyte blastogenesis and aging (*5*), results obtained in the group of marihuana smokers may be interpreted as being indicative of cellular hyporesponsiveness. Supporting this conclusion is the close similarity between the depressed MLC and PHA responsiveness of marihuana users and that of cancer (*6*), uremia (*7*), and immunosuppressed transplant patients in whom impairment of T (thymus derived) cell immunity is known to occur. Furthermore, we observed that in vitro inhibition of PHA-induced blastogenesis of normal human lymphocytes started with 1.6 μM THC and was complete with 20 μM.

The major psychologically active constituent of *Cannabis sativa* is Δ^9-THC. This substance, as well as its metabolites, is insoluble in H_2O, but is very fat soluble, and has a half-life of several days in tissues where it might exert a cumulative and pharmacological effect (*8*). Such an effect might be related in a still unknown way to the depressed cellular immune response in vitro of chronic marihuana smokers. The effect of THC on adrenergic receptors (*9*) might also play a role in its immunosuppressive activity, as was suggested for other drugs administered continuously over a long period (*10*).

This inhibition of blastogenesis might result from an impairment of DNA synthesis. One of us (A.M.) sampled lymphocytes from four marihuana smokers, cultivated the cells for 72 hours, and then observed a decreased number of cells during the period of DNA synthesis (S period of the cell cycle). There was also an increased incidence of chromosomal breakages,

Table 1. Comparative cellular mediated immunity of normal subjects, marihuana smokers, and patients with impairment of T cell immunity. The in vitro blastogenic response of lymphocytes was studied by the MLC and the PHA tests. The incorporation rate of [^{3}H]thymidine of the T lymphocytes is given in counts per minute ± the standard error.

Subjects	MLC		PHA	
	No. tested	[^{3}H]Thymidine incorporated (count/min)	No. tested	[^{3}H]Thymidine incorporated (count/min)
Normal controls	81	26400 ± 200	81	23250 ± 210
Cancer patients				
Primary tumors	16	14894 ± 792	16	17501 ± 124
Regional spread	23	15816 ± 420	23	13345 ± 540
Distant spread	21	8968 ± 459	21	10516 ± 580
Uremic patients	26	12001 ± 272		
Transplant patients*	24	12307 ± 357		
Marihuana smokers†	34	15679 ± 499	51	13779 ± 169

* After 1 to 4 years of immunosuppressive therapy. † At least 1 year, at least once a week; no other drug taken.