Table 1. Influence of metabolic inhibitors on membrane potentials in *Neurospora*.

Additions to reference medium	Concentration (molar)	Average potential* measured ± S.D. (mv)
None		126 ± 7
Sodium fluoride	10^{-2}	124 ± 7
Sodium azide	10^{-2}	28 ± 2
Sodium azide	10^{-3}	29 ± 8
Sodium azide	10^{-4}	37 ± 5
Sodium azide	10^{-5}	78 ± 19
Nystatin	2.2×10^{-5}	24 ± 7
Nystatin	2.2×10^{-6}	19 ± 9
Nystatin	2.2×10^{-7}	30 ± 5

*All averages refer to measurements on 5 to 15 cells 8 mm from the margin of the colony.

periphery, a more abrupt drop to about 25 mv at the growing hyphal tips (Fig. 1*A*).

The influence of ionic environment has been explored for cells in the 8-mm region. When the components of the reference medium were varied singly, only potassium displayed a clear effect on the potential (Fig. 1*B*). Increasing the external potassium (by adding either KCl or K_2SO_4) from 1 to 60 m*M* produced a fall in the membrane potential which was linear with the logarithm of the potassium concentration and which had a slope of 32 mv per log unit. Correction for pipette tip potentials might increase this slope by 10 mv per log unit (*5*). Below 1 m*M* potassium, the relationship was no longer linear, which suggested that other ions might determine the membrane potential under these conditions. Indeed, in the absence of potassium the membrane potential was sodium-sensitive, with a slope of about 40 mv per log unit. In the reference medium containing 36.8 m*M* potassium, however, variations of NaCl or Na_2SO_4 between 0.1 and 60 m*M* had no measurable effect. Although media for the above experiments were not adjusted to constant total osmolarity, control measurements indicated that this was not necessary. Thus, essentially the same stable potentials were measured for cells in distilled water (222 ± 15 mv) and for those in 200 m*M* sucrose (229 ± 12 mv). The sharp decline in membrane potential produced by 60 to 100 m*M* NaCl or KCl was duplicated by high concentrations of sucrose, and was presumably an osmotic effect.

Among other ions tested, Mg^{++}, NH_4^+, NO_3^-, and $H_2PO_4^-$ did not influence the membrane potential. Addition of 1 m*M* $CaCl_2$ to the reference medium raised the measured potentials from 130 to 175 mv, and noticeably stabilized the cells against mechanical injury. On the other hand, 10 m*M* (sodium) citrate, in the absence of added calcium, diminished the potentials from 130 to about 30 mv.

In an attempt to obtain further information concerning ionic contributions to the membrane potential, we assayed intracellular potassium and sodium by flame photometry. One-day cultures grown at 25°C in liquid minimal medium were found to contain approximately 160 mmole of potassium and less than 20 mmole of sodium per kilogram of cell water. These values are based on inulin determinations of extracellular space, and they are in agreement with previous potassium and sodium measurements for *Neurospora* (*6*). It is concluded that, under the present conditions, young *Neurospora* cultures concentrate potassium at least 4.3-fold from minimal medium, and may exclude sodium slightly.

Finally, the effects of several metabolic inhibitors were studied (Table 1). Sodium azide, at concentrations as low as 10^{-4} *M*, reduced the membrane potentials to about 35 mv; the maximum effect was already evident in the first cells measured, two minutes after the addition of inhibitor. In preliminary experiments sodium cyanide and dinitrophenol lowered the potentials with similar rapidity. Sodium fluoride (10^{-2}*M*), however, had no apparent effect, even after 1 hour preincubation with the cells. The polyene antibiotic nystatin (*7*) proved to be the most potent compound tested, being maximally effective at a concentration of 2.2×10^{-7}*M*, again within 2 minutes. It has previously been suggested, on the basis of other types of evidence, that this antibiotic acts by damaging the selective permeability of the cell membrane (*8*).

The observations contained in this preliminary report are presently being extended. In addition, experiments are now in progress to correlate altered membrane properties in *Neurospora* with specific genetic mutations (*9*).

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4. The reference medium (36.8 m*M* KCl, 25.3 m*M* NaCl, and 1 percent sucrose) was designed as a simplification of minimal medium. Similar potentials were measured in both media, those in minimal medium being 129 ± 10 mv for cells 8 mm behind the growing tips.
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9. We wish to thank H. K. Hartline and E. L. Tatum, in whose laboratories this work was done.

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Effect of Background Illumination on the Retinal Action Potential

Abstract. A study of the relation between the membrane potential and the amplitude of the slow component of the retinal action potential obtained from inside a single insect photoreceptor cell suggests that the slow component is equivalent to the change in the membrane potential caused by background illumination.

In recent years retinal action potentials have been recorded from single cells in the compound eye of the insect (*1*, *2*). The retinal action potential presumably recorded from the inside of a photoreceptor cell or retinula cell is composed of two components, a fast transient or spike-like "on" response and a slow component maintained during illumination. This short report describes the effects of background illumination on the retinal action potential.

The materials used were the compound eye of a dragonfly, *Agriocnemis*. Methods involved were the same as reported elsewhere (*3*). Both the stimulating and background illumination obtained from tungsten lamps gave diffused light over the whole surface of the compound eye. Intensities of both lights were continuously varied by controlling the current through the lamps. This variation changed the spectral distribution of the light but probably did not affect the results of the experiment. Both intensity and duration of the stimulus were monitored by a photodiode and were displayed on the lower beam of an oscilloscope.

Figure 1*A* is a typical response from a single receptor cell obtained by intermittent stimulus light of 0.1-second

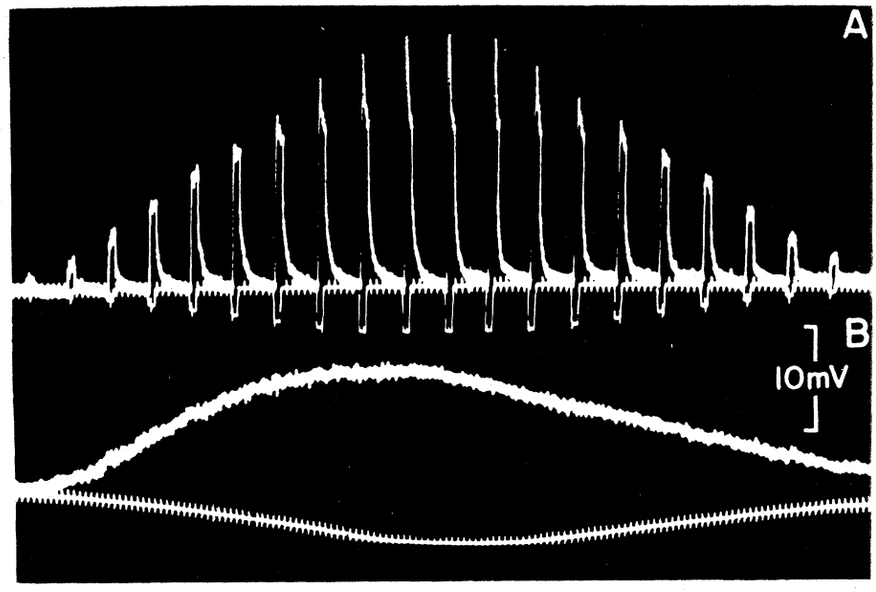

Fig. 1. Response of a single retinula cell to intermittent (*A*) and continuous (*B*) illumination. Both upper and lower traces are superimposed in *A*. Pips indicate 0.1-second intervals.

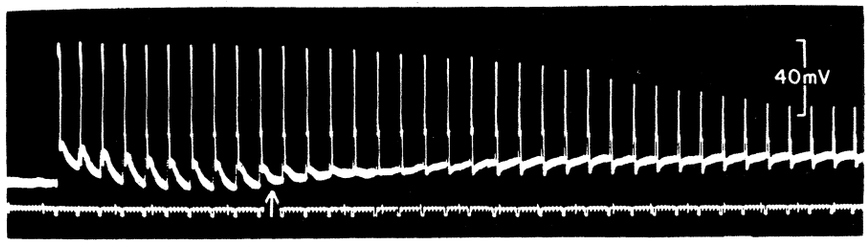

Fig. 2. Response of a single retinula cell to intermittent illumination of 0.1-second duration. The arrow indicates onset of background illumination. Pips are 0.1 and 1 second apart.

duration (without background illumination). The intensity was increased toward the middle of the record and then decreased. The low intensity response was mainly composed of the slow component, as is apparent from the flat top of the response. Increase in the intensity of illumination brought forth the initial transient component. However, as is shown by the hump on the falling phase of the response, the amplitude of the slow component reached a level of saturation by the 8th flash and stayed at that level (15 mv) while the transient component still increased in amplitude. The light intensity used in this experiment was apparently supramaximal for elicitation of the slow component in this cell. Figure 1*B* was obtained by continuous light (not intermittent) of the same intensity as that used to obtain the data shown in Fig. 1*A* and varied at the same rate. As the intensity of light was increased, a decrease was observed in the membrane potential. Up to a certain point corresponding roughly to the saturation level of the slow component shown in Fig. 1*A*, the change in the membrane potential was the same as that of the slow component obtained by the intermittent stimulation. But the shape of the response shown in Fig. 1*B* was not symmetrical as in the case of the response to intermittent light shown in Fig. 1*A*. This asymmetry in the response to continuous stimulation was apparently due to the effect of adaptation to continued supramaximal high intensity light.

Figure 2 was obtained by adding background illumination to intermittent stimulation of constant intensity. The response to the first few flashes shows the characteristics of a dark-adapted cell: a large after-potential and a prolonged transient potential which masked the slow component. With repeated illuminations, the hump due to the slow component began to appear on the falling phase of the response and the after-potential became somewhat smaller in amplitude and shorter in duration. When the response became stable, background illumination was added (from arrow), and the intensity was gradually increased to the end of the record. The background illumination first brought forth a decrease in the membrane potential which, in turn, resulted in decrease in the amplitude of the after-potential. When the membrane potential decreased by 6 mv from the resting level under dark-adaptation, no after-potential was observed. Further increase in the intensity of background illumination gave rise to a polarity reversal of the after-potential. The amplitude of the transient component also showed marked decrease due to light-adaptation. By the end of the record the membrane potential had decreased by 17 mv and the amplitude of the transient component also had decreased, from 80 to 27 mv. However, the level of the hump on the falling phase of the response stayed at a level of 26 mv, indicating that the amplitude of the slow component remained unchanged.

An absence of response was also obtained with decrease of membrane potential to the level of the maximum amplitude of the slow component. These observations show that when the membrane potential was decreased by background illumination the amplitude of the slow potential decreased by nearly the same amount. However, unlike the transient component which depolarizes the cell membrane to zero (*3*), the range of membrane potential controlled by steady illumination was limited, usually one-half to one-third of the membrane potential under dark-adaptation.

If the intensity of illumination was supramaximal and was maintained too long, another phase of light-adaptation occurred; the cell membrane became repolarized and the cell lost its ability to respond to light stimulus. Apparently at this stage of light-adaptation photochemical substances become exhausted and the control over the membrane potential of the retinula cell by the reaction products is lost. It is not known whether this state of light-adaptation occurs under physiological conditions.

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Urinary Excretion of Amines in Phenylketonuria and Mongolism

Abstract. Children with phenylketonuria excrete considerably less serotonin and tryptamine and somewhat less normetanephrine and *p*-tyramine than normal children. The excretion of these amines was not decreased in mongolism. Even during monoamine oxidase blockade, *o*-tyramine could not be detected in the urine of phenylketonurics. These findings are discussed in relation to the mental defect of phenylketonuria.

Increasing attempts have recently been made to elucidate the mechanism of the mental defect in phenylketonuria. One theory suggests that a toxic substance is produced in the brain. Mitoma *et al.* (*1*) found that *o*-tyrosine enters rat brain in vivo and is decarboxylated to *o*-tyramine, and they suggested that overproduction of this amine might cause the mental defect in phenylketonuria. Jepson *et al.* (*2*) reported that phenylketonurics treated with monoamine oxidase inhibitors excrete large amounts of phenylethylamine in the urine and lesser amounts of *o*-tyramine. Both these amines might damage brain tissue, especially during its period of active growth.

A second possible mechanism for the mental defect of phenylketonuria is failure of production of certain neurohumoral agents essential for normal brain function. Several metabolites of phenylalanine present in increased concentrations in the tissues of phenylketonurics inhibit the in vitro decarboxylation of 5-hydroxytryptophan to serotonin (*3*), and phenylpyruvate inhibits the in vitro decarboxylation of 3,4-dihydroxyphenylalanine to dopamine (*4*). Pare *et al.* (*5*) reported that serum concentrations of serotonin, and urinary excretion of its terminal metabolite, 5-hydroxyindoleacetic acid, are decreased in phenylketonurics, and they suggested that underproduction of serotonin might contribute to the mental defect in these patients. Nadler and Hsia (*6*) recently showed that plasma concentrations of norepinephrine and epinephrine, and urinary excretion of these two amines as well as of dopamine, are low in phenylketonurics.

A third possible explanation for the mental defect in phenylketonuria is based on the discovery of Chirigos *et al.* (*7*) that uptake of tyrosine by rat brain in vivo is inhibited by high concentrations of certain amino acids. If a high plasma concentration of phenylalanine competitively inhibits the transport of aromatic amino acids across the blood-brain barrier in man, the phenylketonuric brain might well suffer a deficiency of serotonin and catecholamines, as well as other consequences.

Recently the discoverers of the trisomy of the 21st chromosome in mongolism reported (*8*) diminished urinary excretion of 5-hydroxyindoleacetic acid in mongoloid children. This suggests that diminished production of serotonin might also contribute to the mental defect in mongolism. The investigation reported here attempts to clarify these possibilities by measuring the urinary excretion of biologically active amines in children having phenylketonuria and mongolism and in suitable controls.

Urine was collected for 24 hours, both before and during administration of a monoamine oxidase inhibitor, from six normal subjects, five untreated phenylketonurics, five mongoloids, and five unclassified mental defectives. All were children aged 8 to 16 years and were in good physical health and well nourished. The 15 mentally defective patients lived under uniform ward conditions and ate a standard hospital diet, while the six normal children lived at home. Subjects received no drugs except pheniprazine (12 mg/day) or nialamide (100 mg/day) as a monoamine oxidase inhibitor.

Amines were separated from other urinary constituents and were measured semiquantitatively by two-dimensional paper chromatography (*9*). Briefly, the amines in each urine specimen were adsorbed before and after acid hydrolysis onto the resin Amberlite CG-50 H^+ and were then eluted with 4*N* acetic acid. After the eluates had been lyophilized, the amines were separated from salts and basic amino acids by extraction with ethanol and acetone. Two-dimensional paper chromatograms were prepared from the final extracts, and the amines were developed with ninhydrin, diazotized *p*-nitroaniline, dimethylaminocinnamaldehyde, and dichloroquinonechloroimide. Amines were identified and quantitated by comparison with appropriate amounts of the authentic compounds similarly chromatographed on paper. Since accurate 24-hour urine collections are unreliable in mentally defective children, the excretion of amines was calculated in relation to urinary creatinine.

The results (Table 1) indicate that

Table 1. Urinary excretion of amines before and during monoamine oxidase (MAO) inhibition, in micrograms of free base per 100 mg of creatinine. C, control; MAO, after 5 to 7 days administration of MAO inhibitor.

Subject	Serotonin		Tryptamine		Normetanephrine		*p*-Tyramine		Phenylethylamine	
	C	MAO	C	MAO	C	MAO	C	MAO	C	MAO
					Normal children					
1	1.3	4.6	5.1	50	2.4	13.2	28	77	0	0
2	1.9	5.2	10.7	91	0.8	6.3	16	60	0	0
3	4.4	9.1	10.4	102	2.2	10.8	29	85	0	0
4	7.7	10.6	10.4	55	2.2	9.2	52	81	0	0
5	4.4	7.7	11.6	88	3.3	13.8	49	115	0	0
6	4.2	6.9	8.1	62	3.0	9.2	27	59	0	0
					Phenylketonurics					
7	0.2	0.9	1.3	42	2.5	10.4	17	40	46	51
8	0	0.9	0.3	8	0.5	5.7	6	26	3	6
9	0.1	1.8	1.0	54	0.5	6.8	11	59	5	77
10	0.2	1.4	2.1	25	2.2	4.4	16	28	0	11
11	0.5	1.8	3.9	38	0.6	3.1	37	42	0	15
					Mongoloids					
12	4.3	12.0	7.0	41	2.2	5.7	85	31	0	0
13	6.8	11.2	8.7	47	3.7	7.8	25	44	0	0
14	4.1	8.7	11.2	47	4.4	6.3	29	51	0	0
15	10.2	15.5	15.6	65	6.1	13.1	67	62	0	0
16	6.2	9.2	11.1	54	7.2	11.5	63	75	0	0
					Undifferentiated mental defectives					
17	7.7	13.3	18.0	130	4.2	7.3	37	95	0	0
18	6.4	7.6	15.8	53	2.7	10.5	52	47	0	0
19	5.3	6.9	18.3	73	2.4	4.2	61	62	0	0
20	6.2	7.5	11.9	73	3.8	4.5	69	53	0	0
21	5.7	8.7	8.2	62	3.1	8.3	40	59	0	0