head-shake response, and it does not return when the reserpine is withdrawn.

A third group does not develop the symptom on solitary confinement. This group generally consists of 30 to 40 percent of the population of most mouse strains that we have tried, but in some cases it is much less.

It appears from the foregoing, that a head-twitch response may be produced in mice by solitary confinement. This response is in all known respects identical to that produced by LSD injected intravenously. The response can be made "permanent" either by treating the mice with indole followed by LSD or by long exposure (3 weeks) to solitary confinement. When it is produced in a "permanent" fashion, the response may be temporarily relieved or may be cured by treatment with reserpine.

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Distinction between Effects on Metabolic Transport and Passive Transfer of Ions

Studies showing that drugs, ions, metabolic inhibitors, and substrates induce or alter the net movement of sodium and potassium in nerve fibers have led to the recognition that such effects are brought about either by altering the metabolic reactions responsible for "active transport" or by modifying membrane permeability or the other electrochemical conditions for ion exchange and diffusion involved in "passive transfer" (*1–3*). A reliable method for distinguishing between effects on metabolism and on the passive properties of biological systems is increasingly necessary in the light of growing evidence for an intimate relationship between ionic movement and alterations in the physiological functioning of many cells (for example, *2, 4*).

It was previously found that while the net movements of ions are suggestive, they do not suffice as a definitive basis for such discrimination (*3*). On the other hand, research currently in progress with M. D. Berman on the unidirectional fluxes of ions in metabolically inhibited and uninhibited sciatic nerves of the toad (*5*) appears to provide one satisfactory approach to this problem. The purpose of this preliminary report is to provide two examples of the technique that demonstrate different types of results and to call attention to erroneous conclusions that may be drawn if metabolic and physical effects are not both considered as possibilities.

The approach consists of a comparison of the action of a given experimental agent or condition before and after metabolic inhibition on ionic flux. Anoxia combined with iodoacetate poisoning has been used as the standard procedure for inhibition because of evidence that this causes cessation of energy turnover (*6*). If, now, the result of treatment of an otherwise normal tissue duplicates that of metabolic inhibition, and this effect is absent when the tissue has been previously inhibited by anoxia and iodoacetate, then the original result is considered to be a consequence of interference with metabolic reactions. The effect of lowering the sodium content of the medium on potassium influx is in this category and is shown in Table 1.

The data in Table 1 were obtained by first exposing desheathed toad (*Bufo marinus*) sciatic nerves for 2 hours to normal or low-sodium Ringer's solution in the presence or absence of oxygen and 1 mmole/lit of sodium iodoacetate, then replacing these solutions for another 2 hours with similar solutions, except for the presence of K^{42}. Glass units identical with those previously described (*5*) assured good stirring and replacement of solutions without oxygen contamination when necessary. Conventional extraction and radioisotope-counting procedures served for measurement of the activity gained by individual nerves. The activity taken up, corrected for that in the extracellular space and for the small backflux, was converted to the equivalent uptake of potassium and divided by the time of exposure to K^{42} to give the influxes in Table 1 (and Table 2).

Our earlier studies demonstrated that metabolic inhibition depresses potassium influx in the desheathed toad sciatic nerve to one-third or one-fourth of that of controls (*5*). This can be verified in Table 1 by comparing the influx in oxygen with that in helium combined with iodoacetate treatment. In addition, Table 1 shows that reduction of the sodium content of the medium to 10 percent of the normal level under aerobic conditions also reduces the influx of potassium. This is true whether choline replaces sodium or whether sucrose replaces both sodium and chloride. The extent of the reduction of influx is about one-half as great as that produced by the combination of anaerobiosis and iodo-

Table 2. Potassium influx (μmole/g min) in paired desheathed toad sciatic nerves compared in Ringer's and in Ringer's with 0.1-percent cocaine with and without prior metabolic inhibition. As in Table 1.

Additional conditions	K^{42} exposure period	Control	Cocaine	Δ
O_2	2 hr	0.031 ± 0.0015	0.027 ± 0.0032	−0.0041 (6) ± 0.0025
O_2	4 hr	0.032 ± 0.0023	0.027 ± 0.0028	−0.0045 (6) ± 0.0028
He + IAA	2 hr	0.0113 ± 0.0005	0.0082 ± 0.0005	−0.0030 (8) ± 0.0004

Table 1. Potassium influx (μmole/g min), on a wet-weight basis, in desheathed toad sciatic nerves compared in Ringer's with normal sodium content and with 90 percent of the normal sodium replaced with choline or sucrose with or without metabolic inhibition. Δ is the mean difference with its standard error based on the differences of individual paired nerves on the same horizontal line. All variability is expressed as the standard error of the mean. The parenthesized figures give the number of experiments. The data in the last row are the only unpaired sets for Δ given to the right.

Other conditions	Choline chloride replacing sodium chloride			Sucrose replacing sodium chloride		
	100% Na	10% Na	Δ	100% Na	10% Na	Δ
O_2	0.042 ± 0.0019	0.030 ± 0.0022	−0.012 (16) ± 0.0024	0.048 ± 0.0019	0.034 ± 0.0021	−0.014 (6) ± 0.0022
He + 1 mmole/lit IAA	0.014 ± 0.0005	0.019 ± 0.001	+0.0054 (16) ± 0.0011	0.013 ± 0.0004	0.026 ± 0.001	+0.013 (4) ± 0.0008
		0.016 ± 0.0013			0.022 ± 0.0007	+0.0064 (4) ± 0.0018
		0.019 (20) ± 0.0011			0.024 (8) ± 0.0009	+0.005 ± 0.0014

acetate poisoning. But it is highly significant, as may be seen from the small standard error for the differences of paired nerves.

The reduction in aerobic influx in low sodium may therefore be the result of depressed oxygen consumption. Inhibition of respiration of nerve (*7*) by low sodium is known.

This interpretation gains further support from the absence of a decrease in potassium influx when the sodium is lowered while the nerves are being metabolically inhibited by the standard procedure. Indeed, the effect of decreasing sodium by 90 percent during inhibition is to produce a small but significant *increase* in potassium influx, which is slightly but significantly larger in sucrose than in choline.

The results in Table 1, therefore, lend themselves to the interpretation that a reduction of the sodium content of the medium reduces aerobic potassium influx by an inhibition of metabolism. In keeping with this, preliminary experiments have shown that under certain conditions potassium outflux can be increased by lowering sodium, as has also been found with metabolic inhibition (*5*). Since recent studies on the influence of external ion concentration on outflux assume that effects are due to the operation of ionic interchange, the present findings would suggest a careful evaluation of such interpretations. This is equally true for the effects of changes in the potassium content of the medium, since metabolic effects by the potassium ion are also well known.

The same approach to the action of a "stabilizer" such as cocaine presents an example of an effect on "passive" ionic transfer with little or no effect on active transport. Data demonstrating this are given in Table 2. Under conditions of metabolic inhibition, cocaine reduced potassium influx in each of eight paired preparations; the decrease is seen to be about 30 percent and is highly significant. Under normal aerobic conditions, whether measured over 2 or 4 hours, potassium influx is reduced by cocaine but proportionately much less; this decrease in influx in cocaine, although probably real, is on the borderline of significance. The absolute decrease in influx of the uninhibited nerves is of the same order as in inhibited preparations, as might be expected if the cocaine acts in the former on a passive influx that is of the same magnitude as the residual influx following inhibition. This is in keeping with other data, such as those in Table 1, which suggest that potassium influx in respiring nerves is the sum of a large metabolically dependent fraction and a small passive fraction (*5*).

The finding that cocaine does not markedly affect ionic transport through alteration of metabolic processes is consistent with the early demonstration of negligible respiratory effects by this and other "stabilizers" at blocking concentrations (*8*). It is also consistent with stabilizer action on the resting membrane potential being clearly demonstrable only under conditions of metabolic inhibition (*9*). The effect is probably on permeability, since other measurements demonstrate a comparable reduction of potassium outflux as well (*5*).

These experiments therefore appear to provide a valid means of discriminating between experimental effects by way of metabolic inhibition and through changes in permeability or other physical characteristics of living biological systems. It may be anticipated that both actions occur under some conditions.

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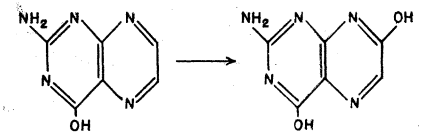
Conversion of 2-Amino-4-Hydroxypteridine to Isoxanthopterin in D. Melanogaster

A number of pteridines occurring in Drosophila (*1, 2*) have been isolated and characterized, and their relation to one another and to the red eye pigments has been discussed (*3*). One method of establishing metabolic relationships among them would be to demonstrate interconversions in cell-free extracts. Since these compounds increase greatly in amount during pupal life (*2*), this stage of development was studied first.

Young pupae of *Drosophila melanogaster* (Canton-S wild type) were ground with sand in a chilled mortar with an equal weight of 0.1*M* potassium phosphate buffer, *p*H 7.5, and the resulting mixture was centrifuged at 100,000*g* for 30 minutes. To 0.1 ml of the supernatant solution were added 0.1 ml of buffer and 0.02 ml of a solution (1 mg/ml) of the pteridine under examination in water or 0.05*N* sodium hydroxide. An aliquot (about 4λ) of the mixture taken prior to and following an incubation period of 1 hour at 25°C was chromatographed on Whatman No. 1 filter paper for 2.5 hours in propanol-1-percent ammonia (2/1). After it had dried in air, the chromatogram was examined under ultraviolet light (Keese lamp; maximum radiation at 360 mμ) for the presence of fluorescent materials. No changes were observed when 2-amino-4-hydroxy-6-(1,2-dihydroxypropyl)-pteridine, 2-amino-4-hydroxy-6-carboxypteridine, or 2-amino-4-hydroxy-6-carboxy-7,8-dihydro-N^8-lactylpteridine was added to the extract. However, the enzymatic oxidation of 2-amino-4-hydroxypteridine to isoxanthopterin (2-amino-4,7-dihydroxypteridine) was readily demonstrated.



2-AMINO-4-HYDROXY-PTERIDINE — ISOXANTHOPTERIN

In order to measure the rate of the reaction, the volume of the reaction mixture was increased in proportion to that described in the preceding paragraph, and 0.3-ml aliquots were deproteinized with 0.7 ml of 10-percent trichloroacetic acid (TCA). The amount of isoxanthopterin formed was measured by the increase in optical density at its absorption maximum, 340 mμ, using a Beckman model DU spectrophotometer. Treatment of the homogenate with charcoal (Norite-A), followed by a heat treatment of 10 minutes at 50°C, produced extracts with low blank readings, and these procedures were used to prepare the enzyme. Under the conditions described here, the enzyme was saturated with the substrate, and the rate of reaction was proportional to enzyme concentration. The *p*H optimum for the oxidation was about 7.5. Only a slight loss of activity was observed when the original extract was dialyzed; complete losses occurred when the extract was placed in a boiling water bath for 5 minutes. The enzyme is also present in larvae and adults.

This enzyme preparation from *Drosophila* pupae will also oxidize xanthopterin to leucopterin and xanthine to uric acid, all of these at approximately the same rate. It thus seemed likely that the activity was caused by xanthine oxidase, and, indeed, a preparation of this enzyme from fresh cream (*4*) oxidized 2-amino-4-hydroxypteridine to isoxanthopterin, and xanthine to uric acid, again at about the same rate. It has been reported (*5*) that 2-amino-4-hydroxy-