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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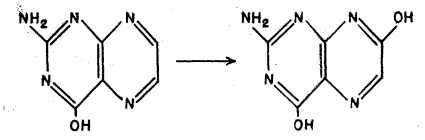
14 September 1956

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-

pteridine is oxidized by xanthine oxidase, although the product was not identified; and recently the production of isoxanthopterin by this reaction has been postulated to take place in the elytra of beetles, although no experimental details were given (*6*).

Since it is likely that a variation in the amount of isoxanthopterin would manifest itself during the synthesis of the eye pigments, 44 eye-color mutants of *Drosophila* (*7*) were tested qualitatively for the presence of the enzyme, in the following way. Larvae (15–20) which were just about to pupate were ground in a small test tube with a small amount of water (0.1 ml) and sand. 2-Amino-4-hydroxypteridine (0.02 ml of a solution of 1 mg/ml in 0.05*N* sodium hydroxide) was added, and aliquots were spotted on Whatman No. 1 filter paper immediately and at the end of 1 hour. The chromatogram was developed and examined in the same way as before. The great majority of the mutants had enzyme activity comparable to the wild-type controls. However, it appeared that maroonlike (*ma-l*) and maroon (*ma*) contained greatly reduced amounts of enzyme activity (*8*). Work is continuing on these phenomena.

There remains the question of the significance of this enzyme in the biosynthesis of pteridines. In order to show that the enzyme is active *in vivo*, white-apricot (w^a) larvae, which normally contain very small amounts of 2-amino-4-hydroxypteridine and isoxanthopterin (*2*, and unpublished data), were allowed to feed on powdered cellulose saturated with an aqueous solution of the former; after they had pupated, they were chromatographed according to the method of Hadorn and Mitchell (*2*). The chromatograms showed that 2-amino-4-hydroxypteridine had been ingested and that isoxanthopterin had been produced. It seems probable, therefore, that this enzyme is important in the biosynthesis of isoxanthopterin, and that 2-amino-4-hydroxypteridine is the immediate precursor.

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8. Since we submitted this paper, Hadorn and Schlink [*Nature* 177, 940 (1956)] have reported that the mutant rosy lacks isoxanthopterin. We have been able to show that this mutant also lacks xanthine oxidase. Further experiments in collaboration with Hadorn are in progress.

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13 June 1956

Modification of the Menstrual Cycle in Rhesus Monkeys by Reserpine

Reproductive function in the female rat is modified by reserpine. Gaunt *et al.* (*1*) found an alteration in the estrous cycle and a reduction in fertility. Barraclough (*2*) obtained inhibition of ovulation. Because of these observations, the possible influence of reserpine on the primate reproductive cycle was considered worthy of investigation. The dose used was in excess of that employed in clinical practice but was comparable to the dose used experimentally in rats and monkeys (*1*). Our purpose was to determine whether or not a maximal tranquilizing dose exerted a demonstrable effect on the menstrual cycle.

Adult, rhesus monkeys weighing between 4.4 and 8.6 kg were used. Three of the animals (909, 940, 921) had been pregnant, thus demonstrating their reproductive capacity. Reserpine (Serpasil, *3*) was administered subcutaneously to six monkeys in a dose of 1 mg/kg daily between 11 and 11:30 A.M. for periods ranging from 8 days to more than 100 days, Sundays excluded. The pattern of injection was of two types: (i) in some monkeys, daily injections of the drug were made for more than 100 days; (ii) in other monkeys, the drug was injected for a period of only 8 to 10 days, early in the cycle. Observations were made on (i) duration of the menstrual cycle, (ii) ovulation as ascertained by rectal palpation and checked by laparotomy, (iii) histological examination of ovarian and uterine tissues, and (iv) vaginal desquamation. Measurements of rectal, basal body temperature demonstrated no cyclic fluctuation. Three animals served as controls. Two of these animals later received a placebo, reserpine vehicle, in a volume equivalent to that which they would have received if the drug were being administered. Part of the remainder of the Carnegie colony served as additional controls with regard to length of the menstrual cycle.

Administration of reserpine daily for more than 100 days to three of the experimental monkeys produced a suppression of menstruation in each case (Fig. 1). In monkeys L52 and L53, in which the treatment was initiated toward the end of the summer anovulatory period, the expected bleeding occurred, but it was not followed by another menstruation until the drug was withdrawn. Laparotomy performed at the termination of the reserpine treatment revealed a failure of ovulation in each case (Fig. 1). Histological examination (*4*) in one monkey (L52) revealed a uterus under

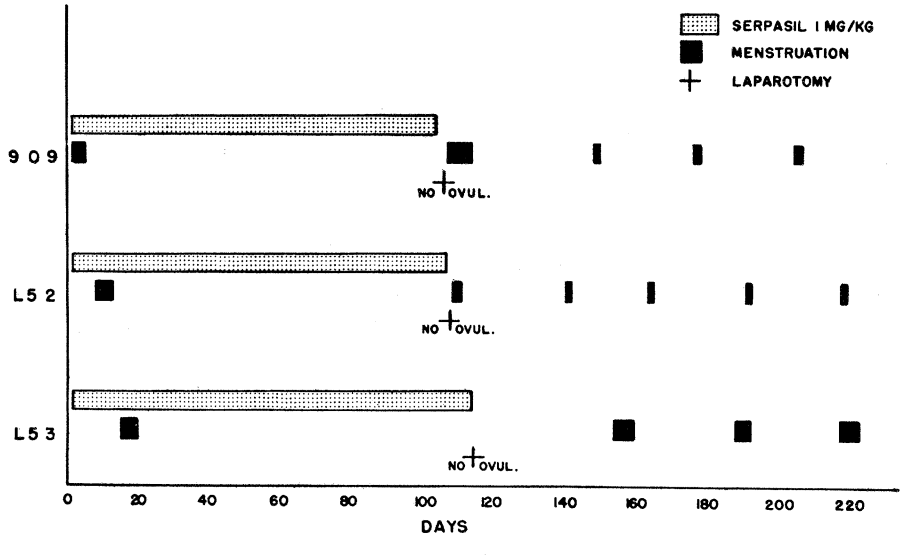

Fig. 1. Prolongation of the menstrual cycle following treatment with reserpine for more than 100 days. Width of the black bar indicates duration of bleeding.