

dicade clearly that the addition of sulfate is not very effective as a means of reducing radiostrontium pickup by crops grown on contaminated soils. Although the addition of soluble strontium does seem to have some effect, the principal reduction observed was that effected by the addition of potassium; for potassium, in amounts as low as about 60 lb per 2 million lb of soil (or about 30 lb per acre for normal 2-in. depth of penetration of water-soluble fallout), something like a 40-percent reduction of radiostrontium uptake was observed.

Although these experiments show that radish plants in certain kinds of soil certainly can utilize the strontium in strontium sulfate, and that the formation of radiostrontium sulfate does not necessarily reduce the uptake of radiostrontium, the positive effect of potassium is established. It is possible that other fertilizers or other additives may have a more marked effect than either the fertilizer or the Vermiculite used in this investigation.

The effects observed by Russell, Alexander, and Reitemeier may involve effects other than those tested here. Certainly one knows that, as strontium lies in the soil, it is very likely eventually to be incorporated into large crystals, in which form it will become physically unavailable to the plants. And so the possibility of chemical aging, taking place slowly over several years, exists. It does not seem likely, however, that this process will be of sufficient magnitude to restore heavily contaminated soil to a useful condition, and further work needs to be done on methods of quick beneficiation.

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#### References and Notes

- Hearings before the Special Subcommittee on Radiation of the Joint Committee on Atomic Energy, on "The Nature of Radioactive Fallout and Its Effects on Man" (27-29 May and 3 June 1957).
- R. Scott Russell, UNESCO Conference on Radioisotopes and Scientific Research, paper No. 175 (Paris, 1957).
- L. T. Alexander, U.S. Department of Agriculture, personal communication.
- R. F. Reitemeier, U.S. Department of Agriculture, personal communication.
- W. M. Latimer, *Oxidation Potentials* (Prentice-Hall, Englewood Cliffs, N.J., ed. 2, 1952).
- R. Bradfield, *J. Phys. Chem.* 36, 340 (1932).
- W. O. Robinson, R. H. Whetstone, G. E. Edgington, *U.S. Dept. Agr. Tech. Bull. No. 1013* (1950).
- M. Peech, C. Beck, K. Larson, R. Scott Russell, personal communications.
- E. T. York, Jr., R. Bradfield, M. Peech, *Soil Sci.* 76, 481 (1953).
- "On the Behavior of Fission Products in Soil, Their Absorption by Plants and Their Accumulation in Crops," *U.N. Rept. No. A/AC.82/G/R41* (24 Oct. 1956), translated from the Russian.

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## Purine Catabolism in *Drosophila melanogaster*

Recently, it has been demonstrated that an eye color mutant, *rosy*<sup>2</sup> (*ry*<sup>2</sup>), does not contain isoxanthopterin which occurs widely in *Drosophila* (1). It has been reported that 2-amino-4-hydroxypteridine (AHP) is oxidized to isoxanthopterin by an enzyme prepared from *Drosophila* (2, 3) and named pterine dehydrogenase (3); xanthine is also converted into uric acid by the same enzyme (2), and xanthine oxidase is also capable of oxidizing AHP to isoxanthopterin (4).

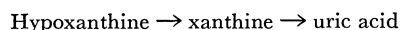
I have found that the mutant *ry* does not contain a trace of uric acid at any developmental stage. Therefore, purine compounds and the activity of xanthine oxidase both in a wild type (Oregon-R) and in the mutant *ry* of *D. melanogaster* were investigated. Purine compounds were detected by paper chromatography. The results are shown in Table 1.

It was discovered that *ry* does not contain isoxanthopterin at any developmental stage, but rather contains a larger amount of AHP than does the wild strain at the pupal stage. It is well known that, as a rule, uric acid is a final product of purine catabolism in insects. On the other hand, mutant *ry* accumulates a larger amount of hypoxanthine, instead of uric acid. The occurrence of hypoxanthine was identified by the absorption spectrum of material isolated from *ry*, and xanthine in pupae and adults of *ry* was also demonstrated by paper chromatography.

The uric acid content of *D. melanogaster* was determined by the reduction of optical density at 295 mμ (4). The wild strain has the enzyme, but the *ry* strain does not. Furthermore, it seems that the enzyme is a true dehydrogenase, because it requires methylene blue or diaphosphopyridine nucleotide (DPN) as an electron acceptor.

In some double mutants homozygous for *ry*, such as *v : ry*, *cn : ry*, *bw : ry*, and *se : ry*, neither isoxanthopterin nor uric acid is found to any extent in any developmental stage. Among them, *v : ry* and *cn : ry* have a light pinkish-red eye pigment, but *bw : ry* is similar in phenotype to *bw*, and *se : ry* is similar to *se* phenotypically. However, these strains have the same amount of hypoxanthine in each pupal stage as does the *ry* strain.

From these results, it seems that in *Drosophila* uric acid is produced from xanthine and hypoxanthine along the general pathway (5) shown in the following scheme.



The deficiency of both isoxanthopterin and uric acid in *ry* strains may be due to the lack of xanthine oxidase. There is

Table 1. Pteridines and purines occurring in strains Oregon-R and *ry* of *D. melanogaster*.

Substance	Larvae	Pupae	Adults
<i>Strain Oregon-R</i>			
AHP	±	+	±
Isoxanthopterin	±	++	+
Hypoxanthine and xanthine	±	±	±
Uric acid	±	+	+
<i>Strain ry</i>			
AHP	±	++	+
Isoxanthopterin	-	-	-
Hypoxanthine and xanthine	±	++	+
Uric acid	-	-	-

still a problem whether or not xanthine oxidase and pterine dehydrogenase are the same enzyme, and further researches are being carried out along this line (6).

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#### References and Notes

- E. Hadorn and I. Schwinck, *Nature* 177, 940 (1956); —, *Z. Induktive Abstammungs- u. Vererbungslehre* 87, 528 (1956); E. Hadorn, *Cold Spring Harbor Symposia Quant. Biol.* 21, 363 (1956).
- H. S. Forrest, E. Glassman, H. K. Mitchell, *Science* 124, 725 (1956).
- S. Nawa, T. Taira, B. Sakaguchi, *Proc. Japan Acad.* 34, 115 (1958).
- H. M. Kalkar, *J. Biol. Chem.* 167, 429 (1947).
- E. Baldwin, *An Introduction to Comparative Biochemistry* (Cambridge Univ. Press, Cambridge, England, 1949).
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## Cobalt Activation of Fatty-Acid Synthesis in Yeast Homogenates

**Abstract.** The incorporation of acetate into lipids in homogenates of *Saccharomyces cerevisiae* was inhibited at low concentrations of ethylenediaminetetraacetate, under both aerobic and anaerobic conditions. Of various cations tested, none could effectively reverse this inhibition. However, Co<sup>++</sup> completely restored the synthesis of fatty acids, but not of non-saponifiable lipids.

Previous reports from this laboratory have dealt with the synthesis of lipids in yeast cells (1) and in extracts prepared from yeasts (2). It has been shown that cell-free preparations incorporate acetate into various cellular lipids and that a particulate fraction consisting of uniform particles, of the order of 30 mμ in diameter, plus the soluble supernatant is required for this activity (2).

During the course of the studies de-

scribed in this report (3), it was found that the addition of ethylenediaminetetraacetate (EDTA) to crude homogenates severely inhibits the incorporation of acetate into lipids, and that cobalt reverses this effect specifically with regard to fatty acids. For these experiments, cells of *Saccharomyces cerevisiae*, strain LK2G12, were cultivated, harvested, and broken according to methods previously described (2). The resulting crude homogenates contained a large particle fraction, necessary for maximal respiration of the extracts, as well as the two fractions mentioned above. The homogenates were incubated for 4 hours in air, or under 100-percent CO<sub>2</sub>, as indicated in Table 1, after which they were hydrolyzed and assayed for radioactivity in the fatty acids and the nonsaponifiable lipids, as described earlier (2).

The results of representative experiments are recorded in Table 1; from these, several conclusions can be drawn. The addition of EDTA in final concentrations as low as 0.0025*M* resulted in drastic inhibition of acetate incorporation into lipids without significant lowering of the rate of oxygen uptake. Indeed, there was usually a concomitant increase in the rate of respiration in the presence of EDTA (Table 1, experiments 1 and 3). That the primary effect of this chelating agent is not on the energy-generating system of these homogenates was further indicated by the fact that EDTA

was similarly effective under anaerobic conditions (Table 1, experiment 2).

Various cations were added to this system in the presence of 0.0025*M* EDTA in order to ascertain whether any one of them would reverse the inhibitory effect, and the following proved to be ineffective at final concentrations of up to 0.005*M*: Ca<sup>++</sup>, Zn<sup>++</sup>, Fe<sup>++</sup>, Mg<sup>++</sup>, Cu<sup>++</sup>, Fe<sup>+++</sup>, Al<sup>+++</sup>, Ba<sup>++</sup>, and Sr<sup>++</sup>. Under these conditions, Ni<sup>++</sup> and Mn<sup>++</sup> were somewhat active in potentiating the effects of EDTA, while Co<sup>++</sup> consistently reversed the inhibitory effect of EDTA on the incorporation of acetate into the fatty-acid fraction but not its effect on the incorporation of acetate into the nonsaponifiable lipids (Table 1, experiments 2 and 3). Indeed, under aerobic conditions, the addition of Co<sup>++</sup> alone frequently increased the level of incorporation into fatty acids above that of the controls. It is interesting to note that, under anaerobic conditions, the concentration of cobalt in the system may be very critical. For example, the presence of 0.0025*M* cobalt alone routinely inhibited acetate incorporation significantly (Table 1, experiment 2). Upon the addition of an equimolar amount of EDTA to such a system, cobalt became a potent activator of fatty acid synthesis, thus suggesting that the EDTA effectively tied up the excess (inhibitory) cobalt.

Earlier studies on the effect of chelating agents on the synthesis of lipids by

intact rat liver cells (4) indicated that EDTA was ineffective in reducing acetate incorporation. However, since the possibility exists that the cells were impermeable to this substance, it may be unwarranted to compare the results of that study to those reported here. Of greater interest are the recent observations (5) that EDTA causes a deterioration or degradation of the particulate matter of microbial homogenates and that certain cations protect against this effect. It may well be, therefore, that acetate incorporation is reduced, in these studies, because of the loss of particle structure, rather than because of a direct effect on one or more enzymes concerned in the biosynthesis of lipids. Nevertheless, studies are now in progress to test the effect of EDTA and cations on enzymes involved in fatty-acid synthesis. The first series of experiments, designed to test the acetate-activating enzyme (6) in these homogenates, revealed this enzyme to be relatively insensitive to EDTA. For example, on the addition of EDTA at a final concentration of 0.0025*M*, acetate activation was decreased by about 20 percent. Furthermore, cobalt did not protect against this small degree of inhibition. Thus, this enzyme does not appear to be directly involved in the cobalt stimulation of fatty-acid synthesis in these homogenates.

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Table 1. Effect of ethylenediaminetetraacetate and cobalt on acetate incorporation into lipids in extracts of *Saccharomyces cerevisiae*. Vessels for these experiments were set up in duplicate and contained 1.5 ml of yeast homogenate (25 to 30 mg of protein), 5  $\mu$ mole of adenosine triphosphate, 3  $\mu$ mole of acetate ( $5 \times 10^{-5}$  count/min), and additions, as indicated, in a total volume of 1.9 ml. All experimental values were obtained by averaging the results from each set of duplicates.

Additions	Gas Phase	Acetate incorporation		$Q_{O_2}^*$
		Nonsaponifiable lipids (count/min)	Fatty acids (count/min)	
<i>Experiment No. 1</i>				
None	Air	16,200	23,500	4.2
EDTA (0.0006 <i>M</i> )	Air	15,000	15,280	8.2
EDTA (0.0009 <i>M</i> )	Air	9,800	8,800	8.1
EDTA (0.0013 <i>M</i> )	Air	7,240	5,000	9.1
EDTA (0.0025 <i>M</i> )	Air	2,450	1,860	8.2
EDTA (0.005 <i>M</i> )	Air	1,100	1,200	8.4
<i>Experiment No. 2</i>				
None	CO <sub>2</sub>	31,400	96,500	
EDTA (0.0025 <i>M</i> )	CO <sub>2</sub>	2,300	12,700	
EDTA (0.0025 <i>M</i> ) + CoCl <sub>2</sub> (0.0025 <i>M</i> )	CO <sub>2</sub>	4,300	218,000	
CoCl <sub>2</sub> (0.0025 <i>M</i> )	CO <sub>2</sub>	16,400	47,000	
<i>Experiment No. 3</i>				
None	Air	7,600	14,400	3.7
EDTA (0.0025 <i>M</i> )	Air	800	2,200	5.8
EDTA (0.0025 <i>M</i> ) + CoCl <sub>2</sub> (0.005 <i>M</i> )	Air	1,800	27,400	5.7
CoCl <sub>2</sub> (0.005 <i>M</i> )	Air	6,200	21,300	3.1

\* Q<sub>O<sub>2</sub></sub> refers to microliters of oxygen consumed per hour per milligram of protein.

#### References and Notes

1. H. P. Klein, N. R. Eaton, J. C. Murphy, *Biochim. et Biophys. Acta* 13, 591 (1954); H. P. Klein, *J. Bacteriol.* 69, 620 (1955).
2. H. P. Klein and Z. K. Booher, *Biochim. et Biophys. Acta* 20, 387 (1956); H. P. Klein, *J. Bacteriol.* 73, 530 (1957).
3. This study was supported, in part, by a research grant (H2421) from the National Institutes of Health, U.S. Public Health Service. The technical assistance of Helen Berman is also gratefully acknowledged.
4. G. L. Curran, *Proc. Soc. Exptl. Biol. Med.* 88, 101 (1955).
5. F.-C. Chao, *Arch. Biochem. Biophys.* 70, 426 (1957); E. T. Bolton, *Abstr. Biophys. Soc.* 15 (1958); W. Gillchrist and R. M. Bock, *ibid.* 15 (1958).
6. F. Lipmann, M. E. Jones, S. Black, R. M. Flynn, *J. Am. Chem. Soc.* 74, 2384 (1952).

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#### Inhibition of Human Plasma Cholinesterase in vitro by Extracts of Solanaceous Plants

While attempting to determine residues of organic phosphorus insecticides in various plant tissues by the method of cholinesterase inhibition, we found that potato (*Solanum tuberosum* L.) tissue presented an unexpected problem (1). Aqueous extracts of foliage and tubers which had not been treated with insecticide still gave a positive test—that is,