Evidence that Molybdenum Is a Nondialyzable Component of Xanthine Oxidase[1, 2]

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Isolation and purification of xanthine oxidase from the milk of cows that have received Mo^{99} has provided evidence that molybdenum is an integral part of the xanthine oxidase of milk and, presumably, of other sources. This finding is in accord with the evidence of Westerfeld and Richert (*1*) for the existence of a nutritional factor essential for the production and maintenance of rat intestinal xanthine oxidase and the identification of this factor as molybdenum by De Renzo *et al.* (*2*).

Labeled sodium molybdate was injected intravenously into a dairy cow and xanthine oxidase was isolated by the method of Ball (*3*) from milk collected one to several days thereafter. This procedure was repeated with the same cow 2 wk later. In all cases after the initial removal of excess molybdenum by one or two treatments with 25–40% ammonium sulfate, the ratio of Mo^{99} to xanthine oxidase activity remained essentially constant despite rigorous purification procedures, as indicated below for a typical sample. Xanthine oxidase was estimated by Kalckar's method (*4*) and Mo^{99} by radioassay procedures (Table 1).

That the Mo^{99} was not removed from, and probably did not enter, the xanthine oxidase merely by exchange was demonstrated by addition of 2 g of ammonium molybdate to a sample of milk from the treated cow and subsequent isolation of the enzyme. This treatment with inert molybdenum did not change the ratio of Mo^{99} to xanthine oxidase. In another dilution experiment, nonradioactive milk (126 units of xanthine oxidase) was mixed with an equal amount of radioactive milk (46 ct/sec/unit of xanthine oxidase; 10.6 ct/sec/unit of xanthine oxidase after purification; 91 units of xanthine oxidase). After isolation of the enzyme and dialysis against water for 40 hr, the xanthine oxidase had a value of 4.23 ct/sec/unit in good agreement with the value of 4.43 expected if there were no exchange of the excess Mo^{99} with the inert molybdenum of the nonradioactive xanthine oxidase.

Spectrophotometric data suggest a molar ratio of flavin to molybdenum of 2:1.

Molybdenum has been recognized for a long time as an essential nutrient for plants, serving metabolic functions involved in the utilization of nitrogen. It would now seem that molybdenum also plays an essential role in animals, at least to the extent that xanthine oxidase is concerned with necessary metabolic processes. In addition, the toxic behavior of excess molybdenum in the copper-molybdenum imbalance may possibly be explained in terms of oxidase activity.

TABLE 1

	Mo^{99} Ct/sec/unit xanthine oxidase
Milk	1095
Cream	71
Na_2HPO_4 extract	67
Na_2HPO_4 extract after $CaCl_2$ treatment	88
Xanthine oxidase after 1st $(NH_4)_2SO_4$ ppt	12.1
Xanthine oxidase after 2nd $(NH_4)_2SO_4$ ppt	12.6
Xanthine oxidase after 20-hr dialysis	10.9
Xanthine oxidase after 40-hr dialysis	10.6
Average for 4 different purified samples (7 milkings)	10.61 (range 10.4–10.78)

References

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Manuscript received July 14, 1953.

[1] Work at the Oak Ridge National Laboratory performed under contract No. W-7405-eng-26 for the Atomic Energy Commission.

[2] Publication approved by the Director of the University of Tennessee Agricultural Experiment Station.

Intracellular Localization of Labeled Nucleic Acid Determined with Autoradiographs[1]

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Autoradiographs have been used to detect incorporation of phosphorus (P^{32}) into the deoxyribose nucleic acids (DNA) of individual nuclei (*1, 2*). In this way the time of synthesis of DNA in relation to the division cycle during mitosis and meiosis has been determined. The method is also applicable to the study of phosphorus incorporation into the ribonucleic acids (RNA) of single cells. By applying a thin photographic emulsion layer to serial sections of large cells and making autoradiographs as previously described (*2*), the radioactive element can be located with sufficient precision to determine its intracellular distribution. For example, in the large cells of the gastric caeca and salivary glands of the third instar larvae of *Drosophila,* the relative rates of incorporation of P^{32} into the RNA of cytoplasm, chromatin, and nucleolus can be followed.

Early third instar larvae of *Drosophila repleta* were

[1] The work reported here is supported by a grant from the Atomic Energy Commission, Contract AT(30-1)-1304.