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### Minerals in the Livers of Protein Deficient Rats

SPECIFIC protein deficiencies can be induced in animals kept on a strict diet lacking protein but otherwise complete. There is little information in the literature about the mineral content of the organs of such animals, especially concerning trace elements. The present work compares the mineral content of the liver of protein deficient rats with that of control rats fed a non-deficient diet.

Mineral analyses were conducted as follows. Samples of liver from animals on normal and experimental diets were decomposed by boiling with sulphuric acid and nitric acid in a reflux apparatus. The extracts were evaporated. A reagent blank was prepared using a known volume of potassium sulphate which was spectrographically pure. Trace elements were estimated by a semi-quantitative spectrographic method using external standards<sup>1</sup>. The dried extracts were mixed with powdered graphite and placed in a graphite electrode which was placed in a continuous arc of 10 Å. The spectrum was photographed in the regions from 2500 Å to 4000 Å with a Hilger spectrograph with quartz optics, and from 4000 Å to 9000 Å using a Huet spectrograph with glass optics. Samples were compared with appropriate standards.

Potassium and sodium were estimated by flame spectrometry. A Hilger medium spectrograph with an electron photomultiplier receiver was used.

In the first experiment, which lasted 50 days, the animals were separated in ordinary metallic cages. Three animals received the protein deficient diet and three were fed one of the control diets<sup>2</sup>. Liver analyses showed a higher content of rubidium and molybdenum in animals fed the control diet. A second experiment was carried out on a larger scale; ten rats were given the deficient diet and nine the control diet, while four were allowed to eat as much as they could. In addition, they were kept in individual glass and hard plastic cages to avoid any contact with metal. After 50 days the mean body weight of the deficient rats fell from 163 to 103 g, the haemoglobin concentration from 84.4 to 66.1 per cent, and the total protein serum from 65.6 g/l. to 42.7 g/l. The livers were analysed for mineral content and the results are given in Tables 1 and 2.

Only three results show a statistically significant difference between the animals on protein deficient and control diets with regard to sodium molybdenum and rubidium content. Analyses of the diets showed that the differences

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Table 1. TRACE ELEMENTS IN LIVERS OF ANIMALS FED PROTEIN DEFICIENT AND CONTROL DIETS (p.p.m.)

Experimental diet	Number of animals	Trace elements										
		Manganese	Molybdenum	Tin	Vanadium	Copper	Silver	Nickel	Cobalt	Chromium	Lithium	Rubidium
No protein	10	3.70	0.57*	0.74	<0.05	3.88	<0.05	0.31	<0.07	0.49	<0.05	0.38*
Control, fed to the first nine rats of deficient group	9	3.76	0.79	0.57	<0.05	3.98	<0.05	0.33	<0.06	0.46	<0.05	1.48
Control, fed freely as much as they wanted	4	3.63	0.84	0.51	<0.05	3.96	<0.05	0.27	<0.05	0.59	<0.05	1.12

\* Average statistically different from that of animals on control diet (variance analysis).  
The method used is not sensitive enough for the determination of caesium.

Table 2. ALKALI ELEMENTS IN LIVERS OF RATS FED DEFICIENT AND CONTROL DIETS

Experimental diet	No. of animals	Sodium			Potassium			Rubidium			Ratio of potassium/sodium	Rubidium/potassium × 1,000
		Fresh liver (% <sub>100</sub> )	Total liver (mg)	In liver/100 g of animal	Fresh liver (% <sub>100</sub> )	Total liver (mg)	In liver/100 g of animal	Fresh liver (p.p.m.)	Total liver (γ)	In liver/100 g of animal		
No protein	10	0.088*	3.55	3.44	0.298	12.00	11.65	0.38*	1.60	1.55	3.3	0.12
Control, fed to the first nine rats of the deficient group	9	0.079	3.76	2.29	0.311	14.65	8.93	1.48	6.85	4.17	3.9	0.47
Control, fed freely as much as they wanted	4	0.093	7.92	2.63	0.317	25.39	8.43	1.12	8.90	2.96	3.2	0.35

\* Average statistically different from that of animals on control diet.

in molybdenum and rubidium were not due to a different intake of these elements. The minerals were estimated in whole fresh liver and so we cannot deduce the relationship between sodium and water content. The small but significant difference in molybdenum content is easily explained by the decrease in xanthine oxidase concentration, which is known to accompany the protein deficiency<sup>3</sup>. The differences in rubidium concentration are important; there is about four times as much in the livers of control rats as in those of protein deficient ones, and the potassium content remains about the same.

The observed differences are difficult to interpret. Relman<sup>4</sup> and other workers have noticed in their experiments a preferential retention of rubidium compared with potassium. In the present work the turnover in protein deficient animals is likely to be slower than in controls, which could explain, assuming the hypothesis of preferential retention of rubidium, the lower level of rubidium compared with potassium in protein deficient animals.

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<sup>1</sup> Pinta, M., *Recherche et Dosage des Éléments Traces*, 382 (Dunod, Paris, 1962).

<sup>2</sup> Gaudin-Harding, F., and Jacquot, R., *Ann. Nutr. Aliment.*, **19**, 1 (1965).

<sup>3</sup> Litwak, G., Williams, J. N., Feigelson, P., and Elvehjem, C. A., *J. Biol. Chem.*, **187**, 605 (1950).

<sup>4</sup> Relman, A. S., Lambie, A. T., and Burrows, B. A., *J. Clin. Invest.*, **26**, 1249 (1957).