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Mineral Metabolism—Copper and Iron.

S. W. KLETZIEN, K. W. BUCHWALD AND L. HUDSON.
(Introduced by R. S. Hubbard.)

From the State Institute for the Study of Malignant Disease, Buffalo, N. Y.

Iron and copper have long been known to play important rôles in respiratory phenomena, oxidation and reduction, enzyme action, pigment formation and in other vital phenomena. The importance of these elements, particularly the former, has been emphasized by the work of Warburg on the respiratory processes occurring in neoplastic tissues. Scott and co-workers[1] have shown that an apparent abnormal iron metabolism obtains in neoplasms. These findings, and those relating to hemochromatosis involvement in primary carcinoma of the liver and the failure in the treatment of neoplasms generally by means of specific chemical agents, have caused us to seek more information as to the metabolism of these elements in the animal body.

The work reported here deals with the influence of large amounts of copper sulphate on the magnitude of the iron assimilation of the various tissues of rats. The copper sulphate was fed as an integral part of an otherwise normal diet. Control animals received the same diet without the addition of copper sulphate. Daily consumption of the respective diets was equalized and recorded. Hence the diets of the 2 groups of animals differed only in the amount of copper as copper sulphate ingested. Distilled water was available to the animals at all times. Three feeding series were run, involving the use of 62 animals, of which 32 served as controls, the remainder receiving the high copper containing diet. All animals were maintained on screens and in individual cages throughout the feeding period of 12 weeks. In each series particular attention was given to securing uniformity of animals in the respective groups, distributing them not only according to weight and age, but also according to sex and litter. The animals were 4 to 5 weeks old when placed on experiment.

The iron intake for all animals averaged 2 mg. per day over the 12-week period. The copper intake of the controls, derived from the natural components of the diet and an inorganic salt mixture, averaged 0.033 mg. per day over the same period. The daily cop-

[1] Scott, G. H., and Horning, E. S., *Am. J. Path.*, 1932, **8**, 329.

per intake of the animals on the high copper diet averaged 5.43 mg. or approximately 165 times that ingested by the controls.

After 12 weeks the tissues of these animals were prepared according to the technique described in the previous paper. Iron determinations were made according to Elvehjem's modification[2] of Kennedy's method.[3] Copper determinations were made according to Elvehjem's and Lindow's modification of the Biazzo method as further modified by Gebhardt and Sommer.[4] Our results follow. In the first 2 series insufficient material prevented our obtaining both copper and iron values on all tissues. With the introduction of the use of chloroform to remove the suspended copper sulphide on precipitation with hydrogen sulphide we are now able to make copper and iron determinations on the same sample of material.

TABLE I.
Iron and Copper Content of Tissues from Rats Receiving a High Intake of Copper as Copper Sulphate (Dry Weight Basis).

		Controls				Copper-fed		
No. Animals	Tissue	% H_2O	Fe mg./100 gm.	Cu mg./kilo	No. Animals	% H_2O	Fe mg./100 gm.	Cu mg./kilo
12*	Liver	73.8	68.20	12.40	10*	74.0	82.40	665.00
	Spleen	76.9	†	27.70		77.5	†	20.90
11 (Z)	Liver	75.9	61.00	16.30	11 (Z)	75.0	63.40	735.00
9 (Z)	Bones	21.1	6.15	1.65	9 (Z)	24.9	7.21	1.67
	Brain	77.0	8.90	10.40		76.2	13.10	10.60
	Heart	73.6	34.60	21.20		74.3	50.30	20.70
	Kidney	80.0	22.60	25.60		80.3	24.30	58.50
	Liver	73.2	88.00	10.30		71.0	91.10	301.00
	Lung	76.1	21.60	17.40		78.8	27.30	16.70
	Muscle	72.5	9.55	1.55		71.5	10.50	1.44
	Spleen	74.5	286.00	32.80		74.6	303.00	20.20
	Testicle	86.0	23.40	15.80		84.5	29.40	14.30

* Bled from carotid. (Z) Perfused. † No iron determination made.

As to our findings we wish to point to the small, nevertheless distinct and consistent increases in the iron content of all the tissues analyzed and the definite decrease in the copper content of the spleens of the animals on the high copper diet as compared to the control animals. Whether this signifies an increased utilization of iron by the tissues is not of course established by this limited amount of data. The authors are inclined to believe, however, that the find-

[2] Elvehjem, C. A., *J. Biol. Chem.*, 1930, **86**, 463.

[3] Kennedy, R. P., *J. Biol. Chem.*, 1927, **74**, 385.

[4] Gebhardt, H. T., and Sommer, H. H., *Ind. and Eng. Chem., Anal. Ed.*, 1931, **3**, 24.

ings do so signify. If iron is better utilized by the tissues in the presence of copper then we may ascribe to copper an additional function in iron metabolism other than its function in making iron available for hemoglobin synthesis. We offer no explanation for the decrease in the copper content of the spleens of animals receiving the high copper diet except that they seem unusual. Pending further experiments involving the use of higher levels of iron intake and more varied intakes of copper we reserve the privilege of making a more definite interpretation of the results here reported.

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Production of Exclusively Thecal Luteinization and Continuous Oestrus with Anterior-Pituitary-Like Hormone.

H. SELYE AND J. B. COLLIP.

From the Department of Biochemistry, McGill University, Montreal, Canada.

The fact that rats do not respond to anterior-pituitary-like hormone (A.P.L.) in the first days of life has been observed repeatedly, and the conclusion has been drawn that they should not be used as test objects for this hormone earlier than the 18th to the 21st day of life. In an attempt to find an explanation for this fact we injected a series of 30 rats daily with A.P.L., starting on the 6th day of life. Although no mature follicles or corpora lutea had been formed after 10 injections and the ovary did not differ macroscopically from that of an untreated rat of the same age, histological examination of this organ reveals very pronounced changes. The thecal cells were very much enlarged and assumed the appearance of corpus luteum cells, whereas the granulosa cells were not luteinized and no signs of ripening of the follicles could be detected. These experiments show that at a very early age A.P.L. is unable to induce follicular maturation or the formation of normal or atretic corpora lutea; however, it does lead to luteinization of the thecal cells and thereby to the formation of thecal corpora lutea. These structures are not very prominent and therefore they can be readily overlooked upon macroscopical examination. (Fig. 1.) Histologically they are composed of a peripheral ring of corpus luteum cells and a central part of a few rows of normal granulosa cells in the center of which the ovum is included. (Fig. 2.)

It is difficult to find a satisfactory explanation for these observa-