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The Rôle of Boron in the Diet of the Rat.*

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Boron is of practically universal occurrence in the plant kingdom. Hence it is apt to be present in almost all classes of foodstuffs, and therefore occurrence of this element might be equally widespread in the animal kingdom and perhaps might play an equally important rôle in the physiology of the animal organism. At present little is known regarding the action or even the distribution of boron in animals. There are but few reports on the distribution of this element in animal tissues.[1-4] These few briefly-reported experiments indicate the necessity for further knowledge of the rôle of boron in the animal organism. As far as was known at the time this investigation was started, no data were on record as to whether boron is a dietary essential for animals. It seemed advisable, therefore, to determine whether boron is required for the growth and development of the rat or whether it is present in the animal body as an accidental constituent ingested with all foods.

Experimental. Experience with the study of other trace elements has shown that to do so effectively, a diet extremely deficient in the element must be used. The natural foods commonly used in purified rations were too abundant in boron. Cow's milk, although lower in this element, is not as low as is desirable and furthermore, a dry ration is preferable for such studies. Hence an attempt was made to produce such a diet depleted of its boron content. Naftel's[5] micro method, using a photoelectric colorimeter, was employed for the determination of boron in the various foods studied. The ashing procedure was somewhat modified by using the overhead heater which has proven advantageous in the matter of time saved and in the prevention of loss of boron due to excessive smoking and swelling

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1 Bertrand, G., and Agulhon, H., *Compt. rend.*, 1912, **155**, 248; 1913, **156**, 732, 2027.

2 Wright, N. C., and Papisch, J., *Science*, 1929, **69**, 78.

3 Blumberg, H., and Rask, O. S., *J. Nutr.*, 1933, **6**, 285.

4 Drea, W. F., *J. Nutr.*, 1934, **8**, 229; **10**, 351; **16**, 325.

5 Naftel, J. A., *Ind. and Eng. Chem. Anal. Ed.*, 1939, **11**, 407.

during the preliminary heating of the material. The overhead heater was also used instead of the water bath described in the original method, for it was found that the temperature could be much more easily controlled by this means. First, a survey was made of purified natural foodstuffs used in the diet of the rat. The foods containing the smallest amounts of boron were selected and the removal of their boron was attempted. Strong acid and methyl alcohol were used for this purpose in an attempt to remove the boron in the form of the methyl borate. Investigation of the extraction of boron with methyl alcohol showed first that quantitative recovery of boron used by this means was seldom obtained. By increasing the number of extractions or decreasing the amount of food to be extracted the results were not improved. Furthermore, this procedure was found unsatisfactory, first because complete elimination of the element appeared impossible, and secondly, in the cases where the boron was largely separated from the food, particularly in the case of protein, the food was so altered in its nutritive properties that it was not satisfactory for nutritional studies. It was evident at this stage, therefore, that the best solution to this problem was the preparation of an adequate diet composed of foodstuffs as low in boron as could possibly be found without attempting the removal of the element.

At about this time the report of Hove and associates[6] on their studies of boron in animal nutrition appeared. Observations were then available in this laboratory, using a diet in connection with an experiment conducted for other purposes, but the boron content of which was practically the same as that of the Wisconsin experimenters. The various components of this diet along with others used in our laboratory had been tested for boron. The composition of this ration was considerably different from that used by Hove and his co-workers.[6] Its total boron content was 163 μg per kg as compared with 155 μg of the diet described by the above mentioned investigators.[6] It was thought, therefore, that it might be of interest to record our findings as further evidence of the results already reported,[6] particularly since the animals fed the boron-low diet in this laboratory have been observed for a longer period of time and in somewhat greater detail.

The lactalbumin was prepared by the Harris Laboratories from fresh centrifuged milk. It is a product of high chemical purity, its ash content being but 0.72%. It was found to be extremely low in boron. Wheat gluten and gelatin contained about the same concentration

[6] Hove, E., Elvehjem, C. A., and Hart, E. B., *Am. J. Physiol.*, 1939, **127**, 689.

Composition of Diet	
Lactalbumin	10.0
Wheat gluten	4.0
Gelatin	4.0
Salt mixture No. 22	5.7
Purified sweet butter fat	8.0
Dextrose	to 100
Viosterol 15 drops per kilo	
Thiamin hydrochloride — 20 μg per rat per day	
Liver concentrate ≡ 3.5 g fresh liver per rat per day	
Vitamin E concentrate 3 mg per rat per day	

Composition of Salt Mixture No. 22	
$CaCO_3$	1.08
$CaHPO_4 . 2H_2O$	.72
KCl	.85
$MgSO_4$	.50
$NaH_2PO_4 . H_2O$	1.14
NaCl	1.24
NaI	.00015
$Na_2SiO_3 . 9H_2O$	.035
$FeSO_4(NH_4)_2SO_4 . 6H_2O$	.07
$CuSO_4 . 5H_2O$	.025
$MnSO_4 . 4H_2O$	.005
$ZnCl_2$	.01
	5.7 g in 100 g diet

of boron as this highly purified lactalbumin; therefore these 3 were selected as the sources of protein for this diet. Dextrose was the carbohydrate found to be lowest in boron. Sweet butter fat remelted and filtered served as the source of fat and vitamin A. Viosterol, a liver concentrate† especially prepared by the Lederle Laboratories and vitamin E concentrate prepared by the Mackenzie method[7] did not, in the amounts analyzed, show the presence of boron. The salt mixture contributed the greatest amount of boron of any of the ingredients of this diet.

Litter mates weighing 35-40 g were used in this experiment. These animals were housed in galvanized wire cages in the regular animal room of this laboratory. Monel metal or porcelain feeding cups and soft glass‡ drinking tubes were used.

Growth and reproduction on this diet were studied.

Extensive histological studies were also made on the tissues of these animals.

Results. The growth in body weight was observed and the food consumption and water intake were recorded; the average daily intake for the experimental period of 34 weeks being 9.6 g of diet per male and 7.8 g per female. The average daily boron intake was 1.56 μg per male and 1.27 μg per female. The general appearance of these animals was good and the rate of growth was normal. The growth of rats on this ration is shown in Table I. It is of interest to note that during the first 6 weeks these animals averaged a daily food intake of 5.2 g containing 0.85 μg boron. This compares well with the observations of the Wisconsin experimenters.

† I wish to thank the Lederle Laboratories, Inc., for supplying this material.

7 Mackenzie, C. G., Mackenzie, J. B., and McCollum, E. V., *U. S. Public Health Rep.*, 1939, **53**, 1779.

‡ Common soft glass is reported to be free from boron.[8]

8 Berger, K. C., and Truog, E., *Ind. and Eng. Chem., Anal. Ed.*, 1939, **11**, 540.

TABLE I.
Growth of Rats on Boron-low Diet.

	No. of rats	Avg wt in g per day	Avg daily food consumption in g	No. weeks avg
Males	12	3.4	9.6	34
Females	11	2.2	7.8	34

The females on this synthetic diet were allowed to carry through 3 litters each, averaging 7 young per litter which were born living, and normal in appearance and weight and which they successfully raised. Further matings were not carried out.

Detailed pathological studies of the tissues of the rats at the end of 34 weeks' experimental period showed that they were normal in every respect.

Since the diet has been used primarily in another investigation, the effect of supplementing it with boron in various concentrations has not been observed and, hence, no comparison can be made between the animals fed the diet described and rats receiving boron in greater concentrations.

The data presented here confirm the findings of Hove, Elvehjem and Hart[6] that if boron is actually essential for growth and development of the rat, it must be in extremely small amounts since an average of 1.27-1.56 μg per rat daily for a period of 34 weeks apparently satisfied its requirement for normal function. It is possible that a deficiency of this element would become apparent in the second or later generations when the boron store might become markedly depleted.

Summary. A synthetic diet is described which has a boron content of 163 μg per kg. This diet supports good growth and development in the rat. Food consumption compares well with that of animals on good purified diets used in this laboratory. The reproductive processes are normal. The young born to mothers on this boron-low diet appear normal and are successfully raised. Pathological studies showed the tissues of these rats to be normal. No evidence is obtained under the experimental conditions that boron is essential in the nutrition of the rat.