A Nutritional Requirement for Bromine. (22431)

JESSE W. HUFF, DAVID K. BOSSHARDT, ORIN P. MILLER, AND RICHARD H. BARNES.
Merck, Sharp and Dohme Research Laboratories, West Point, Pa.

It has been shown by Bosshardt and Huff (1) that the incorporation of an iodinated casein with thyroid activity (Protamone)* into a low-fat diet caused a growth retardation of mice. This depression of growth rate could be prevented partially by feeding fat and completely prevented by feeding cottonseed meal.†

The present study was undertaken to elucidate further the nature of the factor, or factors, in natural materials required by hyperthyroid mice.

Methods. The basal rations employed in these studies had the composition shown in Table I. All substitutions were made at the expense of equal weights of white dextrin. The various diets were fed *ad libitum* to groups of 20-day-old weanling male albino mice obtained from the Sharp and Dohme colony. All studies were of 12 day duration except for one study that was carried for 25 days. In any given experiment groups of 16 mice were employed. The extent of replication of a group is evident from the Table where total number of mice is given.

Results. Diet A (Table I) supported excellent growth in mice. However, when Protamone and Succinylsulfathiazole (SST) were added to this diet a growth retardation resulted. It is to be noted that Diet B, containing Protamone and SST, also contained 0.5% ribose nucleic acid. The ribose nucleic acid was added since it has been shown(2) that under certain dietary conditions an SST-induced growth inhibition in mice could be prevented partially by feeding ribonucleic acid or certain purines or purine derivatives.

It was found that if whey was added to Diet B, a partial reversal of the Protamone-SST induced growth retardation was obtained. The results shown in Table II indicate that the factor(s) in whey responsible for this reversal is water soluble, not adsorbed by Norit or a cation exchange resin (IR 120), and is present in the ash of whey. A dried sea salt preparation, Trace Element Sea Salt (TESS)‡ was found to possess activity similar to that of whey ash (Table II). Since this product was readily obtainable and a very complete mineral analysis was available, it was used as a reference substance for further study.

Chemical studies on TESS that are summarized in Table III show that the activity was adsorbed from water by an anion exchange resin (Dowex-1) and was not adsorbed by a cation exchange resin (IR 120), suggesting that the active material was either an anion or a cation capable of forming an anion. An analysis of TESS, obtained from the Trace Elements Corp., showed the presence of 39 elements. From this list were eliminated those elements that were present in the basal diet, those cations that could not form anions and those anions or anion formers present in quantities so small as not to be expected to stimulate growth under the test conditions. There remained the following 5 elements: aluminum, boron, bromine, fluorine, and rubidium. A mixture of salts of these 5 elements was prepared and fed to mice receiving Diet B in an amount equivalent to that supplied by the addition of 1% TESS to the diet. As shown in Table III this mixture was effective in reversing the Protamone-SST induced growth retardation. The components of this mixture were fed individually in amounts equivalent to those supplied by the addition of 1% TESS to the basal diet. No effect was obtained with the salts of aluminum, boron, fluorine, or rubidium. However, a complete reversal of the Protamone-SST induced growth retardation was obtained with bromine supplied as sodium bromide. Although most studies were of 12 days duration the effects of TESS and bromine were also evident in studies carried for 25 days.

Since it was improbable that a deficiency of a mineral could be induced by a bacterio-

* Cerophyll Laboratories, Inc., Kansas City, Mo.

† Proflo, Traders Oil Mill Co., Fort Worth, Texas.

‡ Trace Elements Corp., Houston, Tex.

TABLE I. Composition of Basal Diets.

	A	B	C
Hydrogenated cottonseed oil*	10.0	10.0	—
Linseed oil	1.5	1.5	—
Hydrogenated coconut oil (Hydrol)†	—	—	10.0
Soybean protein‡	25.0	25.0	25.0
DL Methionine	.3	.3	.3
White dextrin	56.6	50.1	56.1
Salt mix (Jones & Foster) (14)	4.0	4.0	4.0
Cellulose§	2.0	2.0	2.0
Choline methionine tartrate	.6	.6	.6
Iodinated casein (Protamone)	—	4.0	2.0
Succinylsulfathiazole	—	2.0	—
Ribose nucleic acid	—	.5	—

All diets supplemented to contain, per 100 g, 4 mg of α-tocopherol, 900 U.S.P. units vit. A, 180 units vit. D, 1 mg menadione, 0.8 mg thiamine hydrochloride, 1.6 mg riboflavin, 0.8 mg pyridoxine hydrochloride, 4 mg niacin, 4.4 mg calcium pantothenate, 4 mg para-aminobenzoic acid, 20 mg inositol, 0.2 mg folacin, 0.02 mg biotin, and 0.03 mg vit. B_{12}.

* Primex, Procter and Gamble, Cincinnati, O.
† Hydrol, Durkee Famous Foods, Chicago, Ill.
‡ Drackett Assay Protein C-1, Drackett Products Co., Cincinnati, O.
§ Celluflour, Chicago Dietetic Co., Chicago, Ill.

static agent (SST) a study was made to determine the effect of bromine on the growth retardation produced by feeding a diet containing Protamone as the sole deficiency-inducing agent. The results in Table IV show that when a diet free of essential fatty acids and containing Protamone (Diet C) is fed to mice a growth retardation results. This depression of growth rate was prevented partially by feeding linseed oil as a source of linoleic and linolenic acids or by feeding bromine. However, a complete reversal resulted only when bromine and linseed oil were fed in combination. The effect of the unsaturated fatty acids and bromine appears to be additive.

TABLE II. Effect of Whey and Whey Fractions on Growth of Mice Receiving Diets Containing Protamone and Succinylsulfathiazole.

Basal diet	Supplement	Avg gain in wt, 12 day period (g)
A		13.9 (66)
B		8.6 (181)
B	10% Delactosed Whey (DW)*	10.4 (61)
B	H_2O ext. of DW ≎ 10% DW	11.0 (29)
B	Norit filtrate of H_2O ext. of DW ≎ 10% DW	12.4 (198)
B	Ash of Norit filtrate of DW ≎ 10% DW	11.8 (16)
B	IR 120 H filt. of Norit filt. of DW ≎ 10% DW	13.1 (16)
B	1% Trace Element Sea Salt	11.9 (75)

* Consolidated Products Co., Danville, Ill.
Figures in parentheses indicate No. of surviving mice.
Growth increments obtained with all supplements are statistically significant at $P = 0.05$ or less.

Discussion. The results that have been obtained in this study suggest that bromine may

TABLE III. Effect of "Trace Element Sea Salt" and Some of Its Components on Growth of Mice Receiving Diets Containing Protamone and Succinylsulfathiazole.

Basal diet	Supplement	Avg gain in wt, 12 day period (g)	Avg gain in wt, 25 day period (g)
A		13.8 (32)	18.4 (16)
B		10.0 (78)	14.6 (31)
B	.25% Trace Elements Sea Salt (TESS)	12.1 (15)	16.1 (15)
B	.5% TESS	12.5 (16)	17.5 (16)
B	1.0% "	12.1 (32)	16.3 (16)
B	2.0% "	12.8 (16)	16.2 (16)
B	Dowex-1 filt. of TESS ≎ 1% TESS	10.2 (32)	
B	IR 120 filt. " " " " "	11.8 (32)	
B	Mix of Al, B, Br, F, and Rb* ≎ 1% TESS	13.4 (32)	
B	B ≎ 1% TESS	10.0 (16)	
B	Al *Idem*	9.5 (16)	
B	Rb "	9.9 (16)	
B	F "	9.6 (16)	
B	Br ≎ 0.25% TESS, 3.75 ppm.†	12.9 (16)	18.3 (16)
B	Br ≎ 0.5% " 7.5 "	12.6 (32)	16.8 (16)
B	Br ≎ 1.0% " 15 "	13.0 (32)	16.6 (16)
B	Br ≎ 2.0% " 30 "	14.1 (16)	18.9 (16)

* Fed as $Al_2(SO_4)_3 \cdot 18\ H_2O$, $Na_2B_4O_7 \cdot 10\ H_2O$, NaBr, NaF, and RbCl.
† μg of Br/g of diet.
Figures in parentheses indicate No. of surviving mice.

TABLE IV. Effect of Bromine and Linseed Oil on Growth of Protamone-Fed Mice.

Basal diet	Supplement	Avg gain in wt, 12 day period (g)
A		14.9 (15)
C		9.0 (15)
C	15 ppm bromine as NaBr	11.6 (15)
C	1.5% linseed oil	11.5 (15)
C	1.5% linseed oil + 15 ppm bromine	14.5 (16)

Least significant difference for P = .05 = 1.7 g.
Idem P = .01 = 2.2 g.

Figures in parentheses indicate No. of surviving mice.

be a dietary requirement for mice. Bromine has never been implicated as a dietary essential for any animal. Winnek and Smith(3) studied this element a number of years ago and concluded that it was not required by the rat. Bromine is widespread in nature and although few analyses are available it is to be expected that it would be present as a contaminant in many ingredients that are commonly used in the so-called "purified" diets. The analyses of Winnek and Smith(4) illustrate this wide occurrence of bromine in food ingredients. These same authors have shown that feeding bromine to rats causes an increase in the bromine level of such tissues as blood, liver, kidney, muscle, spleen, and brain. It is probable that without special dietary precautions animal tissues will contain bromine as a result of maternal transfer to the offspring or due to the ingestion of bromine-containing foods prior to the usual 21 day weaning period that frequently marks the start of an experimental animal on a "purified" dietary regimen. The growth-promoting effects of bromine that were observed in the present study could be obtained only under conditions of a hyperthyroid-induced deficiency. It is conceivable that the effect of bromine under these conditions was due to a specific reversal of the thyrotoxic activity of the iodinated casein. In view of the small dietary addition providing a response (3.75 ppm) this does not appear to be a logical conclusion.

Abelin and Poretti(5) have shown that in hyperthyroid rats there is an increased uptake of bromine by the liver, kidney, adrenals, brain, and blood. In the present study the effect of hyperthyroidism is interpreted as inducing a partial deficiency of bromine by increasing tissue requirements. The final establishment of a bromine requirement for the mouse probably will have to depend upon the use of weanling mice that have been exposed to a very low bromine intake during their growth period both *in utero* and post-partum up to the time of weaning. The serious difficulties involved in establishing an uncomplicated requirement for this element are reminiscent of the unusually stringent experimental design that was employed by McClendon and Gershon-Cohen when a fluorine requirement for rats was established recently(6).

In those experiments in which a thyroid active material was incorporated into a diet free of essential fatty acids the partial reversal of growth inhibition by linseed oil confirms the experiments of Greenberg(7). The growth stimulation was due undoubtedly to linoleic and linolenic acids, as was shown earlier by these investigators. The effect of bromine was independent of and additive to the fatty acid effect and is similar in certain respects to the "anti-stress" responses of liver fractions that have been reported by Ershoff (8).

An unknown trace element requirement for chicks has been studied recently by several investigators(9-12). Bromine was mentioned specifically in the study of Morrison *et al.* (12) as inactive in supplying the unknown requirement for chicks. A report from this laboratory(13) concerned with the effect of bromine on chick growth deals with this question in more detail.

Summary. Addition of an iodinated casein with thyroid activity to a purified diet adequate in all known essential nutrients produced a growth retardation in mice. Growth inhibition was reversed by fractions of whey and by whey ash. The active inorganic component was found to be bromine.

1. Bosshardt, D. K., and Huff, J. W., *J. Nutrition,* 1953, v50, 117.

2. Huff, J. W., and Bosshardt, D. K., *J. Am. Chem. Soc.,* 1952, v74, 4472.

3. Winnek, P. S., and Smith, A. H., *J. Biol. Chem.,* 1937, v121, 345.

4. Winnek, P. S., and Smith, A. H., *J. Biol. Chem.*, 1937, v119, 93.

5. Abelin, I., and Poretti, G., *Helv. Physiol. et Pharmacol.*, 1952, Acta 10, C7-9.

6. McClendon, J. F., and Gershon-Cohen, J., *J. Agr. and Food Chem.*, 1953, v1, 464.

7. Greenberg, S. M., *J. Nutrition*, 1952, v47, 31.

8. Ershoff, B. H., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 209.

9. Norris, L. C., *Proc. Dist. Feed Conf.*, 1955, v10, 39.

10. Couch, J. R., Reid, B. L., Camp, A. A., Dannenburg, W. N., and Rozacky, E. E., *ibid.*, 1955, v10, 47.

11. Edwards, H. M., Jr., Chin, D., Norris, L. C., and Heuser, G. F., *J. Nutrition*, 1955, v57, 329.

12. Morrison, A. B., Scott, M. L., and Norris, L. C., *Poultry Science*, 1955, v34, 738.

13. Bosshardt, D. K., Huff, J. W., and Barnes, R. H., PROC. SOC. EXP. BIOL. AND MED., in press.

14. Jones, J. H., and Foster, C., *J. Nutrition*, 1942, v24, 245.

Received April 26, 1956. P.S.E.B.M., 1956, v92

Effect of Bromine on Chick Growth. (22432)

DAVID K. BOSSHARDT, JESSE W. HUFF, AND RICHARD H. BARNES.

Merck, Sharp and Dohme Research Laboratories, West Point, Pa.

Huff *et al.*(1) have shown that under certain dietary conditions a hyperthyroid-induced growth retardation in mice could be prevented by feeding selected whey fractions including whey ash or a dried sea salt preparation, Trace Element Sea Salt (TESS).* The active inorganic ingredient was found to be bromine. In view of the interest centered around whey as a source of unidentified growth factors for chicks, the present study was undertaken to determine the effect of bromine on chick growth.

Methods. In 2 of the experiments (1 and 2) one-day-old cockerels (New Hampshire Silver Cornish Cross)† were employed. In experiment 3 one-day-old Nichols strain New Hampshire‡ cockerels and in experiment 4 an unidentified strain of New Hampshire cockerels§ were used. They were housed, in groups of 15, on wire-mesh floors in electrically heated battery brooders. The brooders were lighted continuously. All studies were carried for 31 days. Feed and water were supplied *ad libitum*. All chicks were identified with numbered wing bands and weighed individually at the start of the experiment and weekly thereafter.

The basal diet, semi-synthetic in nature, employed in these studies had the composition shown in Table I. Whey and TESS substitutions were made at the expense of equal weights of glucose. The sodium bromide supplement was prepared by lyophilizing a water solution with dextrin to give a final concentration of 1.5 mg/g dry weight and was added at the expense of white dextrin in the basal diet.

The results obtained in Exp. 1, 2, and 3 (Table II) showed significant growth responses with whey and bromine. In Exp. 1, a positive effect was obtained with 1% TESS. The addition of 1% TESS to the diet supplied 15 ppm. bromine.

In Exp. 4 no significant growth response was obtained with either whey or bromine.

Discussion. A number of investigators [Norris(2), Couch *et al.*(3), and Edwards *et al.*(4)] have shown that chick growth can be stimulated by a mineral supplement in the form of an ash of natural products such as liver, whey, and distillers solubles. It appears possible from the results obtained in this study that bromine may be an active inorganic material. This suggestion is supported by the work of Huff *et al.*(1) who showed that bromine replaced whey ash or sea salt in the hyperthyroid mouse. On the other hand Morrison *et al.*(5) have reported

* Trace Elements Corp., Houston, Texas.

† Shaw Hatchery, West Chester, Pa.

‡ Moyer Hatchery, Quakertown, Pa.

§ Price Hatchery, Souderton, Pa.