Effect of Bromobenzene and Cystine Administration On Vitamin A Deficient Rats.* (19494)

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Outside of its demonstrated role in the mechanism of vision, the biochemical function of vit. A is still unknown. Meunier, Ferrando and Perrot-Thomas(1) investigated the possibility that vit. A might be directly implicated in the synthesis of cystine. In order to investigate this possibility, they made use of the fact that mammalian organisms can be depleted of cystine by administration of bromobenzene; this substance conjugates with cystine to form bromophenyl mercapturic acid, which is then excreted in the urine(2,3). It had been shown previously by Haley and Samuelson(4) that adding 2% bromobenzene to an otherwise normal diet resulted in a decrease of the vit. A content of the liver of the rats of the order of 60% after 3 months. Meunier and his co-workers used 5 groups each of 4 or 5 animals fed a basal, vit. A-free diet containing 15% casein. The diet of one group a) was left unsupplemented. The diet of the other 4 groups was supplemented as follows: b) 1% bromobenzene, c) 1% bromobenzene and 0.12% cystine, d) 2.5 μg of vit. A per animal per day, and e) 1% bromobenzene and 20 μg vit. A per day. No differences were found between groups b and c as regards weight loss or time of survival (average survival time for both groups, 25 days). Groups d and e survived normally and showed average weight gains of 4.9 and 6.9 g per week respectively. Survival in group a (unsupplemented) was 33 days. Meunier and his associates concluded that vit. A was specifically responsible for the synthesis of cystine. However, it must be noted that the level in the diet of the sulfur-containing amino acid corresponding to 15% casein(5) was apparently sufficient to nullify the effect of the cystine supplement in the case of groups b and c, and that their other groups lacked proper controls. No record of the food intake was mentioned.

High levels of dietary protein seem to have a sparing effect on vit. A deficiency(6); epithelial tissues, which are particularly sensitive to vit. A deficiency, show special affinity for administered radioactive cystine(7); it appeared, therefore, useful to study again the possible interrelationship of vit. A and cystine metabolism in bromobenzene intoxication. It seemed important, in this study, to limit the cystine content of the diet to the lowest level compatible with some growth, to increase the bromobenzene effect by increasing its concentration in the diet to the highest level compatible with absence of acute toxic effects and to keep records of the food intake of the different groups.

Experimental. Forty-eight weanling (21-day-old) male rats, weighing on the average 50 g and obtained from the Charles River Stock, were divided into 8 groups of 6 animals. They were fed the following basal diet: casein (Labco), 9%; sucrose, 82%; corn oil, 5%; salt IV, 4%; and choline, 0.1%. The following vitamin supplements were added per kg of ration: thiamine, 10 mg; riboflavin, 10 mg; pyridoxine, 10 mg; nicotinamide, 20 mg; calcium pantothenate, 20 mg; folic acid, 1 mg; biotin, 0.2 mg; para-aminobenzoic acid, 100 mg; inositol, 100 mg; menadione, 15 mg; and alpha tocopherol, 40 mg. Vit. A, when given, was administered every week in doses corresponding to 400 I. U. per day. Bromobenzene (A. R.) when given was added to the diet daily (in solution in the corn oil) at a concentration of 0.5% during the first 10 days and 2% after that time to the end of the experiment; cystine, when given, was incorporated in the diet at a concentration of 0.2%. The groups received the following supplements: vit. A: groups A, AC, AB, ABC; bromobenzene: groups B, BC, AB, ABC; cystine: groups C, AC, BC, ABC; group O received no

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TABLE I. Wt Gains and Food Intake of Non-Deficient and Vit. A-Deficient Rats Receiving Bromobenzene and Cystine.

Groups	Treatment	Wt gain at 40th day, g	Avg food intake (23rd-38th day), g per day
AC	Vit. A, cystine	116.3 ± 18	13.8
A	Vit. A	58.8 ± 22.9	12
ABC	Vit. A, cystine, bromobenzene	80 ± 16.8	10.8
AB	Vit. A, bromobenzene	32.8 ± 3.6	8
C	Cystine	64.7 ± 23.9* (60 ± 21)	9.4
O	—	36.6 ± 17.6	9.7
BC	Bromobenzene, cystine	54.5 ± 18.6* (53.3 ± 22.4)	9.9
B	Bromobenzene	21.4 ± 5.5	7.9

* Figure in the column in this case is the max wt gain; the figure in parenthesis is the wt gain at 40 days.

Stand. dev. calculated by the formula $\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$.

Student's t computed for comparison between groups by formula $t = \frac{(M_1 - M_2)\sqrt{N_1 + n_2 - 2}}{\sqrt{\Sigma d_1^2 + \Sigma d_2^2}}$.

supplement. All animals were kept in individual screen-bottomed cages at constant temperature and under regular conditions of illumination. Food intake was recorded every day and weight every two days.

Results and discussion. Essential results, concerning maximum weight gains of the deficient animals, rates of weight gain of the non-deficient animals and average food intake are recorded in Table I. Groups O and B reached their maximum weight gain 40 days after the start of the experiment; group BC after 39 days; group C after 35 days. Averages and standard deviations of the weight gains of all groups are therefore given at the 40th day for all groups. In the case of groups BC and C, maximum weight gains are also given.

All deficient animals lost weight and declined rapidly after the 40th day and showed typical signs of vit. A deficiency. The severity of these symptoms was in close relation to previous rates of growth.

It is apparent from consideration of the weight gains of non-vit. A deficient animals, that, at the level of protein used, cystine was the limiting factor in growth. Weight gains of animals receiving cystine, (AC, ABC) were twice as great as those of corresponding animals (A, AB) whose diet was left unsupplemented. ($p = 0.002$ and $p = 0.001$ respectively). Administration of bromobenzene caused definite limitation of weight gain, in a manner compatible with its known effect on cystine metabolism. Groups ABC and groups AB showed a weight gain very significantly ($p = 0.002$) smaller than that of AC and A respectively; the addition of bromobenzene to the diet supplemented with cystine caused a reduction in weight gain of the order of 31% and, in the case of the diet not supplemented with cystine, of 44%. The supplement of 0.2 cystine would permit a theoretical maximum of 16% of the bromobenzene to be detoxified as bromophenyl mercapturic acid (2). The fact that group ABC grew significantly ($p = 0.05$) better than group A, seems to indicate that all the cystine supplement was not conjugated with bromobenzene.

In the deficient group cystine was again the limiting factor as regards maximum weight reached. (Difference between C and O, $p < 0.02$; difference between BC and B, $p = 0.002$). The depressing effect of bromobenzene on growth was definite, particularly in the case of the animals receiving no cystine. (Difference between O and B, $p = 0.002$). Decreases due to administration of bromobenzene were somewhat smaller than in the case of the animals receiving vit. A, doubtless because of smaller over-all growth and resulting smaller cystine requirements.

The food intake values reflected observation on growth: cystine increased food intake (AC, ABC, as compared to A, AB), bromobenzene depressed it (A, AC as compared to AB, ABC). The differences between deficient groups (C and O, for example) were masked by the fact that C showed deficiency signs and anorexia before O; ABC, although it grew more than A, did present some periods of anorexia and arrest in weight gains which

were compensated for in the following periods. The values given in Table I correspond to the 15 days comprised between the 25th and the 38th day of the experiment, before any group showed a decline in food intake. Such a decline started on the 38th day for group C, on the 40th day for other deficient groups.

These results do not support the hypothesis that vit. A intervenes directly in the synthesis of cystine, as claimed by Meunier and his associates. For one thing, while the inclusion of cystine in the diet did increase the maximum weight gain of the animals fed vit. A deficient diets, it did not delay the onset of the deficiency. As a matter of fact, weight loss occurred earlier in group C and symptoms were more acute among the animals of this group. Secondly, adding bromobenzene to the vit. A-deficient diets did not cause a more drastic effect than in non-vit. A-deficient animals. If vit. A was specifically instrumental in the synthesis of cystine, one would expect the vit. A-deficient animals, whose capacity of cystine synthesis should already be impaired, to be more immediately and more severely affected by the bromobenzene.

Summary. A study of the effect of bromobenzene administration to vit. A-deficient animals on low-protein diets, supplemented or not supplemented with cystine, and to their controls, does not support the theory, postulated by some authors, that vit. A is directly implicated in the synthesis of cystine.

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Manometric and Histochemical Demonstration of Tyrosinase in Foetal Guinea-Pig Skin. (19495)

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At least some of the skin and hair pigments of mammals (melanins) appear to be produced from the precursor, tyrosine, and it has recently become possible to demonstrate tyrosinase activity in normal mammalian skin. A histochemical demonstration of melanin formation from tyrosine was made in the case of non-pigmented human skin subjected to erythema doses of ultra-violet irradiation(1), and tyrosinase activity has also been demonstrated in skin homogenates from mice and foetal guinea pigs by means of manometric measurements of oxygen consumption(2-4). Since this pigment-forming process has been demonstrated either histochemically or manometrically, it was considered desirable to attempt to demonstrate both aspects of the process in the same sample of tissue.

Methods. In the experiment reported here the intense brown portion from the back of an approximately 52-day-old brown-and-white-spotted foetal guinea pig was separated from the white portion, placed in a frozen mortar surrounded by dry ice, ground to a frozen powder by means of a previously frozen pestle, and this frozen powder was subsequently suspended in pyrex-redistilled water. Four Warburg reaction vessels, each with a volume of approximately 10 ml, were used. Each vessel contained 0.2 ml of 20% KOH in the center

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