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Toxic Effects of an Excess of Vitamin B_1 in Rats.

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Incidental to other studies on the effect of an excess of vitamin B_1 on resistance, certain unusual toxic manifestations of an excess were observed. We have been unable to find any report in the literature describing similar effects.

The rats used in these experiments were of Wistar stock, raised in our laboratories and free from *Bartonella muris*. Three groups of animals, 4 weeks old, were used. All 3 groups were fed a complete diet consisting of 15 gm. per rat per day of a basic mixture of hominy 100 parts, rolled oats 25 parts, fine meat and bone 25 parts, salt 1½ parts and dried skimmed milk 16 parts, to which was added a few drops of cod liver oil, 0.3 mg. of wheat germ and 0.3 gm. of crude Fleischman's brewer's yeast per rat. This yeast product contains 6 I.U. of B_1 per gram. This is equivalent to about 2 I.U. per rat per day. In addition, the animals received fresh greens twice a week and fresh milk every day. On this complete diet, it has been our experience that the rats maintain a good growth curve, reproduce well and rear all their young without loss of any members of their litters.

In the present experiment, one group containing 8 females was fed this complete diet, but supplemented with Mead Johnson's brewer's yeast in amounts equivalent to 50 I.U. of vitamin B_1 per rat per day. A second group containing 6 females was given supplements of vitamin B_1 concentrate (adsorbate, Eli Lilly) in amounts equivalent to 50 I.U. per day. The vitamin B_1 adsorbate was subsequently replaced by synthetic vitamin B_1 (Betaxin) and this was given in equivalent unitage, administered subcutaneously. A third group in which there were 9 females maintained on the standard complete diet alone, was used as controls. The experiment was continued for several generations.

Of the control group, within a period of 4 months (in the winter) 5 became pregnant and had normal litters of 7 to 10 young, all of which grew to maturity. The other 4 did not conceive in this period. The offspring continued to breed at maturity as usual. In common with the experience of others, we have observed fluctuations in the rate of reproduction dependent on season, but only rarely have we observed instances in which a litter was not normally raised.

The rate of growth of the animals fed an excess of B_1 concentrate, or synthetic vitamin B_1, was no greater than that of the group of animals fed the normal complete diet. The females of these groups bred more regularly than the controls. However, in the first generation of rats fed the excess B_1 concentrate, 2 of their 7 litters were born dead, apparently due to premature parturition; 2 were born alive, but died and were eaten within 24 hours; and 3 litters of 3 to 8 young grew to maturity. Before the second mating, the B_1 concentrate was replaced by synthetic vitamin B_1 (Betaxin) in equivalent unitage injected subcutaneously. One mother that had eaten its first litter, when given Betaxin instead of B_1 concentrate, became pregnant a second time and threw a litter of 4 healthy young that grew to maturity. The second generation on Betaxin, however, when bred, showed the same toxic effects. In 3 litters the young were born alive but the mothers neglected their young and within 48 hours had devoured most of the offspring.

The animals fed an excess of vitamin B, in the form of Mead Johnson's brewer's yeast, thrived much better than the controls or those fed vitamin B_1 concentrate as far as the rate of growth was concerned. Throughout the experiment, the animals weighed on an average of 50% more than did the members of the other 2 groups. (This is consistent with the findings of Williams, Waterman and Keresztesy[1] who noted improved size of rats fed increasing amounts of vitamin B_1 up to a limit of 100 times the minimal protective dose.) Seven of 8 females conceived and gave birth to litters of 6 to 11 young at a time of the year when breeding in the control stock was at a minimum. However, in 2 litters, the young were ignored by the mothers, and they died of starvation, a defect of lactation apparently being present. The other 5 litters grew to maturity, their growth curve exceeding the controls. This second generation grew well and showed no toxic effects from the excess. However, when they became mature, some were inbred with their litter mates and of the 9 litters of this second generation, in 2 the mothers showed no interest in the young and failed to lactate adequately. As a result, the young became emaciated and starved to death. In 4, the mothers ate the young within 24 hours. When it was noted that the first 6 litters born of the mothers in this second generation were lost, the excess yeast was discontinued for a period of 2 weeks and then readministered in amounts of 20 I.U. per day to the remaining pregnant females. Of the next litter born after this change in quantity

[1] Williams, R. R., Waterman, R. E., and Keresztesy, J. C., *Science*, 1935, **81**, 535.

of excess yeast administration, 3 of the young survived. The remaining young of this litter were not nursed and starved to death. Litters born within a period of 4 weeks after the reduction of the excess of yeast were perfectly normal, all surviving. The mothers lactated adequately and showed the normal interest in their young. It is interesting that new-born rats of the stock given an excess of yeast for 2 generations, injected daily subcutaneously with 20 to 50 I.U. of synthetic vitamin B_1, did not show any perceptible difference in the time of weaning from those of the control normal stock fed our standard adequate diet.

Since the groups receiving excess yeast, concentrated B_1 and synthetic B_1 showed similar toxic effects, it is definite that the common responsible factor was B_1 and not other elements in the B_1 adsorbate or other factors in the yeast.

Leong[2] observed that the maximum storage of vitamin B_1 is attained following the administration of 30 I.U. per rat per day in a period of 4 to 6 weeks. From our own observations, it would seem that an excess of vitamin B_1 in amounts exceeding 20 or 30 I.U. a day during a period of more than one generation may interfere with lactation and the normal maternal instinct, resulting in high litter mortality and cannibalism. Nakahara, Inukai and Ugami[3, 4] have recently described an "L-factor" in liver and yeast which they state is a specific dietary factor necessary for lactation. It is not present in the concentrated vitamin B_1 adsorbate. It is possible that vitamin B_1 in great excess may inhibit the action of such a lactation factor.

Summary. Additions of Mead Johnson's brewer's yeast in amounts equivalent to 50 I.U. of vitamin B_1 per animal per day will cause a marked increase in the weight curve for several generations. This amount of yeast, however, results in a disturbance in lactation and a loss of the nursing instinct evident in the first generation, but almost universal in the second generation, with consequent starvation of the young, and a high incidence of cannibalism. The effect was more pronounced in those animals fed B_1 concentrate (adsorbate). Among these, still-births were common as well. Synthetic vitamin B_1 was somewhat less toxic in excess amounts than the vitamin B_1 adsorbate but similar interference with lactation and the nursing in-

2 Peng Chong Leong, *Biochem. J.*, 1937, **31**, 367.

3 Nakahara, W., Inukai, F., and Ugami, S., Abstr. in *Ber. ü. d. g. Physiol. u. Exp. Pharm.*, 1937, **98**, 148; *Sci. Pap. Inst. Physic. Chem. Res.*, 1935, **28**, 152.

4 Nakahara, W., Inukai, F., Kato, S., and Ugami, S., Abstr. in *Ber. ü. d. g. Physiol. u. Exp. Pharm.*, 1937, **98**, 149; *Sci. Pap. Inst. Physic. Chem. Res.*, 1936, **29**, 47.

stinct in the second generation occurred. It is interesting that vitamin B_1 in amounts equivalent to 40 times the maintenance requirement seems to have a similar effect in interfering with the capacity of the mother to rear her young and the nursing instinct as does a relative insufficiency of this vitamin. This should in no way discourage the generous use of vitamin B_1 in the diet of human beings, as the difference between the optimal requirement and the amount which may prove toxic after several generations is considerable. The excess amount given to our rats would be equivalent to the administration of between 5,000 and 10,000 I.U. of vitamin B_1 per day for several generations (assuming that the vitamin B_1 requirement of humans is between 100 and 200 units per day).

I am indebted to Mead Johnson for the supply of brewer's yeast for these and other experiments, to Eli Lilly for vitamin B_1 concentrate, and to Winthrop Chemical Company for the generous supply of Betaxin (synthetic vitamin B_1).

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Lysis of *Vibrio comma* by Bacteriophage and by Immune Serum.*

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The very simple idea that a bacteriophage may be a minute particulate living being capable of growing and multiplying within a larger living cell and able there to produce soluble enzymes which may act upon the bacterial structure and may eventually diffuse in solution through the medium has been postulated by d'Herelle and has been supported by ever-increasing experimental evidence assembled by his disciples. Certainly this conception is in accord with what we know about larger organisms such as the coccidia, the malarial plasmodia, and the yeasts. The morphological observations upon the bacteriophage particles have been technically difficult and the results not wholly satisfactory. The study of other filterable viruses has met with similar difficulty.

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