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FURTHER EVIDENCE OF A NEW FACTOR IN THE B COMPLEX

W. R. WYATT AND V. E. NELSON

The vitamin B complex is generally accepted as containing at least five distinct components for the rat; B₁ (thiamin), flavin, nicotinic acid, B₆, and the filtrate factor. Work by Jukes and Babcock (1) and by Oleson and co-workers (2) indicates that a distinct antiparalytic factor (very likely Reader's "B₄") exists. Stockstad (3) has reported a growth factor "U" for the chick. One of the authors (4) has given evidence of the existence of still another factor, the "8B factor," in the B complex. That chondroitin sulfuric acid is a growth factor for the rat has been suggested by Robinson and co-workers (5). This paper deals with further study of the 8B factor and its relation to the other factors.

EXPERIMENTAL

Three-weeks-old rats of thirty to thirty-five grams weight were placed in groups of six in screen bottom metal cages and received weighed amounts of the supplemented diets furnishing the desired quantity of each supplement per day. The basal ration contained sixty per cent alcoholic extracted washed casein eighteen per cent, salt mixture three and seven-tenths per cent, cod liver oil (Squibb) four per cent, and sucrose to 100 per cent. Three and one-half per cent of water was added to the ration to prevent rancidity. The washed casein was prepared by washing commercial casein for fourteen days with 0.15 per cent of acetic acid and decanting daily. From this casein was prepared the alcohol extracted casein by extracting in a continuous extractor with sixty per cent of alcohol for four days.

Concentrates of filtrate I (B₆) and filtrate II (FII) were prepared from liver by the method of Lepkovsky, Jukes, and Krause (6). The B₆ concentrate was purified further by readsorption and elution before concentration. Both B₆ and FII fractions were found necessary in addition to B₁, flavin, and nicotinic acid for the growth and well being of the rat.

When rats received the basal ration supplemented with adequate B₁, flavin, and nicotinic acid, along with 0.30 g. alfalfa meal (as a source of B₆, and FII) restricted growth (four grams per week

for eight weeks) and an unhealthy appearance resulted. A doubled growth rate (eight grams per week for eight weeks) was observed when Feaster and Nelson's (7) B₁ concentrate 8B (0.05 gram) was used in place of crystalline B₁ and a normal healthy appearance was maintained. Addition of a tested B₆ concentrate to the alfalfa diet gave no improvement in growth or appearance. Further evidence that B₆ was not the factor involved was given by the response of a B₆ free group, during the fifth week of depletion, to the addition of 0.15 gram of alfalfa. This group was protected from the B₆ deficiency symptoms (acrodynia) and grew five grams per week for a period of six weeks while the B₆ free control group stopped gaining in the first week of the test, three of the six animals developing severe acrodynia and dying in the third and fourth weeks.

A doubled level (0.10 gram) of the 8B concentrate failed to support growth and life as the sole source of the FII fraction. Very little growth (one gram per week for five weeks) occurred after the fifth week. The rats developed severe deficiency symptoms such as alopecia, dermatitis, and bleeding of the paws and the nose. In some cases the eyelids stuck together, and there developed paralysis and a puffing up of the skin on the face with hemorrhages beneath the skin. Only one of six rats survived the tenth week on this diet. This observation along with the fact that a doubled level of alfalfa (0.60 gram) gave growth inferior to 0.30 gram alfalfa + 0.05 gram 8B proved that the effect of 8B on the alfalfa diet was not merely additive.

Molasses (0.15 gram) was found to be very effective as a source of the 8B factor in supplementing 0.30 gram alfalfa. This distinguishes the 8B factor from the growth factor "U" which is reported to be abundant in alfalfa and absent (or very deficient) in molasses.

The stability of the 8B factor to heat in alkaline solution was investigated. The active principle was eluted from the fuller's earth adsorbate by means of a mixture of one part pyridine, one part methyl alcohol, and four parts of water and concentrated. Half of the concentrate was fed unheated while the other half was heated four hours at pH 10.0 in a boiling water bath and then fed. Addition of heated or unheated concentrate to the 8B deficient diet gave identical marked improvement in growth and appearance showing that the 8B factor is not destroyed by this heat treatment in alkaline solution. The stability of the 8B factor to heat in alkaline solution verifies the conclusions drawn from

comparative distribution, adsorbability and symptoms — that the 8B factor differs from the chick antidermatitis factor, from W (8), and from the antiparalytic factor (which is probably the old alkaline heat labile "B₄").

The 8B factor deficiency is not the unsaturated fatty acid deficiency since four per cent of cod liver oil (Squibb) was included in the basal ration and all of the animals received at least three grams of the basal ration per day which would provide a minimum of 120 mg. cod liver oil daily. This is twice the quantity which Burr and Burr (9) found ample to maintain rats for a year with only slight symptoms, some scales on feet and tail, but otherwise healthy.

Yellow corn (0.375 gram) gave more than one-half of the supplementary effect of 0.05 gram 8B when added to 0.30 gram of alfalfa as a source of FII. The effect of so low a level of corn distinguishes the 8B factor from chondroitin sulfuric acid which is reported to have a supplementary effect on rations containing over fifty per cent of corn.

The lack of the characteristic symptoms of the "spectacled eye" factor and "antihemorrhagic" factor deficiencies on the 8B deficient diet containing alfalfa and the suggestion of these symptoms on the diets containing the doubled level of 8B as the sole source of filtrate factor indicates that the 8B factor must differ from these factors.

SUMMARY

The feeding of 0.30 gram alfalfa as a source of "filtrate factor" (B₁, flavin, nicotinic acid, and B₆ added) produces restricted growth (four grams per week) and an unhealthy appearance in the rat.

With the addition to the diet of the "8B factor," present in a fuller's earth adsorbate from rice polish extract, doubled growth and normal appearance result.

The new water soluble factor is stable to heat in alkaline solution.

The new factor is shown to be distinct from B₁, flavin, nicotinic acid, B₆, chick antidermatitis factor, antiparalytic factor, U, W, and unsaturated fatty acid.

The new factor appears to differ also from the "spectacled eye factor," antihemorrhagic factor, and chondroitin sulfuric acid.

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