of lower initial pH will also show a lower osmotic pressure than one of higher pH (Oakley[8]). Since the composition of the acacia solutions used by Dodds and Haines[1] is not given in sufficient detail, it is impossible to say more than that very likely the explanation of the difference between their values for the colloid osmotic pressure of 6% acacia in 0.9% NaCl and the values of the various investigators cited here lies in differences in inorganic composition of the initial acacia solutions. Presumably the same explanation applies to the marked differences among the values reported for the colloid osmotic pressure of 6% acacia solutions by various investigators (quoted by Amberson[9] and Butt and Keys[7])—values ranging from 120 to 3740 mm H_2O; some of the differences arose, however, because measurements were made on solutions now of one salt content, now of another. Recent measurements made in the United States on 6% acacia in 0.9% NaCl or similar solutions seem to be in satisfactory agreement.

Summary. 1. The colloid osmotic pressure of several samples of 6% acacia in 0.9% NaCl has been determined to be 246 to 260 mm H_2O at 20° C. 2. This value is approximately the same as the average colloid osmotic pressure of human sera ranging in protein concentration from 6 to 8%, namely, 276 mm H_2O. 3. Acacia solution processed by the procedure of the Lilly Research Laboratories appears to be a stable and uniform product.

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Evidence of Another Factor in the B Complex for Rats.

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B_1 (Thiamin), flavin, nicotinic acid, B_6, and the filtrate factor are generally accepted as distinct components of the B complex for rats. Recent work on other species of animals by Morgulis, Wilder, and Eppstein[1] and by Jukes and Babcock[2] indicates that antimuscular dystrophy and antiparalytic factors exist, while Stockstad[3] reports evidence of a distinct growth factor U for the chick.

9 Amberson, W. R., *Biol. Rev.*, 1937, **12**, 48.

1 Morgulis, S., Wilder, V. M., and Eppstein, S. H., *J. Nutr.*, 1938, **16**, 219.

2 Jukes, T. H., and Babcock, S. H., *J. Biol. Chem.*, 1938, **125**, 169.

3 Stockstad, E. L. R., and Manning, P. D. V., *J. Biol. Chem.*, 1938, **125**, 687.

The work herein reported has been confined to the differentiation of the various factors of the B complex which are necessary for the rat. Three-weeks-old rats of 30 to 35 g weight were placed in groups of 6 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 60% alcoholic extracted washed casein 18.0, salt mixture 3.7, cod liver oil (Squibb) 4.0, and sucrose to 100%. Three and one-half percent of water was added to prevent rancidity. The washed casein was prepared by washing commercial casein 14 days with 0.15% acetic acid and decanting daily. From this casein was prepared the alcoholic extracted casein by extracting in a continuous extractor with 60% alcohol for 4 days.

Concentrates of filtrate I (B_6) and filtrate II (FII) were prepared from liver and from rice polish by the method of Lepkovsky, Jukes and Krause.[4] The B_6 concentrate was purified further by readsorption and elution before concentration. Both B_6 and FII fractions were found necessary in addition to B_1, flavin, and nicotinic acid for the growth and well being of the rat. Omission of nicotinic acid in diets with 0.3 g of alfalfa plus washed casein or with 0.3 g alfalfa plus 8B as a source of total filtrate resulted in decreased growth showing that nicotinic acid is essential for rat growth and that this substance is deficient in 0.3 g of alfalfa, in washed casein, and in 8B concentrate.

The following are the results of studies to investigate the possible multiple nature of FII. The adsorbate and filtrate obtained by treatment of the FII fraction with norite gave no supplementary effect to one another, although about ¼ of the total activity was adsorbed. Adsorbed and unadsorbed fractions prepared by continuous (chromatographic) adsorption of total filtrate from liver (filtrate from flavin adsorption) on 1 kg English fuller's earth or on 3 kg of this earth gave no significantly better growth when fed in combination as a source of FII than when fed alone. Less than ½ the activity was adsorbed on 1 kg of the fuller's earth and more than ½ on 3 kg of the fuller's earth.

When concentrates of B_6 and FII (prepared from liver) were fed together as a source of the total filtrate (B_1, flavin supplied) poorer growth and appearance of the animals resulted when crystalline B_1 was employed than when Feaster and Nelson's[5] fuller's earth 8B adsorbate from rice polish extract was used to supply B_1. Again, in

[4] Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, 1936, **115**, 557.
[5] Feaster, J. F., and Nelson, V. E., *Am. J. Physiol.*, 1936, **115**, 147.

groups receiving the adsorbed portion from the continuous adsorption treatment or the combination of adsorbed and unadsorbed portions as a source of FII a marked stimulation of growth resulted by the addition of the 8B concentrate.

The supplementary effect of the 8B concentrate was also strikingly shown when it was added to 0.3 g of alfalfa daily as the sole source of the total filtrate. Without 8B, 0.3 g alfalfa supplemented with adequate B_1, flavin, and nicotinic acid gave poor growth and the rats appeared unthrifty and exhibited alopecia and dermatitis. With 8B in place of crystalline B_1 growth was twice as rapid and the animals maintained a healthy appearance. The effect of 8B on the alfalfa diet was not due to its B_6 content as was shown by supplementing the alfalfa diet with a tested B_6 concentrate, in which case little or no beneficial effect resulted. The possibility that the 8B effect might be merely additive was eliminated when it was found that twice the level of 8B failed to support growth and well being as a source of FII, while twice the level of alfalfa did not give nearly as good results as the combination of alfalfa and 8B.

The use of washed casein in the alfalfa ration in place of alcohol extracted washed casein resulted in increased growth but hastened the development of dermatitis, indicating a growth factor and an antidermatitis factor may be involved in the supplementary effect of 8B.

Molasses (0.15 g) and yellow corn (0.375 g) were also found effective in supplementing 0.3 g alfalfa as a source of the filtrate factor (B_1, flavin, nicotinic acid added).

The results show that rats require at least 2 factors in addition to B_1, flavin, nicotinic acid, and B_6. One of these factors is supplied by the alfalfa; whereas the other factor is present in the fuller's earth adsorbate from rice polish extract (fraction 8B) as well as in molasses and yellow corn. The distribution of the 8B factor, the effects of its deficiency and its adsorbability indicate that it differs from the chick antidermatitis factor, the antiparalytic factor, and factors U and W.[6]

[6] Frost, D. V., and Elvehjem, C. A., *J. Biol. Chem.*, 1937, **121**, 255.