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## A Highly Satisfactory Vitamin B Deficient Ration.\*

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For a number of years we have experienced considerable difficulty in securing uniform and reliable results on so-called vitamin B deficient rations. In all of our vitamin B studies we have negative as well as positive controls. Very frequently the negative control, receiving no vitamin, would continue growing for many weeks, which was quite distressing, particularly in the biological assay of concentrates which necessitated discarding appreciable amount of data, thus involving much waste of time and labor. After numerous attempts to arrive at the source of the troubles we finally discovered 2 main factors responsible for animals growing after the vitamin reserves have been depleted. First, our method of purifying casein by washing for 7 to 10 days in acidulated water is inadequate and second, subjecting dried baker's yeast to such drastic treatment as autoclaving for 8 hours at 20 pounds pressure did not always insure the destruction of all traces of vitamin B. Furthermore, such drastic autoclaving destroyed appreciable amounts of vitamin G.

The purification of commercial casein, purchased from Atterbury Bros., New York, is carried out as follows: 3 kg. of casein are suspended in 30 liters of tap water to which are added 50 cc. glacial acetic acid and 5 cc. chloroform and 5 cc. toluene as preservatives. The water, acetic acid, and preservatives are changed daily for a period of 14 days. During the last 3 days of this period distilled water is used instead of tap water. The casein is then dried at 100°C. and ground. Five kilograms of the dried acid washed casein are subsequently suspended in 25 liters of 25% ethyl alcohol, by volume, and soaked for 24 hours. During this period the casein is stirred in the alcohol for 12 hours with a mechanical stirrer. This operation is conducted at room temperature. The casein is then filtered through a large Buchner funnel, dried at 100°C. and ground.

Our ration deficient in the vitamin B complex (No. 1751) consists of casein (purified according to the method described), 20;

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agar-agar, 2; McCollum's salts No. 185, 4; filtered butter fat, 10; and dextrin, 64. On this ration, deficient only in the vitamin B complex, we secure depletion in 2 and generally in not more than 3 weeks.

For our uncomplicated vitamin B studies we have introduced autoclaved round steak as a source of vitamin G, because muscle meats are known to be deficient in the antineuritic and abundant in the antipellagric factor. The autoclaving is carried out as follows: Thirty pounds of round steak are cut in small cubes and heated for 6 hours at 20 pounds pressure, dried at 100°C., and ground. The composition of the ration deficient only in vitamin B (No. 2345) is as follows: Casein (purified with acidulated water and 25% alcohol), 10; salts No. 185, 4; autoclaved dried round steak, 15; butter fat, 10; dextrin, 61. On this ration depletion of vitamin B reserves generally take place 2 to 3 weeks after all storage of the vitamin B complex has been depleted on Ration No. 1751. The most satisfying feature about ration 2345 is that all negative controls lose weight on this diet rapidly and invariably die.

We have determined that this ration is abundant in vitamin G for optimum growth with a large number of animals, receiving supplementary additions of small amounts of highly concentrated vitamin B preparations.

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### Further Studies on Production of Thecal Luteinization by Means of A. P. L.

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We have reported that if A.P.L (anterior-pituitary-like hormone) is given to very young rats (6-8 days old) no follicle maturation or corpus luteum formation occurs, but the thecal cells increase in size and store large amounts of fat, so that their appearance becomes very similar to that of real corpus luteum cells. Normal reactions to A.P.L—that is, follicle maturation and corpus luteum formation—is regularly found in prepubertal rats if the injections are started around the 21st day of life. We found, however, that rats treated daily with this hormone, beginning at the age of 6 days, react with