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Value of Urea in the Diet of Rabbits.

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The rabbit shows some very interesting peculiarities in its nutrition in that it does not require a dietary source of riboflavin, pantothenic acid, biotin or folic acid.[1] On the basis of the excretion of these vitamins it is apparent that there is considerable microbial synthesis in the digestive tract of the rabbit. Rabbits show a normal functional coprophagy which presumably enables them to make better utilization of the vitamins synthesized in the digestive tract. It has been shown that more than 50% of the feces produced by the rabbit is normally reingested.[2]

The literature on the synthesis of the B vitamins and of protein by microorganisms in the paunch of ruminants has been previously reviewed.[3-5] It is well established that elemental forms of nitrogen such as urea can be converted into protein by microorganisms in the rumen of polygastric animals and that the protein is utilized by the host animal. The fact that the rabbit has a large cecum favorable to microorganisms and the recent observations showing that they do not require a dietary source of some of the B vitamins prompted us to investigate the possibility of amino acid synthesis by the rabbit and its utilization during the normal process of feces ingestion. Observations have also been made in the course of these studies on the effect of decreasing the level of protein on the requirements for vitamin A.

Experimental. Weanling rabbits, 8 weeks old, of the New Zealand White breed and weighing 800 g to 1200 g were used in these studies. There were 5 rabbits in each group with an equal number of males and females. These were kept in wire bottom cages. This type of cage does not prevent coprophagy in the rabbit. Group I was fed a diet composed of purified casein (Labco) 10%, *DL*-methionine 0.30%, cerelose 68.2%, cellulose 10%, Salts mixture[6] 3%, corn oil 8%, A & D Oil (Nopco XX) 0.5%. The following amounts of vitamins were added per 100 g of diet: mixed tocopherols 50 mg, choline chloride 200 mg, niacin 20 mg, inositol 10 mg, pyridoxine hydrochloride 0.7 mg, thiamine hydrochloride 0.7 mg, riboflavin 0.7 mg, calcium pantothenate 1 mg, and 2-methyl, 1,4-naphthoquinone 0.075 mg. Group 2 was fed a diet similar to the above except that 3.43% of urea was added at the expense of an equal amount of dextrose to make the protein equivalent (N x 6.25) to 20%. The third group received 20% of casein and no methionine. At the end of the 4th week the diets were slightly modified by replacing wood pulp for the cellophane as a source of cellulose. The methionine was added at levels so that the methionine content of the diets containing 10% of casein was equivalent to that of the diet containing 20% of casein. Since methionine has been shown to favor the synthesis of amino acids in ruminants[7] it was added to eliminate this as a factor of variation with regards to the control group receiving 20% casein. Water and food were given *ad libitum*. The rabbits were kept on experiment for 10 weeks and weighed at weekly intervals.

The average growth response of the animals in each group is shown in Fig. I. As can be

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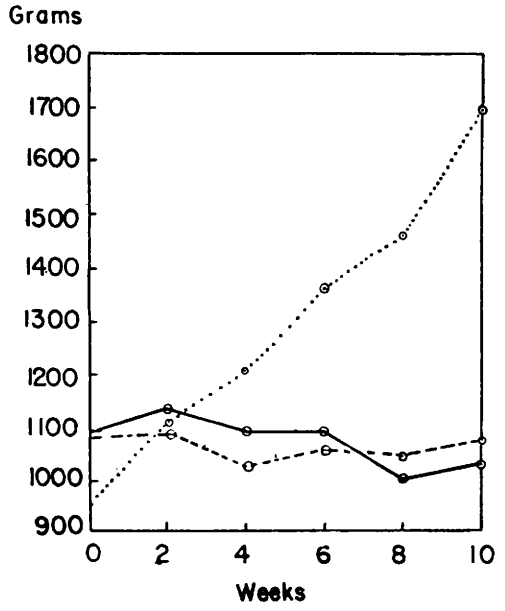

FIG. 1.
Growth of rabbits on diet containing 20% casein is indicated by dotted line. Broken line shows weight of rabbits receiving diet containing 10% casein and solid line weight of rabbits fed diet containing 10% casein plus 3.43% urea.

readily seen the group receiving 20% casein was the only group that made satisfactory gains. The groups receiving either the 10% casein alone or 10% casein plus 3.43% urea both lost weight. Since the rabbits receiving urea as a supplement to a low protein diet made no more gain than those on 10% of casein it is apparent that there is no significant synthesis of protein by microorganisms in the gastrointestinal tract of the rabbit.

At the end of the 3rd week 3 rabbits in each of the groups on the low protein diets showed some eye lesions. The main symptom was a conjunctivitis. A viscid conjunctival secretion was present and the lids were stuck together. Usually these symptoms became noticeable in both eyes at the same time. Oral administration of A and D oil for a period of one week relieved the animals of these early symptoms of vitamin A deficiency but when the administration was stopped the symptoms reappeared in one week. Again the A and D oil was administered for 1 week and the symptoms disappeared but they reappeared as soon as the administration of the A and D oil was discontinued on the 7th week. None of the rabbits on the diet containing 20% casein showed vitamin A deficiency symptoms. Since the administration of the extra vitamin A did not influence the growth of the rabbits it may be assumed that the failure of the rabbits on the low protein diets to grow was due to a deficiency of protein *per se*. The fact that a vitamin A deficiency occurred with the rabbits fed low protein diets is very significant and suggests an interrelationship in the diet between proteins and fat soluble factors. This may be related to the preservation of vitamin A.

Although no carefully controlled experiments have been reported on vitamin A deficiency produced by the partial replacement of protein concentrates by urea, some contradictory evidence exists in the literature with regards to the effect of adding urea to silage. While Cullison[8] has reported an increased content of carotene in silage which had received urea supplementation, Wise, *et al.*[9] found losses of carotene in silage that was fed with urea as a supplement. On the other hand, Woodward and Shepherd[10] have found no appreciable differences in the carotene content of silage containing urea and silage not containing urea.

Destruction of vitamin A can not be attributed to the addition of urea to the diet low in protein, because, although the rabbits fed this diet developed vitamin A deficiency symptoms, similar symptoms were shown by the rabbits fed the low protein diet that did not contain urea. The ill effects are therefore attributable to the lower protein level in the diet. Since it is very doubtful that the lower protein intake interferes with the absorption of fat-soluble factors, the mechanism by which the additional protein produces its beneficial effects is probably related to the synergistic effect of the amino acids in the stabilization of the fat. Clausen, Lundberg and Burr[11] have

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9 Wise, G. H., Mitchell, J. H., La Master, J. P. and Roderick, D. B., *J. Dairy Sci.*, 1944, **27**, 649.

10 Woodward, T. E., and Shepherd, J. B., *J. Dairy Sci.*, 1944, **27**, 648.

11 Clausen, D. F., Lundberg, W. O., and Burr, G. O., *J. Am. Oil Chem. Soc.*, 1947, **24**, 403.

shown that by the addition of adequate amino acid synergists the effectiveness of natural anti-oxidants, which are present in the fats in appreciable quantities, may be greatly increased. This stabilization of the fat may be an important factor in reducing vitamin A and carotene destruction.

Summary. The addition of urea to a diet low in protein did not increase the rate of gain of rabbits. From the data it is apparent that this species cannot use urea in the diet as a replacement of part of the protein. Rabbits fed diets containing 10% of casein as the sole source of protein or 10% casein plus 3.43% urea developed typical vitamin A deficiency symptoms as compared with the control group fed a diet similar except that it contained 20% of casein. The results suggest that protein has a protective action on the oxidation of vitamin A in the diets or that it has a sparing action on the vitamin A requirements of the rabbit.

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Pantothenic Acid and the Metabolism of Amino Acids by Bacteria.*

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Dorfman, Berkman and Koser,[1] and Hills[2] first observed that pantothenic acid functions in the pyruvic acid metabolism of *Proteus morganii*. More recently, Lipmann, *et al.*,[3] and Novelli and Lipmann[4] have found that pantothenate is a part of a coenzyme which is concerned with an acetylation system in tissues and with the breakdown of pyruvate to acetate and other products. They isolated this coenzyme, which they termed "coenzyme A", and were able to recover pantothenic acid from it. They also found that the amount of pantothenate bound in the cells closely correlated with the increased stimulation of pyruvate oxidation, and devised a method for the assay of coenzyme A.[5]

The present study was undertaken to determine if pantothenic acid has any effect on the metabolism of amino acids or other related compounds by bacteria.

Technic. The chemically-defined medium employed in this study for growing the bacteria has already been described.[6] The organism used was *Proteus morganii*. Pantothenate deficient cells were prepared as follows: A small amount of growth was removed from a 6-hour agar slant culture by means of a sterile needle and cultured in 10 ml of a chemically-defined medium for 24 hours at 37°C. One ml of this culture was then used to inoculate larger quantities of the same basal medium containing in this case only 0.01 μg of Ca-pantothenate per ml. The "pantothenate-deficient" cells from 4 liters of a 24-hour culture were then harvested by means of a Sharples centrifuge, and washed twice with 50 ml of sterile saline by the ordinary centrifuge technics. From this quantity of medium it was possible to obtain yields of cells ranging in dry weight from 150

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