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Is Pantothenic Acid Essential for the Growth of Rats?

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The identification of the chick antidermatitis factor as pantothenic acid[1, 2] raises a question as to the need of this vitamin in the ration of rats. Lepkovsky, Jukes, and Krause[3] showed that rats required a factor in addition to riboflavin and vitamin B_6 and since it remained in the filtrate it was called the "filtrate factor." Their preparations were also active for chicks. Elvehjem, Koehn and Oleson[4] demonstrated the need for an additional factor for rats which was designated as factor W. Factor W was separated rather completely from the chick factor (pantothenic acid) but no evidence was presented to show that the rats required the chick factor in addition to factor W. Recently Subbarow and Hitchings[5] have obtained evidence that the active factor in their filtrate preparations is pantothenic acid. El Sadr, *et al.*,[6] have reported that their liver filtrate factor shows great resemblance to both pantothenic acid and factor W. Hoffer and Reichstein[7] have reported a growth response with β-alanine which they consider to be the active principle of pantothenic acid.

In our studies the following basal ration, which is similar to those used in our earlier work[8] has been used: sucrose 76%, purified casein 18%, salts III[9] 4%, corn oil 2%, supplemented with 1.2 mg thiamin*; 2.0 mg riboflavin, 2.5 mg synthetic vitamin B_6* (Merck),

1 Woolley, D. W., Waisman, H. A., and Elvehjem, C. A., *J. Am. Chem. Soc.*, 1939, **61**, 977.

2 Jukes, T. H., *J. Am. Chem. Soc.*, 1939, **61**, 975.

3 Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, 1936, **115**, 557.

4 Elvehjem, C. A., Koehn, C. J., and Oleson, J. J., *J. Biol. Chem.*, 1936, **115**, 707.

5 Subbarow, Y., and Hitchings, G. H., *J. Am. Chem. Soc.*, 1939, **61**, 1615.

6 El Sadr, M. M., Hind, G. H., Macrae, T. F., Work, C. E., Lythgoe, B., and Todd, A. R., *Nature*, 1939, **144**, 73.

7 Hoffer, M., and Reichstein, T., *Nature*, 1939, **144**, 72.

8 Oleson, J. J., Bird, H. R., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1939, **127**, 23.

9 Arnold, A., and Elvehjem, C. A., *Am. J. Physiol.*, 1939, **126**, 289.

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300 mg nicotinic acid,* and 300 mg choline per kilo. Each rat received 2 drops of haliver oil* per week.

A relatively crude preparation of pantothenic acid was used, which was prepared in the following way.† The liver extract was made alkaline and treated with norit. The filtrate and washings were acidified to pH 3 and again treated with norit. The factor was eluted from the second adsorbate with ammonia, readsorbed from acid solution and eluted again with a mixture of alcohol and pyridine. The eluate fraction was concentrated, $BaCO_3$ added to pH 8, and the mixture poured into a large volume of 95% alcohol. The precipitate was dissolved in water and reprecipitated and washed. The combined filtrates of the alcohol soluble Ba salts were concentrated to a thick syrup and thoroughly extracted with absolute alcohol. The combined filtrates were again concentrated to a syrup and the Ba salts were precipitated with acetone. These acetone insoluble Ba salts were freed from barium in the usual way. The final concentrate contained about 10% pantothenic acid as determined by bacterial assay.[10]

To inactivate the pantothenic acid, 1 cc of the above concentrate (containing about 70 mg of solids and 7 mg pantothenic acid), was added to 5 cc of normal NaOH and heated at 95° on the steam bath for one hour. The mixture was carefully neutralized and made up for feeding.

Rats placed on the basal ration at weaning failed to grow and plateaued at weights varying from 40 to 60 g. When 250 mg of liver extract was fed per day the growth rate was 3.4 g per day. A growth of only 2.4 g per day was obtained when the basal ration was supplemented with an amount of the crude preparation sufficient to supply 100 micrograms of pantothenic acid per rat per day. When the alkali-treated concentrate was fed no growth above that obtained with the controls was obtained. In another series of rats the average daily gain was 1.5 g when an amount equivalent to 50 micrograms per day was used and 1.7 g when the level was equivalent to 150 micrograms per day. Thus no significant improvement resulted when the intake was increased 3-fold. These records are calculated for a growth period of 6 weeks and if we study the weekly growth increments we find that the rats on the concentrate grew best the first 2 or 3 weeks and then showed a considerable decrease in the growth rate. Similarly a poorer response was obtained if the supplement

† A large proportion of the pantothenic acid was prepared by Dr. David Klein of Wilson Laboratories.

10 Snell, E. E., Strong, F. M., and Peterson, W. H., *Biochem. J.*, 1937, **31**, 1789.

was added after the rats had been on the basal ration for 3 weeks. Evidently the rats become depleted in factor W during this time.

A small sample of crude crystalline material, made from the lactone of the hydroxy acid portion of the pantothenic acid molecule and reconjugated with β-alanine gave a growth of 1.1 g per day. The material was fed at a level equivalent to 50 micrograms of pantothenic acid per rat per day. Thus none of the concentrates gave growth equivalent to that obtained with liver extract, due to the lack of factor W, especially in the case of more purified preparations. Experiments on the supplementary action of pantothenic acid and factor W will be published later.

It is evident that the rat required in addition to the crystalline factors supplied in our basal ration an alkali labile factor, which is probably pantothenic acid. This factor will not completely replace the original liver extract or crude filtrate preparations, showing further the need for factor W.

The rat apparently requires the intact pantothenic acid molecule since the alkali-treated concentrates contain both the β-alanine and dihydroxy acid fractions. Stimulation due to β-alanine as present in our alkali-treated concentrates was not significant.

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The Metabolism of Human Spermatozoa.*

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Though several papers appear in the literature describing some aspects of the metabolism of mammalian spermatozoa, little information is available on the metabolic behavior of *human* spermatozoa. McCarthy, *et al.*,[1] investigated glycolysis in human semen by measuring the decrease in sugar concentration at various intervals after incubation of the semen at 38°C. After 24 hours of incubation, they found that 75 to 90% of the sugar had disappeared but that, in some cases, the amount of lactic acid formed was not enough to account for the sugar used. They concluded that lactic acid is not

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1 McCarthy, J. F., Stepita, C. T., Johnston, W. B., and Killian, J. F., PROC. SOC. EXP. BIOL. AND MED., 1927, **25**, 54.