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Observations on the Multiple Nature of the "Rat Filtrate Factor."

D. W. Woolley. (Introduced by Kenneth Goodner.)

From the Hospital of the Rockefeller Institute for Medical Research, New York City

The filtrate factor for rats is that material present in fuller's earth filtrates of liver extract which promotes the growth of rats maintained on a basal diet of only pure chemicals. It has been generally assumed that the growth response is due to the presence of one vitamin in the filtrate, although there have been some dissenters from this view.[1, 2] It has recently been shown[3, 4, 5] that relatively pure preparations of the chick antidermatitis factor or pantothenic acid will produce a good growth response in rats in the classical filtrate factor assay. Hence the question arises as to whether pantothenic acid is to be identified with the "rat filtrate factor."

Results of the present investigation show clearly that there are other unknown growth factors for the rat. Pantothenic acid promotes growth in a filtrate-factor-deficient animal only for a relatively short period. It appears that a growth response is obtained only because the young rat has a sufficient store of other growth factors to meet his needs for the relatively short duration of the usual filtrate factor assay. If young male rats are fed a synthetic ration for 4-6 weeks, they will no longer show a growth response when pantothenic acid is subsequently supplied to them. They still respond dramatically when certain liver concentrates in addition to pantothenic acid are added. If the pantothenic acid is added after 3 weeks, rather than after 4-6 weeks, on the synthetic ration, about half of the animals will grow for a week or 2 and then the growth again ceases. Addition of even 200 mg per day of pantothenic acid fails to produce a response, whereas 50 mg will produce good growth in the usual filtrate factor assay. The synthetic ration consisted of sucrose 75, Labco

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

2 Mohammed, A., Emerson, O. H., Emerson, G. A., and Evans, H. M., *Science*, 1939, **90**, 377.

3 Hitchings, G. H., and Subbarow, Y., *J. Nutrition*, 1939, **18**, 268.

4 Oleson, J. J., Woolley, D. W., and Elvehjem, C. A., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 151.

5 Lepkovsky, S., private communication.

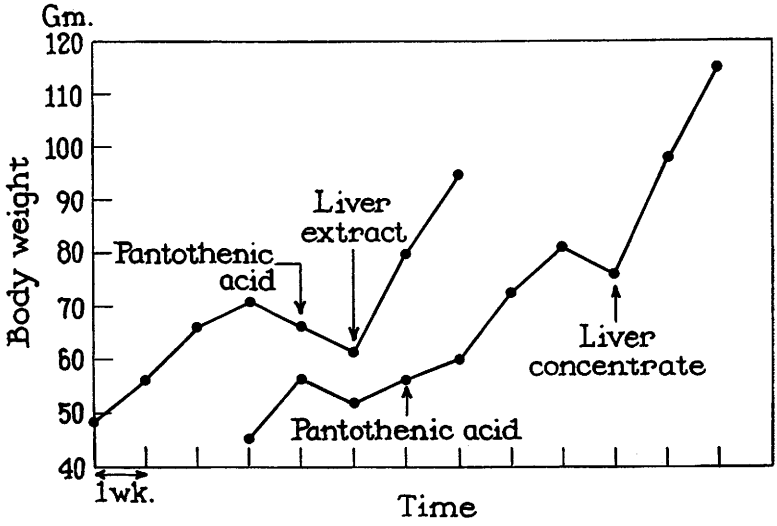

FIG. 1.
Growth responses to pantothenic acid and liver fractions.

casein 18, salts 1[6] 5, cod liver oil 1, corn oil 1, thiamin 200 γ per 100 g, riboflavin 500 γ per 100 g, nicotinic acid 1 mg per 100 g, Merck's synthetic B_6 200 γ per 100 g, β-alanine 1 mg per 100 g, and choline 1 mg per 100 g. Typical growth curves are shown in Fig. 1.

Several sources of the unknown growth factors were compared, and it was found that the fraction of aqueous liver extract which was soluble in 95% alcohol was the richest source. We wish to thank Dr. David Klein of Wilson Laboratories for gifts of this material. One percent of this substance when added to the ration of rats which had failed to grow after the addition of 100 γ per day of pantothenic acid produced a weekly gain of 15 g.

The best method for concentrating the unknown growth factor or factors was found to be as follows: 100 g of the liver extract were extracted thoroughly with butanol. The extracts were treated with an equal volume of acetone, and the filtrate was adsorbed with 100 g of norit A. The active filtrate was concentrated under reduced pressure to a syrup and thoroughly extracted with acetone. The active acetone residue was then dissolved in a small volume of water, made alkaline with barium hydroxide, and treated with 10 volumes of alcohol. The precipitated barium salts were decomposed with H_2SO_4 and were found to contain most of the activity of the original material.

Attempts have been made to show that biotin stimulates the growth of rats. For this work, a highly potent biotin concentrate was made

[6] Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 657.

by the method of Woolley, McDaniel and Peterson.[7] When this material was added at a level equivalent in biotin content to 2% of rice bran extract, no growth response was obtained. The same assay technic was used here as with the liver fractions; that is, the rats were fed the synthetic ration for 5 to 6 weeks, then given 100 γ per day of pantothenic acid, and subsequently the biotin concentrate. The ability of the animal to respond was finally proven by addition of one of the potent liver fractions described above. While this does not prove that the rat does not require biotin, it shows that this substance is not the sole remaining growth factor for this species.

It is not justifiable to ascribe the growth-promoting activity of the above liver concentrate solely to one substance. The experience of the past has emphasized the need for caution in attributing "filtrate factor" activity of concentrates to one compound. However, the above method is a simple and effective means of purifying the remaining unknown rat growth factors. The final answer as to whether only one substance is concerned must await crystallization of the active factor.

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Acute Circulatory Failure (Shock) Following Subcutaneous Injection of Hypertonic Sodium Chloride Solution.

HARRY A. DAVIS. (Introduced by A. G. Eaton.)

From the Department of Surgery, School of Medicine, Louisiana State University, New Orleans.

Intraarterial injection of hypertonic solutions of NaCl is followed by a marked rise in blood pressure which persists for several hours.[1,2,3] The present study is concerned with the effect of the subcutaneous introduction of hypertonic NaCl solutions upon the circulation of the blood, the purpose being to create an area of excessive osmotic pressure and thus induce a flow of fluid out of the capillaries into the site of injection.

Materials and Methods. Seven dogs, weighing from 5 to 8 kg,

[7] Woolley, D. W., McDaniel, L. E., and Peterson, W. H., *J. Biol. Chem.*, 1939, **131**, 381.

[1] Davis, H. A., Jermstad, R. J., and Choisser, R. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 144.

[2] Davis, H. A., *M. Ann. District of Columbia*, 1937, **6**, 344.

[3] Kendrick, D. B., *Surgery*, 1939, **6**, 520.