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Failure of Nicotinic and Yeast Nucleic Acids in "Filtrate Factor" Deficiency in Rats.*

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An aqueous extract of fresh hog's liver will stimulate growth in rats, cure a specific dermatitis in chicks, cure black-tongue in dogs, and cure human pellagra, when fed in conjunction with diets that would otherwise produce a deficiency disease.

Elvehjem, Madden, Strong and Woolley¹ have shown that nicotinic acid (or its amide) will cure black-tongue. This finding has been confirmed.^{2, 3} Likewise nicotinic acid has been shown with high probability to be the anti-human pellagic factor.⁴ Results with chicks and rats have been negative.^{5, 6}

Frost and Elvehjem,⁷ however, obtained a definite growth response in rats by the addition of adenine nucleotide and nicotinic acid or its amide to their basal ration, K₁₂, supplemented with flavin.

The experiment herein reported was designed to test the possible growth-stimulating action of nicotinic acid and yeast nucleic acid when added to a diet deficient only in the factors supplied by liver filtrate. Diet No. 805 was employed:

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The following materials were generously supplied:

Cod liver oil by E. R. Squibb and Sons.

Thiamin Chloride (Betaxin) and Riboflavin by Winthrop Chemical Company.

Hog liver by the H. Moffat Company.

Molasses by Waialua Agricultural Company.

¹ Elvehjem, C. A., Madden, R. J., Strong, F. M., and Woolley, D. W., *J. Am. Chem. Soc.*, 1937, **59**, 1767.

² Dann, W. J., *Science*, 1937, **86**, 616.

³ Street, H. R., and Cowgill, G. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **37**, 547.

⁴ Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **37**, 405.

⁵ Cook, C. A., Clarke, M. F., and Light, A. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **37**, 514.

⁶ Macrae, T. F., and Edgar, C. E., *Biochem. J.*, 1937, **31**, 2225.

⁷ Frost, D. V., and Elvehjem, C. A., *J. Biol. Chem.*, 1937, **121**, 255.

Casein, extracted ⁸	24
Sucrose	67
Fresh lard	3
Cod liver oil	2
Salts (McCollum 185)	4

Twenty-four female rats were placed at weaning on the above diet supplemented with 10 micrograms of thiamin chloride 6 times weekly.

After 4 weeks on the ration, all animals had plateaued in weight. They were then segregated, one littermate in each of 4 groups. All rats then received the following supplements 6 times weekly:

Thiamin chloride	10 micrograms
Riboflavin	40 micrograms
Molasses eluate	44 mg. total solids, equivalent to 4 gm. molasses

The eluate was assayed previously for vitamin B₆ potency.

The additional supplements were as follows:

Group I a—negative controls—no supplements.

I b—after 15 days, 3 of the above group received liver filtrate equivalent to 5 gm. original liver.

Group II—5 mg. nicotinic acid (Eastman) recrystallized to m.p. 228-230°.

Group III—5 mg. yeast nucleic acid (Eastman).

Group IV—5 mg. yeast nucleic acid + 5 mg. nicotinic acid.

After 15 days on the supplements all 4 groups had evidenced like growth responses, and 3 of the negative controls were given a previously assayed multiply adsorbed liver filtrate prepared according to Lepkovsky, Jukes and Krause,⁹ except that 6 rather than 5, adsorptions were made. The total gains were as shown in Table I.

TABLE I.

Preparation fed 6 times weekly	No. of rats	Days after initial depletion period	Gain in gm.	Aver. daily gain, gm.
None	3	1-56	27	0.48
Liver filtrate equivalent to 5 gm. original liver	3	15-56	82	2.00
5 mg. nicotinic acid	6	1-56	26	0.46
5 mg. yeast nucleic acid	6	1-56	25	0.45
5 mg. yeast nucleic acid + 5 mg. nicotinic acid	6	1-56	20	0.36

Nicotinic acid and yeast nucleic acid, fed separately or combined, failed to stimulate growth in rats supplied with basal diet 805, thiamin chloride, riboflavin and vitamin B₆ as a molasses eluate.

A liver filtrate multiply adsorbed on fuller's earth to remove B₁

⁸ Halliday, N., and Evans, H. M., *J. Nutrition*, 1937, **18**, 657.

⁹ Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, 1936, **115**, 557.

(thiamin chloride), riboflavin and vitamin B₆ produced an immediate growth response. The rôle of nicotinic acid as a component of the original filtrate may be masked by a double deficiency. It is possible that although adenine nucleotide is utilized, this is not the case with yeast nucleic acid, and it is also possible that the K₁₂ ration fed by Frost and Elvehjem conceivably supplies factors lacking in our ration.

All rats except those receiving liver filtrate showed a non-specific type of skin lesions dissimilar to the florid acrodynia type of dermatitis characteristic of vitamin B₆ deficiency. The fur was dull and without luster.

Summary. Female rats received a diet in which all the known factors of the vitamin B₂ complex were supplied except that found in a multiply adsorbed liver filtrate. They failed to show a growth response on the addition of nicotinic acid, yeast nucleic acid, or a combination of the two to their ration. Liver filtrate, on the other hand, produced an immediate and marked stimulus to growth.

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Growth-Stimulating Action of Alpha Tocopherol.*

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It would now appear to be well established that in the rat a continuance beyond the fourth month of normal general body growth requires a nutritive factor which is either vitamin E or a substance closely related to it.¹⁻⁵ The growth-stimulating factor was found to be present in wheat germ and cottonseed oils and in their non-saponifiable fractions, a distribution which parallels that of vitamin

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¹ Evans, H. M., *J. Nutr.*, 1928-29, **1**, 23.

² Blumberg, H., *J. Biol. Chem.*, 1935, **108**, 227.

³ Olecott, H. S., and Mattill, H. A., *J. Biol. Chem.*, 1936, **114**, lxxvii.

⁴ Olecott, H. S., and Mattill, H. A., *J. Nutr.*, 1937, **14**, 305.

⁵ Emerson, G. A., and Evans, H. M., *J. Nutr.*, 1937, **14**, 169.