

and hemorrhages into the small intestine. Acute necrosis of the liver may result from doses of 2.0 g per kg or larger. 3. Rats tolerate daily doses of 1.4 g per kg by mouth for about a month without inhibition of growth or pathological changes.

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Lactobacillus casei ϵ Factor in the Nutrition of the Chick.*

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The activity of a norit eluate fraction required by *Lactobacillus casei* ϵ , in promoting growth in chicks receiving purified rations, was recently reported from this laboratory.¹ Further studies have been made on the rôle of this fraction in chick nutrition and the results are presented here. It has been found that the *Lactobacillus casei* ϵ eluate fraction contains a factor or factors essential for growth, hemoglobin formation, and normal feathering in growing chicks.

The basal ration used had the following percentage composition: dextrin 59, purified casein 18, cartilage 10, kidney residue 2, salts IV² 5, CaHPO₄ 1, and soybean oil 5. Each kg of ration also contained thiamin 3 mg, riboflavin 4 mg, pyridoxine 4 mg, Ca pantothenate 15 mg, nicotinic acid 100 mg, choline 1.5 g, and inositol 1 g. Each chick received 2 drops of haliver oil per week. In more recent work 10% gelatin and 0.2% cystine were substituted for cartilage in order to produce a more deficient ration. It has been found that gelatin and cystine can replace cartilage in promoting growth.³

Day-old White Leghorn chicks were obtained either from a commercial hatchery or from the University poultry department. They

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¹ Hutchings, B. L., Bohonos, N., Hegsted, D. M., Elvehjem, C. A., and Peterson, W. H., *J. Biol. Chem.*, 1941, **140**, 681.

² Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1941, **138**, 458.

³ Unpublished data.

were divided into groups of 6 and placed in electrically heated brooders with raised screen bottoms. Water and the experimental ration were supplied *ad libitum*.

The kidney residue used in these rations is prepared by extracting dried defatted kidney with large volumes of hot water and 50% alcohol with additional refluxing with 50% methanol. It is used as a source of biotin.

Hemoglobin was determined by the method adapted for the Evelyn photoelectric colorimeter, with the following slight modification: The samples were centrifuged at high speed for 1 minute after addition of the alkali in order to clarify the solutions and readings were made immediately.

The results are summarized in Table I. Chicks on the basal ration grew very poorly, averaging 124 g at 4 weeks of age. Addition of solubilized liver extract at a level of 2% of the ration supplied all other essential factors for the chick, since optimum growth was obtained. A norit eluate prepared from solubilized liver extract by the method of Hutchings, *et al.*,⁴ also gave marked growth responses, but the maximum weight was about 40 g below that obtained with the solubilized liver extract. The difference is still more marked when a biotin concentrate is substituted for kidney residue. This difference indicates the existence of at least one other unidentified factor in the solubilized liver extract.

Very poor feathering was also noted on chicks receiving the basal ration. The poor condition of the feathers first becomes noticeable at 3 weeks. After this period, feather formation is very slow or absent, and more and more feathers break off until few large feathers remain. Fig. 1 shows a chick maintained on the basal ration for 5 weeks. At this time the feathers are rough, shaggy, sparse and brittle; many are broken off. The bird in Fig. 2 is the same age, but received 2% solubilized liver extract in the ration. The feathering is normal

TABLE I.
Effect of Supplements on Growth and Anemia.

Supplement to basal ration	No. of chicks	Avg wt.		Hemoglobin at 4 wks g/100 cc	Feathering
		3 wks g	4 wks g		
None	30	105	124	6.13	Poor
Norit eluate equivalent to 5% solubilized liver extract	18	161	189	8.09	Good
2% solubilized liver extract	60	172	232	8.40	"
2% glycine + 1/2% arginine	6	107	120		Poor

⁴ Hutchings, B. L., Bohonos, N., and Peterson, W. H., *J. Biol. Chem.*, 1941 **141**, 521.



FIG. 1.
Chick on basal ration. Age 5 weeks. Note poor feathering.



FIG. 2.
Chick on basal ration plus 2% solubilized liver extract. Age 5 weeks.

and complete. The norit eluate also produces normal feathering.

The poor feathering on this basal ration is distinct from that ascribed to a glycine and arginine deficiency;⁵ the typical lacy appearance of the feathers on the amino acid deficiency is absent here. A group of chicks was given arginine and glycine at adequate levels. No improvement in feathering above the basal was noted, and there was no increase in growth.

Another symptom noted on the basal ration at 4 weeks is a reduced hemoglobin content of the blood. The average hemoglobin content of the blood of chicks on the basal ration was 6.13 g per 100 cc with some values as low as 3 g. The chicks receiving solubilized liver extract had an average level of 8.40 g, and those getting the norit eluate, 8.09 g. The anemia becomes more severe as the chicks grow older.

The properties of the *Lactobacillus casei* ϵ eluate fraction are similar to those reported for several other chick factors. Factor U,⁶ the alcohol precipitate factor,⁷ Hogan's antianemia factor,⁸ and the norit eluate fraction are all alcohol insoluble and are all adsorbed on Fuller's earth at an acid pH. The identity of "folic acid" and the norit eluate factor has already been discussed.⁴ The occurrence of anemia on our basal ration and its prevention by the norit eluate is suggestive of the identity of the norit eluate fraction with Hogan's antianemia factor.

Summary. Further studies on the rôle of the *Lactobacillus casei* ϵ eluate factor in chick nutrition are reported. Purified preparations of the factor promote growth, hemoglobin formation, and normal feathering.

⁵ Hegsted, D. M., Briggs, G. M., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1941, **140**, 191.

⁶ Stokstad, E. L. R., and Manning, P. D. V., *J. Biol. Chem.*, 1938, **125**, 687.

⁷ Schumacher, A. E., Heuser, G. F., and Norris, L. C., *J. Biol. Chem.*, 1940, **135**, 313.

⁸ Hogan, A. G., and Parrott, E. M., *J. Biol. Chem.*, 1940, **132**, 507.