

Effect of *Lactobacillus casei* € Eluate Fraction on Reproduction in the Domestic Fowl.

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Hutchings, Bohonos, Hegsted, Elvehjem and Peterson¹ have reported that a norite eluate fraction required by *Lactobacillus casei* € is essential for the normal growth of chickens. In further studies Mills, Briggs, Elvehjem, and Hart² found that the eluate fraction is essential for hemoglobin formation and normal feathering in young chicks. Schumacher and Heuser³ have reported that mature hens require a factor, termed the alcohol-precipitate factor, for egg production, hatchability and maintenance of body weight. The possible relation of the *Lactobacillus casei* € factor and the alcohol-precipitate factor, as well as other chick factors, is suggested by Mills, *et al.*²

In studies on the effect of various members of the vitamin B complex on reproduction in poultry we have observed that a norite eluate of solubilized liver extract prepared by a modification of the method of Hutchings, Bohonos and Peterson¹ contains a factor or factors essential for normal embryonic development in the domestic fowl. The results of these studies are reported here.

Single Comb White Leghorn Pullets were housed in individual laying cages and artificially inseminated. Eggs were incubated weekly. In the first experiment the basal ration had the following composition: Sucrose, 60; Casein,[†] 18; gelatin, 5; salts

IV,[‡] 5; soybean oil, 5; whey concentrate, 5; and fish oil, 2 (3000 I. U. vitamin A, 400 A.O.A.C. units of D per g). Each kg of ration also contained thiamin, 3 mg;[‡] riboflavin, 5 mg; calcium pantothenate, 15 mg; pyridoxin, 8 mg; nicotinic acid, 100 mg; and choline, 2 g. Oyster shell was supplied *ad libitum*. After the birds had been on the basal ration for 7 weeks, 3% kidney residue replaced the whey concentrate as a source of biotin. The kidney residue used in these rations was prepared by extracting dried defatted kidney with large volumes of hot water and 50% ethanol. Dextrin was substituted for the sucrose during the tenth week.

After 9 weeks on the basal ration hatchability was at zero. The birds were continued on the basal ration for an additional 3 week depletion period, then supplements were added as shown in Table I. Since the inositol content of the basal ration was unknown one hen was given 0.2% inositol. One hen was fed the norite eluate fraction of solubilized liver extract, 2 birds were given 0.2% inositol plus the norite eluate fraction and 2 birds were given 4% solubilized liver extract. The results are given in Table I.

The results of this experiment furnish evidence that both solubilized liver extract and a norite eluate fraction of solubilized liver extract contain a factor or factors essential for normal development of the

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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 Mills, R. C., Briggs, G. M., Elvehjem, C. A., and Hart, E. B., *Proc. Soc. Exp. Biol. and Med.*, 1942, **49**, 186.

³ Schumacher, A. E., and Heuser, G. F., *Poul. Sci.*, 1940, **19**, 315.

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

[†] Borden's H.5P.

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

[‡] We are indebted to Borden and Company, Special Products Division, New York City, for the fish oil and whey concentrate; to Merck and Company, Rahway, N.J., for the crystalline vitamins; and to Wilson Laboratories, Chicago, Ill., for the solubilized liver extract and gelatin.

TABLE I.
Supplements to Basal Ration and Percent Hatch of Fertile Eggs.

Week	Group and supplements to the basal ration							
	1		2		3		4	
	0.2% inositol		Norite eluate equivalent to 10% solubilized liver extr.		0.2% inositol + norite eluate equivalent to 10% solubilized liver extr.		4% solubilized liver extr.	
	Fertile Eggs	% Hatch	Fertile Eggs	% Hatch	Fertile Eggs	% Hatch	Fertile Eggs	% Hatch
12	11	0.0	0	—	18	0.0	0	—
14	8	0.0	1	0.0	17	23.5	0	—
16	10	0.0	4	100.0	20	70.0	0	—
18	8	0.0	0	—	20	75.0	2	100.0
20	10	0.0*	0	—†	20	75.0‡	8	87.5
22	10	60.0	0	—	17	70.6	4	100.0
24	8	75.0	0	—	21	47.6	18	83.4
26	9	88.9	1	100.0	16	31.2	15	80.0
28	5	80.0	7	85.7	19	5.3	19	57.9
30	6	100.0	10	80.0	19	5.3	19	94.6

*Norite eluate equivalent to 10% solubilized liver extract added.

†0.2% inositol added.

‡Norite eluate fraction discontinued.

chicken embryo. The bird receiving only the norite eluate came into production 3 weeks after the supplement was added then stopped laying until 4 weeks after the inositol was added. Obviously with only one bird no final conclusions can be drawn. The bird receiving inositol alone continued to lay

but all her eggs failed to hatch until the norite eluate was added. The response to the latter supplement was almost immediate.

The birds receiving only the solubilized liver extract responded very slowly because they were in such poor condition at the time the supplements were added. They each

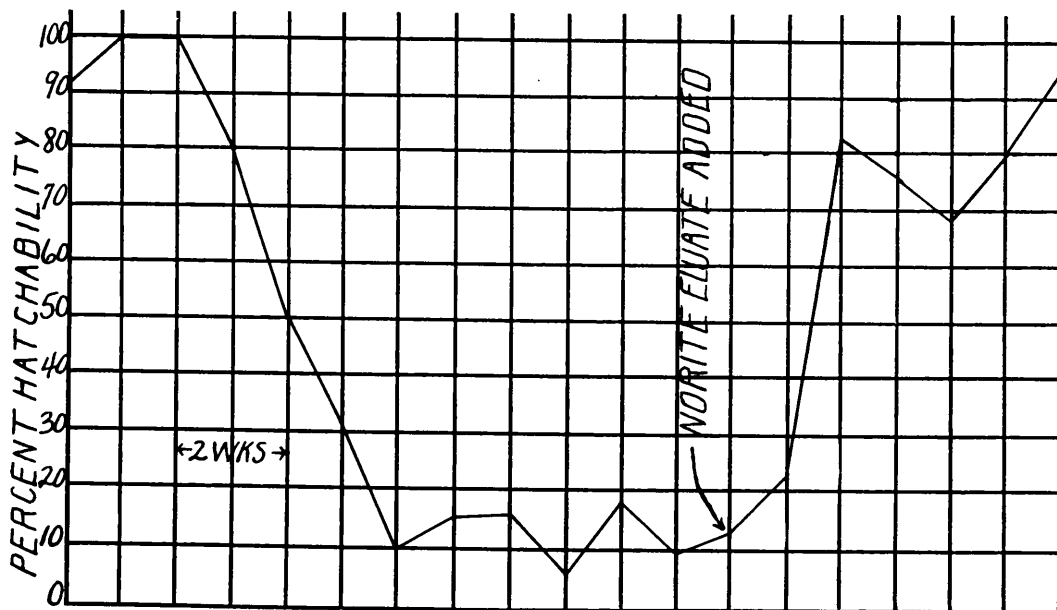


FIG. 1.
Hatchability of Fertile Eggs.

weighed 950 g when the supplement was added and at the time they started laying again their weight had increased to approximately 2 kg each.

The basal ration used in the second experiment was identical to that used in the first except that 0.1% inositol was added.

In this experiment 4 birds were fed the basal diet. Their average hatchability dropped from above 90% to less than 20% in 6 weeks. They were continued on the basal diet for 6 weeks longer to insure thorough depletion and then were fed the norite eluate fraction preparation equivalent to 10% solubilized liver extract. The results are given in Fig. 1.

The results of this experiment are in agreement with the results of the first experiment and show conclusively that the

norite eluate of solubilized liver extract contains a factor or factors essential for normal development of the chicken embryo.

A deficiency of this factor resulted in a less pronounced decline in egg production than in hatchability. Some birds continued to lay when fed the basal ration while others would go out of production and lose weight. Such results suggest that the basal ration contained a suboptimal amount of the factor involved. Further studies are in progress to clarify this point.

Summary. Evidence is presented to show that the *Lactobacillus casei* ϵ eluate fraction is essential for normal development of the chicken embryo.

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Chemotherapy in *Cl. welchii* Infection in Mice.

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The importance of *Cl. welchii* infection in war wounds has given impetus to work in this field during the past year. The early literature on the subject has been well reviewed by Bliss, Long and Smith.¹ Recently, Sewell, Dowdy and Vincent² have been able to produce an infection in dogs which closely simulated human infection. They found that sulfadiazine therapy gave the best results.

In this study, a comparison of the efficacy of sulfanilamide, sulfapyridine, sulfathiazole and sulfadiazine‡ in experimental *Cl. welchii* infection in mice has been made. By feeding

the animals a moist cooked diet in which the drugs had been incorporated, it was possible to maintain blood concentrations comparable to those used in the treatment of patients ill with serious infections.

The drug-diet was prepared in the following manner: The required amount of drug was dissolved in 1000 ml of water at 100°C. To this was added slowly 280 g of a modified Sherman dry diet and the mixture cooked with stirring until it was the consistency of thick mush. It was then allowed to cool, weighed and the amount of drug calculated as mg per g of food. The per cent of drug

* A. B. Kuppenheimer Foundation.

† Joseph B. DeLee Fellow 1941.

¹ Bliss, E. A., Long, P. H., and Smith, D., *War Med.*, 1941, **1**, 799.

² Sewell, R. L., Dowdy, A. H., and Vincent, J. G., *Surg., Gyn. and Obst.*, 1942, **74**, 361.

‡ The sulfonamides used in this study were furnished through the courtesy of Lederle Laboratories, Inc. (sulfadiazine), Merck and Company (sulfapyridine), and the Department of Medical Research, Winthrop Chemical Company (sulfanilamide and sulfathiazole).