tom of the container. This substance was dissolved in water; the insoluble part was filtered off and the clear solution was treated with norite and evaporated in a water bath to a small volume. Alcohol was added gradually until a precipitate began to be formed. While standing in the cold overnight, slightly yellow crystals were formed. By repeated crystallization from diluted alcohol, using the technic as described, colorless crystals were obtained. They had a corrected melting point of 223° and after drying at 110° gave the following analysis:

40.07 and 39.95% C; 7.04 and 7.10% H; 0.0% N. The substance showed no optical rotation and gave the Scherer reaction.

The theoretical values for inositol are 40% C and 6.67% H. A sample of commercial inositol was recrystallized and then gave a melting point of 223°. A mixture of the crystals from thyroid and inositol had the same melting point. The substance thus was identified as inactive inositol.

The quantity contained in the glandular material was not determined exactly, as the repeated crystallizations lead necessarily to losses. However, it is estimated that about 0.3% of free inositol was extracted from the thyroid powder.

The presence of inositol in aqueous extracts obtained from thyroids in a manufacturing process has been reported before by Tambach.[1] The quantity contained in the thyroid is suggestive of a special significance in the organism. Most of our attempts to find physiological effects of inositol which are not limited to the mere correction of a deficiency, have proved unsuccessful.

Brissemoret and Chevalier[2] have observed a toxic action of inositol on the rabbit's heart perfused by the Langendorff method. They added 0.05 and 0.1% of inositol to the perfusion fluid. In our experiments by the Langendorff method we replaced 10 and 20% of the 0.1% glucose in Locke's solution with inositol to avoid differences in ionic concentration that alone may cause a change in the rhythm of a perfused heart. The effect of both inositol products, the commercial and that isolated from thyroid, was identical in 6 different experiments and consisted in an immediate decrease in the auricular and ventricular amplitude followed by arrhythmias. Final death of the heart could be prevented by change to standard Locke solution as perfusion fluid, which effected prompt, but not quite complete, recovery. The concentrations of inositol of 0.01 and 0.02% are higher than occur physiologically for which reason no conclusion as to the significance of inositol in the function of the heart can be drawn.

Summary. Substantial quantities of optically inactive free inositol were extracted from dried thyroid glands. In the perfused rabbit's heart inositol causes a decrease in the amplitude and arrhythmias.

[1] Tambach, R., *Pharmazeutische Centralhalle*, 1896, **17**, 167.

[2] Brissemoret, A., and Chevalier, J., *C. R. Acad. Sciences*, 1908, **147**, 217.

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Absence of Appreciable *L. casei* Factor Effect in Anti-Pernicious Anemia Liver Extract.

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The absence of considerable quantities of "free" *L. casei* factor from concentrated anti-pernicious-anemia liver extracts[1] is in contrast with the effect of the "liver" form of *L. casei* factor in producing remission of pernicious anemia.[2-4] The possibility remained that "free" *L. casei* factor could be liberated

[1] Clark, G. W., *Am. J. Med. Sci.*, 1945, **209**, 520.

[2] Vilter, C. F., Spies, T. D., and Koch, M. B., *South. Med. J.*, 1945, **38**, 781; Moore, C. V., Bierbaum, O. S., Welch, A. D., and Wright, L. D., *J. Lab. and Clin. Med.*, 1945, **30**, 1056.

in vitro or *in vivo* from the maturation factor which is present in such liver extracts.

In the present investigation, concentrated parenteral liver extract, 15 U.S.P. units per cc, was incubated with dried chicken pancreas, which contained "vitamin B_c conjugase." The content of "free" *L. casei* factor after incubation was found to be 1.0 μg per cc by *L. casei* assay and 0.8 μg per cc by *S. lactis R* assay, against synthetic "liver" *L. casei* factor standard.

The effect of various samples of the same type of liver extract on chicks deficient in *L. casei* factor was then measured. Day-old New Hampshire Red chicks were placed on the diets. Ten chicks were used in each group. The basal diet had the following composition: glucose (cerelose) 58.5 g, purified casein (Labco) 20 g, gelatin 8 g, calcium gluconate 5 g, cystine 0.4 g, choline chloride 0.2 g, inositol 0.1 g, bone ash 2 g, NaCl 0.6 g, KH_2PO_4 0.45 g, K_2HPO_4 0.6 g, $MgSO_4$ 0.25 g, $MnSO_4,4H_2O$ 0.05 g, ferric citrate 0.05 g, $CuSO_4,5H_2O$ 2 mg, $Al_2(SO_4)_3,18H_2O$ 1.6 mg, zinc acetate 1.4 mg, KI 0.6 mg, cobalt chloride 0.4 mg, nickel chloride 0.2 mg, calcium pantothenate 5 mg, niacinamide 5 mg, riboflavin 1 mg, pyridoxine HCl 1 mg, thiamine HCl 1 mg, p-aminobenzoic acid 1 mg, 1-acetoxy-2 methyl-4 naphthyl sodium phosphate 0.5 mg, (dl) biotin .04 mg* to which were added vitamin A 1500 U.S.P. units, vitamin D 200 A.O.A.C. units, mixed tocopherols 34 mg, dissolved in corn oil (Mazola) to a total of 3 g. Table I illustrates the results of a typical experiment in which concentrated parenteral liver extract was injected into chicks at a rate which corresponded to approximately 0.4 U.S.P. unit of maturation factor per day. This level of dosage had no effect on the chicks under conditions in which a marked growth response was obtained with 0.5 or 1.0 mg of "liver" *L. casei* factor added per kilo of diet, which resulted in a daily intake of 5 to 20 μg of added *L. casei* factor.

TABLE I.
Effect of Various Supplements on Growth of Chicks on Diet Deficient in *L. casei* Factor.

Supplement, or other treatment	Gain in g	
	In 3 wk	In 4 wk
None	47	98†
0.1 cc liver extr., 15 units per cc injected twice weekly	48	66‡
1.0 mg synthetic "liver" *L. casei* factor added per kilo of diet*	113	222

* Other experiments showed that the growth response with 0.5 mg was as great as with 1.0 mg.
† 6 survivors.
‡ 4 "

It has been shown elsewhere[5] that chicks under similar conditions respond markedly to daily injections of 5 to 10 μg of "liver" *L. casei* factor.

Results with pernicious anemia patients[3,4] indicate that an effect corresponding approximately to 1 U.S.P. unit of maturation factor in injectable liver extract is obtained by injecting or feeding 1 to 10 mg of "liver" *L. casei* factor daily, while a level of 0.35 mg produced an incomplete response.[4] From this it would seem that 0.4 U.S.P. unit of maturation factor would be equivalent to at least 400 μg of "liver" *L. casei* factor in the treatment of pernicious anemia. The present investigation indicates that *L. casei* factor was not liberated in appreciable quantities from concentrated liver extract by treatment with vitamin B_c conjugase as supplied by dried chicken pancreas. The maturation factor as present in liver extract was ineffective for chick growth when injected at a level which would correspond, on an anti-pernicious-anemia basis, to an estimated dosage of an equivalence of 400 or more μg of "liver" *L. casei* factor daily; moreover, chicks under these conditions showed a marked growth response to a daily intake of 5 to 10 μg of "liver" *L. casei* factor.†

[3] Doan, C. A., Wilson, H. E., Jr., and Wright, Claude-Starr, *Ohio State Medical J.*, 1946, **42**, 139; Spies, T. D., *J. A. M. A.*, 1946, **130**, 474.

[4] Welch, A. D., Heinle, R. W., and Moore, C. V., paper presented at Atlantic City meeting, American Chemical Society, April, 1946.

* Kindly provided by Dr. B. R. Baker.

[5] Frost, D. B., Dann, F. P., and McIntire, F. C., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 65.

† Unpublished results by Daft and co-workers (personal communication from Dr. F. S. Daft) indicate that concentrated parenteral liver extract, 15 U.S.P. units per cc, was found to be ineffective against a deficiency of *L. casei* factor as produced in rats by feeding a purified diet to which a sulfonamide had been added.