additional supplements for a period of 9-11 days, following which the animals were sacrificed for lipid analyses. These supplements were always given at the following levels, riboflavin 9 mg, pyridoxine hydrochloride 6 mg, calcium pantothenate 21 mg, niacin 21 mg, and biotin 60 γ per 1200 g of sucrose. Choline chloride and inositol were administered as recorded in the accompanying table.

The animals were rapidly exsanguinated by decapitation at the end of the supplemental feeding period. Their livers were removed, weighed, and after grinding in a mortar, analyzed individually for neutral fat and cholesterol. The intestinal tract was removed and the animal skinned, after which the neutral fat and cholesterol content of both the bodies and skins from each group was collectively determined. The analytical procedures have been described previously.[5,6]

From the experimental results, which are recorded in Table I, it will be seen that the lipotropic action of inositol in the absence of biotin from the diet is confirmed. The effect, however, is much less than one would expect from choline under the same conditions. The effect of choline plus inositol is greater than of either alone but the data are not sufficient to show whether they act synergistically or not. The effect on liver cholesterol is, on the whole, less than on the neutral fat. The small amount of biotin used in experiments 3, 4 and 5 did not apparently influence the lipotropic action of the inositol, neither did it increase the lipid content of the livers in the absence of inositol. These results are contrary to those obtained by McHenry and his associates[1] but it is possible that if we had used larger amounts of biotin, choline-resistant inositol-sensitive fatty livers might have been obtained.

The neutral fat content of the body and skin of those animals receiving both choline and inositol is greater than that of the corresponding animals receiving these individually. Whether this is due to an increased food intake, as suggested by the greater rate of growth of these animals, or to a stimulating effect on fat synthesis, cannot be stated at this time as food intake was not sufficiently accurately controlled to justify definite conclusions.

Summary. The lipotropic action of inositol in the presence of other members of the vitamin B-complex has been confirmed. The effect of inositol plus choline is considerably greater than that of either alone.

[5] Outhouse, E. L., and Forbes, J. C., *J. Lab. and Clin. Med.*, 1940, **25**, 1157.

[6] Forbes, J. C., *J. Nutrition*, 1941, **22**, 359.

14316 P

Preparation of the Pituitary Corticotrophic Hormone.*

A. H. NEUFELD. (Introduced by J. B. Collip.)

From the Research Institute of Endocrinology, McGill University, Montreal, Canada.

The procedure used for the preparation of the corticotrophic hormone from whole and from dissected calf anterior pituitary tissue was based on methods recommended by Lyons, by Li, Simpson and Evans, and by Sayers, White and Long.[1-3] It has been our experience that most of the posterior lobe potency present in the starting material remains with the fraction showing corticotrophic activity. Attempts made to separate these factors by their solubilities at various pH and salt concentrations were without success. The same held true in utilizing dialysis

* Aided by a grant from the Dazian Foundation.

[1] Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 645.

[2] Li, C. H., Simpson, M. E., and Evans, H. M., *Science*, 1942, **96**, 450.

[3] Sayers, G., White, A., and Long, C. N. H., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 199.

TABLE I.
The Assay Results of the Pituitary Corticotrophic Preparations.

Preparation	NH_4OH temp.-time, hr	Amt given daily, mg	Wt of single adrenals; in brackets—left, control adrenal, mg	Oxytocin μ/mg	Antidiuretic μ/mg
Step 5—ant. pit.	—	0.1	7 (5); 7 (4); 6 (5); 5 (4)	.04-.07	.05-.09
		0.2	9 (6); 8 (5); 9 (5); 7 (5)		
		0.25	15 (7); 13 (7); 12 (6); 16 (7); 11 (7); 10 (7)		
		0.5	9 (6); 15 (7); 13 (6); 16 (5); 11(6); 18 (5)		
Step 5—whole pit.	—			3.8-4.0	3.3-3.9
Step 7—ant. pit.	37°, 4	0.25	6 (4); 7 (3)	.02-.03	.02-.04
		0.5	18 (9); 7 (3); 10 (4); 11 (7); 16 (8); 12 (7)		
		1.0	19 (5); 16 (6); 12 (5); 13 (6); 12(7); 21 (6)		
Step 7—ant. pit.	37°, 10	0.25	9 (5); 11(7)	.002-.003	.002-.003
		0.5	12 (8); 15 (7); 11 (6); 8 (5); 9 (6)		
		1.0	15 (7); 18 (6); 16 (6); 11 (5); 12 (7)		
		2.0	16 (5); 19 (6); 21 (8); 14 (6)		
Step 7—whole pit.	37°, 10	0.25	7 (6); 8 (5); 7 (3); 9 (6)	.12-.15	.12-.16
		0.5	10 (6); 14 (5); 9 (6); 8 (5); 10 (4)		
		1.0	16 (4); 17 (7); 12 (7); 15 (5); 14 (6)		
Step 7—ant. pit.	25°, 6	0.5	12 (7); 10 (6); 9 (5); 9 (6); 12 (5)	.02-.04	.03-.04
		1.0	16 (7); 18 (6); 12 (5); 16 (5); 16 (4)		
Step 7—ant. pit.	25°, 14	0.25	8 (6); 12 (7); 6 (5); 8 (5)	.003-.005	.004-.006
		0.50	10 (6); 14 (8); 9 (5); 16 (6); 10 (4)		
		1.0	12 (5); 17 (7); 17 (4); 13 (5); 12 (6); 18 (5)		
Step 7—whole pit.	25°, 14	0.5	12 (6); 18 (5); 11 (6); 16 (4); 14 (8)	.18-.19	.17-.19
		1.0	18 (6); 21 (4); 23 (8); 16 (8); 13 (4)		

with membranes of various porosities. This led us to attempt inactivation of the posterior lobe hormones with alkali.[4] Treatment with NaOH under varying conditions of concentration and time, when successful in removing posterior lobe activities, almost invariably resulted in appreciable losses of corticotrophic potency. The same held true for NH_4OH treatment at higher temperatures. NH_4OH treatment at 37° and at 25°C resulted in the inactivation of most of the posterior lobe hormones without any appreciable loss of corticotrophic activity (Table I).

The method utilized for the preparation of the corticotrophic hormone containing only traces of posterior lobe contamination was as follows: (1) An 80% acidified acetone extraction was made from thoroughly minced tissue. Two such extractions, 6 hours each, were sufficient to remove all the active material; the residues being subjected to pressures in each instance. This was carried out at room temperatures, all subsequent procedures were done at 0° to 3°C. (2) The active material was precipitated from the combined filtrate by raising the acetone concentration to 90%. This was thoroughly washed with cold acetone and dried. (3) The powder, containing the corticotrophic and prolactin hormones, was extracted repeatedly with 0.1 M Na_2HPO_4. This was brought to half saturation with $(NH_4)_2SO_4$, the precipitate formed taken up in water and dialyzed. (4) The dialyzed solution (made to a concentration of 0.5%) was adjusted to pH 3 and NaCl added to 0.36 M. The precipitate formed, containing the prolactin activity, can be further purified. The filtrate

[4] Collip, J. B., *Edinburgh Med. J.*, 1938, **45**, 782.

was brought to half saturation with $(NH_4)_2SO_4$, filtered, taken up in water and dialyzed. (5) The pH was adjusted successively to 8.0, 6.6, and 5.4 and the precipitates formed discarded. (6) One-half volume of concentrated NH_4OH was added and this allowed to stand at 37° or 25°C for varying lengths of time (Table I). Then acetone was added to a concentration of 90%, the precipitate formed taken up in water and dialyzed. (7) Step 5 was repeated, the final solution dialyzed until free of salt and lyophilyzed. The yield of material has varied between 0.6 to 1.2 g per kg (with whole glands the yield was 0.6 to 0.88 g, with dissected anterior lobes, 0.76 to 1.2 g).

When assayed for growth, prolactin, thyreotrophic and gonadotrophic activities this material showed negative results with the amounts used. The assays for corticotrophic activity were done on young rats weighing 100 to 120 g, 14 to 18 days after hypophysectomy. The left adrenal was removed and weighed prior to injection. Daily injections were given for 9 days and the right adrenal weighed on the 10th day. The daily amounts of the final material necessary to restore the right adrenal size to normal ranged between 0.25 and 0.5 mg (Table I). This is of the same order of potency as that reported by Li *et al.* and Sayers *et al.* The preparations made from whole glands showed a similar corticotrophic potency, but still retained appreciable posterior lobe contamination (more than 0.1 μ per mg).[4]

14317 P

Congenital Malformations Induced in Rats by Maternal Nutritional Deficiency. V. Effects of a Purified Diet Lacking Riboflavin.

JOSEF WARKANY AND ELIZABETH SCHRAFFENBERGER.

From the Children's Hospital Research Foundation and the Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, O.

The appearance of congenital malformations in the offspring of female rats fed a deficient diet (Diet I) has been described.[1,2] Diet I had the following percentage composition: Yellow corn meal 76, wheat gluten 20, calcium carbonate C.P. 3, and sodium chloride C.P. 1. Sixty International Units of vitamin D as viosterol were administered to each female by pipette every tenth day. The malformations could be prevented in the offspring when the maternal diet was supplemented by 2% dried pig liver.[3] It was concluded that a substance deficient in Diet I and present in large amounts in liver was responsible for the presence or absence of the congenital defects.

Several investigators have reported satisfactory growth and reproduction of rats on highly purified diets in which synthetic factors of the vitamin B-complex (thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid and choline) were employed.[4-7]

We have studied the reproduction of female rats fed a similar purified diet which, however, did not contain riboflavin.

Twenty-one female rats of the Sprague-

1 Warkany, J., and Nelson, R. C., *Science*, 1940, **92**, 383.

2 Warkany, J., and Nelson, R. C., *Anat. Rec.*, 1941, **79**, 83.

3 Warkany, J., and Nelson, R. C., *J. Nutrition*, 1942, **23**, 321.

4 Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 625.

5 Unna, K., *Am. J. Med. Sci.*, 1940, **200**, 848.

6 Unna, K., Richards, G. V., and Sampson, W. L., *J. Nutrition*, 1941, **22**, 553.

7 Henderson, L. M., McIntire, J. M., Waisman, H. A., and Elvehjem, C. A., *J. Nutrition*, 1942, **23**, 47.