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]

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

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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.

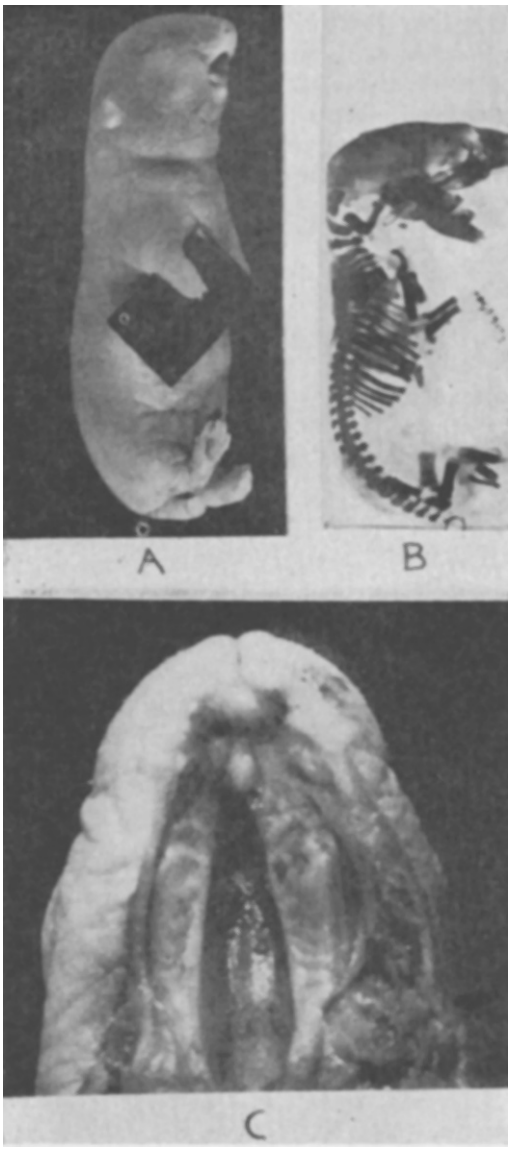

FIG. 1 A.
Abnormal newborn rat showing short mandible, protruding tongue, short extremities, and syndactylism of the hand.

1 B.
Cleared specimen of an abnormal newborn rat showing short mandible and short tibia, and fusion of ribs.

1 C.
Cleft palate of an abnormal newborn rat.

Dawley strain, reared on an adequate diet and weighing from 170-240 g were put, at the age of 3 months, on a purified diet of the following percentage composition: Sucrose 68, Vitamin-test Casein 18, Vegetable Oil 10,* Salt Mixture 4.† One hundred grams of this diet were supplemented by 0.8 mg thiamine hydrochloride, 0.8 mg pyridoxine hydrochloride, 1 mg calcium pantothenate, 10 mg nicotinamide (Merck), and 100 mg choline chloride (SMACO). Every tenth day 1-2 drops of a 1:4 dilution with olive oil of "Drisdol with vitamin A in oil" (Winthrop) were administered by pipette.

During the first 2 weeks the rats maintained their weight on this diet or gained from 4 to 15 g. They also showed regular estrous cycles for several weeks. They were mated to normal males of the same strain which had been fed an adequate stock diet. All but 2 of the females became pregnant within 3 weeks. Of these 19 pregnancies 11 ended in resorption while 8 females delivered their litters at term. The pregnancies caused such great weight losses in 11 animals that they were considered unfit for further breeding. Of the 8 rats that remained in the experiment 4 did not show estrous cycles within 8 weeks after the first pregnancy. Four rats underwent a second pregnancy. One of these resorbed, while 3 delivered their litters at term.

A total of 74 young was obtained from these female rats fed the purified diet lacking riboflavin. Sixty-four of these young were anatomically normal on external inspection and after clearing. Ten of the newborn rats showed congenital malformations of the "pattern of Diet I"[8] (Fig. 1A and B). Three of the abnormal young were the result of a first pregnancy and 7 the result of 2 second pregnancies. The malformations consisted in shortness of the mandible, tibia, fibula, radius and ulna, fusion of ribs and sternal centers of ossification, syndactylism, brachydactylism and cleft palate (Fig. 1C). The frequency of the osseous defects in the 10

* The mixture of sucrose, casein, and vegetable oil was furnished by S. M. A. Corporation.

† Salt Mixture No. 351 as recommended by Hubbel, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition*, 1937, **14**, 273.

[8] Warkany, J., Nelson, R. C., and Schraffenberger, E., *Am. J. Dis. Child.*, 1943, **65**, 882.

abnormal specimens is indicated in the following tabulation:

Tibia	7	Hand	6
Mandible	8	Sternum	2
Ribs	6	Ulna	4
Fibula	5	Hindfoot	4
Radius	5	Cleft palate	2

One of the abnormal young showed in addition to the abnormalities of the "pattern of Diet I" a defect of the spine.

Summary. Congenital malformations were observed in the offspring of female rats fed a purified diet lacking riboflavin. The malformations were anatomically identical with those described previously as the "pattern of Diet I."

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Cephalin, Protamine and the Antithromboplastic Activity of Normal and Hemophilic Plasmas.

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Normal human plasma, collected and separated with especial precautions, has the property of reducing the clot accelerating activity of homologous brain tissue extracts. The clot decelerating activity of hemophilic plasma is several times greater than normal.[1] The thromboplastin of aqueous brain-tissue extracts, on which this activity of the plasma has been tested, is a lipoprotein; its clot accelerating properties reside chiefly in the lipid moiety, the active constituent of which is a cephalin.[2] From the evidence here presented it appears that cephalin is the constituent of the lipoprotein vulnerable to the antithromboplastic action of the plasma. When the lipoprotein is stripped of most of its lipid content by extraction with fat solvents, it loses most of its susceptibility to the antithromboplastin.

Methods for separating the plasma and testing antithromboplastin activity have been given in detail elsewhere.[1] Only clear, "platelet-free" plasmas may be used. The thromboplastic lipoprotein solution (Sol. A) was prepared by extracting 300 mg of acetone dried human brain powder with 5 cc of 0.85% NaCl.[3] Cephalin was obtained from acetone dried human brain by extraction at 5°C overnight, with ether of low peroxide content (1 g brain powder plus 20 cc ethyl ether). In view of the rapid loss of potency of cephalin solutions[4] the time of extraction was made short and only a small amount was prepared each time. The clear ether extract was rapidly concentrated *in vacuo* and poured into ice cold absolute ethanol. The white flaky precipitate was then washed with acetone, dried to a waxy consistency and suspended at once in 10 cc of saline. In a few instances the cephalin was purified further by repeating the passage of the lipid through absolute alcohol 2 or more times before suspending it in saline. A pale bluish grey suspension was obtained (Sol. B), 0.1 cc of which gave a clotting time between 60 and 80 seconds on 0.3 cc recalcified citrated normal human plasma. As is well known, the cephalin obtained by ether extraction of the thromboplastic lipoprotein does not have the potency of the latter (0.1 cc of a concentrated aqueous extract of brain tissue clots 0.3 cc of recalcified human plasma in 14-15 seconds). To the dried residue that remained after the twice-repeated ether extraction of 1 g of brain powder, 10 cc of 0.85% NaCl was added (Sol. C). All extracts were allowed to stand until the coarse particles had settled; the supernatant fluid was used for the

[1] Tocantins, L. M., *Am. J. Physiol.*, 1943, **139**, 265.

[2] Howell, W. H., *Am. J. Physiol.*, 1912, **31**, 1.

[3] Quick, A. J., *Science*, 1940, **92**, 113.

[4] McLean, J., *Am. J. Physiol.*, 1917, **43**, 586.