TABLE I.
Yields of Radioactive Arsenic^{76} from Pile Irradiated Cacodylic Acid (Szilard-Chalmers Reaction).

Amt. cacodylic acid irradiated (mg)	NaCl %	Activity/cc (mc)	Arsenic/cc (mg)	Total vol. (cc)	Total activity (mc)	Specific activity (mc/mg)
300	10	2.37	0.09	17.5	41.5	26.4
700	14	4.26	0.26	28.0	119.0	15.2
500	13.0	4.35	0.35	24	104.4	12.0
600	7.7	4.50	0.35	20	90.0	12.8
700	10.0	8.50	0.34	17	142.0	25.0
750	16.0	5.60	0.32	20	112.0	17.5
800	20.0	4.57	0.26	16	73.2	17.5
500	16.0	2.77	0.17	17	47.1	16.4
800	14.0	4.0	0.26	25	100.0	16.9
650	14.0	6.48	0.20	25	162	32.4
700	11	6.82	0.18	8	54.6	30.3

TABLE II.
Comparison of Specific Activities of Several Arsenic Compounds Exposed to Pile Irradiation.

Material	mc As^{76} / mg As^{75}	Enrichment
As_2O_3	0.5	1
Cacodylic acid $(CH_3)_2AsO(OH)$	15.0	30
Arsenilic acid $C_6H_4NH_2AsO_3H$	0.1	0.2

Values are representative ones.

preparations of arsenic^{76} from pile irradiated cacodylic acid. There is wide variation in the specific activities because (1) time and power levels of pile irradiation were not uniform, and (2) activities were calculated to time of preparation of the arsenic solution.

It will be seen, however, that specific activities of arsenic^{76} obtained by pile irradiation of cacodylic acid are considerably higher than those obtained by pile irradiation of arsenic trioxide. Table II compares specific activities following irradiation of cacodylic acid, arsenic trioxide, and arsenilic acid. In addition to the high specific activity of the resulting product, as can be seen from Table I, irradiation of cacodylic acid yields a solution of high total activity, this being a function of the high neutron capture cross section for As^{75}.

Summary. A method of obtaining As^{76} of high specific activity by pile irradiation of cacodylic acid is described.

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Relation of Folic Acid and Vitamin A to Incidence of Hydrocephalus in Infant Rats.*

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Richardson and Hogan[1] observed hydrocephalus among infant rats and ascribed it to the inadequacy of the maternal diet. The diet, which was of the usual synthetic type, was supplemented with all of the recognized

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[1] Richardson, L. R., and Hogan, A. G., *J. Nutrition*, 1946, **32**, 459.

vitamins except pteroylglutamic acid, and the abnormality occurred in about 2% of the offspring. When the diet was supplemented with aqueous liver extracts, or with an eluate prepared from liver extracts, the abnormality was not observed. These concentrates would be expected to contain folic acid, but it was observed that crystalline "*L. casei* factor" would not cure the defect once it had developed. More recently Richardson and De Mottier[2] have produced hydrocephalus in an unrelated colony of rats.

The purpose of this communication is to show the relationship of pteroylglutamic acid and vitamin A to the incidence of hydrocephalus among the offspring of rats that consume a synthetic diet.

Methods. Adult albino rats from the colony described by Richardson *et al.*[1] were placed on the experimental diets only a short time before mating since it was found that depletion periods of several weeks had no noticeable effect on the incidence of hydrocephalus. After pregnancy occurred the rats were transferred to individual round cages, containing wood shavings as litter, where the offspring were born. All of the offspring were allowed to remain with the dam until weaning. The young rats were observed frequently for abnormalities and were weighed at weekly intervals until the end of the fourth week, when they were weaned and discarded if no abnormalities occurred.

At the end of the second, third, and fourth weeks the rats were examined for symptoms of hydrocephalus by means of a light-transmission test. The head of the rat was placed over a small opening in a box containing an incandescent bulb, with the size of the orifice so adjusted that the head of a normal rat of comparable size transmitted no light. If transmission of light could be detected the animal was considered hydrocephalic, the severity being measured by the amount of light transmitted.

The basal synthetic diet was composed of acid-washed casein, 30%; cerelose, 52%; wood pulp, 3%; salts,[1] 5%; lard, 10%; vitamin A, 2000 I.U.; vitamin D, 285 I.U.; (Vitamins A and D were supplied in the form of oleum percomorphum, Mead Johnson & Co.) α-tocopherol,† 2.5 mg; 2-methyl-1, 4-naphthoquinone, 2.5 mg; thiamine hydrochloride, 1.6 mg; riboflavin, 1.6 mg; pyridoxine hydrochloride, 1.6 mg; and calcium pantothenate, 4.0 mg. This diet was supplemented with various combinations of the following nutrients: Niacin, 5 mg; biotin, 0.020 mg; choline chloride, 100 mg; inositol, 100 mg; and ascorbic acid, 10 mg. Since none of these vitamins had any effect on the incidence of hydrocephalus, all of the rations containing any of these vitamins were grouped together and designated as Diet A. Diet A was supplemented with mcg of pteroylglutamic acid,‡ 5% liver extract or 5% brewers' yeast. The vitamin A content of the diet was varied from 2000 I.U. to 100 I.U. per 100 g of diet.

Results. The data in Table I indicate that the addition of pteroylglutamic acid to Diet A markedly decreases the occurrence of hydrocephalus among the offspring.

During 1947 there were 1001 young born to dams maintained on Diet A and 10 or 1.9% of those weaned showed symptoms of hydrocephalus. Of the 1890 young from dams which received 50 mcg of pteroylglutamic acid per 100 g of diet only 2 developed the abnormality. During this same period 3 out of 1826 young rats born in the stock colony, which received a natural feedstuffs diet and served as a control, became hydrocephalic.

When it was observed that pteroylglutamic acid decreased the occurrence of hydrocephalus it was considered worthwhile to present all of our earlier data[1] as they have some bearing on the relation of pteroylglutamic acid to the

[2] Richardson, L. R., and DeMottier, Jeanne, *Science*, 1947, **106**, 644.

† The α-tocopherol, thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, calcium pantothenate, choline chloride, niacin, biotin, inositol, ascorbic acid, and 2-methyl-1, 4-naphthoquinone were supplied through the courtesy of Dr. D. F. Green, Merck and Co., Rahway, N. J.

‡ The pteroylglutamic acid was supplied through the courtesy of Dr. T. H. Jukes, Lederle Laboratories, Pearl River, N. Y.

TABLE I.
Relation of Pteroylglutamic Acid in the Maternal Diet to Hydrocephalus in the Offspring.

Experimental period	Supplements to diet A	No. of litters	No. born	No. weaned	Hydrocephalus No. cases	Hydrocephalus %*
1947	None	157	1001	532	10	1.9
	Pteroylglutamic acid (50 mcg)	218	1890	1437	2	0.1
	Stock ration†	230	1826	1212	3	0.2
1944-1946	None	895	5026‡	2986	52	1.7
	Liver extr.§	180	1197‡	937	0	0.0
	Brewers' yeast	83	488‡	309	3	1.0

* % of those weaned.
† A diet of natural feedstuffs, used as a control.
‡ The number of young retained, since in the earlier experiments the litters were reduced to 8.
§ The liver extract supplement consisted of either 5% of an aqueous extract of pork liver or 1% of a fullers' earth eluate of this extract.

TABLE II.
Relation of Vitamin A in the Maternal Diet to Hydrocephalus in the Offspring.

Vitamin A content*	No. of litters	No. born	No. weaned	Hydrocephalus No. cases	Hydrocephalus %†
I.U. per 100 g					
2000	51	335	226	5	2.2
1000	33	220	122	3	2.5
500	38	217	145	3	2.0
100	40	244	145	1	0.7

* The vitamin E content of the diet at each vitamin A level was varied from 0.5 mg to 2.5 mg but since the differences were of no consequence the data are combined.
† % of those weaned.

abnormality. From 1944, when the first case was observed, through 1946 there were 5026 young retained from dams which consumed diets that contained no added folic acid. Fifty-two animals, or 1.7% of those weaned, developed the syndrome while of the 1197 retained from dams which received liver extracts rich in folic acid, none showed symptoms. There were 488 offspring retained from dams which received 5% dried brewers' yeast in the diet and 1% of those weaned developed hydrocephalus. The folic acid content of the brewers' yeast is not known but it must have supplied some of the vitamin. It seems probable that the quantity was insufficient to prevent the syndrome.

The occurrence of hydrocephalus among young rabbits has been observed by Hyde[3] and by Greene,[4] and it was believed by Greene to be the result of a vitamin A deficiency. In view of the possibility that destruction of vitamin A might occur during storage of the rations, lower levels of vitamin A were fed in an attempt to increase the incidence of hydrocephalus. The results are shown in Table II.

The incidence of hydrocephalus was not increased by lowering the vitamin A content of the diet from the usual level of 2000 I. U. to as low as 100 I.U. per 100 g.

Although the incidence of hydrocephalus

TABLE III.
Moisture Contents of Rat Brains.

Type Diet	Brains Analyzed No.	Moisture Content %
Stock	23	79.93
Synthetic	23	79.91
"	6*	88.10

* Brains from hydrocephalic rats.

[3] Hyde, R. R., *Am. J. Hyg.*, 1940, **31**, 1.

[4] Greene, H. S. N., Department of Pathology, Yale University, personal communication.

TABLE IV.
Cerebrospinal Fluid Pressure Determined by Cisternal Puncture.

Type Diet	No. of Observations	Cerebrospinal fluid pressure mm of water	Stand. dev.
Stock	30	77	26
Diet A	39	67	29
" A	3*	220	—
" A + PGA	34	78	29

* Rats showing symptoms of hydrocephalus.

on Diet A was 10 times as high as on the stock ration, it was still too low to permit rapid progress in discovering the cause. It was hoped that other less obvious symptoms of the deficiency might occur. Therefore a search was made for chemical and physiological differences between rats reared on synthetic and on practical rations. The moisture content of the brains from hydrocephalic rats is usually about 90%. That there was not a significant difference in moisture contents of brains of normal appearing rats from the synthetic and from the stock rations is shown by Table III. Twenty-three rats, 4 to 5 weeks of age, from each of the 2 types of rations were paired according to age, weight and sex, and their brains removed and dried for 24 hours at 65°C in a vacuum oven.

Hydrocephalus may be classified as internal or external depending on whether the fluid accumulates in the ventricles or in the subdural space and as communicating if there is free flow of liquid between the subdural space and the ventricles. In the communicating type there is a marked increase in cerebrospinal fluid pressure as determined by cisternal puncture.[5] Using the technic described by Griffith and Farris the cerebrospinal fluid pressure of the hydrocephalic rats was found to range from 150 to 350 mm of water. Thus the hydrocephalus appears to be the communicating type. Cerebrospinal fluid pressure determinations were made on rats, 4 to 6 weeks of age and weighing from 60 to 150 g, reared on the stock diet and on synthetic diets with and without added pteroylglutamic acid. The results are shown in Table IV. The variation within a group was quite large and there was no significant difference between groups.

Summary. About 2% of the young rats weaned from dams fed a synthetic type diet deficient in folic acid developed hydrocephalus. This abnormality could be largely prevented by the addition of pteroylglutamic acid to the maternal diet. Decreasing the vitamin A content of the diet to a low level did not increase the incidence of the hydrocephalus. There was no difference in either the moisture content of the brains or in the cerebrospinal fluid pressure of rats from the stock colony and those from dams receiving a synthetic type ration.

[5] Griffith, J. O., and Farris, E. J., J. B. Lippincott Co., 1942, pp. 192-196.