itiate but not to continue growth may be owing to the preformed thiamine contained within the organism at the time of transfer. Growth ceases or becomes extremely slow after this supply is exhausted. The growth of *Microsporum audouini* from infected hairs in concentrations of neopyrithiamine higher than that which completely inhibits pure culture transplants may be brought about by higher concentrations of thiamine present on the hair.

Unfortunately Sabouraud's dextrose agar is not devoid of thiamine. Stokes and coworkers(7) have found 0.11 to 0.28 μg of thiamine per gram of peptone. Assuming that the peptone used in this study contained the lower concentration of thiamine, each milliliter of medium would already contain 0.001 μg of thiamine. It seems reasonable to assume that on a purely synthetic medium entirely lacking in thiamine, still lower concentrations of neopyrithiamine would inhibit growth. An attempt has not been made to calculate the inhibition index from these data as the exact concentration of thiamine was not known. Experiments are being carried out on a synthetic medium to determine the index. It appears, with one exception, that the concentrations of neopyrithiamine employed did not kill all the *Microsporum audouini* during 14 days' contact. Strain No. 2 originally planted on medium containing 5 μg per ml indicates, however, that in higher concentrations neopyrithiamine may be fungicidal in the same period.

Summary. The ability of neopyrithiamine to inhibit growth of *Microsporum audouini in vitro* in very low concentrations suggests its use in cases of tinea capitis caused by this fungus. The application of neopyrithiamine alone or in combination with other agents or vitamin antagonists now being studied may prove helpful in combating these infections.

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Received December 6, 1950. P.S.E.B.M., 1951, v76.

Vitamin B_{12}, a Factor in Prevention of Hydrocephalus in Infant Rats.* (18487)

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During a study of the adequacy of a synthetic-type, casein diet for reproduction and lactation in the rat, Richardson and Hogan (1) observed that about 2% of the offspring were hydrocephalic. They ascribed this abnormality to a nutritional deficiency and showed that it could be prevented by an aqueous extract of liver. O'Dell, Whitley and Hogan(2) showed that the addition of folic acid to a diet containing casein as the source of protein usually, but not always, prevented the abnormality. More recently Hogan, O'Dell and Whitley(3) observed hydrocephalus in about 20 percent of the offspring when a folic acid inhibitor, crude methylpteroylglutamic acid (Methyl PGA), was added to a diet that contained soybean oil meal as the source of protein. The type of diet consumed during the pre-experimental period had a marked effect on the incidence of hydrocephalus among the first few litters produced by each dam, a fact which suggested that a protective factor may be stored in the

* Contribution from the Department of Agricultural Chemistry, Mo. Agric. Exp. Station, Journal Series No. 1243. This investigation was supported in part by a grant from the U. S. Public Health Service.

1. Richardson, L. R., and Hogan, A. G., *J. Nutrition*, 1946, v32, 459.

2. O'Dell, B. L., Whitley, J. R., and Hogan, A. G., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 272.

3. Hogan, A. G., O'Dell, B. L., and Whitley, J. R., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 293.

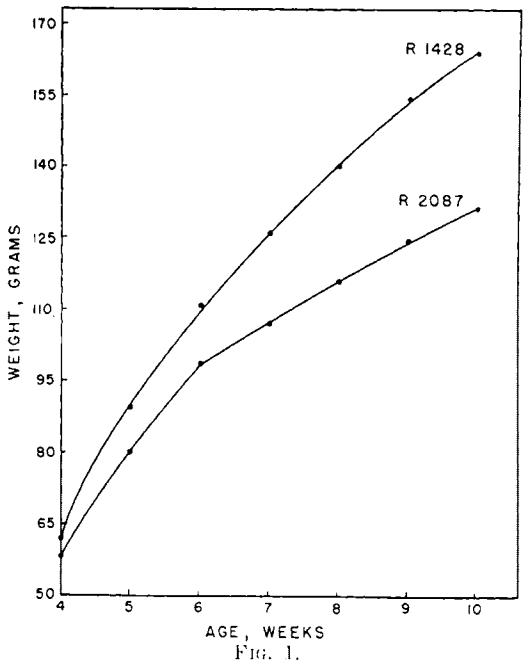

FIG. 1.

Age-weight curve of female rats. The experimental rats were transferred at 4 wk of age from the stock colony diet, R1428, to the depletion diet, R2087, and their litter mate controls were continued on R1428. There were 22 animals on each diet.

body of the mother rat. The purpose of this communication is to show the value of vit. B_{12} in the prevention of hydrocephalus.

Experimental. (a) Depletion of the protective factor. After it was observed that the pre-experimental diet has a profound effect on the incidence of hydrocephalus, all female rats used subsequently were depleted by maintaining them from weaning to maturity on the basal ration, No. 2087(3), which has the following percentage composition: Soybean oil meal 70, cerelose 22, lard 4, and salt mixture(1) 4. All recognized vitamins were added to this diet except ascorbic acid, niacin, folic acid, inositol, and vit. B_{12}.† The adequacy of Ration 2087 for growth is shown in Fig. 1 which compares the growth of female rats, taken from the stock colony at 4 weeks of age and fed Ration 2087, with litter-mate controls fed the stock ration, No. 1428. It has been shown by data not presented here that when Ration 2087 is supplemented with a vit. B_{12} concentrate that supplies 2.2 μg/100 g ration, the rate of growth equals that obtained on the stock ration. Thus the basal ration is low in vit. B_{12} but does not show any other serious deficiency for growth of the rat.

(b) Addition of a vit. B_{12} concentrate to the diet. Since the basal ration is deficient in vit. B_{12} and since one factor important in the prevention of hydrocephalus is stored for relatively long periods in the animal body, it seemed probable that vit. B_{12} is important in the prevention of the abnormality. To test its value three groups of animals were used. Group I was maintained on the basal ration for a period of at least three months in order that the animals might be thoroughly depleted of the protective factor. The animals then received, during the experimental period, Ration 2087 to which was added 1, 2, or 4 mg of crude Methyl PGA per 100 g of ration. Group II was not depleted but received the same experimental diets as Group I. Group III was not depleted and received, in addition to the Methyl PGA, a vit. B_{12} supplement in the form of a concentrate‡ which supplied 2.2 μg per 100 g of diet. Since there was no difference in response to different levels of antagonist in any group, the results of the different levels are combined in each case to simplify presentation. The results are presented in Table I.

The incidence of hydrocephalus was 23% among the offspring from depleted dams and 15% from undepleted females. This difference was striking among first and second litters but after the third litter there was little difference. When the dams received vitamin B_{12} in the diet there was no hydrocephalus among the 1261 offspring observed although some individual females bore a total of seven litters during the experimental period. When vit. B_{12} was omitted a low percentage of the offspring was weaned, with most of the

† The folic acid was supplied through the courtesy of Dr. T. H. Jukes, Lederle Laboratories, Pearl River, N. Y., and all other B-complex vitamins through the courtesy of Dr. D. F. Green, Merck and Co., Rahway, N. J.

‡ The vit. B_{12} concentrate was Merck's Animal Protein Factor Supplement which contained according to microbiological assay 12.5 mg per lb.

TABLE I. Relation of the Incidence of Hydrocephalus to the Vit. B_{12} Content of the Diet.

Additions to ration 2087	Litters born		No. born	% weaned at 4 wk*	Hydrocephalic offspring	
	Total	With hydrocephalic young			No.	%
	Group I—Pre-experimental depletion					
Methyl PGA†	144(6)‡	77	949	17	222	23
	Group II—No pre-experimental depletion					
Methyl PGA	241(8)	96	1568	21	237	15
	Group III—No pre-experimental depletion					
Methyl PGA + B_{12} conc.	166(7)	0	1261	81	0	0

* The % weaned is based on the number born alive.

† Crude methylpteroylglutamic acid supplied by Dr. T. H. Jukes, Lederle Laboratories, Pearl River, N. Y.

‡ Numbers in parentheses indicate maximum number of litters produced by any one female during the experimental period.

mortalities occurring during the first 2 to 3 days of life. The young rats had gross symptoms similar to those described by Schultze(4). According to Schultze uremia was prevented by the subcutaneous injection of vitamin B_{12} in infant rats, but their weaning weights were not improved. Under our conditions the subcutaneous injection of vit. B_{12} did not decrease the rate of mortality among the offspring of B_{12}-depleted dams. Of 20 new-born rats injected with 0.1 microgram of vit. B_{12} according to the technic described by Schultze only 2 survived to 1 week of age and of 21 untreated litter-mate controls 4 survived. It seems that there is some defect at birth which is not readily corrected by administering vit. B_{12} at that time. The offspring of dams that received vit. B_{12} in the diet were vigorous and 81% of those born alive were weaned. In addition to the high mortality and hydrocephalus which occurred among the offspring, other abnormalities have been observed. These include occasional cases of spina bifida, cranium bifidum, edema, anophthalmia or microphthalmia, hare lip, cleft palate, and short lower mandible. The total or partial absence of the eyes as well as other eye abnormalities seems to be closely associated with the incidence of hydrocephalus.§ Yudkin(5) reported the occurrence of congenital anophthalmia in a family of albino rats but its cause was not determined.

(c) Parenteral administration of crystalline vit. B_{12}. The vit. B_{12} used in the feeding trial described above was in the form of a concentrate and there was thus the possibility that the activity was due to another substance in the concentrate. In order to test crystalline vit. B_{12} 5 female rats were selected that had produced at least 3 consecutive litters with hydrocephalic young. In these 3 litters there were 97 young born, 43% of which were hydrocephalic and only 16% of which survived to 4 weeks of age. The females then received parenterally 1 μg of crystalline vit. B_{12} (Cobione) each week beginning at least 2 weeks before conception and continuing throughout gestation. The results are shown in Table II. Each female produced 2 litters while receiving crystalline vit. B_{12}. No hydrocephalic offspring were observed and 75% of the young survived to 4 weeks of age. The hydrocephalus observed in these infant rats is congenital and is almost always detectable at birth. It seemed of interest therefore to determine at approximately what stage of embryonic development the damage to the brain occurs. For this study female rats which had consistently produced hydrocephalic offspring were injected parenterally with 1 microgram of vit. B_{12} at different stages of gestation. The results of these treatments are shown in Table II. One group of 7

4. Schultze, M. O., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 613.

§ Mr. Fred Ransdell has undertaken a histological study of the eyes of hydrocephalic animals.

5. Yudkin, A. M., *Am. J. Ophthalmology*, 1927, v10, 341.

TABLE II. Effect of Parenterally Administered Vit. B_{12} on the Incidence of Hydrocephalus.

Pre-experimental period					Experimental period				
					Litters				
No. of females	No. of litters	No. born	% weaned	% hydrocephalic	Total	With hydrocephalus	No. born	% weaned	% hydrocephalic
Treatment: 1 μg vit. B_{12} weekly for 5 wk before parturition									
5	15	97	16	43	10	0	67	75	0
Treatment: 1 μg of vit. B_{12} on 7th day of gestation									
7	21	147	3	39	7	0	42	14	0
Treatment: 1 μg vit. B_{12} on 14th to 16th day of gestation									
5	15	126	21	37	5	4	28	14	50

TABLE III. Effect of Folic Acid on Incidence of Hydrocephalus When Fed in the Absence of Vitamin B_{12}.

Additions to ration 2087	Litters		No. born	% weaned	% hydrocephalic
	No.	With hydrocephalic young			
1 mg % methyl-PGA*					
5 mg % folic acid	7	7	30	17	40
0.5 mg % folic acid	20	10	155	11	28

* The females used in this group had previously produced 3 consecutive litters with hydrocephalic offspring.

females was treated on the 7th day of gestation and they produced 7 litters with a total of 42 young none of which was hydrocephalic. Thus it appears that 1 μg of B_{12} administered on the seventh day of gestation is sufficient to prevent hydrocephalus in at least the majority of cases, but it does not allow the production of viable offspring since only 14% were weaned. Five females were treated with 1 μg of vit. B_{12} on the fourteenth to sixteenth days of gestation and 4 out of 5 litters contained hydrocephalic young. Two females not included in the table were treated on the twelfth and thirteenth days respectively and their litters were free of hydrocephalus. The data available are insufficient to draw conclusions as to the exact time when vit. B_{12} must be administered to prevent the abnormality, but it is clear that the damage occurs after the seventh day of gestation and probably around the twelfth to fourteenth day.

(d) Addition of folic acid without vit. B_{12}. Folic acid is effective in decreasing the incidence of hydrocephalus when the females consume a diet that contains 30% of casein(2), but this protein is usually contaminated with a significant amount of vit. B_{12}. It seemed desirable to determine whether folic acid is still effective when the diet is practically devoid of vit. B_{12}. For this purpose folic acid was added to Ration 2087 and to a similar ration containing crude Methyl PGA and these rations were fed to female rats that had been depleted of vit. B_{12}. The results are shown in Table III. Hydrocephalus was not prevented in vit. B_{12} depleted dams by the addition of folic acid to the basal diet, and it made little difference in the incidence whether the antagonist was added or omitted. Hogan, *et al.*(3) reported that the incidence of hydrocephalus on the basal diet was low unless the folic acid antagonist was added. These observations were made on the offspring from the first 3 litters of females that had not been depleted. When these females were then fed the diet supplemented with the antagonist the incidence of hydrocephalus rose sharply, suggesting that the antagonist induced a folic acid deficiency. It is reasonable to believe that the antagonist did precipitate the occurrence of hydrocephalus but it is unlikely that a severe folic acid deficiency existed since Franklin, *et al.*(6) found the

6. Franklin, A. L., Stokstad, E. L. R., Belt, Margaret, and Jukes, T. H., *J. Biol. Chem.*, 1947, v169, 427.

ratio of antagonist to folic acid necessary to produce deficiency symptoms in rats fed succinylsulfathiazole to be 3000. According to microbiological assay, the basal ration contained at least 0.3 mg of folic acid per 100 g. The data in Table III show that if female rats are depleted of vit. B_{12} they produce litters with a high incidence of hydrocephalus, even though the basal diet is supplemented with folic acid.

Discussion. In our early work casein was the source of protein and the incidence of hydrocephalus was under 2%. When folic acid (O'Dell *et al.*)(2), was added to the diet the incidence of the abnormality was practically zero indicating that folic acid is an important preventive factor. The importance of folic acid was supported by the observation of Hogan *et al.*(3) that when female rats consumed a soybean oil meal diet the addition of a folic acid inhibitor precipitated the occurrence of hydrocephalus. However, the failure of folic acid to reverse the inhibition made it seem doubtful that folic acid was the only factor involved. According to microbiological assay casein usually contains more vit. B_{12} than does soybean oil meal, and this suggested that a deficiency of vit. B_{12} may be implicated in the abnormality. Our recent studies show that when this vitamin is administered to female rats on a soybean oil meal diet which contains low levels of Methyl PGA their offspring are not hydrocephalic. Since the level of inhibitor used is unlikely to induce a marked folic acid deficiency it is uncertain whether or not an adequate level of vit. B_{12} in the absence of folic acid will prevent hydrocephalus. It is certain, however, that folic acid, in the absence of vit. B_{12}, will not prevent the abnormality. Studies are now under way to determine whether both of these vitamins are required.

Summary. After female rats, on a diet that contained soybean oil meal as a source of protein, became depleted of the factor that prevents hydrocephalus, the incidence of the abnormality among the offspring was 28%. The addition of a vit. B_{12} concentrate to the diet or the injection of crystalline vit. B_{12} in the dams during the early stages of gestation prevented the abnormality in the young animals and increased their viability. Hydrocephalus was not prevented by folic acid alone, but it has not been determined whether vit. B_{12} alone is effective or whether both folic acid and vit. B_{12} are required.

Received January 15, 1951. P.S.E.B.M., 1951, v76.

Effect of Treatment with ACTH or Cortisone on Anatomy of the Brain.* (18488)

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Much evidence indicates that adrenocortical hormones exert important effects on the central nervous system. In rats sensitized by unilateral nephrectomy and feeding of high salt diets, Selye(1) induced inflammatory vascular lesions and edema in the brain by the administration of cortisone. In man, euphoria, psychotic reactions, restlessness, convulsions(2), and irregularities in the electroencephalogram(3) have been observed

* Supported (in part) by a research grant from the National Cancer Institute of the National Institutes of Health, Public Health Service.

† Student Research Fellow of the University of Michigan Medical School.

‡ The authors with to express their appreciation to Dr. Elizabeth C. Crosby for her generous advice during the course of this study.

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2. Rome, H. P., and Braceland, F. J., *Proc. Staff Meeting, Mayo Clinic,* 1950, v25, 495.