where, for instance the thalamus may be completely spared and the cortical lesions very scant(11).

Summary. A study of the neuropathology of Teschen disease reveals it to be a diffuse encephalomyelitis with characteristic lesions both as to type and distribution. The lesions are, with minor exceptions, confined to the grey matter of the brain and cord. Their widespread distribution in the CNS, and particularly their striking density in the cerebellum serve to distinguish Teschen disease from poliomyelitis, although spinal cord lesions may be similar in the 2 diseases.

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Methylation of Homocystine by Chicks Deficient in Vitamin B_{12}.* (18661)

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Gillis and Norris(1,2) and Schaefer, Salmon and Strength(3) have shown that chicks partially depleted of their reserves of vit B_{12} have an increased requirement for compounds which furnish labile methyl groups. Jukes, Stokstad and Broquist(4) have presented evidence which suggests that chicks deficient in vit B_{12} are unable to utilize homocystine plus betaine for the synthesis of methionine. Oginsky(5) concluded that the livers from vit B_{12}-deficient rats exhibit a lower ability to form methionine from homocystine and either choline or betaine as compared with livers from animals dosed with vit B_{12}. The work of Jukes *et al.*(4) and Oginsky(5) appeared to be in disagreement with the unpublished results of an earlier experiment conducted at this laboratory which showed that the methylation of homocystine by means of choline or betaine was not hampered by a deficiency of vit B_{12}. In view of this discrepancy the original study has been repeated and extended. The results of both experiments are presented in this report.

Experimental. The chicks used in this investigation were hatched from eggs laid by White Leghorn hens fed a diet containing no animal products or vit B_{12} and housed in pens with wire-mesh floors. The hatchability of the eggs was low, and the chicks were found in a preliminary experiment to be markedly responsive to vit B_{12} administration. The chicks were fed a vit B_{12} deficient diet containing 25% vegetable protein for a pre-experimental period of 10 days. They were then uniformly distributed on the basis of weight among the experimental lots. Thereafter they were weighed daily at the same hour. The basal diet furnished approximately 0.2% methionine and was deficient in vit B_{12} and choline. In Exp. 1 it contained corn starch 65.55; isolated soybean protein 20; gelatin 4; L-cystine 0.15; soybean oil 2; ground cellophane 3; dicalcium phosphate 2; calcium carbonate 1; sodium chloride 0.5; dibasic potassium phosphate 0.5; magnesium sulfate 0.25; trace mineral mixture 0.05; and vitamin-starch mixture 1%. In Exp. 2 the basal diet was the same except that it contained 22% water extracted, isolated soybean protein and no gelatin, and hydrogenated vegetable fat was used in place of soybean oil. In Exp. 1 each lot contained 6 chicks and the experimental treatment lasted 5 days. In Exp. 2 each lot contained 5 chicks and the experimental period was of 9 days' duration.

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TABLE I. Growth of Vit. B_{12}-deficient Chicks Fed Various Supplements in a Diet Deficient in Methionine, Choline, and Vit. B_{12}.

Supplement, %	Avg wt gained 5-day period, g
0	—1.6
.3 choline Cl	3.7
.3 betaine HCl	0.9
.5 DL-homocystine	6.8
.5 DL-methionine	23.4
.5 DL-homocystine + .3 choline Cl	22.5
.5 DL-homocystine + .3 betaine HCl	23.2

TABLE II. Growth of Vit. B_{12}-deficient Chicks Fed Various Supplements With and Without Vit. B_{12}.

Supplement	Avg wt gained, 9 day period: B_{12}-deficient diet, g	Same + B_{12}/100 g diet, g	Increase due to B_{12}, g
0	6.6	23.6	17.0
.15% DL-homocystine	4.6	34.6	30.0
.15% DL-methionine	24.6	43.2	18.6
Homocystine + .172% betaine HCl	23.6	42.4	18.8
Homocystine + .156% choline Cl	31.4	53.4	22.0

The experimental plans are presented in Tables I and II respectively. To obtain a better conception of the relative efficiency of methionine and homocystine plus choline or betaine, the amounts of methionine and homocystine fed in Exp. 2 were reduced to 0.15% of the diet and the additions of choline chloride (0.156%) or betaine hydrochloride (0.172%) were made to correspond to the theoretical amounts necessary to methylate 0.15% homocystine.

Results. The chicks in Exp. 1 which received the unsupplemented basal diet failed to grow (Table I). Little or no effect was obtained by adding either choline or betaine alone, but the addition of homocystine alone resulted in a small improvement in growth. On the other hand when methionine, homocystine plus choline, or homocystine plus betaine were added growth improved significantly. Moreover, the results obtained with either of the two latter regimens were equally as good as those obtained with methionine. Vit B_{12}, therefore, did not appear to be directly concerned with the transfer of methyl groups from either choline or betaine to homocystine to form methionine. The small response obtained with homocystine alone indicates that methyl groups needed in this instance for the conversion of homocystine to methionine were obtained by synthesis.

In Exp. 2 the chicks which received the unsupplemented diet deficient in vit B_{12} grew very little and the addition of homocystine resulted in no improvement in growth (Table II). On the other hand, supplementing the deficient diet with either methionine, homocystine plus betaine, or homocystine plus choline significantly improved growth. The response due to supplementation with homocystine plus betaine was the same as that obtained with an equivalent amount of methionine, while supplementation with homocystine plus choline was slightly more effective, indicating that the choline deficiency was being partially overcome.

The basal diet with added vit B_{12} promoted significantly better growth than that obtained in the comparable lot fed the vit B_{12}-deficient diet. Adding homocystine alone to the vit B_{12}-adequate diet stimulated further growth. Since homocystine had no effect on growth in this experiment when added to the basal diet containing no vit B_{12}, this is regarded as evidence that in the chick, vit B_{12} is concerned in the utilization of homocystine in the absence of known methyl donors. Stekol and Weiss(6) have reported that rats are able to grow when fed a diet free of methionine and all known labile methyl donors, providing the diet contains vit B_{12} and homocystine. The results of the present work indicate that methyl synthesis also occurs at a suboptimal rate in chicks receiving vit B_{12}.

The effect of including vit B_{12} in the basal diet appears to have been strictly additive except where homocystine was supplied alone, and provides no evidence that the vitamin is required for the transfer of preformed methyl groups from either betaine or choline to homo-

6. Stekol, J. A., and Weiss, K., *J. Biol. Chem.*, 1950, v186, 343.

cystine for the formation of methionine. The results obtained in this experiment, therefore, confirm the results obtained in Exp. 1.

Discussion. The results reported in this paper indicate that chicks severely depleted of their vit B_{12} reserves retain the ability to perform the over-all reaction: homocystine + labile methyl = methionine. If vit B_{12} is needed for the completion of this reaction, the amount required must be exceedingly small. It seems probable, however, in view of the results obtained with rats by Stekol and Weiss (6) and others, and the results presented here that vit B_{12} is concerned in the synthesis of methyl groups or the utilization of such synthesized groups by the chick.

The results presented here, showing that methionine formation occurred in vit B_{12}-deficient chicks supplied homocystine plus choline or betaine do not agree with those of Jukes *et al.*(4). This may be due to the fact that the diet used by the latter workers contained only 0.1% of betaine hydrochloride, an amount which on theoretical grounds could not methylate more than 0.087% of homocystine. The amount of homocystine furnished by these investigators was greatly in excess of this amount (0.6%). Furthermore, the basal diet used by Jukes *et al.*(4) contained 0.2% dimethylaminoethanol hydrochloride, a compound which presumably competes with homocystine for methyl groups. It appears, therefore, that the amount of methionine which could have been formed and made available for growth under these conditions must have been very small. This conclusion is supported by the fact that homocystine alone gave as good growth in the presence of vit B_{12} as did homocystine plus 0.1% betaine hydrochloride. There is thus no evidence that the efficiency of methyl transfer was increased by vit B_{12} in the work reported by Jukes *et al.*(4). Rather their evidence suggests a considerable synthesis of methyl groups in the presence of vit B_{12}.

Although Oginsky(5) reported that the livers of vit B_{12}-deficient rats exhibited a lower ability to form methionine from homocystine and either choline or betaine as compared with those from animals dosed with vit B_{12}, attempts by this investigator at *in vitro* activation of the methionine forming system by addition of solutions of crystalline vit B_{12} proved to be unsuccessful. Moreover it is clearly evident from Oginsky's(5) results that a very considerable synthesis of methionine occurred in the livers of the vit B_{12}-deficient rats, particularly in the presence of betaine.

Conclusions. Chicks very deficient in vit B_{12} grew as well when homocystine and betaine were added to a methionine-deficient diet as when a comparable amount of methionine was added. No evidence was obtained that vit B_{12} increased the efficiency with which preformed methyl groups were tranferred to homocystine since the effects of vit B_{12} were additive to those of methionine or its equivalents of homocystine plus labile methyl. It appears likely, however, that when the chick's diet lacks methyl groups vit B_{12} is concerned in the synthesis of such groups and their combination with homocystine to form methionine. Formation of methionine by this procedure, however, is limited and insufficient to meet the needs of the chick.

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