action of these compounds.

Summary. 1. Mast cell counts were made on the cheek pouches of hamsters subjected to total-body X-irradiation. 2. Following irradiation there is a marked increase in the number of atypical mast cells accompanied by a reduction in the total number of mast cells. 3. Repopulation of the cheek pouch with mast cells is relatively slow, being incomplete even at the end of 33 days.

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Vitamin B_{12} and Transmethylation in the Baby Pig.* (20863)

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It has been stated by Schaefer *et al.*(1,2) that vit. B_{12} is required by the chick and the rat for the synthesis of choline from methionine. Stekol *et al.*(3) have stated, however, that "vit. B_{12} deficiency had no effect on the extent of transmethylation to choline or creatine". Johnson *et al.*(4,5) have shown that baby pigs raised on a synthetic milk diet containing adequate vit. B_{12} and 0.8% methionine require 0.1% choline in the diet and that this choline requirement can be eliminated by increasing the methionine level to 1.6%(6) as is true for the rat(7). Since a severe vit. B_{12} deficiency can readily be produced in the baby pig(8,9), the effect of this deficiency on choline synthesis by transmethylation from methionine was studied in this species.

In the previous work in which a high

TABLE I.

Basal diet	
Alpha-protein	29.4
DL-methionine	1.3
Cerelose	30.5
Lard	30.5
Minerals	8.3

The above materials were made into a synthetic milk containing 13% solids according to a modification of the W. M. Clark method(10).

Vitamins added/liter synthetic milk	mg
Thiamine	.65
Riboflavin	1.30
Pyridoxine HCl	1.30
Nicotinic acid	2.60
Ca pantothenate	7.80
Folic acid	.052
Biotin	.010
2 methyl-1,4-napthoquinone	.26
Vit. A	2000 I.U.
Vit. D	200 I.U.

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The vit. B_{12}, choline, thiamine, riboflavin, pyridoxine HCl, nicotinic acid, and calcium pantothenate used were generously supplied by Merck and Co, Rahway, N. J., through the courtesy of Dr. Harold H. Draper; the folic acid by Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y., through the courtesy of Dr. T. H. Jukes; the "water-soluble" vit. A and D, as A CON and D CON, by Endo Products Co., Richmond Hill, N. Y. through the courtesy of Dr. S. M. Gordon; the DL-methionine by the Dow Chemical Co., Midland, Mich., through the courtesy of Dr. J. E. Johnson; and the lard, as a stabilized 60% lard in water emulsion, by Armour and Co., Chicago through the courtesy of Mr. Bryon M. Shinn.

TABLE II. Growth, Liver B_{12}, Liver Choline, and Urinary Choline of Pigs.

Group	Avg initial wt, kg	Avg 4 wk wt, kg	Avg liver choline $-B_{12}$ mg/g fresh	Avg liver choline $+B_{12}$* mg/g fresh	Avg liver B_{12} $-B_{12}$ mμg/g fresh	Avg liver B_{12} $+B_{12}$* mμg/g fresh	Avg urinary choline excr. $-B_{12}$ mg/24 hr	Avg urinary choline excr. $+B_{12}$* mg/24 hr
1 Positive control ($+B_{12}$)	1.93	10.19	—	2.03	—	290	—	—
2 Basal	1.91	5.41	2.63	2.37	18.6	39.7	2.84	2.31
3 Basal + dimethylaminoethanol	1.60	4.71	2.61	2.38	18.1	48.6	25.84	17.85

* Four pigs, 2 in Group 2 and 2 in Group 3, given one dose of 50 μg of vit. B_{12} by injection at 4 wk. The $+B_{12}$ refers to these pigs in Groups 2 and 3.

(1.6%) methionine diet replaced choline(6), casein served as protein source and all the animals received vit. B_{12}. In the present experiments, alpha-protein supplemented with methionine, again to a level of 1.6%, was used as protein source to produce a B_{12} deficiency. It was found that the high level of methionine served as an adequate choline source even in animals moribund with B_{12} deficiency.

Experimental. Two groups (2 and 3) of 5 pigs each were fed *ad libitum* a high methionine alpha-protein synthetic milk diet, the composition of which is given in Table I. The choline content of the diet was determined to be less than .01% by the method of Horowitz and Beadle(11) using *Neurospora crassa* cholineless. The pigs in Group 2 received only the basal diet; and those in Group 3 received 0.1% dimethylaminoethanol to supply a methyl acceptor in the event that B_{12} deficiency blocked its synthesis. Group 1 was a positive control group; these 5 pigs receiving the alpha-protein basal with 0.5% methionine added (instead of 1.3%) as well as 0.1% choline and vit. B_{12} at the rate of 0.8 μg/kg body wt/day. After 4 weeks on the diet, 4 of the deficient pigs were injected with a single dose of 50 μg B_{12}/kg B.W. All the animals were killed when it became evident they were near death due to the deficiency. Liver and kidney samples were taken for histological examination and liver samples for choline and vit. B_{12} assay. Twenty-four-hour urines were collected prior to and after injecting the 4 deficient pigs and assayed for choline.

Results. The growth data given in Table II illustrate the effect of the B_{12} deficiency. The 4 pigs, which were injected with 50 μg B_{12}/kg B.W. after 4 weeks on the deficient diet, gave a characteristic reticulocyte response which reached a peak at 8 to 10 days as shown in Table III.

Choline assays of the urines (Table II) gave significantly higher results for the group receiving 0.1% dimethylaminoethanol due to its excretion and failure of the *Neurospora* assay to differentiate between it and choline. Injection of B_{12} failed to produce any difference in choline excretion.

The deficient pigs were killed during the fifth week of the experiment when they refused food, vomited repeatedly, and became very weak and unsteady. It has been repeatedly noted that B_{12} deficient pigs die soon after these symptoms are observed.

Histological examination of liver and kidney gave no evidence of fatty infiltration or renal damage indicative of a choline deficiency. Choline assay of the liver samples as indicated in Table II further shows that a choline deficiency did not exist in either Groups 2 or 3 as the values are essentially equal to those of Group 1 which received choline.

Marked differences were found in assays of the livers for B_{12}(12) (Table II). Controls receiving 0.8 μg B_{12}/kg B.W./day by weekly injection contained approximately 10 times more B_{12} than did the deficient animals. Slight increases were found in the 4 treated pigs.

Growth data and liver assays for B_{12} indicate that a definite B_{12} deficiency was produced while histological examination of livers and kidneys gave no evidence of a choline deficiency. It thus appears that in the pig, B_{12} is not involved in the synthesis of choline from methionine.

TABLE III. Reticulocyte Response to B_{12}. (Reticulocytes/100 red cells.)

Days after B_{12}	Basal		Basal + dimethylaminoethanol	
0	1.5	2.8	2.0	1.9
4	2.9	5.5	4.8	4.4
6	6.4	8.5	5.8	5.0
8	9.9	10.5	7.8	10.0
10	8.4	12.1	5.2	8.7
13	6.1	6.5	3.3	3.8

Summary. The effect of a B_{12} deficiency on transmethylation from methionine to choline has been studied. The deficiency did not influence the level of choline in the liver nor the excretion of urinary choline. Histological examination of livers and kidneys did not reveal the fatty infiltration or renal damage characteristic of a choline deficiency. From this study it appears that vit. B_{12} is not involved in this direct transmethylation.

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Histochemical Changes in Succinic Dehydrogenase Activity in Rat Kidney Following Administration of Mercurial Diuretics.* (20864)

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It is a well known fact that the sulfhydryl-containing enzymes are inhibited by compounds of heavy metals such as mercury(1). Consequently, succinic dehydrogenase activity has been shown to be inhibited by mercurial diuretic agents *in vitro* in the heart (2) and *in vivo* in the kidney(3). Such enzymatic inhibition is concomitant with the diuretic action of mercurials and may be related to the depression of tubular reabsorption, since both enzymatic inhibition and diuresis can be prevented by the administration of 2,3 dimercaptopropanol (BAL)(4).

It is generally assumed that mercurial diuretics act on some specific portion of the kidney tubule, although there is no satisfactory physiological evidence on this point (5). Recently, Mustakallio and Telkkä(6) using a histochemical method, reported that the "distal segment" of the tubule is inhibited by mercuhydrin. Since pathological and other evidence is not in agreement with their conclusions, we restudied the problem by an improved histochemical method and, in addition, investigated the activity of BAL, cysteine, and glutathione in suppressing the mercurial inhibition.

Methods. Nearly all of our work has been done on young female rats (175-200 g) of the Holtzman strain although kidneys of a few adult males and immature animals of this strain also have been studied. The animals were killed by decapitation, a kidney removed immediately, and sectioned at 50 μ on the freezing microtome. Sections were placed in physiological saline solution and within a period of 30 minutes transferred to small,

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