tube have begun to die before the end of the 2-week period. Therefore, it would appear that when fed a ration deficient in an essential amino acid the young animals used in these experiments are unable to cope with normal quantities of food intake. Thus they begin to die after about 10 days when fed a constant amount of food by stomach tube. Animals of similar weight and age fed *ad libitum*, however, appear to adjust to some extent to the deficient ration by reducing their food intake and consequently survive for longer periods of time.

Summary. 1. A lysine-free ration fed to weanling rats either *ad libitum* or by a forcible feeding procedure reduces the activity of liver xanthine oxidase, succinic oxidase, and choline oxidase to somewhat over half that of normal. 2. A lysine deficiency has little effect on liver endogenous respiration. 3. The deficiency produces an entirely different pattern of liver enzyme activity from that produced by a methionine or histidine deficiency.

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Received March 8, 1954. P.S.E.B.M., 1954, v85.

Nutritional Studies with the Guinea Pig. B-Vitamins other than Pantothenic Acid.* (20947)

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The only B-vitamins for which there is unequivocal proof of dietary essentiality for life in the guinea pig are thiamine(1-3), folic acid(4-7), and pantothenic acid(8). There is considerable indication that a dietary supply of niacin also is essential(9,10).

A semi-synthetic diet recently developed for guinea pigs(11) has proven useful in evaluating the essentiality of several of the B-vitamins for these animals. In the present investigations deficiencies of thiamine, choline, folic acid, pyridoxine, niacin, and riboflavin have been demonstrated in the guinea pig, some for the first time. This report is concerned chiefly with a brief description of the gross deficiency symptoms and the effects of the deficiency on the growth rate and survival time. The effect of lack of a dietary supply of para-aminobenzoic acid, inositol, biotin, and vit. B_{12} has also been studied. Supplements of these substances were found

* Part of the data in this paper was presented at the American Institute of Nutrition in Chicago, April 1953 (*Fed. Proc.* 1953, v12, 472.)

TABLE I. Summary of Results with B-Vitamins other than Pantothenic Acid.

Vitamin	Control group: No. of animals	Control group: Avg wt (g) 42nd day	Deficient guinea pigs: No. of animals	Deficient guinea pigs: Avg wt (g) of survivors 42nd day	Deficient guinea pigs: No. of survivors	Deficient guinea pigs: Avg survival time (days) of animals which died and day of 1st death	Deficient guinea pigs: Deficiency symptoms, if any, other than retarded growth, anorexia, and weakness
Thiamine	28	333 ± 8*	20	209	1	26 (17)	Footnote [1]
Choline	18	334 ± 6	36	165 ± 10*	10	24 in 4 exp. (16, 21, 13, 18).† In other exp. all living with avg wt 181 g	" [2]
Folic acid	32	343 ± 5	32	281 ± 8	6	27 (22, 26, 16, 25)†	" [3]
Pyridoxine	12	339 ± 9	12	214 ± 6	7	28 (18, 29)	" [4]
Niacin	22	338 ± 8	23	245 ± 13	15	29 (27)	" [5]
Riboflavin	12	341 ± 15	12	284 ± 20	7	25 (18)	" [6]
PABA	13	336 ± 9	13	328 ± 11	13	—	No symptoms
Inositol	20	340 ± 8	20	311 ± 13	19	—	" "
Biotin	18	341 ± 7	18	328 ± 9	17	—	" "
B_{12}	22	346 ± 6	22	328 ± 10	22	—	" "

* Stand. error. † Day of first death in different experiments.

[1] Emaciation, tremor, and spasm.

[2] Clear fluid frequently found in body cavities, kidneys pale and atrophic, some with scarred surface but no acute hemorrhage. No visible fattiness of liver.

[3] Decrease in No. of erythrocytes and leucocytes, an almost completely aplastic condition of bone marrow in animals which died early, tendency to fatty infiltration of liver, and hemorrhagic adrenals.

[4] Enlargement of adrenals, atrophy of sex organs, disturbances in gastrointestinal tract such as hemorrhagic condition of cecum.

[5] Drooling, paleness of feet, nose, and ears. Soiled coats.

[6] Paleness of feet, nose, and ears. Rough coats.

to be non-essential under our experimental conditions.

Procedure. Animals of the Hartley strain, 2 to 4 days old, with equal numbers of each sex and weight ranges of 95 to 115 g, were reared in screen-bottomed cages and were fed the basal semi-synthetic diet previously described(11). For study of the effects of a vitamin deficiency, the particular vitamin chosen for a study was omitted from the diet and the appearance, growth, and survival of the animals at the end of 6 weeks were compared with those of a group which had been maintained on the complete diet. In most of the experiments there were 6 to 8 animals in a group.

Results. The total number of animals employed in all the control groups was 96 and their average weight on the 42nd day was 339 g. Only one death occurred in the control animals. The means of the weights for the control groups in different experiments were very similar and are indicative of a consistent growth-rate on the control diet. The absence from the diet of thiamine, choline, folic acid, or pyridoxine caused pronounced deficiency symptoms. Table I shows that the weight gain of all the deficient groups was less than that of the controls and that the survival after 6 weeks was much less, particularly with respect to thiamine, choline, and folic acid. The data in the table show that a retardation in growth occurs also in the absence of a dietary supply of niacin or riboflavin, and that a considerable number of the animals succumbed to the deficiency as compared to almost 100% survival in the control groups. Some of the survivors in each of these 2 groups grew fairly well, however, and it appeared that some of them might have survived for an indefinite time. No specific symptoms characteristic of niacin and riboflavin deficiencies, such as dermatitis, were observed. In niacin deficiency there was no swelling of the tongue or reddening of the mouth, and in riboflavin deficiency there was no evidence of corneal opacity or vascularization.

No significant differences were found between the weights of the groups lacking a

dietary supply of PABA, inositol, biotin, or vitamin B_{12} and their respective control groups. In the early phases of these studies the diet contained 3 mg of folic acid per kg. During this period the addition of PABA appeared to have beneficial results on growth but, in the later studies in which the content of folic acid was increased to 10 mg, there was no longer a growth response to PABA (see table, also reference 6). The results herein shown suggest strongly that under the present experimental conditions no additional inositol, biotin, or vit. B_{12} is essential for the maintenance of life in the guinea pig. Since, however, the weights of the control groups were slightly, though not significantly,† higher than those of the groups not supplied with the vitamins, it would be necessary to conduct further studies to obtain positive proof that these vitamins are not necessary for maximum growth. Mention should be made of the fact that Hogan and Hamilton(12) observed that some benefit resulted from the addition of inositol to purified diets for guinea pigs.

Discussion. There may be unidentified factors needed by the guinea pig which, if present, would improve the growth, but thus far we have not succeeded in demonstrating any benefit from feeding supplements of fresh kale (6 g daily of the green mesophyll tissue) or addition of a forage juice factor(13). Possibly these supplements would improve the reproductive performance but thus far, in most cases, the studies have been continued only through the first 6 to 8 weeks of growth. The data herein presented demonstrate, however, the need of the guinea pig for certain of the B-vitamins and afford the first clear-cut demonstration of a requirement of a dietary supply of choline, riboflavin, and pyridoxine. The guinea pig appears to be similar to the chick in its requirement for many of the B-vitamins, including a high requirement for folic acid. In of both of these animals folic acid deficiency symptoms are produced in very young animals by the absence of the vitamin from the diet without resorting to the use of anti-metabolites. The guinea pig's requirement of a dietary supply of certain B-vitamins is quite unlike that of the rabbit (14). This is somewhat surprising since both animals subsist naturally on green forage. The practice employed in the present experiments of removing the guinea pigs from the mother when only 2 to 4 days old may have influenced the bacterial flora of the gastro-intestinal tract, thereby indirectly affecting the vitamin requirements. As compared to rabbits (left with the mother 21 days or more), this difference in post-natal time spent with the mother, together with apparently a greater tendency to coprophagy in the rabbit (15), may have an important bearing on the dietary requirement of B-vitamins in the 2 types of animals.

B-vitamin studies with the guinea pig are being continued using the basal diet herein employed and a determination of the quantitative requirement, if any, of each of these vitamins is being made.

Summary. A determination has been made of the essentiality or non-essentiality of vitamins of the B group as components of a semi-synthetic diet developed especially for guinea pigs. Clear-cut deficiencies of choline, pyridoxine, and riboflavin were produced for the first time. In addition, a dietary need of thiamine, niacin, and folic acid has been confirmed. It appears that in the presence of an adequate supply of folic acid, PABA is not essential in the diet. Under our conditions no requirement has yet been demonstrated for inositol, biotin, or vit. B_{12}.

† According to the T-test of Fisher.

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Received March 11, 1954. P.S.E.B.M., 1954, v85.

Autoradiographical Distribution and Excretion Studies with S^{35}-Labelled Penicillin. (20948)

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Previous investigations of the pharmacology of penicillin have depended in general on bacteriological methods of penicillin estimation. In the present investigation autoradiography has made possible a more detailed localization of the radioactive substances in histological sections, so that the distribution and fate of penicillin in normal mice could be studied. Some preliminary observations have also been made on the distribution of penicillin in diseased tissue, and on the inhibitory effect of probenecid* on the excretion of penicillin by the kidneys.

Methods. Radioactive (S^{35}-labelled) penicillin was prepared biosynthetically according to methods similar to those described by Smith and Hockenhull(1) but using stirred bottles and aeration. Crystalline sodium penicillin was obtained via the ethylpiperidine salt. Paper chromatography showed almost entirely benzylpenicillin. The histological technic was complicated by the solubility of penicillin in water and fixation fluids. The labelled penicillin was injected intravenously in a tail vein. A dose of one millicurie per g of body weight was employed, which corresponds to 8 μg of penicillin per g of body weight. Animals were killed at different times after injection. In most cases the entire body of the mouse was rapidly frozen in isopenthane at —170°C. The frozen mice were mounted on large stages and allowed to reach —10°C, when they were sectioned. All of the sections were dried at —10°C. In some of the mice small pieces were taken from organs and treated by the usual freeze-drying methods with subsequent paraffin-embedding and sectioning at room temperature. Contact autoradiographic technic was used with exposure for some weeks in iron presses at —10°C.

Results. Distribution in normal tissues. Penicillin is rapidly distributed to the tissues from the blood stream. In all cases the relative distribution of penicillin concentration in the various tissues was similar from 5 minutes after the injection until the penicillin began to disappear from the tissues.

Fig. 1 shows the distribution of penicillin in an adult mouse 20 minutes after injection. The numbers indicate the relative degrees of radioactivity in the organs as a percentage of that of the blood. These were obtained by measuring on a dried section with a mica-window G-M tube, when the whole section, except for the measured area, was covered.

The greatest penicillin concentration is found in the kidneys, which are responsible for the elimination of most of the penicillin, but the liver is not to be disregarded as an excretory organ, as can be seen from the high activity in the biliary system. All the activity in the lumen of the intestine appears to come from the biliary system. The subcutis

* Probenecid is the generic name for *p*-(di-n-propylsulfamyl)-benzoic acid supplied under the trademark 'Benemid' (U.S.A.) and 'Probecid' (Sweden).