tion product of α-ketoglutarate). Further, since glyoxylate is readily converted to glycine by transamination (17), glycine would also be expected to have a relatively high specific activity. Finally, the oxidation of injected glyoxylate was much more limited than acetate. These data are in agreement with the observations of Madsen (18), who was unable to demonstrate activity of key enzymes of this pathway in tissue of chick embryos just prior to hatching.

All of the data presented here are consistent with the metabolism of the injected acetate primarily via the citric acid cycle as it is in other animals. No evidence was obtained which would indicate that the chick has some mechanism to conserve glucose, since injected glucose was readily oxidized (Table I), although slightly slower than acetate. The relatively large amount of isotope associated with protein after acetate-1-^{14}C was injected probably reflects the rapid growth rate of the day-old chick. In the absence of any evidence of special pathways of acetate utilization, it is concluded that the lack of ketosis in the chicken is the result of a more subtle control mechanism of ketone body biosynthesis, either through reduced enzyme activity *in vivo* or control of substrate availability.

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Vitamin B_6 Requirement in the Hypothalamic-Hyperphagic Rat* (33392)

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Vitamin B_6 requirement has been related to the dietary intake of both protein and fat. Twenty-three years ago, Cerecedo and Foy (1) showed that increasing the dietary protein level caused a more rapid onset of typical acrodynia and a reduced survival time of rats provided with a diet deficient in vitamin B_6. In humans, Baker *et al.* (2) observed that xanthurenic acid excretion, a symptom

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of vitamin B_6 deprivation, was directly proportional to dietary protein level. A high-fat diet delays the appearance and reduces the severity of acrodynia in vitamin B_6-deprived rats (3). Since recommended dietary allowances of certain vitamins, notably thiamine, riboflavin, and niacin, have been based on calorie intake, it was wondered if a similar relationship might exist for vitamin B_6. To examine this possibility, use was made of hypothalamic-hyperphagic rats. These animals demonstrate a marked increase of food intake leading to obesity (4, 5). As a biochemical criterion of vitamin B_6 nutriture, measurement of erythrocyte glutamic-pyruvic transaminase activity was performed. The sensitivity and specificity of this enzyme to vitamin B_6 intake has been demonstrated (6, 7).

Methods. Male rats of the Wistar strain were housed in individual, wire screen cages at an environmental temperature of 24 ± 1° with 12 hr of light and 12 hr of darkness each day. Food and water were provided *ad libitum* and food intake and body weight were measured at regular intervals. Composition of the basal diet was as follows (in per cent by weight): vitamin-free casein, 16; sucrose, 64; corn oil, 10; vitaminized casein, 4; salts mixture, 4; alphacel, 2; choline, 0.4; and inositol, 0.2. The vitaminized casein was prepared by mixing in 800 g of casein, the following vitamins: thiamine chloride, 100 mg; niacin, 900 mg; riboflavin, 200 mg; pyridoxine hydrochloride, 250 mg; *p*-aminobenzoic acid, 400 mg; calcium pantothenate, 400 mg; biotin, 20 mg; folic acid, 20 mg; menadione, 10 mg; alpha-tocopherol, 2 g; and vitamins A and D 30 ml of Ostogen. The vitamin B_6-deficient diet differed by omission of pyridoxine hydrochloride from the vitaminized casein.

Under pentobarbital sodium anesthesia (5 mg/100 g of body wt.) given intraperitoneally, hyperphagia was induced by bilateral electrolytic ablation of the ventromedial region of the hypothalamus (2 mA for 10 sec) with the use of a Horsley-Clarke stereotaxic instrument. The coordinates used for placing the electrode were anterior 6 mm, vertical 8.6 mm and lateral 1 mm. The rats weighed 200–220 g at the time of operation. Operated animals along with unoperated controls were provided with the basal diet and water *ad libitum* for a period of 12 days. The 43 operated animals showing the greatest hyperphagia were then selected. To reduce their body weight to approximately that of controls, these animals were fasted for 24 hr and food intake was then restricted to 10 g/rat per day for a 4-day period. They were then fed *ad libitum* for 48 hr and divided into six comparable groups on the basis of 48-hr food intake. Control animals were divided similarly into six groups. Mean body weights after grouping were 295 and 325 g for control and hyperphagic rats, respectively.

Following grouping, all animals were provided *ad libitum* with the vitamin B_6-deficient diet. For each of the next 32 days, animals of each group were injected intraperitoneally with 0.5 ml of an aqueous solution of pyridoxine hydrochloride to provide 10, 40, 80, 120, 160, or 200 μg/rat. At the end of this experimental period, cardiac blood (heparinized) was obtained from the exposed heart under pentobarbital sodium anesthesia. Erythrocytes were separated by centrifugation, washed with an equal volume of 0.9% aqueous sodium chloride and hemolyzed by mixing with twice their volume of distilled water. The hemolyzate was frozen and stored until time of assay. Glutamic-pyruvate transaminase (GPT) activity was measured by the procedure of Caldwell and McHenry (8) as adapted by Cheney *et al.* (7). Statistical significance of difference between means was determined by application of Student's *t* test.

Results and Discussion. As shown in Table I, average food intake of unoperated control animals was not affected by dosage level of pyridoxine hydrochloride over the range 10–200 μ. At all dosage levels of pyridoxine hydrochloride, hyperphagic animals exhibited greater body weight gains per 100 g of initial body weight than did intact controls (Fig. 1). Body weight gain of intact controls attained a maximum at a dose of approximately 80–120 μg followed by a decline at higher doses. In operated groups, maximum body weight response occurred at about 80 μg dose

TABLE I. Average Daily Food Intake of Intact Control and Hypothalamic-Hyperphagic Male Rats Given Various Amounts of Pyridoxine Hydrochloride by Daily Intraperitoneal Injection.[a]

Group	Pyridoxine hydrochloride (μg/rat per day):	Food intake (g/rat per day)					
		10	40	80	120	160	200
Prior to experiment (48 hr)							
Intact		16	14	17	16	16	15
Hyperphagic		30	26	26	27	27	24
Days 1–32							
Intact		17	18	20	20	19	19
Hyperphagic		21	27	28	28	25	25

[a] Results are expressed as the mean value for groups of 6–8 rats.

level. In both groups, a tendency for body weight gain to plateau was noted with a daily dose level of about 40 μg (Fig. 1). It should be emphasized that body weight gain is not a specific criterion and indeed, in hyperphagic animals the excess gain is composed of fat and water (5) and is associated with altered metabolism in the direction of increased lipogenesis.

Results of erythrocyte GPT activity measurements are shown in Fig. 2. When plotted on a semilogarithmic scale, the relationship of erythrocyte GPT activity to vitamin dosage was essentially linear in both groups of animals although somewhat more variable in hypothalamic–hyperphagic rats. At no level of pyridoxine hydrochloride was there a signifi-

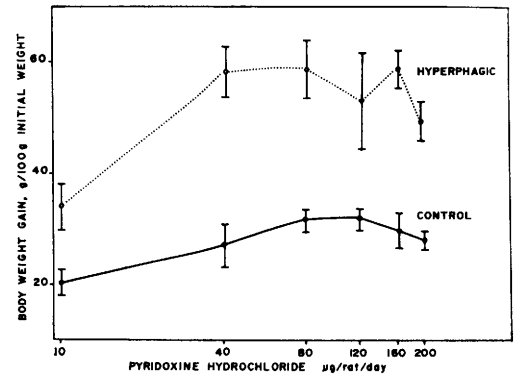

FIG. 1. Body weight gain of intact and hyperphagic rats injected daily (intraperitoneal) with pyridoxine hydrochloride at doses of 10, 40, 80, 120, 160, and 200 μg/rat. Each point represents the mean of 6–8 rats; the vertical bar represents the standard error of the mean. The experimental period was 32 days.

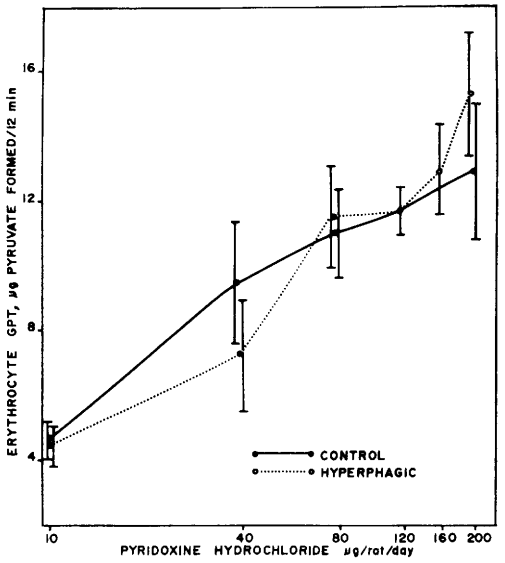

FIG. 2. Erythrocyte glutamic-pyruvic transaminase (GPT) activities in intact and hyperphagic rats injected daily (intraperitoneal) with pyridoxine hydrochloride at doses of 10, 40, 80, 120, 160, and 200 μg per rat. Each point represents the mean of 6–8 rats; the vertical bar represents the standard error of the mean. The experimental period was 32 days.

cant difference in erythrocyte GPT activity between intact and operated animals. Correlation coefficients were calculated between pyridoxine dosage and erythrocyte GPT activity with the following results: for intact control rats $r = 0.678$ (not significant); for hyperphagic rats $r = 0.961$ ($p<0.001$); for both groups considered together $r = 0.865$ ($p < .02$). Thus a relationship between vitamin dosage and enzyme response was established.

These observations on body weight gain and erythrocyte GPT response to pyridoxine hydrochloride administration suggest that in the rat: (a) pyridoxine hydrochloride requirement for maximum body weight gain is in the range 80–120 μg for both control and hyperphagic rats; and (b) a daily dose of at least 200 μg of pyridoxine hydrochloride is required to elicit maximum erythrocyte GPT activity in control and hyperphagic rats.

Our observations on apparent vitamin B_6 requirement in intact rats are in general agreement with those of Cheney and Beaton (6). It would appear that hypothalamic-hyperphagia in the rat does not alter the requirement and it may be concluded therefore, that increasing daily calorie intake on the average from 78 (control) to 115 (hyperphagic) calories per rat does not alter vitamin B_6 requirement. Coincident with increased calorie intake as a consequence of hyperphagia, there was also an increased absolute intake (about 47%) of protein, carbohydrate, and fat, yet daily requirement for vitamin B_6 was apparently unaltered. Had one or more of these components been increased to a greater extent, an altered requirement might have been observed; however, Cheney *et al.* (7) failed to observe any differences in whole blood GPT activities among intact rats fed 5, 10, 20, or 40% casein diets either in the presence or absence of dietary pyridoxine.

Agnew and Mayer (9) fed diets deficient in vitamin A or in thiamine to intact and hypothalamic-hyperphagic rats and observed an apparent increased requirement for thiamine but not for vitamin A as a consequence of hyperphagia. This result might be expected in view of the accepted relationship of thiamine, but not vitamin A, requirement to calorie intake. From the results reported here, and based on a specific biochemical criterion, it would appear that the requirement for vitamin B_6 in the rat is not related to calorie intake.

Summary. Control and hypothalamic-hyperphagic rats were injected daily with pyridoxine hydrochloride at dosage levels of 10, 40, 80, 120, 160, and 200 μg/day for 32 days. Body weights and food intakes were measured throughout the experimental period; erythrocyte glutamic-pyruvic transaminase activities (GPT) were measured at the termination of the experiment. Based on body weight changes, it is suggested that vitamin B_6 requirement is in the range 80–120 μg/day for both control and hyperphagic rats. However, a daily dose of at least 200 μg is required to elicit maximum erythrocyte GPT activity. A significant correlation between vitamin intake and enzyme activity was observed thus indicating a direct relationship. There appeared to be no evidence of an increased requirement for vitamin B_6 as a consequence of hyperphagia and therefore, it is concluded that in the rat, requirement for this vitamin is not related to calorie intake.

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