to pass the Shope rabbit fibroma to woodchucks and to squirrels have been unsuccessful.

Other experiments have further indicated immunological relationship between squirrel and Shope rabbit fibromas. In current studies 2 domestic rabbits were inoculated with fibroma emulsions, 2 inoculations having been given intracutaneously a week apart. The fibroma-emulsions were from 2 of the original squirrels, each rabbit receiving material from only one squirrel. When bled 10 days after the last inoculation, both rabbits had developed neutralizing antibodies against the OA strain of rabbit fibroma, as indicated by neutralization tests performed in the skin of a fresh rabbit. Preinoculation sera had no neutralizing capacity. Although one of the rabbits died, the second one failed to develop tumors when challenged with virulent OA fibroma virus. Only 2 serum specimens have been obtained from grey squirrels bearing tumors. Both of these sera had a definite capacity to neutralize OA fibroma in dilutions of 10^{-3} and 10^{-4}; whereas sera from 10 normal squirrels showed no neutralizing capacity.

Discussion. Although difficulties in obtaining grey squirrels and woodchucks for laboratory use have somewhat limited the extent of the investigations, our studies demonstrate a close relationship between squirrel and Shope rabbit fibromas. Further studies are in progress to determine how the agent of the squirrel tumor may be transmitted in nature, to define more clearly similarities and differences between it and the Shope fibroma virus, and to investigate the histochemical nature of cellular inclusions encountered.

Summary. Six grey squirrels collected in Maryland were found to have multiple small cutaneous nodules which histologically resembled Shope's rabbit fibroma. The squirrel fibroma was carried through 2 intracutaneous passages in grey squirrels, 4 consecutive passages in woodchucks, and from woodchucks for 2 passages in domestic rabbits. Cross-neutralization tests provided immunological evidence that the squirrel and Shope fibromas are related.

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Effect of Vitamin B_6 Deficiency on Hepatic Transaminase and Cysteine Desulfhydrase Systems. (20100)

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The relationship of vit. B_6 to transamination has been demonstrated in studies on animals(1-3) and microorganisms(4,5) deficient in this vitamin, and also in experiments with isolated enzyme systems(6-11). Schlenk and Snell(1) found that the tissues of vit. B_6-deficient rats exhibited decreased glutamic-aspartic transaminase activity. These findings were confirmed by Ames, Sarma and Elvehjem(2) and similar observations have been made by Shwartzman and Hift in studies on hamsters(3). *In vitro* experiments showed that pyridoxal phosphate activated resolved transaminase preparations(6-11) and recent evidence suggests that pyridoxamine phosphate may also function as a coenzyme for transaminase(12).

Previous communications from this laboratory(13-15) have described an hepatic transaminase system capable of catalyzing the following reaction: L-glutamine + α-keto acid → α-ketoglutaric acid + L-α-amino acid + NH_3. The available evidence indicates that a) a wide variety of α-keto acids may par-

ticipate in this reaction, b) the ammonia formed is derived from the amide group of glutamine, c) deamidation does not precede transamination, d) a similar reaction between asparagine and α-keto acids is also catalyzed by liver preparations, and e) the α-keto analogues of glutamine (α-ketoglutaramic acid) and asparagine (α-ketosuccinamic acid), possible intermediates in these reactions, are hydrolyzed to ammonia and the corresponding dicarboxylic α-keto acids by liver preparations. Although vit. B_6 has been implicated in the mechanism of a number of transaminase systems there is as yet no evidence that this vitamin or its phosphorylated forms is involved in the mechanism of the glutamine-α-keto acid transamination-deamidation reaction. We have therefore examined the livers of vit. B_6-deficient rats for this activity as well as for the classical glutamic-pyruvic transaminase activity. For comparative purposes studies have also been made on cysteine desulfhydrase since it has been reported that this system is reduced in activity in vit. B_6-deficient animals(16), and it is probable that pyridoxal phosphate is a coenzyme for cysteine desulfhydrase in *Proteus morganii*(17).

Experimental. Adult rats of the Buffalo strain(18) weighing 150 to 220 g were housed in individual cages and given water *ad libitum,* and the following vit. B_6 deficient diet* expressed in grams per kg of diet; Labco brand vitamin-free casein 180, cerelose 570, salt mixture 45(19), lard 200, choline chloride 5, and vitamin mixture 0.1. The vitamin mixture contained in grams per kg of completed diet folic acid 0.01, biotin 0.001, thiamine hydrochloride 0.04, riboflavin 0.02, niacin 0.2, and calcium pantothenate 0.14. Vit. A ester (10,000 units per kg of diet) and α-tocopherol (0.1 g) were dissolved in a portion of the lard and incorporated into the diet. In an effort to hasten development of the vit. B_6 deficiency syndrome(20), the rats were given 2.4 mg of desoxypyridoxine† (2,4-dimethyl-3-hydroxy - 5 - hydroxymethylpyridine) per week by intraperitoneal injection in 6 equal doses. All of the rats developed characteristic symptoms of vit. B_6 deficiency with severe dermatitis, scaliness of paws and moist snouts in 73 to 91 days, at which time they were sacrificed. The animals lost between 40 and 78 g (average weight loss = 57 g) during the experimental period. Control animals received the same diet supplemented with 25 mg pyridoxine hydrochloride per kg of diet. No significant differences in the levels of enzymatic activity were noted between control animals receiving the pyridoxine-supplemented diet and rats fed a stock purina chow diet. The animals were sacrificed by decapitation and exsanguination, and the livers were rapidly removed, chilled, and homogenized with 3 volumes of ice-cold distilled water. The determinations of transaminase activity were carried out as described previously(13,14). Cysteine desulfhydrase was determined according to Greenstein and Leuthardt(21). Pyruvate(22), glutamine, and glutamate(23) were determined as described. Crystalline pyridoxamine and pyridoxal phosphates were employed(24).

Results. The results, which are summarized in Table I, indicate that vit. B_6 deficiency, induced by dietary restriction of this vitamin and desoxypyridoxine injection, led to a marked decrease of cysteine desulfhydrase activity as indicated by considerably reduced formation of ammonia and pyruvate from cysteine. The lack of agreement between pyruvate and ammonia formation may be ascribed to further metabolism of pyruvate (25). The glutamic-pyruvic transaminase activity was reduced to about 40% of the control values, while the glutamine-pyruvic and the glutamine-phenylpyruvic reactions proceeded equally well in the livers of deficient and control animals. As indicated in Table I, addition of pyridoxal phosphate to the homogenates resulted in the restoration of most of the cysteine desulfhydrase activity. In several cases pyridoxamine phosphate was added and a comparable activation was observed. Conversion of pyridoxamine phosphate to pyridoxal phosphate would be expected to occur in crude liver preparations. Pyridoxal phosphate also increased the gluta-

* This diet is similar to one used previously in studies on mice(26).

† Generously supplied by Merck and Co.

TABLE I. Effect of Vitamin B_6 Deficiency on Several Hepatic Enzyme Systems.

Enzyme system	Deficient: No. of rats	Deficient: Range (μmoles)	Deficient: Avg	Control: No. of rats	Control: Range (μmoles)	Control: Avg
Glutamic-pyruvic transaminase*	14	2.27–3.10	2.64	14	5.35–7.09	6.36
reaction†	9	3.87–5.70	4.53	9	3.74–4.96	4.39
-phenylpyruvic reaction‡	14	4.88–7.50	6.41	14	5.24–7.45	6.22
-phenylpyruvic reaction§	10	5.52–6.76	6.22	10	6.00–7.14	6.44
Cysteine desulfhydrase;‖ NH_3 formed	10	0 –1.44	0.39	10	2.18–3.60	2.89
Cysteine desulfhydrase; pyruvate formed	10	0 – .59	0.19	10	.88–3.02	1.38
Cysteine desulfhydrase; pyridoxal phosphate (100 γ) added; NH_3 formed	5	2.10–3.48	2.63	5	2.44–3.77	3.25
Cysteine desulfhydrase; pyridoxal phosphate (100 γ) added; pyruvate formed	5	1.08–1.36	1.16	5	1.38–2.28	1.78

* Reaction mixtures consisted of 1 cc homogenate (diluted 1:10), 1 cc 0.1 M veronal-acetate buffer (pH 7.2), 1 cc 0.01 M L-glutamate, and 1 cc 0.02 M sodium pyruvate; incubated 30 min. at 37°. Values expressed in terms of disappearance of glutamate.

† Reaction mixtures consisted of 1 cc homogenate, 1 cc 0.1 M veronal acetate buffer (pH 7.2), 1 cc 0.01 M L-glutamine, and 1 cc 0.02 M sodium pyruvate; incubated 4 hr at 37°; values given in terms of ammonia formation.

‡ Reaction mixtures consisted of 1 cc homogenate, 1 cc 0.1 M veronal-acetate buffer (pH 8.2), 1 cc 0.01 M L-glutamine, and 1 cc 0.01 M sodium phenylpyruvate; incubated 3 hr at 37°; values given in terms of ammonia formation.

§ Experimental details given in footnote ‡; values given in terms of glutamine disappearance.

‖ Reaction mixtures consisted of 2 cc homogenate, 1 cc 0.1 M potassium phosphate buffer (pH 7.5), and 1 u 0.03 M L-cysteine; incubated 2 hr at 37°.

mic-pyruvic activity of liver homogenates obtained from deficient rats, but not those of normal rats, a finding similar to that of Ames *et al.*(2) on the glutamic-aspartic system of vit. B_6-deficient rats.

Discussion. The present demonstration of reduced glutamic-pyruvic transaminase activity in liver homogenates of vit. B_6-deficient rats is in accordance with *in vitro* studies indicating the participation of pyridoxal phosphate in this reaction. Glutamic-pyruvic transaminase was reduced to approximately 40% of the control values in vit. B_6-deficient rats, which is about the same magnitude of effect observed for the glutamic-aspartic system in vit. B_6-deficient rats(2) and hamsters(3). The loss of cysteine desulfhydrase activity was striking and it should be noted that this activity could not even be demonstrated in the livers of 6 of the deficient animals studied. In the light of these findings the absence of a significant decrease in the activity of the glutamine-α-keto acid system in the deficient rats is of interest. This result may be interpreted as compatible with the concept that vit. B_6 is not involved in the glutamine-α-keto acid reaction, although the participation of vit. B_6 is not conclusively excluded. It is possible that the affinity of the glutamine system for vit. B_6 is greater than that of the other enzymes studied and that even under the severe conditions of vitamin deprivation and inhibition employed, sufficient coenzyme was retained for maximal activity.‡ Evidence derived from *in vitro* studies may be expected to aid in elucidating this problem.

Summary. 1. The glutamic-pyruvic, glutamine-pyruvic, and glutamine-phenylpyruvic reactions, and cysteine desulfhydrase activity were determined in homogenates of livers of vit. B_6-deficient rats and vit. B_6-supplemented control rats. 2. Glutamic-pyruvic transaminase activity was considerably reduced in livers of the deficient animals, while the glutamine-pyruvic and glutamine-phenylpyruvic reactions proceeded at approximately equal rates in control and deficient rats. 3. Cysteine desulfhydrase activity was markedly reduced in the livers of the deficient animals. Addition of pyridoxal phosphate to homogenates of the

‡ It is of interest in this connection that dialysis (24 hours against running water at 15°) of liver homogenates resulted in almost complete loss of cysteine desulfhydrase activity (almost completely reversed by addition of pyridoxal phosphate), while the glutamic-pyruvic and glutamine-α-keto acid systems were not significantly affected.

livers of the deficient animals resulted in restoration of most of the cysteine desulfhydrase activity. 4. The implications of these findings in terms of the participation of vit. B_6 in the glutamine-α-keto acid reactions are discussed.

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Response of Preputial and Adrenal Glands of the Rat to Sex Hormones.* (20101)

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Paired preputial glands are present in both male and female rats. They are considered accessory reproductive glands, since it is well known that they are stimulated by androgens and to some degree by other steroid hormones (1). Indeed, since they are well developed in both sexes, their weight has often been used as a measure of the endogenous androgen production of gonads or adrenal glands(2). Only recently has it become apparent that in certain aspects the response of the preputial glands to ACTH parallels that of the adrenal glands. Thus, highly purified preparations of ACTH are reported to exert a direct, growth-promoting action on the preputial glands of adrenalectomized-ovariectomized rats(3,4). Furthermore, such a growth response need not be attributed to a contaminating principle present in the ACTH preparation, since it has also been shown that preputial gland hypertrophy follows adrenalectomy, an operation known to evoke a hypersecretion of ACTH(5). It appears, therefore, that the direct action of ACTH is not limited to the cells of the adrenal cortex, but extends also to those of the preputial glands. Such a concept is in accord

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