salicylates required for effective displacing activity are in the range of 2×10^{-4} to 1×10^{-3} molar. This is equivalent to 5.6-28.0 and 3.6-18.0 mg per 100 ml of sulfamethoxypyridazine and acetylsalicylic acid, respectively. The most effective concentrations are in the upper range achieved with customary chemotherapeutic doses of these drugs(15,16). It remains to be determined whether these or similar agents will prove useful as adjuncts to penicillin chemotherapy.

Summary. A series of compounds, many of which had been shown in dialysis studies to be effective in displacing penicillins from binding to human serum, have been tested for ability to enhance the antimicrobial activity of various penicillins and other antibiotics in the presence of 50% human serum. Enhanced antimicrobial activity in serum was shown in the case of sulfonamides to be only partially reversible by para-amino benzoic acid, suggesting that augmentation of penicillin activity was due to serum displacing effects as well as to the intrinsic antibacterial activity of sulfonamides. Evidence is presented that the "R" groups of penicillins are the most important determinants of binding to serum. The specificity of the penicillin binding sites was shown by the failure of displacing agents shown to be effective against the penicillin site(s), to effect binding of novobiocin and tetracyclines, and by the lack of effect of probenecid, known to be highly bound, to displace penicillins from serum.

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Effect of Pyridoxine Deficiency on Rat Adrenal Phosphorylase.* (29500)

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Pyridoxal 5-phosphate, the prosthetic group of muscle polysaccharide phosphorylase, is essential for catalytic activity as well as for maintenance of the stable tetramer form of the enzyme(1,2). The phosphorylase molecule upon removal of pyridoxal 5-phosphate exhibits little enzymatic activity; however, this can be restored almost completely by recombining the apoenzyme with pyridoxal 5-phosphate(3). Illingworth *et al*(4) have shown that pyridoxine deficiency in the rat causes a reduction of total muscle phosphorylase but found no change in phosphorylase

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a activity. Eisenstein(5), however, demonstrated decreases in both forms of muscle phosphorylase in pyridoxine deficient rats. The latter investigator observed a concomitant reduction of liver phosphorylase and suggested that pyridoxal 5-phosphate is a cofactor for liver phosphorylase. Since pyridoxal phosphate may be a common cofactor for different tissue phosphorylases we thought it of interest to determine the effects of pyridoxine deficiency on rat adrenal phosphorylase. A further stimulus to carry out this study was the postulate of Haynes and Berthet(6) who suggested that ACTH enhances adrenocortical hormone secretion by increasing phosphorylase activity of the gland.

Materials and methods. One hundred female, Holtzman rats were used in 2 separate experiments. In Experiment I, 60 rats weighing 70 to 80 g were randomly divided into 2 groups and housed in individual cages. One group was fed a pyridoxine deficient diet while a control group consumed the same diet to which pyridoxine (30 mg/kg) was added. Daily food intake of control animals was limited so that average body weight of this group would be approximately equal to that of deficient rats. Animals were maintained on diets for 35 to 60 days during which each animal was weighed and examined 3 times a week. Physical signs of pyridoxine deficiency, including porphyrin staining of fur and whiskers and scaling of paws, tail and ears, began to appear by the 35th day. Gain in body weight of deficient rats ceased by the 50th day. Five animals from each group were killed on days 35, 40, 45, 50, 55 and 60. All rats were given sodium pentobarbital intraperitoneally prior to decapitation. Both adrenals were quickly removed, dissected free of fat and connective tissue, weighed and homogenized in cold 0.1 M sodium fluoride. Essentially the same procedure was carried out in Exp. II in which 40 rats, weighing 50 to 60 g were used. Feeding was continued for 80 days and animals were killed on days 72, 78, 79 and 80.

Pyridoxine deficient and control diets were purchased from General Biochemicals Inc.; glucose-1-phosphate dipotassium salt, glycogen, 5'AMP, glucose-6-phosphate dipotassium salt and TPN trisodium salt were obtained from Sigma Chemical Co.

Adrenal phosphorylase was assayed in the presence or absence of 5'AMP according to the method of Sutherland and Wosilait(7); however, incubation was extended to 60 minutes. Inorganic phosphorus was determined by the method of Fiske and Subbarow(8) as adapted to the Klett-Summerson photometer. One unit of phosphorylase activity was defined as that amount of enzyme liberating 31 μg of inorganic phosphorus from glucose-1-phosphate in 60 minutes. Specific activity was expressed as units per 100 mg tissue protein. Adrenal protein was measured by the method of Lowry *et al*(9). Glucose-6-phosphate dehydrogenase was assayed spectrophotometrically in the 13,000 $\times$ *g*, 30 minute supernatant of adrenal homogenates, according to the method of Glock and McClean(10) with minor modifications. Rate of reduction of TPN at 340 mu at 25°C was followed spectrophotometrically at one minute intervals in the Model DU Beckman spectrophotometer. The reaction mixture consisted of 0.1 ml clear supernatant; 1.0 ml glycylglycine buffer 0.08 M, pH 7.5; 0.5 ml $MgCl_2$ 0.1 M; 0.2 mg TPN and 1.1 ml glass distilled water. The reaction was started by addition of 0.1 ml 0.05 M glucose-6-phosphate. One unit of glucose-6-phosphate dehydrogenase activity was that amount of enzyme which at 25°C, in a 3.0 ml assay mixture, changes optical density at 340 mu by 0.001 in one minute. Specific activity was expressed in units per 100 μg adrenal protein.

Results. In pyridoxine deficient rats, adrenal phosphorylase activity measured in the absence of 5'AMP was 26.5% lower ($P=<0.01$) than that of control animals and phosphorylase activity in the presence of 5'AMP was reduced by 28.9% ($P=<0.001$) in Exp. I (Table I). In Exp. II (Table II), the activities of adrenal phosphorylase measured without 5'AMP and phosphorylase assayed in the presence of 5'AMP of pyridoxine deficient rats were reduced 27.4% ($P=<0.05$) and 29.0%

TABLE I. Effect of Vitamin B_6 Deficiency on Adrenal Phosphorylase Activity.
Mean values ± S.E. of mean.

Group*	Days deficient	Adrenal wt (mg/100 g body wt) C	Adrenal wt (mg/100 g body wt) D	Phosphorylase activity (units/100 mg protein) −AMP C	−AMP D	−AMP % Difference	+AMP C	+AMP D	+AMP % Difference
1	35	32.5 ± 1.54	29.2 ± 1.90	3.86 ±.15	2.92 ±.13	24.0	4.48 ±.12	3.38 ±.08	24.6
2	40	28.7 ± 1.55	29.5 ± .81	3.43 ±.06	2.48 ±.11	27.4	4.45 ±.21	3.09 ±.07	29.7
3	45	25.8 ± 1.04	25.6 ± 1.05	3.54 ±.10	2.47 ±.08	30.0	4.41 ±.08	2.92 ±.07	29.2
4	50	26.0 ± 2.03	26.1 ± 2.51	2.73 ±.09	2.16 ±.03	21.0	3.61 ±.10	2.58 ±.09	28.6
5	55	32.0 ± 1.23	34.4 ± .94	3.17 ±.21	2.51 ±.14	21.6	3.96 ±.09	3.14 ±.09	20.5
6	60	29.1 ± 1.23	31.4 ± .64	3.20 ±.21	2.11 ±.05	34.0	4.13 ±.22	2.65 ±.08	35.9
Average		29.0 ± 1.2	29.4 ± 1.3	3.32 ±.16	2.44 ±.12	26.5	4.16 ±.14	2.96 ±.13	28.9
Statistical significance		N.S.		P = <.01			P = <.001		

* Control and deficient groups consisted of 5 rats each.

(P = <0.01) respectively. There was no correlation between degree of reduction of enzyme activity and duration of pyridoxine deficiency. In contrast to the reduction of phosphorylase activity, no alteration in glucose-6-phosphate dehydrogenase activity was observed in the second experiment.

TABLE II. Effect of Vitamin B_6 Deficiency on Adrenal Phosphorylase and Glucose-6-Phosphate Dehydrogenase Activity.
Mean values ± S.E. of mean.

Group*	Days deficient	Adrenal wt (mg/100 g body wt) C	Adrenal wt (mg/100 g body wt) D	Phosphorylase activity (units/100 mg protein) −AMP C	−AMP D	−AMP % Difference	+AMP C	+AMP D	+AMP % Difference	Glc-6-P dehydrogenase (U/100 μg protein) C	Glc-6-P dehydrogenase (U/100 μg protein) D
1	72	24.2 ± 1.39	23.4 ± .85	3.01 ±.07	2.45 ±.04	19.6	4.00 ±.09	2.95 ±.05	26.0	—	—
2	78	25.4 ± 1.64	23.4 ± 1.54	2.53 ±.07	1.80 ±.05	28.3	3.39 ±.08	2.53 ±.10	25.6	37.4 ± 2.5	41.9 ± 2.5
3	79	27.5 ± 1.39	25.0 ± 1.57	2.73 ±.04	2.02 ±.04	26.7	3.71 ±.07	2.61 ±.06	29.7	29.0 ± 4.0	30.2 ± 4.5
4	80	29.3 ± 1.70	25.9 ± 1.60	3.31 ±.40	2.12 ±.07	35.3	4.46 ±.25	2.81 ±.10	34.7	29.4 ± 2.3	34.2 ± 4.5
Average		26.6 ± 1.13	24.4 ± .22	2.90 ±.17	2.10 ±.13	27.4	3.88 ±.23	2.72 ±.10	29.0	31.9 ± 2.7	35.4 ± 3.4
Statistical significance		N.S.		P = <.05			P = <.01			N.S.	

* Groups 1 and 2 consisted of 5 control and 5 deficient rats. Groups 3 and 4 consisted of 4 control and 4 deficient rats.

It was of interest that the adrenal weight of vit. B_6 deficient animals did not differ from that of control rats. This may possibly be explained by the fact that control animals were pair-weighed, so that their food intake was restricted and was actually less than that of the deficient group. The restricted food intake may have served as a stress which induced adrenal hypertrophy in these animals, as has been observed by other workers (11).

Discussion. Haynes and Berthet(6) postulated that ACTH enhances steroid synthesis in the beef adrenal gland by controlling the level of phosphorylase activity. According to their hypothesis, ACTH causes formation of 3′5′ AMP which stimulates phosphorylase activity and results in accelerated conversion of glycogen to glucose-1-phosphate. The latter compound is rapidly converted to glucose-6-phosphate which, in the adrenal cortex is predominantly metabolized through the hexose-monophosphate shunt(12). Since most of the glucose-6-phosphate is metabolized in this manner, NADPH is rapidly generated and is available for various hydroxylation reactions in steroid synthesis. This mechanism of action of ACTH has been shown to occur in beef adrenal cortex. In the rat, however, recent studies by Kobayashi *et al*(13,14) have shown that ACTH does not act as postulated by Haynes and Berthet. These workers failed to observe alterations in adrenal phosphorylase and glucose-6-phosphate dehydrogenase activities following ACTH administration even though corticosteroid production was greatly increased, thus suggesting that enhanced phosphorylase activity is not necessary for increased adrenal steroid synthesis in rat. Our observation that rat adrenal phosphorylase activity is diminished in vit. B_6 deficiency, coupled with previous findings that steroid synthesis in the rat is not impaired by pyridoxine deficiency (15) supports the findings of Kobayashi *et al.* Thus, it appears that adrenal steroid synthesis in this animal may not be influenced by changes in phosphorylase activity. It must be pointed out, however, that the decrease in adrenal phosphorylase observed in this study may also reflect changes in phosphorylase levels in the adrenal medulla, since this enzyme has been shown to be present in significant amounts in this tissue(16).

Reduction in adrenal phosphorylase in this study was comparable to the decrease in liver phosphorylase of vit. B_6 deficient rats previously reported(5), but not as marked as that found in muscle. The explanation for this difference in reduction of enzyme activity in pyridoxine depleted animals is not clear. It is established that pyridoxal 5-phosphate is the coenzyme for muscle phosphorylase(1,2,3). However, the only evidence which suggests that the vitamin functions in a similar manner for the hepatic enzyme is that liver phosphorylase activity is significantly reduced in pyridoxine deficient rats (5). Since a significant decrease in adrenal phosphorylase activity was observed in the present investigation, it seems reasonable to suggest that pyridoxal phosphate may also be a cofactor of adrenal phosphorylase in the rat.

Summary. Female, Holtzman rats were fed a pyridoxine deficient diet for periods of up to 80 days while pair-weighed controls consumed the same diet containing pyridoxine. Animals from both groups were killed at intervals beginning on the 35th day and adrenal homogenates were prepared for assay of enzyme activities. In deficient rats adrenal phosphorylase activity was reduced by approximately 30% ($P = < 0.001$), while glucose-6-phosphate dehydrogenase activity was not altered. Reduction in adrenal phosphorylase was comparable to decrease in liver phosphorylase observed in earlier studies but was less than the decline in muscle phosphorylase. It is suggested that pyridoxal phosphate is a cofactor of adrenal phosphorylase.

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Actinomycin D Inhibits Parathyroid Hormone and Vitamin D Activity.* (29501)

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Administration of excessive doses of either Vit. D or parathyroid hormone to animals results in increased absorption of calcium from the gastrointestinal tract, hypercalcemia and dissolution of bone, among other effects (1-3). The hormone, at least, causes increased reabsorption of phosphate by the kidney(4). It has recently been proposed that a number of hormones act by inducing the synthesis of specific DNA directed RNA which directs the synthesis of enzymes which, in turn, cause the observed effects of hormone action (5-7). There is suggestive evidence in this regard in a recent publication in which the action of parathyroid extract on organ cultures was described(8). In these experiments it was demonstrated that addition of parathyroid extract to the culture medium resulted in destruction of bone with release of moieties of collagen into the culture medium. The size of the fragments was such as to indicate that the destruction of the collagen, which forms the bulk of the organic matrix of bone, was due to enzymatic digestion rather than simple solubilization. Such enzymatic digestion could occur either by release of enzymes from sequestered pockets within the cell such as lysozomes, activation of enzymes, or synthesis of new enzymes. If the last were true, the action of the hormone on bone should be inhibited by the action of Actinomycin D, an antibiotic which is considered to inhibit specifically the synthesis of DNA directed or messenger RNA(9-11).

Materials, methods and results. Six-week-old male Swiss-Webster mice were divided into 4 equal groups. One group was injected intraperitoneally with 150 units of parathyroid extract (Eli Lilly & Co.), a second with 0.01 mg of Actinomycin D, a third with 0.01 mg of Actinomycin D and 150 units of parathyroid extract and a control fourth group received an equal volume of 0.9% NaCl. After 18 hours, the animals were anesthetized and blood obtained by decapitation. Hormone activity was assessed by measuring the level of serum calcium using an atomic absorption spectrometer and the production of specific bone lesions, which have been well described(12). Similar experiments were designed to test the ability of Actinomycin D to inhibit the ability of excessive dosages of Vit. D to induce hypercalcemia except that 10,000 units of Vit. D were injected instead of parathyroid extract. Analysis of the data for serum calcium is presented in Table I. Actinomycin completely suppressed the hypercalcemic effects of both parathyroid extract and Vit. D. Histologic studies of the proximal tibial epiphysis of mice injected

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