"net production" of renin. Incubated controls had only slightly higher renin content than did the nonincubated controls, but the addition of epinephrine, norepinephrine or cyclic AMP caused striking increases in "net production" of renin. The effects of these agents on "net renin production" were abolished by cycloheximide, an inhibitor of protein synthesis. It is suggested that the renin-stimulating effect of catecholamines in the intact animal might be due, at least in part, to a direct chemical action on the renal cells. Cyclic AMP may play a role as an intracellular mediator of the action of catecholamines.

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Renal Stones of Calcium Phosphate: Physicochemical Basis for Their Formation (33648)

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Nephrolithiasis resulting from calcium phosphate stones is a frequent complication of idiopathic hypercalciuria and of hypercalciuria resulting from sarcoidosis, hyperparathyroidism or prolonged treatment with Vitamin D (1, 2). New stones are usually not formed after hypercalciuria has been corrected. It has therefore been suggested that the urine of such patients is supersaturated with respect to Ca^{2+} and phosphate ions, and that this leads to precipitation and formation of calcium phosphate stones (1, 3, 4). In this communication, we present experimental verification for this hypothesis.

The calcium phosphate first formed from urine, and the phase which is stable at the normal acid pH of urine, is presumably brushite ($CaHPO_4 \cdot 2H_2O$) (5,6); at basic pH, brushite undergoes hydrolysis to octacalcium phosphate and hydroxyapatite. Thus, brushite is often found in the stones of patients with hypercalciuria. We therefore determined the state of saturation of urine with respect to brushite. This was achieved by calculating the activity product of Ca^{2+} and HPO_4^{2-} of the urine before and after incubation with brushite.

Material and Methods. The calculation of activity product of Ca^{2+} and HPO_4^{2-} requires an estimation of (i) the ionic strength (μ) of urine, (ii) the fraction of total orthophosphate represented by HPO_4^{2-}, and (iii) the activity coefficient of Ca^{2+} and HPO_4^{2-} at varying ionic strengths.

The ionic strength of urine was calculated from the formula: $\mu = 1.056\ (C_{Na^+} + C_{K^+} + C_{NH_4^+} + 4C_{Mg^{2+}} + 4C_{Ca^{2+}})$, where C is concentration in moles/liter and 1.056 is the

TABLE I. Comparison of Three Groups.*

Group	No. of patients	No. of urine samples	Urinary Ca excretion (mg/day)	Activity product ratio
I. Hypercalciuria	15	37	291.5 ± 9.0	2.981 ± 0.264
II. "Normocalciuria" with nephrolithiasis	9	22	136.3 ± 2.9	1.213 ± 0.226
III. Control subjects	19	21	106.6 ± 2.3	0.700 ± 0.171

* Urinary calcium excretion and activity product ratios are presented as mean ± SEM. There was no significant difference in pH, volume of urine excreted per day, or in ionic strength among the three groups (7).

correction factor applied to the μ estimated from the cation concentrations alone to obtain the true μ derived from both cations and anions (7). Levinskas (8, 9) calculated the fraction of HPO_4^{2-} in the total phosphate at the ionic strength of .165. Using his method (7), we have extended this calculation to other ionic strengths. The activity coefficient of Ca^{2+} was calculated from the Debye–Hückel theory; the values used were the averages of those obtained by Moreno and associates (10) and by Frankenthal (11). The activity coefficient of HPO_4^{2-} was calculated by extending the method of Levinskas (8, 9). These calculations will be described in detail in a future communication (7).

The degree of saturation of urine with respect to brushite was determined as follows. The activity product of Ca^{2+} and HPO_4^{2-} of the original urine (*Ksp,i*) was calculated. This was then compared with the activity product of the supernatant of the same urine (*Ksp,f*) which had been incubated with brushite (Mallinkrodt Chem.) for 3–4 days. Two hundred mg of brushite were kept in suspension in 100 ml of urine at 37° with a magnetic stirrer. Chloroform, 0.5 ml, was added to control bacterial growth. The supernatant was obtained by centrifugation of the suspension at 1400*g*. The activity product ratio (*Ksp,i/Ksp,f*) represents the degree of saturation of urine, where the value of 1 indicates saturation, a value greater than 1, supersaturation, a value less than 1, undersaturation. This procedure of estimating the degree of saturation of urine from *Ksp,i/Ksp,f*, "corrected" for the small errors which may be attendant in our method of calculation of the activity product of brushite. The most important of these errors is probably in the calculation of the activity of Ca^{2+}, since we did not consider the complexing of Ca^{2+} by the various ligands in urine (7). Since these errors contributed to both *Ksp,i* and *Ksp,f*, they were largely canceled out in the determination of *Ksp,i/Ksp,f*.

Thirty-seven urine specimens from 15 patients with idiopathic hypercalciuria (2) (Group 1), 22 urine specimens from 9 patients with calcium-containing renal stones but with normal urinary calcium (Group II), and 21 urine specimens from 19 subjects without nephrolithiasis (Group III) were evaluated (Table I). We define hypercalciuria as urinary calcium excretion exceeding 200 mg/day on a diet containing approximately 400 mg/day; normocalciuria then represents urinary calcium excretion of less than 200 mg/day on the same calcium restriction. The patients in Groups I and II passed many renal stones, which were either pure calcium phosphate, or mixed calcium phosphate and oxalate, on X-ray diffraction. Group III consisted of normal volunteers and patients with essential hypertension, idiopathic edema, and adrenal insufficiency (treated). After they had received a diet containing 400–600 mg of calcium/day for 4 or more days, two or three 24-hr urine specimens were obtained from each of the patients in Groups I and II, and one or two specimens from each of the control subjects. The specimens were stored in closed plastic

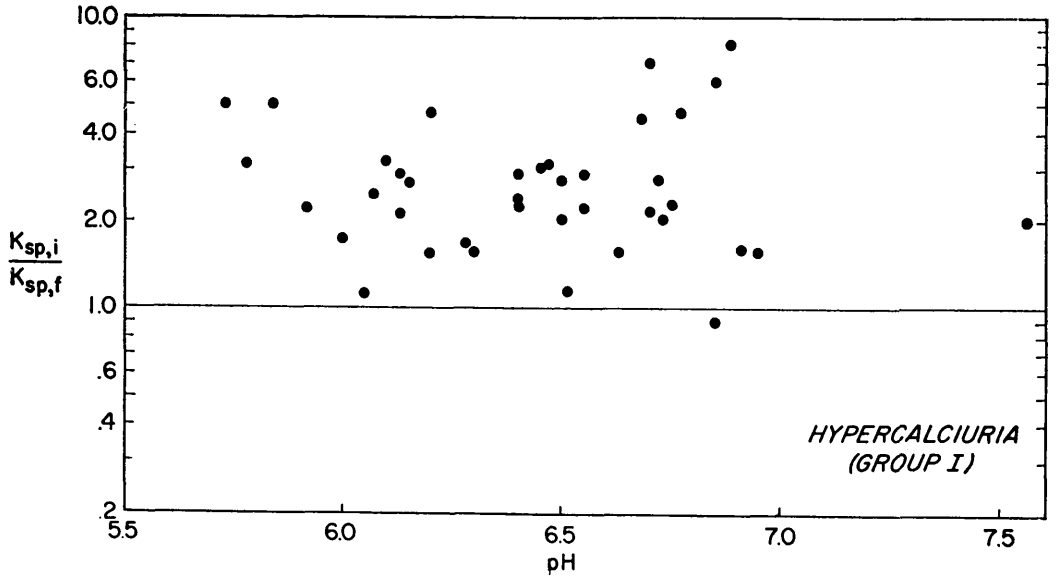

FIG. 1. Activity product ratio (*Ksp,i/Ksp,f*) of the urine of patients with idiopathic hypercalciuria. The horizontal line at *Ksp,i/Ksp,f* of 1 represents the state of saturation with respect to brushite. The values above this line estimates the number of times the urine is supersaturated. Most of the urine specimens were supersaturated.

containers at 10° during collection; those with pH greater than 6.70 were collected under oil.

Results. The 24-hr urinary calcium of patients in Group I was greater than 200 mg in every urine specimen, with a mean of 291.5 ± 9.0 (SEM) mg/day. The urinary calcium in patients of Group II and Group III was 136.3 ± 2.9 and 106.6 ± 2.3 mg/day, respectively; these values were significantly lower than that of Group I ($p < .001$).

The activity product ratio of urine from Group I was greater than 1 except in one specimen; it had a mean of 2.981 ± .264 (Fig. 1). The urine of patients with idiopathic hypercalciuria was therefore supersaturated nearly 3-fold with respect to Ca^{2+} and HPO_4^{2-}. Furthermore, the activity product ratio was not dependent on the pH of the urine (correlation coefficient, .076, Fig. 1).

In contrast, the activity product ratio for the urine from "normocalciuric" groups (II and III) was strongly influenced by pH (correlation coefficient, .87 for Group II and .80 for Group III, Fig. 2). The activity product ratio was greater than 1 only at pH greater than 6.5. Below this pH, *Ksp,i/Ksp,f* was less than 1. Most of the urine specimens were therefore undersaturated with respect to Ca^{2+} and HPO_4^{2-} except at high pH. The principal reason for the supersaturation of urine in idiopathic hypercalciuria appears to be the high urinary excretion of calcium. In contrast, the major cause for the supersaturation of urine among normocalciuric subjects is the passage of urine of high pH.

Discussion. The significance of activity product of Ca^{2+} and HPO_4^{2-} in the pathogenesis of nephrolithiasis resulting from calcium phosphate may be appreciated from the following considerations. First, the initial formation of calcium phosphate stone may require a state of supersaturation of Ca^{2+} and HPO_4^{2-} with respect to brushite, as previously emphasized. It is therefore unlikely that a calcium phosphate stone could form from urine with an activity product ratio of less than 1 (3, 4). The state of saturation of urine with respect to other calcium phosphates (octacalcium phosphate and hydroxyapatite) was calculated by Robertson and associates (4). Such a calculation may be important in explaining the growth and dissolution of an already-formed, octacalcium phosphate or hydroxyapatite stone. However, it would not explain the occurrence of a

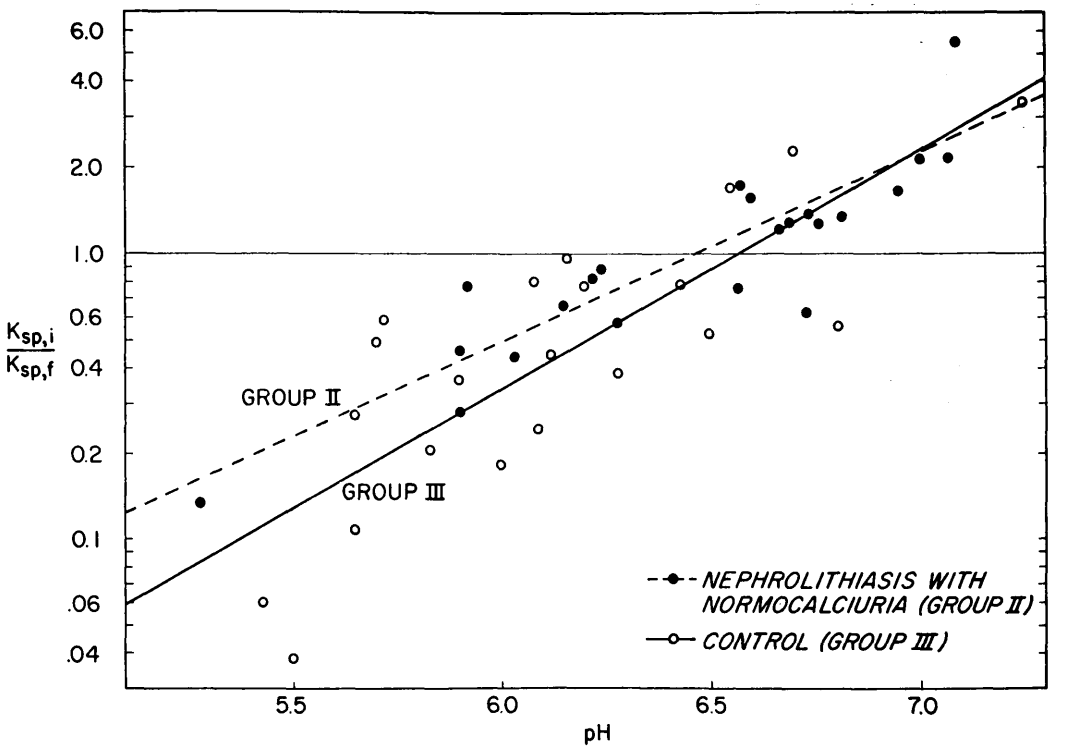

FIG. 2. Activity product ratio ($Ksp,i/Ksp,f$) of the urine of subjects with normocalciuria. The activity product ratio was pH-dependent. The supersaturation ($Ksp,i/Ksp,f>1$) was observed only at high pH.

brushite stone, or the formation of stone at acid pH (which probably involves the brushite phase).

Secondly, once the stone has been formed, its growth and maintainance probably requires a state of supersaturation. In the supersaturated urine, the activity product of Ca^{2+} and HPO_4^{2-} is reduced upon incubation with brushite, indicating an incorporation of Ca^{2+} and HPO_4^{2-} from urine into the solid phase or growth of brushite. In the undersaturated urine, the activity product is increased with incubation, indicating that Ca^{2+} and HPO_4^{2-} have been added to the urine from the solid phase, or a partial dissolution of brushite.

Several clinical observations support the above conclusions. Nephrolithiasis with stones of calcium phosphate develops in patients with hypercalciuria (1,2), and in nomocalciuric subjects who consistently excrete urine of high pH because of therapy with alkali or a urinary tract infection with urea-splitting organisms (1), that is, in the conditions in which we have demonstrated supersaturation of urine with respect to brushite. Stone formation often stops when therapy leads to the conversion of a supersaturated urine to an undersaturated urine (7).

We therefore conclude that the passage of urine supersaturated with respect to brushite, either by excessive urinary excretion of calcium (as in idiopathic hypercalciuria) or by excretion of urine of high pH, is etiologically important in the pathogenesis of renal stone. Other factors which may be involved in the formation of renal calculi, such as inhibition of nucleation, should be reexamined in terms of the degree of saturation of urine with respect to brushite.

Summary. The activity product of Ca^{2+} and HPO_4^{2-} was calculated for the urine before and after incubation with brushite ($CaHPO_4 \cdot 2H_2O$). This permitted an estimation of the degree of saturation of urine in terms of Ca^{2+} and HPO_4^{2-}. The urine of

patients with idiopathic hypercalciuria was supersaturated with respect to brushite at all pH. However, the urine of normocalciuric subjects with stone was supersaturated only at high urinary pH.

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An Effect of Intestinal Motility on Iron Absorption (33649)

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Iron absorption is influenced by numerous intraluminal, mucosal and corporeal factors (1). Most manipulations affecting iron absorption do not produce measurable effects for several days after their initiation (2). Endotoxin is exceptional because it markedly diminishes iron absorption within hours after injection and seems to act by decreasing the transfer of dietary iron from the intestinal absorptive cells into the body (3, 4). The rapid effect of endotoxin upon iron absorption suggested that it triggered a basic regulator of iron absorption and that the response was not mediated by changes in erythropoiesis or the body iron stores. To study this phenomenon, we measured iron absorption in rats dosed with drugs that altered various manifestations of the endotoxin response. Preliminary studies showed that antihistaminics and corticosteroids produced no significant change in iron absorption by either normal or endotoxin treated rats. On the contrary, phenoxybenzamine (Dibenzyline), an alpha adrenergic blocking agent, increased iron absorption in both normal and endotoxin-treated animals. The present paper reports investigations of the effect of phenoxybenzamine upon iron absorption.

Methods. Male albino rats of the Walter Reed Carworth Farm strain, weighing 200–250 g, were used. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed. Rats were raised in a pathogen-free environment and housed in galvanized wire cages. The animals were fed a standard laboratory diet containing 25% protein and 9 mg of iron/100 g of dry weight.

Drugs were administered by intraperitoneal injection. These included: phenoxybenzamine hydrochloride, 3 mg (Smith, Kline & French Laboratories); endotoxin as *E. coli* lipopolysaccharide, 0.1 mg (Difco Laboratories); and atropine sulfate, 30 mg.

Iron absorption studies were performed with a test dose containing 0.5 μCi of ferrous^{59} citrate and 0.25 mg of elemental iron as ferrous sulfate in 0.5 ml of distilled water. The test dose was administered to rats fasted for 16 hr by injection into the stomach with an olive-tipped 17-gauge endoesophageal needle. Whole-body radioactivity (0.8 MeV-∞) was measured in a small animal whole-body liquid scintillation detector (ARMAC, Packard Instrument Co.) 3 hr and 7 days after dosing to determine the percentage of the