Renal Effects of Hypercalciuria in Immobilized Children.* (29993)

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Numerous studies have demonstrated a relationship between hypercalcemia, hypercalciuria, and decreased urinary concentrating capacity(1-9). The underlying mechanisms, however, are unknown. Previous studies, performed during both hypercalcemia and hypercalciuria, could not discriminate between possible separate effects of each of these two conditions. Moreover, the results of some investigations have been complicated by the use of subjects with underlying renal, endocrine, or other disease(1,2,5). Finally, effects of acute and transient hypercalcemia and hypercalciuria induced by means of calcium infusion or administration of calciferol or parathyroid extract(3,4,6-8) may be different from effects on the kidney of prolonged states of calcium excess. In seeking a situation in which these relationships could be investigated in man without exposing subjects to the possible dangers of experimentally induced hypercalcemia or hypercalciuria (9), observations were made on children in whom hypercalciuria could be expected as a result of immobilization(10-12).

Materials and methods. Twenty children aged 18 months to 14 years immobilized for various orthopedic conditions in traction, hip spicas, or spinal casts, were each studied 1-4 times during periods of immobilization lasting up to 90 days. Blood samples and 24-hour urine collections were analyzed for osmolality, Na, K, Cl, Ca, P, urea, and creatinine. A standardized test of urinary concentrating performance was conducted as follows: Except for a dry evening meal, subjects were thirsted and fasted totally from 1 PM until 8 AM the following morning. Urine was collected from 8 PM until 8 AM as a single specimen, designated hereafter as the concentrated urine specimen.†

Osmolality was determined cryoscopically using the Fiske Osmometer. Na and K were measured with the Perkin-Elmer Flame Photometer. Cl was determined by amperometric titration using the Cotlove Chloridometer. Ca, P, urea, and creatinine were measured colorimetrically with the Technicon AutoAnalyzer and modifications of the Technicon N-Methodology.

Results. Although blood samples were not obtained in each subject throughout the entire period of immobilization, all estimations of serum urea, creatinine, Na, K, Cl, and P, as well as Ca were within normal ranges for this laboratory and no upward or downward trends were detected. Values for serum Ca ranged from 9.0 to 10.8 mg per ml with no significant departure at any time from the mean value for all measurements of 9.8.

Mean rates of excretion of urinary solutes at various stages of immobilization are shown in Fig. 1. Except for Ca, the rate of excretion of each solute paralleled closely the rate of excretion of total solutes. A 2-fold increase in rate of excretion of solutes other than Ca, noted within the 2nd to 10th day of immobilization, either was maintained or decreased somewhat during the remainder of the period of observation. In contrast, Ca excretion increased steadily during the first 3 weeks of immobilization and then slowly returned to initial levels. As rates of Ca excretion increased, there was an inverse change in urinary concentrating performance: concomitant with the maximum increase in rate of Ca excretion, the mean osmolality fell

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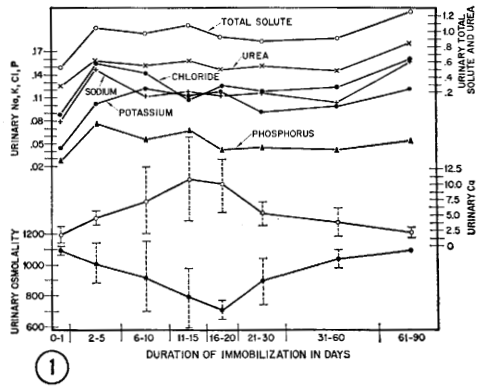
† The following normal values for concentrating performance under these conditions have been established in this laboratory in a study of 181 healthy children ranging in age from 3 to 14 years: 1090 ± 222 mOsm/kg (mean ± 2 SD). For practical purposes, 900 mOsm/kg is considered the lower limit of normal.



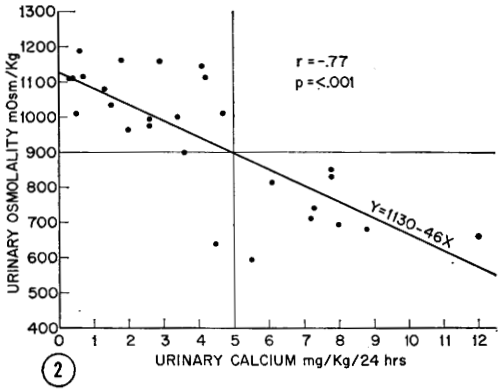


FIG. 1. Rates of excretion of individual and total urinary solutes and osmolality of concentrated urine specimens during immobilization. Na, K, Cl, P, and urea are expressed as mM per mg creatinine; Ca as μM per mg creatinine; total solute as mOsm per mg creatinine; and osmolality as mOsm per kg urinary water. Each point represents the mean of 4-9 observations, except for the last point of the curve of osmolality which represents a single patient. Vertical lines indicate $\pm$ 1 SD.

FIG. 2. Osmolality of concentrated urine specimens as a function of rate of urinary calcium excretion. Curve was calculated by the method of least squares.

from 1100 mOsm/kg at the onset of immobilization to 735, and then gradually returned to normal values by the end of the period of observation (Fig. 1). This relationship is demonstrated more precisely in Fig. 2 in which the osmolality of each concentrated urine specimen is plotted against the corresponding rate of excretion of Ca. A highly significant inverse relationship is demonstrated, expressed by the equation $Y = 1130 - 46X$ ($r = -.77$; $p < .001$). It should be noted that at rates of Ca excretion below 5 mg per kg per 24 hours all values for urinary osmolality save one were above 900 mOsm/kg, whereas above this rate of Ca excretion all values for osmolality fell below 900.

Discussion. The occurrence of prolonged hypercalciuria without frank hypercalcemia permits investigation of the metabolic effects of hypercalciuria. The children reported here, immobilized for orthopedic procedures, developed a progressive impairment in urinary concentrating performance that paralleled a steadily increasing rate of excretion of Ca. That this defect was not due to a solute diuresis is demonstrated (Fig. 1) by (1) minimal change in concentrating performance early in the course of immobilization, at which time total solutes had reached peak rates of excretion, and (2) progressive loss of concentrating performance and subsequent return to normal levels during periods when rates of excretion of solutes other than Ca remained at elevated levels. Moreover, examination of osmolality of concentrated urine specimens showed no correlation with rates of excretion of total solutes ($r = +.30$), whereas there was a highly significant inverse correlation with rates of excretion of Ca (Fig. 2). The impairment of concentrating performance appears to be related specifically to hypercalciuria, since no changes were detected in serum Ca concentrations.

Summary. It is shown that hypercalciuria without frank hypercalcemia develops within a few days in otherwise healthy children immobilized for various orthopedic conditions. This occurrence permits investigation of the metabolic effects of hypercalciuria. In the present study, an inverse correlation has been demonstrated between rate of urinary calcium excretion and renal concentrating performance in such children. In addition to its physiological interest, the degree of impairment of concentrating performance observed may be of considerable clinical significance in immobilized patients not given free access to water.

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Differences in Hepatic Drug Metabolism in Various Rabbit Strains Before and After Pretreatment with Phenobarbital.* (29994)

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Species variation in drug response is a well recognized pharmacological phenomenon. Strain variations within a given species, however, have been characterized to a much lesser degree. These problems assume importance not only when attempting to extrapolate results of animal experiments to man, but also in basic biological research.

Law *et al*(1) have shown variations in beta-glucuronidase activity in various strains of mice. Jay(2) has also shown strain differences in mice with regard to hexobarbital sleeping time. The experiments of Yaffe(3) indicate that strain variation in drug response in mice is a genetic factor which is manifest soon after birth. Strain variation in drug response and hepatic drug metabolism in rats have been reported by Quinn *et al*(4). However, to our knowledge, no studies on strain differences in drug response have been reported using rabbits, a species commonly employed in research.

We have attempted to determine whether the activity of several hepatic microsomal drug metabolizing enzyme systems varies in different rabbit strains. Studies were carried out on strains of rabbits commonly employed in research as well as on wild rabbits. We have also attempted to determine whether the stimulation of the hepatic drug metabolizing enzyme activity by phenobarbital differed in these strains.

Materials and methods. Dutch, New Zealand, English, and California strains were studied as representatives of commonly employed research animals. In addition, wild Jack rabbits were obtained from the Durant Animal Co., Ft. Sumner, New Mexico and wild Cottontails were obtained from the Earl Johnson Farms, Rago, Kansas. Rabbits were maintained on regular Purina Lab Chow and water *ad libitum* during the experiment, and constant conditions were maintained as nearly as possible. In all cases young adult bucks were employed.

To study the effect of pre-treatment with phenobarbital, rabbits were injected intraperitoneally twice daily with 15 mg/kg phenobarbital sodium dissolved in normal saline. Injections were made for 3 days, on the 4th day the animals were not injected, and on the fifth day the animals were sacrificed and livers removed. Control animals were injected with normal saline on the same schedule. The livers were placed immediately on ice, blotted, weighed, and ground in the cold with a Potter homogenizer (teflon pestle) in 2 volumes of ice cold 1.15% KCl solution. This homogenate was then centrifuged at 1-3°C (International portable refrigerated centrifuge, model PR-2) at 9,000 *g* for 30 minutes.

The following metabolic pathways were studied: Side-chain oxidation of hexobarbital

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