1. Beaufay, H., Bendall, D. S., Boudhuin, P., Wattiaux, R., DeDuve, C., *Biochem. J.,* 1959, v73, 628.

2. DeDuve, C., Beaufay, H., Jacques, P., Rahman, L. Y., Sellinger, O. Z., Wattiaux, R., DeCovinck, S., *Biochem. Biophys. Acta,* 1960, v40, 186.

3. Thomson, J. F., Klipfel, F. J., *Arch. Biochem. Biophys.,* 1957, v70, 224.

4. Appelmans, F., DeDuve, C., *Biochem. J.,* 1955, v59, 426.

5. Adams, D. H., Burgess, E. A., *Brit. J. Canc.,* 1957, v11, 310.

6. ———, *Biochem. J.,* 1960, v77, 247.

7. ———, *ibid.,* 1959, v71, 340.

8. Feinstein, R. N., *Arch. Biochem. Biophys.,* 1959, v79, 399.

9. Miller, L. A., and Goldfeder, A., *Cancer Res.,* 1961, v21, 64.

10. Chance, B., Maehly, A. C., *Methods in Enzymology,* Colowick, S. P., Kaplan, N. O., eds., Academic Press, Inc., New York, 1955, vII, p764.

11. Lowry, P. H., Rosebrough, N. J., Farr, L. A., Randall, R. J., *J. Biol. Chem.,* 1951, v50, 182.

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Effect of Variations in Sodium Intake on Calcium Excretion in Normal Humans.* (28821)

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The amount of calcium excreted per unit time represents the balance between the quantity filtered at the glomerulus and the magnitude of reabsorption by the renal tubules. The filtered load is the product of the concentration of diffusible (complexed and free Ca^{++} ion) calcium in the plasma and the glomerular filtration rate, while tubular reabsorption appears to be an active transport process which takes place in both proximal and distal parts of the nephron(1). There is no evidence that net tubular secretion of calcium occurs(1). The relative importance of alterations in filtered load or tubular reabsorption to the homeostatic regulation of calcium excretion has not been defined. However, it is clear that normally greater than 95% of the filtered calcium is reabsorbed. The latter process seems to be influenced by the level of the filtered load (2,3), the concentration of parathyroid hormone in body fluids(3), the overall calcium balance or body "stores," the quantity of organic and inorganic anion which may complex free Ca^{++} ion in luminal fluid, and the nature of the other univalent and divalent cations simultaneously excreted(2,4,5,6).

Walser(6) has conclusively demonstrated in the dog a direct relationship between the renal clearances of calcium and sodium. The clearances of these 2 ions were remarkably similar, and any increase in sodium excretion immediately caused an increase in calcium excretion. It is quite obvious that this relationship could greatly effect the interpretation of the homeostatic regulation of calcium excretion in any clinical or experimental study.

The present study was undertaken to determine whether these observations in the dog could be confirmed in the human.

Methods and procedure. The experiments were carried out on 6 normal young adult volunteers ranging in age from 25 to 35. All were males except subject M.K. Throughout the study each subject was maintained on a near-constant calcium intake by eating the same quantity of dairy foods and leafy vegetables daily. The only significant variable in the diet was the intake of sodium chloride. Twenty-four hour urinary collections were made by each subject while carrying out his normal daily activities. The sodium content of the diet was varied from 20 to 425 mEq/24 hours by addition of weighed quantities of NaCl to diets calculated to contain from 200 mg to 2 g of sodium. Each subject re-

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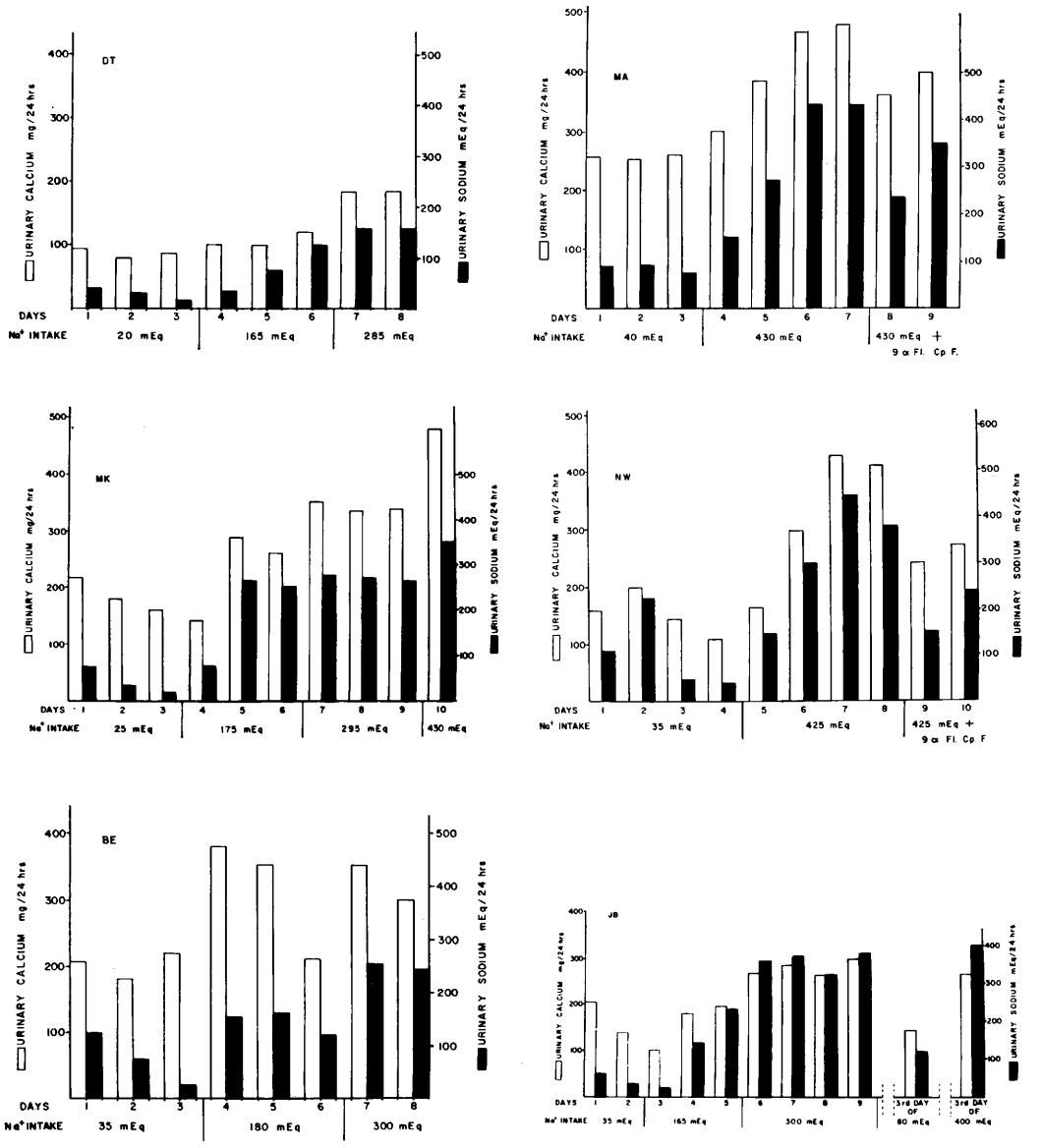

FIG. 1. Effect of varying intake of sodium on renal excretion of calcium. Each curve represents an individual subject.

mained on a given sodium intake for periods ranging from 2 to 6 days. Subjects N.W. and M.A. received 2 mg (0.5 mg, orally, 4 times daily) of 9-alpha-fluorohydrocortisone on the last 2 days of their highest sodium intake. On the last day of any given salt intake, while the subjects sat in a comfortable lounge chair, 2 one-hour urine collections were obtained and a sample of venous blood was collected anaerobically at the end of the first hour. From these were calculated the clearances illustrated in Fig. 2. Calcium, phosphorous and creatinine were measured in plasma and urine by methods previously described(3). Sodium was determined by flame photometry. Diffusible (ultrafiltrable) calcium in the plasma was obtained with the Lavietes anaerobic ultrafiltration unit(7). Ultrafiltration was carried out at room temperature.

Results. The variations in dietary intake of sodium and the administration of 9-alpha fluorohydrocortisone had no significant effect on the serum level of total or diffusible calcium, sodium, inorganic phosphorus, or creatinine, nor on urinary excretion and calculated clearances of creatinine and inorganic phosphorus. Therefore, the data presented are restricted to the relationship of sodium excretion to calcium excretion and clearance in each subject.

Although urinary excretion of sodium and calcium varied considerably from subject to subject, it was apparent in each that these 2 parameters paralleled each other (Fig. 1). This was least evident in subject B.E., but even here calcium excretion was significantly lower when this subject was ingesting the lowest sodium intake (35 mEq/day). When subject J.B. was switched from 35 to 165 mEq of sodium/day, urinary sodium continued to decrease on the first day of the higher salt intake and excretion of calcium paralleled the urinary sodium rather than sodium intake. This relationship was emphasized by the urinary findings in subjects N.W. and M.A. when they received the 9-alpha-fluorohydrocortisone. While ingesting 425 mEq/day of sodium the steroid caused a distinct reduction in sodium excretion and an obvious positive salt balance. Despite the positive salt balance, calcium excretion decreased in both subjects. Definite hypercalciuria was caused by the high salt ingestion in 4 of the subjects (M.A., M.K., N.W. and B.E.).

A more exact representation of the relationship of sodium excretion to calcium excretion is depicted in Fig. 2. During the short collection periods obtained on each subject, the clearance of diffusible calcium varied directly with the simultaneous urinary excretion of sodium.

Discussion. These results indicate that Walser's observations on the dog(6) can be reproduced with normal human subjects. Calcium excretion and clearance are directly related to sodium excretion. Walser has shown that this is an effect of sodium ion and not a non-specific effect of solute or water excretion(6). He has suggested that the renal

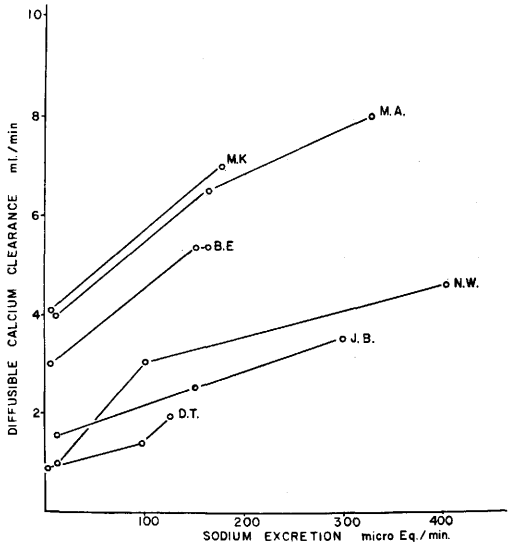

FIG. 2. Relationship between minute excretion of sodium and renal clearance of diffusible calcium. Increase in sodium excretion illustrated on each curve is the consequence of increase in dietary sodium.

tubules attempt to maintain a constant ratio of sodium ion to calcium ion in the tubular fluid, and that calcium shares a common reabsorptive transport mechanism linked to sodium transport.

These observations indicate that the proper interpretation of the urinary excretion of calcium in any clinical or experimental investigation cannot be made without knowledge of the simultaneous excretion of sodium.

Although the effect of varying the sodium content of the diet on the intestinal absorption of calcium was not followed in the present study, it is most unlikely that the correlation between sodium and calcium excretion was due to changes in the absorption of calcium from G.I. tract.

Summary. The effect of variations in excretion of sodium on calcium excretion and clearance has been studied in 6 normal humans. The excretion of sodium was altered by varying the salt content of the diet. A direct relationship between calcium excretion and clearance and the simultaneous sodium excretion was demonstrated. These studies emphasize the importance of measuring sodium excretion in any investigation of the regulation of calcium excretion.

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1. Howard, P. J., Wilde, W. S., Malvin, R. L., *Am. J. Physiol.*, 1959, v197, 337.

2. Chen, P. S., Jr., Neuman, W. F., *ibid.*, 1955, v180, 623.

3. Kleeman, C. R., Bernstein, D., Rockney, R., Dowling, J. T., Maxwell, M. H., *Yale J. Biol. and Med.*, 1961, v34, 1.

4. Chen, P. S., Jr., Neuman, W. F., *Am. J. Physiol.*, 1955, v180, 632.

5. Walser, M., Browder, A. A., *J. Clin. Invest.*, 1959, v38, 1404.

6. Walser, M., *Am. J. Physiol.*, 1961, v201, 769.

7. Lavietes, P. H., *J. Biol. Chem.*, 1937, v120, 267.

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Effect of Neo-Natal and Adoptive Immunization on Tumor Transplant Susceptibility in Mice.* (28822)

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There are numerous reports in the field of tumor immunology concerning administration of immune serum from resistant animals to tumor susceptible animals ("passive" immunization), and also upon the effect of pre-immunization with heated tumor or tumor extracts ("active" immunization)(1). In general these techniques have provided protection if carried out either prior to, or soon after transplantation of tumor. They have had little success in preventing growth either of well established transplanted tumors, or of spontaneous neoplasms. A third type of immunization, called "adoptive" immunization has also been shown to be practicable (2). The technique involves transplanting functioning spleen or lymph node tissue from an immunized animal into an unimmunized animal of the same inbred strain. It has been shown that spleen and lymph nodes transferred in this way continue to produce antibodies in the new host(3). Techniques have recently been developed for rendering unrelated animals graft tolerant by neo-natal transfer of tissue(4). This makes possible the use of "adoptive" immunization between different strains of mice. Spleen and lymph nodes from a tumor resistant strain of mice might then be used to influence growth of a transplanted tumor in a tumor susceptible strain.

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This paper describes neo-natal induction of graft tolerance between tumor resistant strain (DBA/2) and tumor susceptible strain (BALB/C) mice. This was followed by adoptive immunization in later life, using grafts of spleen and lymph node cells from the tumor resistant strain. Growth of previously established tumor transplants under the influence of this adoptive immunization was then studied.

Materials and methods. Mice were obtained from Jackson Memorial Laboratories, Bar Harbor, and were fed on commercial mouse pellets and tap water *ad libitum*. Two strains of mice were used, one (BALB/C) which is susceptible to the transplantable and isologous CD/5 lymphoma, and the DBA/2 mouse which is resistant to this tumor. Mice bearing CD/5 lymphoma for transplantation were kindly supplied by Dr. Morris Barrett, Nat. Cancer Inst., Bethesda, Md. Inbred mice were maintained for one generation by random mating, and for succeeding generations by brother/sister mating.

Neo-natal cross-immunization. The neo-natal cross-immunization procedure is shown schematically in Fig. 1. By using an intermittent mating schedule, numbers of BALB/C and DBA/2 litters were obtained which were born within 4-12 hours of each other. One newborn BALB/C animal was sacrificed, its spleen was removed, and a single cell suspension of spleen cells was pre-