mediated through a decrease in urine alkalinity. Investigations with rats(5) and mink (6) have indicated that the addition of ammonium chloride to the diet prevented urinary calculi formation. Leoschke and Elvehjem (6) reported that the addition of one gram of ammonium chloride daily to a diet fed to mink reduced the incidence of urinary calculi. They attributed the effect to the increase in urinary acidity. Feeding sheep a 10% level of sodium chloride in diets similar to those fed in this experiment has resulted in an increase in urinary acidity from an average pH of 7.95 to 7.30.† Using analysis of variance, this difference was statistically significant (P <.01). The urine pH was determined by collecting a 24-hour urine sample under toluene and immediately measuring the pH of the urine with a glass electrode pH meter.

The effect of the high salt diet may be related to the relationship which exists in the body between sodium, potassium and other minerals. Of course, a combination of several or all of the above mentioned factors may have been responsible for the preventive effect of the 10% level of sodium chloride. Whatever the reason, it should be emphasized that additional investigation is needed before the use of a high sodium chloride diet to prevent urinary calculi can be recommended.

† Elam, C., and Ham, W., unpublished data.

Summary. Sixty wether lambs were divided into 12 lots of 5 each and fed the experimental diets for a 105-day period. The animals were group fed all they would consume twice daily. The variables included in the diets were the presence and absence of potassium acid phosphate (K_2HPO_4), a 10% level of sodium chloride, a 30% level of beet pulp, and the ash of the same amount of beet pulp. Fourteen cases of urinary calculi resulted in the 60 sheep. None of these cases occurred in the 30 sheep which received the 10% level of sodium chloride. Eleven of the 14 cases of calculi occurred in those lots receiving added potassium acid phosphate in the diet, while 3 occurred in its absence. Beet pulp or beet pulp ash had no effect on the incidence of urinary calculi.

1. Lindley, C. E., Taysom, E. D., Ham, W. E., and Schneider, B. H., *J. Animal Sci.*, 1953, v12, 704.

2. Elam, C. J., Schneider, B. H., and Ham, W. E., *ibid.*, 1956, v15, 800.

3. Meyer, J. H., Weir, W. C., Ittner, N. R., and Smith, J. D., *ibid.*, 1955, v14, 412.

4. Shohl, A. T., *Mineral Metabolism*, Reinhold Publishing Co., N. Y., 1939.

5. Vermeulen, C. W., Ragins, H. D., and Grove, W. J., *J. Urol.*, 1951, v66, 1.

6. Leoschke, W. L., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 42.

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Effect of Vitamin D on Retention of Radium by the White Rat.* (23360)

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The effect of vit. D on retention of radium in white rats was studied as part of a general investigation on distribution and behavior of radium in the animal body. It has been believed since the early studies on radium poisoning that radium is deposited and retained in the skeletal structure. Evans *et al.*(1), using radioautographs, confirmed this belief, showing that radium was concentrated in the region of the epiphyses. Norris and Kisieleski (2) found that 3 days after intravenous administration of radium skeletal retention was 63% of the injected dose; retention was still 53% after 30 days. Since total body retention after the 30 days was 55% of the injected dose, practically all the radium re-

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tained was in the skeleton. Qualitatively similar results were obtained with Ca^{45} and $Sr^{89,90}$. Greenberg(3) found that the amounts of Ca^{45} and Sr^{89} retained in the skeletons of rachitic rats 3 days after intraperitoneal injection of the radioisotopes were markedly increased by administration of irradiated ergosterol to the rats. Retention in the soft tissues was insignificant. In addition, vit. D increased absorption of oral doses of the isotopes. Skeletal uptakes of injected (intravenous) Ca^{45} and $Sr^{89,90}$ were shown by Copp and coworkers(4) to be very rapid in young rats, reaching a maximum within an hour. The deposited isotopes were removed only slowly from the bones. Young rachitic (low P) rats showed the same rapid initial uptake as normal animals, but much more rapid loss occurred. Skeletal retention after 2 to 3 days was therefore much lower than in the normal rats, and after 8 days was less than one-third that in normal animals. These workers also found that skeletal retention of Ba^{140} in the low P rachitic rats was only half that in normal rats after 8 days. Dudley and Friedman (5) found that retention of Ga^{72} in the ends of the long bones of rachitic rats was increased 40% by administration of vit. D, which had no effect on deposition in the shafts. Considerable Ga^{72} was readily deposited in the bones of rachitic rats even when no concurrent deposition of calcium salts occurred.

The experiment reported here was designed to study the effect of low P rickets, and vit. D treatment thereof, on radium retention in the rat. Unpublished results of preliminary experiments indicated that rachitic rats on a vit. D-free, high-calcium, low-phosphorus diet retained less radium 30 days after a radium injection than did vit. D-treated rachitic or normal control animals. Similar results of a more extended investigation are presented here.

Procedures. Male rats of the Sprague-Dawley strain, 21 days old, average weight about 40 g, were divided by random selection into 3 groups: (A) control group (14 rats); (B) untreated-rachitic group (20 rats); (C) vit. D-treated rachitic group (13 rats). The animals were quartered in individual cages in a constant temperature room, with free access to water and U.S.P. Rachitogenic Diet No. 2. The animals of the control group were fed, by medicine dropper, 40 International Units of Vitamin D per day as a solution of calciferol in corn oil. The animals of the treated-rachitic group, after 40 days on the rachitogenic diet, were given 80 I.U. of vit. D per day for a period of 4 days prior to injection of radium, then received 40 I.U. per day until sacrificed. All animals not receiving vit. D were given the corresponding amount of corn oil daily. Forty-four days after the animals had been placed on the rachitogenic diet each rat received an intraperitoneal injection of 1 ml of physiological saline solution containing 10.6 μg of radium, as radium chloride. (Radium contents of several samples were estimated by gamma radiation determinations.) The animals were sacrificed by anesthesia with ethyl ether 23 days following injection of the radium, then each carcass was hermetically sealed in a thin-walled metal can, and placed in a deep-freeze. The radium content of each can was estimated after a 30 day period by measurement of gamma radiation with a Geiger counter, and comparison with a radium standard. Five animals from the control group and 6 animals from the untreated-rachitic group were sacrificed at time of injection of radium into the remaining animals of the experiment, and bone ash was determined on dry, fat-free long bones of the hind legs of each animal. The bones were ashed at 750°C in an electric muffle furnace. Per cent of bone ash was calculated as the weight of remaining ash x 100, divided by the weight of the fat-free, moisture-free bone.

Results. The data (Table I) show that the animals of the untreated-rachitic group retained significantly less radium than did the animals of either the vit. D-treated rachitic group or the control group. Greatest individual variations in amount of radium retained were observed in the untreated-rachitic group, where retention ranged from 1.97 to 3.86 μg, while control and vit. D-treated groups did not exhibit such wide variations.

That the untreated animals were rachitic at time of injection of radium is shown by

TABLE I. Radium Retention in Rachitic and Vit. D-Treated Rats.

Group	No. of rats	Bone ash, %	Mean radium retention, μg
I. Normal control	9		3.98±.128* (3.61-4.77)
	5†	60.78 (60.38-61.35)	
II. Untreated rachitic	14		2.90±.141* (1.97-3.65)
	5†	52.93 (45.99-57.40)	
III. Vit. D-treated rachitic	13		4.36±.063* (4.02-4.76)

* Stand. error.
† Killed at same time that radium was inj. into other animals of group.

the bone ash determinations on animals from the rachitic and control groups, sacrificed at time of injection of radium into the rest of the animals of the groups. The rachitic animals showed significantly lower bone ash levels than did the control animals (Table I). Variation between bone ash values of the rachitic group was much greater than in the control group.

The high variation of bone ash levels of the rachitic animals corresponds to the variation in radium retention in this group, in contrast to the relatively small variations in the control and treated groups.

Discussion. Significantly more radium was retained by the animals receiving vit. D than by the untreated rachitic animals. Differences in mean radium retention between Groups I and II, and between Groups II and III, are highly significant, t values being 5.33 and 9.19 respectively. Even the difference between Groups I and III has a probability of only 1%, so is also significant. It was assumed, because of the previous studies by many other workers, that total body retention of radium 23 days after administration of the radium would be analogous to skeletal retention. The results of our experiments do not permit us to decide whether the effect of vit. D is on the initial deposition of radium in bones, or on rate of removal from the bones.

It would appear that retention of radium, therefore, is affected by the absence or presence of vit. D in the diet in the same manner as are the retentions of Ca^{45}, $Sr^{89,90}$, and Ba^{140}. Since it is impossible to demonstrate in rats a requirement for vit. D on diets with normal Ca and P contents, the high Ca-low P diet was used to demonstrate these effects on radium retention. There is of course no assurance that vit. D would affect Ra retention in rats on normal diets, or in humans.

Summary. Total body retention of injected radium was significantly less in rachitic rats than in vit. D-treated rachitic rats, or in non-rachitic control animals.

1. Evans, R. D., Harris, R. S., and Bunker, J. W. M., *Am. J. Roentgenol.,* 1944, v52, 353.

2. Norris, W. P., and Kisieleski, W., *Cold Spring Harbor Symposia on Quantitative Biol.,* 1948, v13, 164.

3. Greenberg, D. M., *J. Biol. Chem.,* 1945, v157, 99.

4. Copp, D. H., Hamilton, J. G., Jones, D. C., Thompson, D. M., and Cramer, C., *Conf. Metab. Interrelations, Trans.,* 1951, v3, 226.

5. Dudley, H. C., and Friedman, L., *Science,* 1952, v115, 146.

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Hypothalamic Lesions Associated with Goldthioglucose-Induced Obesity.* (23361)

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A massive and persistent obesity can be induced in various strains of mice with a single injection of goldthioglucose(1-4). Of those animals surviving the LD_{50} dose about one-third show varying degrees of fat deposition (5). This obesity is associated with a marked

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