have been reached by Rosenfeld and collaborators after perfusion of calf adrenals with ACTH and with growth hormone(5). Recent *in vivo* studies have indicated the probability that the main stimulus of aldosterone secretion is directly or indirectly related to certain changes of sodium, potassium, and water metabolism. Although the results presented here show that alteration of the Na/K ratio of the incubation media is accompanied by an increase in aldosterone secretion, the physiological significance of these experiments seems to be limited by the following considerations: 1) They do not disclose whether alteration of Na or K concentrations or both is the effective stimulus for the observed increase of aldosterone secretion. 2) This increase is not very striking, leaving open the possibility that the alteration of Na and K concentrations *in vivo* may exert major effects by an indirect action which would not be demonstrable by the study of isolated glands.

Conclusion. Rat adrenal glands incubated *in vitro* released a sodium-retaining substance with the properties of aldosterone. Purified growth hormone does not seem to increase secretion of sodium-retaining activity. Two ACTH preparations caused a slight but significant increase of aldosterone production *in vitro*. Alteration of the Na/K ratio of the incubation media, by lowering the Na and increasing the K concentration, produces a moderate enhancement of aldosterone secretion.

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Strontium-Calcium Discrimination Factors in the Rat. (22636)

C. L. COMAR, R. H. WASSERMAN, AND M. M. NOLD.
Medical Division, Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tenn. under Contract with the United States Atomic Energy Commission.

The behavior of strontium in the animal body has become of particular interest in relation to possible hazards from atomic fission debris. Contamination of the food chain by fallout material may create a situation in which the calcium of the dietary becomes associated with essentially carrier-free radiostrontium. The body burden of radiostrontium, in terms of body calcium, attained after ingestion over long periods of time will be governed by the Sr*/Ca of the intake and by the over-all differentiation between strontium and calcium that may take place in such processes as primary absorption from the tract; incorporation into new bone; incorporation into pre-existing bone; renal excretion; endogenous excretion into the gastrointestinal tract; and reabsorption from the tract.

Comar, Whitney, and Lengemann(1) showed that growing rats utilized dietary calcium in a commercial ration for bone growth by a factor of 3.6 over carrier-free Sr 90 that had been incorporated into the feed, *i.e.,* the Sr 90/Ca ratio of the diet divided by the Sr 90/Ca ratio of the bones equaled 3.6. These rats had been raised for one or 2 generations on a diet in which the Sr 90 to calcium ratio was maintained at a constant level so that their bones were formed entirely from the Ca-Sr 90 of the "spiked" diet. This find-

ing was confirmed by Alexander, Nusbaum, and MacDonald(2) who estimated stable strontium-calcium ratios in the bones and diets of experimental animals.

The present paper is concerned with (a) development of shorter methods for comparison of calcium and strontium metabolism by the use of double tracer techniques; (b) demonstration of the effect of normal types of diets on the relative metabolism of calcium and strontium; and (c) estimation of the extent to which the individual processes in the body differentiate between strontium and calcium.

Methods. Previous work(1) had shown that Ca 45 incorporated into the diet was utilized to the same extent as the dietary calcium. Therefore, it was considered that the behavior of Ca 45 and Sr 89 administered in the diet for a matter of weeks would give a true picture of the behavior of dietary calcium and incorporated radiostrontium. This would avoid the long feeding period necessary to obtain animals whose bones had been entirely formed from the "spiked" diet. Three groups of rats were raised on diets as follows: *Milk*—evaporated milk was supplemented to contain 8.5 mg $MnCl_2 \cdot 4H_2O$, 4.2 mg $CuSO_4$, 27 mg $FeSO_4 \cdot 7H_2O$, 14 mg KI and 4 g yeast extract (Difco) per liter of reconstituted milk; *Milk and Corn*—in addition to the milk supplemented as above, each animal received 1 g of ground yellow corn per day; *Commercial*—Rockland rat pellets. The animals on the "Milk" and "Commercial" diets were on experiment between 30 and 50 days of age, whereas the other group was studied between 21 and 81 days of age (Table I). The animals on the "Commercial" diet received the Ca 45 and Sr 89 (in constant ratio) in their drinking water; the other groups received the tracers in the milk. The radioactivity dosage amounted to about 0.4 μc of Ca 45 and 0.2 μc of Sr 89 per animal per day; relatively high specific activity preparations were used. During the latter part of the experiment, the animals were placed in metabolism cages for quantitative collections of feces and urine. At termination, representative animals were killed to provide samples of blood and bone (entire femurs). The 2 radioisotopes were measured by the usual methods for radioassay of mixtures based on differential absorption (3,4). Twelve to 15 rats had been started on each diet. However, the analytical results were in sufficient agreement that only 3 to 4 animals were used and the remainder were carried for another study.

Expression. In the original paper on this subject(1), the results were discussed in terms of preferential utilization of calcium over strontium as already mentioned. Alexander *et al.*(2) then proposed the term "bone retention factor", which is essentially the reciprocal of the preferential calcium utilization. To denote the individual tissues, excretions, or physiological processes involved, it is necessary to have broader terminology. We are therefore proposing the term "Strontium-Calcium Observed Ratio" (OR) to denote the actual differentiation in tissues or excreta and defined as follows:

$$OR_{\text{sample-precursor}} = \frac{\text{Sr/Ca of sample}}{\text{Sr/Ca of precursor}} \quad (1)$$

This observed ratio (OR) does not imply that the tissue in question has exerted any action in causing the discrimination.

To denote the discrimination that is produced by a given physiological process, we are proposing the term "Strontium-Calcium Discrimination Factor" (DF). By definition:

$$OR = (DF_1)\,(DF_2)\,(DF_3)\ldots(DF_n) \quad (2)$$

Thus, for example, the symbol DF_{urinary} is used to designate the contribution to the overall discrimination in the body that resulted from differential handling of the two elements by the kidney.

It is noted that OR and DF as defined here are the conceptual inverse of the preferential calcium utilization, and describe strontium behavior in terms of calcium. They are thus similar to the retention factor; when OR or DF is less than 1, this means that the strontium is discriminated against in favor of calcium.

Data and Calculations: Table I presents data for (a) OR values as measured for bone, blood, feces, and urine in double tracer experiments under conditions of daily intake; (b)

TABLE I. Strontium-Calcium Observed Ratios and Percentage Excretion in the Rat. (Values represent mean ± stand. error of mean.)

Diet	Milk	Milk and corn	Commercial
No. of animals	4	3	4
Age at start and end of study, days	30–50	21–81	30–50
$OR_{bone\text{-}diet}$*	.57± .02	.45± .01	.27± .01
$OR_{retained\text{-}diet}$†	.57	.56	.30
$OR_{blood\text{-}diet}$	.46± .04	.43± .08	.21± .04
$OR_{urine\text{-}}$ „	1.99± .01	1.47± .03	1.1 ± .03
$OR_{feces\text{-}}$ „	1.41± .08	1.33± .05	1.27± .03
% excretion‡			
Ca 45 in urine	4.5 ±1.1	5.7 ± .6	1.8 ± .3
Sr 89 " "	8.7 ±1.9	8.4 ± .6	1.9 ± .2
Ca 45 in feces	44 ±2	50 ±3	71 ±6
Sr 89 " "	62 ±4	67 ±2	90 ±8

* $OR_{bone\text{-}diet} = \dfrac{\text{Sr*/Ca* in bone}}{\text{Sr*/Ca* in diet}}$.

† Calculated by difference from excretion values.

‡ $\dfrac{\text{Excretion during last 4 days of exp.}}{\text{Intake during last 4 days of exp.}} \times 100$.

OR for the entire body as calculated by difference from excretion values; and (c) percentage excretion of Ca 45 and Sr 89 under conditions of daily intake.

As will be discussed later, there appear to be only 2 major discriminatory processes in the rat: absorptive and urinary. Under these conditions, and with the assumption that the urinary excretion of each element is proportional to the amount absorbed, the following equations are easily derived for calculation of the Strontium-Calcium Discrimination Factors:

$$DF_{absorptive} = \frac{100 - \%\ \text{Sr 89 in feces}}{100 - \%\ \text{Ca 45 in feces}} \qquad (3)$$

$$DF_{urinary} = \frac{OR_{retained\text{-}diet}}{DF_{absorptive}} \qquad (4)$$

Table II presents the DF values as calculated in this way as well as the comparison of blood and urine values to indicate the relative renal clearances of calcium and strontium. It will be recognized that the DF values as estimated in this way represent the over-all contribution to the discrimination, and do not take into account such matters as the change in Sr/Ca ratio as the food passes through the gastro-intestinal tract.

Discussion. It is first noted that the $OR_{bone\text{-}diet}$ value of 0.27 reported for the commercial diet in Table I is in excellent agreement with previous values of 0.28(1) and 0.27(2) determined by other methods. Thus, any of the following methods should be valid for prediction of naturally occurring Sr/Ca ratios or Sr*/Ca ratios when the intake is known: (a) analysis and radioassay of bone formed entirely from feed with constant ratio of added Sr* to naturally occurring calcium (1); (b) analysis of natural strontium-calcium ratios in a given diet and bones formed from this diet(2); and (c) radioassay of tissues or excretions from animals on a daily intake of Sr* and Ca* for a matter of weeks. The last method will usually be the most convenient from the experimental standpoint.

The $OR_{retained\text{-}diet}$ values as calculated from the balance results are in fair agreement with the $OR_{bone\text{-}diet}$ values. Differences are probably due to inaccuracies in collection of excreta and analysis or less likely to the bone not having a Sr*/Ca* ratio representative of the entire body. The higher $OR_{bone\text{-}diet}$ values of 0.59 and 0.42 for the animals on the milk-containing diets were anticipated and agree with the findings of Wasserman *et al.*(3), who showed that substances such as lactose and l-lysine that increased calcium absorption as does milk, were even more effective in promoting Sr 89 absorption than Ca 45 absorption. This emphasizes that the nature of the diet is important in governing not only the extent of alkaline-earth absorption, but also the discrimination that occurs.

Processes with minor discrimination: It is convenient first to consider those physiological processes by which little or no differentiation occurs. Any discrimination in movement of calcium and strontium from blood to skeleton

TABLE II. Strontium-Calcium Discrimination Factors in the Rat.

Diet	Milk	Milk and corn	Commercial
$\dfrac{OR_{urine\text{-}diet}}{OR_{blood\text{-}diet}}$	4.3	3.4	5.2
$DF_{urinary}$	.84	.84	.88
$DF_{absorptive}$	.68	.66	.34
$OR_{retained} = (DF_{urinary})(DF_{absorptive})$	.57	.56	.30

can be estimated by comparison of $OR_{blood\text{-}diet}$ and $OR_{bone\text{-}diet}$ values. Table I shows that the strontium rather than being discriminated against was, if anything, preferentially utilized by bone ($0.02 < p < 0.05$). This may be of interest in connection with early observations on strontium retention that led to strontium therapy for osteoporosis(5). For purposes of calculation of over-all discrimination, however, it can be assumed that movement from blood to skeleton does not contribute significantly. This conclusion was also reached by Bauer *et al.*(6). The relatively large uncertainties in the $OR_{blood\text{-}diet}$ values resulted from the low levels of radioactivity present in the blood in these studies; these low levels led to counting difficulties.

It also appears that excretion of calcium and strontium from the blood into the gastrointestinal tract via the bile, pancreatic juices, and secretions of intestinal mucosa would not contribute in a major way to the over-all discrimination. Greenberg and Troescher(7) found little difference in the biliary excretion and total fecal excretion following intraperitoneal injection of Ca 45 and Sr 89 into rats. From short term experiments with dogs, Singer *et al.** found about twice as much Sr* was secreted in the bile as Ca*; the total excretion of Sr* into the gastrointestinal tract, however, exceeded that of Ca* by only about 20%. Also, the percentage of parenterally administered Sr* and Ca* that reaches the intestinal tract is relatively small, 7 to 20%. In the present paper, no attempt is made to distinguish between endogenous and unabsorbed Sr* and Ca* in the fecal excretion values.

Processes with major discrimination: The renal clearance of strontium was 3 to 5 times that of calcium as indicated by the ratios of $OR_{urine\text{-}diet}$ and $OR_{blood\text{-}diet}$ values in Table II. This general behavior has also been observed in man, dog, and bovine(8,9,10). It is also apparent, however, from Table II that the differential absorption contributed more to the over-all discrimination than did the differential urinary excretion. This was more pronounced in the rats on the commercial diet ($DF_{absorptive} = 0.34$: $DF_{urinary} = 0.86$). The contribution of the urinary discrimination was essentially the same in all 3 groups. The $DF_{absorptive}$, however, was about 0.67 for the milk-containing diet. These differences are of the same order as reported for the effects of lactose on relative strontium and calcium absorption.

As an independent confirmation of the ($DF_{urinary}$) value, an experiment was done in which rats on a commercial diet were injected intraperitoneally with Ca 45 and Sr 89 and the excretions studied. Under these conditions $OR_{retained} = 0.85$, $DF_{urinary} = 0.89$ and $DF_{fecal\ excretion} = 0.96$. The discrimination factor due to fecal excretion resulted both from differential endogenous excretion and differential reabsorption from the gastrointestinal tract. As expected the contribution from both of these processes was small. It is of interest to note the agreement between the $DF_{urinary}$ value as obtained in this experiment, and that in Table II from the continuous feeding study.

Comparison of discrimination among species is somewhat difficult because of the variables involved, such as age and dietary intake. Harrison *et al.*(8), however, reported that adult man receiving a somewhat higher stable strontium intake than normal, absorbed calcium about twice as much as strontium. For cattle, it appears that absorptive discrimination is small(10). Values of $OR_{bone\text{-}diet}$ for laboratory animals on commercial diets were reported as follows: mice, 0.35; rats, 0.27; and guinea pigs, 0.22. The ratios for Nevada desert animals were jack-rabbits, 0.20; cottontail rabbits, 0.22; and kangaroo rats, 0.16(2).

The principles as presented here can also be used in studies where lactation or deposition in the fetus provide additional discriminatory processes.

Summary. The term "Strontium-Calcium Observed Ratio" (OR) is proposed and defined to designate the over-all discrimination that occurs in movement of the 2 elements from one phase to another in a biological system. The term "Strontium-Calcium Discrimination Factor" (DF) is proposed and defined

* Singer, L., Maqsood, M., Medlen, A. B., and Comar, C. L., unpublished results.

to characterize the contribution of individual processes to the over-all discrimination. Double tracer studies of Ca* and Sr* ingestion over a 20-day period gave the same OR values as methods based on lifetime feeding. Absorption from the tract and urinary excretion were the major processes by means of which the rat discriminated against dietary strontium in favor of calcium. There is evidence of slight strontium preference in movement of the two elements from blood to bone. Excretion into the gastrointestinal tract did not contribute significantly to the over-all discrimination. Rats on a nonmilk diet ($OR_{bone\text{-}diet} = 0.27$) discriminated more against strontium than did those on a milk diet ($OR_{bone\text{-}diet} = 0.57$). This behavior was related to the absorptive process.

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Effect of Digoxin on Cardiac Glycogen of the Rat.* (22637)

W. O. READ AND F. E. KELSEY.

Department of Physiology and Pharmacology, University of South Dakota School of Medicine, Vermillion.

Non-toxic doses of cardiac glycosides have been reported to increase cardiac glycogen (1,2,3). On the other hand, the data of Bomskov and Kaulla(4) and Liebig(5) indicate non-toxic doses decreased the glycogen level of the heart, while the results of Kimura (6) showed digotoxin to be without effect. Toxic doses of the cardiac glycosides have been reported to cause increased glycogen in the heart of the pigeon(7) and in the rat doses of 3.4 mg of digitoxin per kilo body weight resulted in an increase of the cardiac glycogen(4). Nevertheless, depletion of cardiac glycogen in animals manifesting signs of cardiac glycoside poisoning has been reported by Cherkes(2) and Haendel and Munilla(8).

The recent report of Bloom, Lewis, Schumpert, and Shen(9) that the glycogen of tissues can be divided into acid soluble and acid insoluble fractions which maintain a reasonably constant ratio to each other and the report of Russell and Bloom(10) that most physiological changes in the tissue glycogen take place in the acid soluble (labile) fraction while the acid insoluble (protein bound) remains relatively constant suggested that some of the equivocal results of the digitalis studies could be resolved by a consideration of the effects of cardiac glycosides on each of the two glycogen fractions.

Methods. Female rats, of various ages, were injected subcutaneously with 0.2 mg/kg of digoxin suspended in distilled water. With the exception of the newborn animals, the rats were injected daily for 5 days and sacrificed 5 hours after the last injection on the 5th day. The newborn animals received one

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