TABLE II. Time Course for Correction of Steatorrhea in 6 Bile Fistula Dogs.

Fecal excretion, g total fat/day										
Control diet (10 g fat)				Plus 25 g triolein			Plus 10 g oleic acid			
	Bile return period*				Bile return period*			Bile return period*		
Bile fistula	1	2	3	Bile fistula	1	2	Bile fistula	1	2	3
11.5	1.0									
12.6	6.9	1.6	.8							
10.2	1.4			29.3	10.0	.7				
8.6	9.1	.9		25.0	5.0	1.0	15.0	3.0	1.3	1.8
				15.8	1.9	.8	13.0	4.1	1.0	.7
							21.7	11.6	1.4	

* Bile return period 1: 2- 7 days after reconnecting cannulas.
Idem 2: 7-12 *Idem*
" 3: 12-17 "

turned by stomach tube or other discontinuous technics. This system does not yet appear to be useful for more than 3 months. In many of the operated animals the gall bladder wall around the cannula eroded after this period, followed by leakage of bile and peritonitis. However, the animals exhibited surprising resistance to liver infections. While slight cholangitis was evident, histological sections revealed significant lesions of the liver parenchyma only in 3 dogs. In these 3 animals some evidence of hepatitis was present.

Summary. Eleven bile fistula dogs, with common bile duct sectioned, were permitted to recirculate their bile by periodically joining together polyvinyl cannulas fixed in the duodenum and gall badder and brought outside through stab incisions. In 6 of these dogs, fed varying amounts of triolein or oleic acid, steatorrhea was corrected completely within 12 days after rejoining the cannulas. In 5 other dogs fed similar diets, but not subjected to prior bile deprivation, fecal fat excretion was within normal limits for period up to 30 days. These results show that the steatorrhea of bile deprivation can be corrected by continuous bile return.

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Effect of Dietary Calcium and Phosphorus Levels on Body Burdens of Ingested Radiostrontium.* (25434)

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Entry of radioactive strontium into the biosphere is one of the important consequences of fallout from nuclear explosions and much study has been given to interrelationships in the progression of fallout strontium and natural calcium through the food chain to human population. Details of comparative behavior of strontium and calcium in various species (under normal conditions and also during pregnancy and lactation) have been worked out and many of the findings con-

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TABLE I. Differences in Experimental Conditions of Two Reports on Influence of Dietary Calcium on Retention of Skeletal Sr* in the Rat.

Factors	Wasserman *et al.*(10)	Palmer *et al.*(11)
Sex of rat	♂	♀
Age of rat	Growing, young	Non-growing, mature
Ca supplement	$CaCO_3$	Ca lactate
P level	Ca/P maintained constant	P maintained constant
Sources of isotopes	Drinking water	Diet

firmed by independent methods(1-3). It is important to understand the extent to which dietary modifications can alter the levels of radiostrontium to be attained and to know how one can best predict future radiostrontium levels in various parts of the biosphere. Attention has recently been given to the effect of dietary calcium levels on retention of radiostrontium. In early studies the calcium carrier and radiostrontium usually were administered in a single dose and skeletal retention of radiostrontium measured shortly thereafter; little or no effect of calcium was seen under these conditions(4-7). Subsequently, double tracer technics with radiocalcium and radiostrontium were developed in comparison with lifetime feeding methods for assessment of calcium-strontium relationships(8,9). By use of these technics and also longer term studies, Wasserman *et al.*(10) showed that increased dietary level of calcium would indeed reduce proportionally the skeletal retention of radiostrontium in the growing rat; they also explained why the short-term retention of radiostrontium could not be used to predict steady-state body burdens. Palmer *et al.*(11) then published data to show that under certain experimental conditions an increased dietary level of calcium did not result in proportional decrease in retained radiostrontium in the mature rat. Table I summarizes the differences in experimental conditions used by the 2 groups of workers. The work reported here is an attempt to reconcile these apparently opposed findings, to explore further as to some of the factors that may be involved: comment is also offered on usefulness and limitations in consideration of strontium behavior in terms of calcium.

Methods. Details of procedure have been previously reported(10). Rats were initially placed on experimental diets for about 25 days; at this time the animals, while maintained on their respective diets, were transferred to individual cages and, for an additional 7 days, were given drinking water labeled with about 20 μc Ca^{45} and 10 μc Sr^{85}/liter as chlorides; these isotopes were essentially carrier-free. Water consumption and ingested radioactivity were estimated through use of graduated drinking tubes. In addition, body weight changes and feed consumption were determined during final 7 days. The rats were then killed and amount of Ca^{45} and Sr^{85} retained in both femurs determined by usual radiochemical procedures; the results are expressed as "percentage of ingested dose." The basal diet was as follows: ground yellow corn, 20%; dextrin, 29.2%; casein, 17.7%; brewer's yeast, 4.4%; Wesson oil, 2.9%; calcium-free salt mixture, 1.6%; Vit. mixture containing adequate levels of thiamine, riboflavin, pyridoxine, niacin, calcium pantothenate, choline, inositol, biotin, folic acid, Vit. B_{12}, p-aminobenzoic acid and Vit. A, D and E, 1%. The rest of the diet, 23.2%, was made up of the calcium salt (usually calcium lactate), K_2HPO_4 and cellulose fiber; the elevated levels of calcium and phosphorus salts replaced an equivalent weight of cellulose bulk.

Results. Table II is a summary of a study designed to compare all experimental differences except calcium/phosphate ratio and the source of tracers. Values for Observed Ratio (OR) compare reasonably well with previous data of Wasserman *et al.*(10) and indicate that major differences between the 2 groups of workers could not have been caused by sex, age, or type of supplement. In this study there appeared to be a small increase in OR value as a result of raising calcium and phosphorus level of the diet; this increase, however, was not large enough to have practical significance in relation to the effects of dietary calcium. In agreement with previous findings

TABLE II. Influence of Age and Sex on the Effect of Dietary Calcium and Phosphorus on the Femur Retention of Ca^{45} and Sr^{85} in the Rat.*

Group	Age	Sex	Dietary levels Ca (%)	Dietary levels P (%)	Feed consumption (g/day)	Calcium intake (mg/day)	Final body wt (g)	Level in femur Ca^{45} (% ingested dose)	Level in femur Sr^{85} (% ingested dose)	OR†
I	young	♂	.5	.4	18.2	91	223	4.47 ±.12	1.00 ±.04	.22 ±.01
II	"	"	2.0	1.2	15.7	314	201	1.51 ±.03	.42 ±.01	.28 ±.03
III	"	♀	.5	.4	14.1	71	163	3.89 ±.14	.87 ±.06	.22 ±.01
IV	"	"	2.0	1.2	11.0	220	131	1.44 ±.06	.39 ±.03	.27 ±.01
V	mature	♂	.5	.4	21.5	108	434	.97 ±.07	.14 ±.01	.15 ±.01
VI	"	"	2.0	1.2	21.0	420	415	.41 ±.03	.09 ±.01	.21 ±.01
VII	"	♀	.5	.4	17.3	87	306	.86 ±.09	.13 ±.02	.16 ±.01
VIII	"	"	2.0	1.2	16.8	336	293	.34 ±.03	.07 ±.01	.19 ±.01

* Six rats/group; values represent mean ± stand. error of mean; Ca added as lactate salt and P as K_2HPO_4; animals on diets for 18 days prior to isotopes and 7 days on isotopes.

† $OR = \frac{Sr^*/Ca^* \text{ of bone}}{Sr^*/Ca^* \text{ of diet}}$.

(1), the younger animals showed somewhat less discrimination against Sr* relative to Ca* than did older animals (OR for young was about 0.22 compared with 0.15 for the mature).

Table III presents data from a study in which experimental conditions of Palmer *et al.* (11) were essentially duplicated, and the interaction of calcium and phosphorus further examined. In the mature rat, when dietary level of calcium alone was raised, OR value increased about 3-fold (from 0.13 to 0.38). This agrees with the results of Palmer *et al.* (11). It can also be noted that raising phosphorus alone (Group III *vs.* Group I) had no effect on relative retention. When both calcium and phosphorus were raised, OR value increased only from 0.13 to 0.20 (Group I *vs.* Group IV). These results also indicate that the technic of incorporation of tracers in drinking water gives the same results as incorporation in the diet.

Another study investigated calcium and phosphorus relationships in the growing rat, and, in addition, determined the effects of increased stable strontium on Ca^{45} and Sr^{85} retention (Table IV). It may first be noted that when dietary calcium alone was increased there was only a small increase in OR value (Group II *vs.* Group I) [as expected from earlier work(10) and Table II]; this is in contrast to behavior just described for mature animals. The effect of stable strontium is of particular interest. Rats in Group IV (0.5% Ca, 0.5% Sr, 0.4% P) showed a rachitic condition after 27 days on the diet, as indicated by (a) gross appearance of the femur, (b) decreased femur ash and (c) reduced deposition of both Ca^{45} and Sr^{85}. Simultaneous raising of phosphorus and strontium levels (Group V) overcame to a certain degree the rachitic effects of the strontium. It was of interest to observe that OR values for high strontium with and without additional phosphorus were about the same as for high calcium animals (Groups IV, V *vs.* Groups II, III). This means that stable strontium influenced radiocalcium behavior in much the same way that calcium itself did. Supplemental dietary strontium is thus less useful than calcium for

TABLE III. Interaction of Dietary Calcium and Phosphorus on Femur Retention of Ca^{45} and Sr^{85} in Mature Rat.*

Group	Dietary levels Ca (%)	P (%)	Feed consumption (g/day)	Calcium intake (mg/day)	Final body wt (g)	Level in femur Ca^{45} (% ingested dose)	Sr^{85} (% ingested dose)	OR†
I	.5	.4	20.4	102	256	1.03 ±.10	.13 ±.02	.13 ±.01
II	2.0	.4	19.8	396	262	.32 ±.04	.12 ±.01	.38 ±.04
III	.5	1.2	20.2	101	253	1.21 ±.08	.13 ±.02	.11 ±.01
IV	2.0	1.2	21.8	436	259	.34 ±.01	.07 ±.01	.20 ±.01

* Six rats/group; values represent mean ± stand. error of mean; Ca added as lactate and P as K_2HPO_4; animals on diets for 20 days prior to isotopes administration; 7 days on isotope feeding.

† $OR = \frac{Sr^*/Ca^* \text{ of bone}}{Sr^*/Ca^* \text{ of diet}}$.

reduction of Sr* burdens, because strontium at levels that would produce an appreciable effect also causes toxic side-effects; in addition, the discrimination by the intestinal barrier against strontium in favor of calcium would immediately reduce the parenteral concentration of strontium in contrast to the concentration of absorbed calcium.

Discussion. To predict the effect of dietary calcium or other dietary additives on the body burden of continuously ingested radiostrontium, it is most often necessary to compare retention of radiocalcium with that of radiostrontium. This is especially important when the experiment is done over a relatively short portion of the life span of the animal. Difficulties of interpretation mainly arise as a result of the adaptation of the animal to dietary changes. Although an increased ingestion of calcium may result in an immediate increase in utilization of calcium, the animal eventually will adapt such that the net retention of calcium is essentially independent of dietary calcium, within reasonable limits. By comparing Strontium-Calcium Observed Ratio (OR)† as defined by Comar *et al.*(9), the long term effect of dietary calcium can be predicted as follows: If an elevated dietary calcium does not alter the $OR_{diet\text{-}bone}$, it would then be apparent that, with time, the body burden of Sr* would be decreased inversely to increased calcium. Thus, the Observed Ratio (OR) can be of primary importance in prediction of long-term behavior.

A general comment is in order about variability that might be expected in the differential behavior of calcium and strontium in the mammal. The earliest observation had shown that strontium, in general, had a metabolic behavior similar to that of calcium. Substances that modified calcium behavior, also modified strontium behavior in a similar way (Vit. D, parathormone, lactose). Subsequent quantitative studies showed that there were differences

TABLE IV. Interaction of Dietary Calcium, Strontium and Phosphorus Levels on the Femur Retention of Ca^{45} and Sr^{85} in the Growing Rat.*

Group	Dietary levels Ca (%)	Sr (%)	P (%)	Feed consumption (g/day)	Alkaline earth intake (mg/day)	Final body wt (g)	Femur ash wt (mg)	Level in femur Ca^{45} (% of ingested dose)	Sr^{85} (% of ingested dose)	OR†
I	.5		.4	14.7	73	143	122	2.47 ±.16	.64 ±.06	.26 ±.01
II	1.0		.4	15.1	151	151	121	1.50 ±.06	.48 ±.02	.32 ±.02
III	1.0		.8	12.2	122	145	112	1.72 ±.07	.41 ±.04	.23 ±.01
IV	.5	.5	.4	14.5	145	103	47	.70 ±.05	.22 ±.02	.32 ±.01
V	.5	.5	.8	10.9	109	120	79	1.96 ±.10	.52 ±.04	.27 ±.01

* Six rats/group; values represent mean ± stand. error of the mean; calcium and strontium additions given as acetate salts, phosphorus as K_2HPO_4. Rats on diets for 14 days prior to isotopes and 7 days thereafter.

† $OR = \frac{Sr^*/Ca^* \text{ of bone}}{Sr^*/Ca^* \text{ of diet}}$.

† See Table II.

in behavior possibly related to the type of ion transport, *i.e.*, absorption from the gut, reabsorption in the tubules, transfer across the placenta, and secretion into milk(1).

Examination of data obtained by independent methods in many laboratories and many countries indicated that under usual dietary conditions OR values fall within a reasonably narrow range, with few indications of variation as high as a factor of two(2). It was also shown in early work that certain dietary modifications in the rat such as use of milk, lactose or lysine would decrease the discrimination against strontium relative to calcium by as much as a factor of two(1). In the very young rat, discrimination was also decreased (1). The present work shows that increased calcium with a high Ca/P ratio in the mature rat will also decrease discrimination, *i.e.*, cause an increased OR value.

With the realization that OR values can be varied by unusual conditions in individuals, it seems that the concept can be of value when properly used for the following purposes: (a) estimation of levels of radiostrontium in local areas of bone since the Sr*/Ca of newly forming bone is related to Sr*/Ca of the contemporary diet, (b) estimation of body burdens and local concentrations to be attained in populations from knowledge or estimates of Sr*/Ca ratios to be attained in food sources, (c) estimation of effects of life-time dietary changes from short-term experiments.

It is emphasized that the concepts of Sr-Ca action should be used where they can provide advantages; likewise, data must also be obtained and reported concurrently on absolute levels of calcium and radiostrontium in the biosphere so that these data can also be used as necessary. Such data are needed when it is required to sum the various constituents of a diet to give the Sr-Ca ratio in the total diet.

The reported differences in the effect of dietary levels on radiostrontium behavior(10, 11), can be explained as follows: In young, growing rats increase of dietary calcium, with or without proportionate increases of dietary phosphorus, caused little or no change in relative retention of calcium and strontium. In the mature rat, an increase of dietary calcium alone caused about a 3-fold decreased retention of radiocalcium as compared to radiostrontium retention; when dietary phosphorus was also increased the effects on Ca* and Sr* were more nearly similar. The reasons for this behavior must be related to the mechanisms of handling the 2 elements which probably differ with age of the animal. These aspects are now under active study.

It must be pointed out that these observations should not be used as a basis for recommendation of changes in human or animal dietaries. Consideration would have to be given to such matters as the extent to which the dietary modification proposed would actually change the body burden of radiostrontium under the given conditions. Also, it would have to be determined whether or not the long term feeding of increased calcium would have deleterious effects on a population or individual basis that would outweigh the benefit to be gained by any reduction in the radiostrontium burden.

Summary. It is emphasized that long term effects of dietary constituents on radiostrontium must be determined either by long term experiments or predicted from double tracer technics. Previous reports in disagreement as to the effect of increased dietary calcium on radiostrontium have been reconciled with predictions as follows: (a) in immature rats, elevated dietary calcium levels (within physiological ranges) with or without increased phosphorus levels would almost proportionately reduce the body burden of dietary radiostrontium, (b) in mature rats, elevated dietary calcium levels alone would not proportionately reduce the radiostrontium and (c) in mature rats, simultaneous increases in dietary calcium and phosphorus levels would to some degree reduce the ultimate body burden of radiostrontium.

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Erythropoietic Factor in Kidney Tissue of Anemic Dogs.* (25435)

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In previous communications it was shown that bilateral nephrectomy abolished erythropoiesis in the dog(1) and that ureter ligation did not impair this function despite the same degree of uremia and malnutrition(2). In other studies an erythropoietic factor was demonstrated in plasma and urines of bled dogs(3) and disappearance of this factor was shown to occur after bilateral nephrectomy (4). These results agree with experiments by Jacobson *et al.*(5) on the rat and suggest that the kidney is the source of an erythropoietic stimulating factor. Several attempts to point out the erythropoietic factor in acidified boiled filtrates from kidney and other organs(6,7,8) have been unsuccessful. This study was an attempt to demonstrate erythropoietic stimulating activity in kidney homogenates.

Materials and methods. Fifteen mongrel dogs weighing between 13 and 25 kg were used. Three were acutely bled to a hematocrit of 14—9% in 6 hours at which time they were bled to death by femoral puncture. Twelve were made anemic (hematocrit 19—5%) by bleeding for 3 to 5 days. In this group 6 dogs were unilaterally nephrectomized 10 to 20 days before bleedings; the removed normal kidneys were used as normal controls. Blood volume was maintained by Dextran infusion as described elsewhere(3). Some dogs died from anemia and the kidneys were taken immediately after death; in other experiments the kidneys were removed under nembutal anesthesia before the animal's death. In 8 experiments the liver was also removed and in 5 the spleen. Removed organs were kept frozen until used. Kidney, liver and spleen tissue were ground with saline in a Waring Blendor for 3 to 5 minutes; saline volume added 150% of the weight of each organ; the tissue suspension was filtered through gauze and injected subcutaneously into rats without further treatment. Erythropoietic activity of organ suspensions was measured by Fe^{59} red cell incorporation assay on starved rats(9), as described in previous paper(3). Recipient rats received 3 ml of organ suspension subcutaneously daily for 2 successive days before Fe^{59} injection. Four to 10 rats were used for each determination. Controls consisted of uninjected rats and groups receiving normal kidney suspension, "anemic" liver suspension and "anemic" spleen suspension. After organ emulsion injections all rats developed abscesses at the site of injection and of the 253 rats used, 26 died from the toxicity of the material injected; no difference in toxicity was noticed for the extracts from different tissues. Hematocrits were measured in Wintrobe tubes by centrifugation at 3,000 rpm for 30 minutes. Hemoglobin was deter-

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