siderably higher than to histamine. The relation between maximal response to gastrin and to histamine thus varies both with species and type of gastric preparation studied. The large discrepancies between maximal response to gastrin and to histamine in some of these test objects are difficult to reconcile with the hypothesis that histamine is the final common mediator for stimulation of gastric secretion(6).

Both basal and histamine-stimulated secretion were higher in the present study than in that reported by Lane *et al.*(2), probably because of the higher body weights of our rats. The mean basal rate was 140 μeq per hour in the present study and 58 μeq per hour in the study of Lane *et al.* Similarly, the output of acid during the first hour after subcutaneous injection of 2 mg histamine phosphate was 221 μeq per hour in the present study and 187 μeq per hour in the study of Lane *et al.* The higher secretory rate in the present study could be accounted for almost entirely by higher volume rates. The concentration of acid was not significantly different from that reported by Lane *et al.*

Summary. Gastrin prepared from the stomach of hogs stimulated secretion of acid when injected subcutaneously into rats with gastric fistulas. The highest rate of secretion attained with gastrin was more than double the maximal rate in response to histamine.

1. Gillespie, I. E., Grossman, M. I., *Gastroenterology*, 1963, v44, 301.
2. Lane, A., Ivy, A. C., Ivy, E. K., *Am. J. Physiol.*, 1957, v190, 221.
3. Bollman, J. L., PROC. SOC. EXP. BIOL. AND MED., 1948, v33, 1348.
4. Gregory, R. A., Tracy, H. J., *J. Physiol.*, 1961, v156, 523.
5. Uvnas, B., *Acta Physiol. Scand.*, 1943, v6, 97.
6. Code, C. F., Histamine and gastric secretion. In Ciba Foundation *Symposium on Histamine*, edited by C. E. Wolstenholme and C. M. O'Connor, P. 189. J. & A. Churchill, Ltd., London, 1956.

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Retention of Strontium, Calcium, and Phosphorus in Human Infants. (28124)

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Radioactive strontium produced by tests of nuclear weapons reaches the human population primarily by contamination of the diet. The level of long-lived strontium 90 to be attained in the population depends mainly on 2 factors: the strontium 90 to calcium ratio of the total diet, and the comparative retention of the two substances in the body.

The differential behavior of strontium and calcium in biological systems has been expressed quantitatively by Comar *et al.*(1) as the Observed Ratio (OR):

$$OR_{sample\,/\,precursor} = \frac{Sr/Ca \text{ of sample}}{Sr/Ca \text{ of precursor}}.$$

$OR_{body\,/\,diet}$ values for adults of most mammalian species including man have been determined by a variety of experimental methods to range from 0.2 to 0.5 with most values falling near 0.25(2). For example, extensive analyses of human bones and diets for stable strontium and calcium have given an average population value of $OR_{body\,/\,diet} = 0.25$ in the United Kingdom(3); Spencer *et al.*(4) in studies with 16 adult cancer patients using radiostrontium and radiocalcium as double tracers found a mean $OR_{body\,/\,diet}$ of 0.29. Thus, there is consistently in adults about a 4-fold discrimination against strontium in favor of calcium in the passage of these 2 elements from the diet to bone.

Early studies of factors that might affect the differential behavior of strontium and calcium indicated that substances which increased gastrointestinal absorption of alkaline earths tended to decrease the discrimination against strontium, that is, to raise the OR value(5). It was therefore anticipated that discrimination might be low in very young individuals, who have a high absorptive efficiency. This was confirmed by Comar *et al.* (6) in experiments with very young rats. Bryant and Loutit(7) also concluded from survey studies that the $OR_{body/diet}$ in 1-year-old children was about 0.5 in contrast to an OR value of 0.25 for the adult. A few scattered observations(2) have suggested that the discrimination approaches the adult value by the time the child reaches a few years of age. There are, however, no data for very young infants.

Thus, in order to permit more accurate assessment and prediction of bone levels and radiation dosages it seemed important to obtain direct information on retention of dietary strontium 90 (Sr^{90}) in very young healthy infants on a normal diet. In the present study, estimates were made of retention during a 15-day period of dietary Sr^{90}, stable strontium (Sr), calcium and phosphorus in 14 infants of about 34 days of age.

Materials and methods. General. Data were obtained on 14 healthy children (8 females and 6 males) of ages from 25 to 46 days (average, 34 days) at the beginning of the 15-day observational period. From birth to start of the observational period, the children were fed only a formula of tap water and a commercial cow's milk preparation* hereafter designated as powder. During the observational period all children received formula prepared from distilled water and a single production batch of powder. The first experimental feeding was started on Dec. 28, 1960 and the last was completed on March 9, 1961. Total intake and excretion of strontium 90, stable strontium, calcium and phosphorus were measured over a 15-day period for each child.

The amount of powder ingested was determined by measurement of the weight of formula of known powder content consumed at each feeding. Correction was made for formula lost by spillage or regurgitation by collection on previously weighed sterile towels. Intake of the strontium 90, stable strontium, calcium and phosphorus was determined from the amount of powder ingested and the percentage of each element found on analysis of an aliquot of the powder.

Excretion values were determined by difference from analyses of diapers plus excreta and diapers alone. Total urine and feces were collected on paper diapers over which waterproof pants were worn. At diaper changes, the children were cleaned carefully with moist, clean paper diapers which were added to the soiled diapers as part of the collection; also the pants were washed carefully with distilled water and wiped dry with clean portions of the diapers. Diapers plus excreta for each child were pooled for the 15-day period. The diapers in the collection were counted prior to ashing so that the appropriate correction could be made for their contribution to amounts of the 4 components of interest found in the collected samples of excreta plus diapers. The content per used diaper of strontium 90, stable strontium, calcium and phosphorus was determined by analyzing an aliquot of the ash obtained from 576 diapers randomly selected from the one production batch used in the experiment.

Treatment of samples and analytical procedures. The diapers plus excreta were dried and then ignited in stainless steel trays in a muffle furnace at 500°C until a white ash was obtained. The ash was fused with sodium carbonate. The powdered milk preparation was ashed in a similar manner. The HCl solution of the fused ash from both the powdered milk and the diapers with excreta were analyzed for strontium 90 and calcium by methods described by Harley and Whitney (8). Phosphorus was determined on the HNO_3 solution of the ash by precipitation with ammonium phosphomolybdate in an adaptation of an ASTM method(9). Stable strontium was estimated on the ash by neutron activation.

Results. Weight gains over the 15-day observational period were normal, ranging from 269 to 627 g with a mean of 450 g per infant. Average intake per infant over the 15-day observational period was 1.6 kg of the commer-

* Similac, purchased from Ross Laboratories, Columbus, Ohio.

TABLE I. Composition of Commercial Cow's Milk Preparation.

	Dry wt basis	Ash basis	Ratios
Ash (%)	4.09	—	—
Sr^{90} ($\mu\mu c$/g)	.043	1.05	—
Sr (%)	.00029	.0071	—
Ca (%)	.61	14.9	—
P (%)	.38	9.4	—
Sr^{90}/Ca ($\mu\mu c$/g)	—	—	7
Sr^{90}/Sr ($\mu\mu c$/mg)	—	—	14.8
Sr/Ca (mg/g)	—	—	.47
Ca/P	—	—	1.58

cial preparation (powder) and 11.2 l of distilled water. Table I presents data on the composition of the batch of commercial cow's milk preparation used during the observational period.

The tap water used for preparation of the formula fed from birth to the start of observational period was found after completion of the study to contain 28 mg/l of calcium and 0.18 mg/l of stable strontium. The formula used during the pre-observational period therefore contained about 0.67 mg of Sr per gram of Ca as contrasted to a value of 0.47 for the formula made with distilled water.

Analytical uncertainties were estimated to be ±5% in assays of strontium 90, stable strontium, and calcium, and ±10% in phosphorus determinations. Since excretion of the 4 constituents of interest was determined by difference (diapers with excreta less diapers alone), the analytical error in the estimate of excreted materials was greater than in that of the intake. Similarly, the error in the calculated retention was greater than in either intake or excretion.

Table II presents the measured values of intake and percentage excretion of Sr^{90}, Sr, Ca and P by the individual infants over the 15-day observational period as well as the Sr-Ca Observed Ratio. As expected, the variability was lowest for the intake values, with standard deviation averaging about 10%; next for the excretions, with stable strontium having the highest standard deviation of about 40%; and greatest for the retentions, with the stable strontium having a standard deviation of 100%. Statistical analysis indicated no differences observable due to sex or age of the infant.

The average retentions were 18.6 $\mu\mu c$ and 27.6% for Sr^{90}; –3.2 mg and –71.2% for Sr; 3.0 g and 31.4% for Ca; 1.8 g and 29.9% for P. The $OR_{body/diet}$ values were calculated as $\frac{\%\ Sr^{90}\ \text{retained}}{\%\ \text{Ca retained}}$ to give an average of 0.92 ± 0.27.

Discussion. In this study advantage was taken of the presence of measurable amounts of strontium 90 and stable strontium in the normal infant diet. Theoretically, the Sr^{90} to calcium relationships should be identical with

TABLE II. Intake and Percentage Retention of Strontium 90, Stable Strontium, Calcium, and Phosphorus, and Sr^{90}-Ca Observed Ratios in 14 Infants During a 15-day Observational Period.

		Sr^{90}		Sr		Ca		P		
Sex	Age (d) at start	Intake ($\mu\mu c$)	Retention (%)	Intake (mg)	Retention (%)	Intake (g)	Retention (%)	Intake (g)	Retention (%)	$OR^*_{body/diet}$
♀	35	64.9	27.4	4.4	– 63.7	9.2	20.6	5.8	27.6	1.33
♀	45	78.5	18.6	5.3	– 60.4	11.1	12.6	7.0	21.4	1.48
♀	40	77.0	29.5	5.2	63.5	10.9	37.6	6.9	34.8	.78
♀	38	66.0	19.7	4.5	13.3	9.4	26.6	5.9	27.1	.74
♂	32	67.9	35.3	4.6	19.6	9.6	34.4	6.1	29.5	1.03
♂	37	78.7	22.6	5.3	–100.0	11.2	19.6	7.0	32.8	1.15
♀	46	62.1	38.0	4.2	–109.5	8.8	35.2	5.6	28.6	1.08
♀	32	62.4	25.0	4.2	–145.2	8.9	32.6	5.6	32.1	.77
♂	25	67.5	38.5	4.6	– 56.5	9.6	37.5	6.0	26.7	1.03
♀	27	63.5	21.9	4.3	– 81.4	9.0	35.6	5.7	38.6	.62
♀	33	64.9	22.3	4.4	–120.4	9.2	30.4	5.8	28.6	.73
♂	31	76.3	14.5	5.2	–186.5	10.8	26.8	6.8	25.0	.54
♂	28	63.3	38.2	4.3	– 79.1	9.0	48.9	5.7	33.3	.78
♂	29	62.0	35.5	4.2	– 90.5	8.8	40.9	5.5	32.7	.87
Mean		68.2	27.6	4.6	– 71.2	9.7	31.4	6.1	29.9	.92
(Std. Dev.)		(±6.4)	(±8.2)	(±.43)	(±57.9)	(±.91)	(±9.5)	(±.56)	(±4.3)	(±.27)

* Calculated as % Sr^{90} retention divided by % Ca retention.

those of stable strontium to calcium when measurements are made under appropriate conditions. The loss of stable strontium from the body as contrasted to the net retention of Sr^{90} is accounted for in large measure if not completely by the change from a given level of dietary Sr/Ca to a lower value at the start of the observational period; this was brought about by the change from tap water to distilled water. These findings demonstrate the desirability in this type of study of attaining as near the steady-state condition as possible, and also emphasize the problems raised by adventitious sources of stable strontium. It is noted that in contrast the ratio of Sr^{90}/Ca was essentially constant from birth through the observational period, having been unaffected by the change from tap to distilled water.

Widdowson *et al.*(10) have recently reported negative stable strontium balances in infants of a few days of age fed on breast milk, and proposed that this was caused by the low level of phosphorus intake; they suggested that under these conditions Sr^{90} also would not be retained. The data reported here are not comparable and cannot serve to support or deny the findings of Widdowson and colleagues. However, it does appear that caution is required in predictions about Sr^{90} from stable strontium behavior in studies of this nature.

To determine a meaningful OR value it is especially important that the strontium to calcium ratios as measured truly represent the ratio as actually available to the organism and as actually derived from a single measured precursor(6). The ideal method would have been to use a double tracer technic since then there could have been no interference by contributions of strontium and calcium from two precursors, namely the diet and the pre-existing bone of the infant. But of course this is not permissible in work with human infants. It was considered, however, that because of rapid bone turnover at this age(7) the skeletons of the infants would have approached equilibrium with the diet of constant Sr^{90} to calcium that was fed from birth to the end of the observational period at about 50 days. With this assumption, the Sr^{90}/Ca values expressed as ratios of percentages retained should reflect the Observed Ratio.

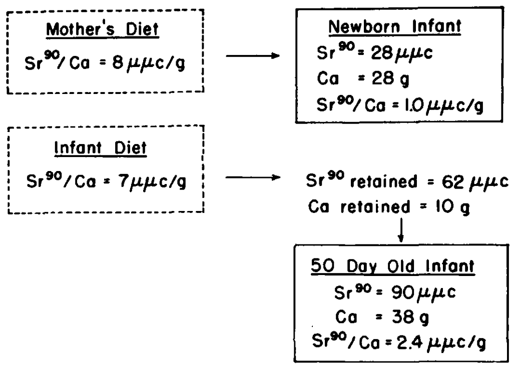

FIG. 1. Typical material balance for Sr^{90} and Ca in growth of infant between birth and 50 days of age.

To permit visualization of the "material balance" of Sr^{90}, and Ca, Fig. 1 presents a simplified model based on average values. Such an analysis can be used to see whether the values obtained are within reasonable expectations as judged by independent data.

Diets consumed during pregnancy by the mothers of these children were not available for analysis. However, from numerous surveys(11) it can be deduced with reasonable confidence that the adult diet in the Detroit area in early 1961 must have contained about 8 $\mu\mu$c Sr^{90} per gram of Ca. According to Mitchell(12) a newborn child will contain about 28 g of calcium. From typical values of the $OR_{body/diet} = 0.25$ and $OR_{fetus/body} = 0.5$ it can be calculated that the newborn infant should have contained about 28 $\mu\mu$c of Sr^{90}. This is quite reasonable since the Sr^{90}/Ca value of 1 is in good agreement with actual measurements of fetuses at this date(13).

As determined by analysis (Table I) the infant diet contained $Sr^{90}/Ca = 7$ $\mu\mu$c/g. Table II presents data on actual amounts of these substances retained or lost over the 15-day balance period and these values are used to calculate the gain or loss over the 50-day period of the infant's life. It is therefore estimated as shown in Fig. 1 that the infants added about 62 $\mu\mu$c of Sr^{90} and 10 g of calcium to their body stores in the first 50 days of life. No large error is likely to be introduced by assuming a constant behavior during the 50 days since the results of Widdowson *et al.*(10) indicate a similar calcium retention in children of about 6 days of age; also a body content of 38 g of calcium at 50 days is consistent with results of Mitchell (12). The calculated Sr^{90}/Ca value of 2.4 is also in agreement with general survey data on individuals of this age group and date.

For purposes of discussion the average $OR_{body/diet}$ of 0.92 has been calculated as the ratio of the percentage retentions of Sr^{90} and Ca. This value is an overestimate and thus if used to predict bone levels would provide an error on the safe side. It is not possible, however, to judge the magnitude of the error because this depends upon the degree of equilibrium attained. Theory and experiment predict the $OR_{body/diet}$ for young infants to fall between 0.5 and 1. For instance, the OR value for the young infant is expected to be greater than 0.5 as deduced by Bryant and Loutit(7) for 1-year-olds, because of the greater absorptive efficiency of the very young which seems to decrease the discrimination against strontium. On the other hand, numerous experiments have shown that strontium should be less efficiently utilized than calcium, since strontium is less readily transported across biologically active membranes; for this reason the $OR_{body/diet}$ should be less than 1.

Whether or not the $OR_{body/diet}$ value of 0.92 is accurate in the sense of the original definition it is clear that this value is the one of practical importance in that it does specify the relative addition of dietary Sr^{90} and Ca to the body of the young infant. Thus in deducing Sr^{90}/Ca ratios or Sr^{90} concentrations in bone of young infants from dietary intake, it is recommended that a value of 0.9 be used instead of the usual 0.25 that has been established for adults. It is not possible to determine unequivocally from this study whether the Sr^{90}/Ca of the new bone of the infant will be 0.9 that of the Sr^{90}/Ca of the diet or whether there will be significant dilution with the alkaline earths released by bone turnover.

Three illustrative situations can be calculated for the 50-day-old infant as follows (Fig. 1): If there were no bone turnover or equilibration, then the original bone containing 28 g of calcium would have a Sr^{90}/Ca ratio of 1 $\mu\mu c/g$, and the new bone with its 10 g of calcium would have a ratio of 6.2; that is, in the 50-day-old infant about 75% of the bone would have a Sr^{90}/Ca ratio of 1 $\mu\mu c/g$ and 25% of the bone would have a ratio of 6.2. At the other extreme, if there were complete bone turnover or equilibration, then all the bone would have a ratio of 2.4 $\mu\mu c/g$. If one assumes a reported turnover rate of about 100% in the first year(7) then about 60% of the bone would have a ratio of 1 $\mu\mu c/g$ and 40%, a ratio of about 4.7 $\mu\mu c/g$.

It is beyond the scope of this paper to consider matters of radiation damage; however, a general statement relative to these findings can be made. If harm to the individual from radiostrontium deposited in bones is dependent upon the long-term dosage, then the lack of discrimination in very early life may not be important. This is because bone turnover is very rapid, and the bone will soon reach a Sr^{90} level that is determined by the characteristic discrimination and dietary levels in the later years of childhood and following. On the other hand, if harm is related to an initial concentration during the first days and weeks of life, then the lower discrimination during this period may be of significance.

Summary. Fifteen-day balance trials were done with 14 healthy infants of about 34 days of age for Sr^{90}, stable strontium, calcium and phosphorus. Average retentions were: 28% Sr^{90}; −71% Sr; 31% Ca; 30% P. The loss of stable strontium from the body as compared to the retention of the other substances most probably is accounted for by the decreased intake of stable strontium that occurred at the start of the observational feeding period. Dietary Sr^{90} and calcium were added to the bodies of the infants in a ratio that was about 0.9 of the Sr^{90} to calcium ratio existing in the diet. This corresponds approximately to an $OR_{body}/_{diet}$ of 0.9 which contrasts with the accepted value of 0.25 for adults and indicates a lesser capability of the very young to discriminate against strontium in favor of calcium.

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1. Comar, C. L., Wasserman, R. H., Nold, M. M., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 859.

2. Wasserman, R. H., Comar, C. L., eds. *Annotated Bibliography of Strontium and Calcium Metabolism in Man and Animals,* U. S. Dept. of Agri., Misc. Publ. No. 821, 1961.

3. Burton, J. D., Mercer, E. R., *Nature,* 1962, v193, 4818.

4. Spencer, H., Li, M., Samachson, J., Laszlo, D., *Metabolism,* 1960, v9, 916.

5. Wasserman, R. H., Comar, C. L., Nold, M. M., *J. Nutr.,* 1956, v3, 371.

6. Comar, C. L., Russell, R. S., Wasserman, R. H., *Science,* 1957, v126, 485.

7. Bryant, F. J., Loutit, J. F., *Human Bone Metabolism Deduced from Strontium Assays,* Atomic Energy Research Establishment, R 3718, 1961.

8. Harley, J. H., Whitney, I. B., U.S.A.E.C. Report No. NYO-4700, 1957.

9. A. S. T. M., *Methods for Chemical Analysis of Metals,* Philadelphia, 1950.

10. Widdowson, E. M., Slater, J. E., Harrison, G. E., Sutton, A., *Lancet,* 1960, 941.

11. *Tri-City Diet Studies,* Fallout Program Quarterly Summary Report U.S.A.E.C., New York Operations Office, N. Y., Health and Safety Lab. Rep. No. 117, p220, Dec. 30, 1961.

12. Mitchell, H. H., Hamilton, T. S., Steggerda, F. R., Bean, H. W., *J. Biol. Chem.,* 1958, v158, 625.

13. Kulp, J. L., Schulert, A. R., *Strontium-90 In Man and His Environment,* Columbia Univ., Palisades, N. Y., 1962.

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Effect of Diet and Sex on Kidney Transamidinase Activity in the Rat.* (28125)

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Transamidinase, which catalyzes the synthesis of glycocyamine, is the first enzyme in the biosynthetic pathway leading to creatine (1). In the rat it occurs in highest concentration in the kidney, but as much as 20% of the glycocyamine required by the animal may come from other tissues(2). The importance of this enzyme in control of creatine synthesis is suggested by an increase in creatine synthesis following administration of glycocyamine(3). This implies that availability of glycocyamine normally limits rate of creatine synthesis.

Various experimental conditions alter kidney transamidinase activity(4-10). Notably creatine feeding depresses the *in vitro* transamidinase activity of normal animals(4-9). Neither the explanation for this effect of creatine nor its significance in physiological control of transamidinase activity are known. Further insight into the mechanisms controlling transamidinase activity might be gained by determining the causes of its variability in normal animals. This variability may be quite large. For instance, results from several separate experiments indicate that the *in vitro* transamidinase activities of female rats receiving a purified diet(4) and of chow-fed male rats(8) are approximately 50% of the activity of male rats receiving a purified diet (9).

This report concerns controlled experiments which demonstrate the differences in kidney transamidinase activity due to diet and sex. Evidence that these differences are not secondary to an effect of creatine is also presented.

Methods. Weanling Sprague-Dawley rats of both sexes were used in these experiments. The male rats were divided into 5 groups: the first group was supplied with a complete purified diet(4), the second group received the

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