parameters were calculated according to the function $\tau = 1/n\Sigma y_i/x_i$ (RAO).

The regeneration rate of despinalized tails was found to be approximatively half the normal rate in both the length and volume.

The real nature of despinalized tail regenerates is discussed and two hypotheses are proposed for their explanation.

## Effects of Stable Calcium and Strontium on Deposition of Calcium-45 and Strontium-89 in Bone

In order to minimize skeletal deposition and retention of radiocalcium and radiostrontium, dilution with their non-radioactive isotopes has been proposed[1]. High dietary levels of non-radioactive calcium depressed absorption of radiocalcium from the gastrointestinal tract[2], but had little effect on bone deposition of radiocalcium or radiostrontium[3]. More recent studies[4,5] have markedly demonstrated that sustained feeding of non-radioactive dietary calcium supplements is accompanied by definite reductions in bone deposition of radiocalcium and radiostrontium. The investigation here reported measured effects of single administration of stable calcium and strontium carrier on deposition of radiocalcium ($Ca^{45}$) or radiostrontium ($Sr^{89}$) in bone.

*Material and Methods.*—480 randomly selected young adult female Sprague-Dawley rats ($232 \pm 32$ g weight) received by intraperitoneal injection or by oral administration 0·25 to 1 ml of aqueous solutions of 10 μc of either $Ca^{45}$ or $Sr^{89}$ mixed with non-radioactive calcium or strontium chlorides at about pH 4. Without added salt the $Ca^{45}$ contained 14 to 130 μg Ca per μc $Ca^{45}$, and the $Sr^{89}$ was carrier-free. Six rats comprised each group given the mixtures of isotopes at dosages which ranged from 3·6 to 3,000 μg Ca/g rat body weight, and from 0 to 1,300 μg Sr/g rat body weight.

The animals were starved for 24 h before radioisotope administration. They were maintained on a standard stock diet during the experimental periods, which amounted to one day for intraperitoneal treatment, and 1–5 days for oral treatment. These differences in the experimental periods after oral treatment had no perceptible effects on the resulting distributions of the radioisotopes, as shown both by direct inspection and by statistical evaluation. The animals were killed by ether anesthesia. The femurs were removed and ashed. Concentrations of $Ca^{45}$ or $Sr^{89}$ in suitable aliquots of the ashed samples were measured and corrected for self-absorption and decay. The concentrations of the radioisotopes in the samples were compared to those of 'standards' prepared from aliquots of the original injection solutions.

*Results.*—The data for deposition of $Ca^{45}$ and $Sr^{89}$ in femur are summarized in Tables I and II. *Table I:* Intraperitoneal $Ca^{45}$ and $Sr^{89}$: $Ca^{++}$ or $Sr^{++}$ added. Deposition of $Ca^{45}$ and $Sr^{89}$ in femur did not vary appreciably with differences in concentrations of stable calcium or strontium administered, and the averages for each isotope and time were associated with relatively small of variability. When stable strontium was injected deposition of $Sr^{89}$ was reduced at each dosage concentration an average of about 30% compared to the average value obtained when stable calcium was administered with $Sr^{89}$. This difference was statistically significant, with $p < 0{\cdot}01$, and contrasts with lack of effect of both stable calcium and strontium on $Ca^{45}$ deposition. *Table II:* Intragastric $Ca^{45}$ and $Sr^{89}$: $Ca^{++}$ or $Sr^{++}$ added. Following oral administration values for $Ca^{45}$ and $Sr^{89}$ femur deposition showed an apparent positive dependence of deposition on inert carrier concentration. With the exception of the $Ca^{45}$–$Sr^{++}$ series, the

*Table I*
Average deposition of $Ca^{45}$ and $Sr^{89}$ in femur following intraperitoneal administration with added stable calcium or strontium[a]

| Radioisotope | % administered radioisotope/femur ± 95% confidence range[b] | |
|---|---|---|
| | $Ca^{++}$ added | $Sr^{++}$ added |
| $Ca^{45}$ | 2·71 ± 0·17 | 2·94 ± 0·38 |
| $Sr^{89}$ | 2·85 ± 0·58 | 1·89 ± 0·34* |

[a] Quantities of added calcium administered ranged up to 155 μg/g body weight. Quantities of added strontium administered ranged up to 375 μg/g body weight.

[b] The 95% confidence interval estimates are based on sample range of mean effect.

* Reduction in deposition compared to $Sr^{89}$-Ca combination statistically significant, with $p < 0{\cdot}01$.

*Table II*
Deposition of $Ca^{45}$ and $Sr^{89}$ in femur following oral administration with added stable calcium or strontium

| Radioisotope | $Ca^{++}$ added μg/gbody wt. | Deposition %/femur | 95% Confidence range[a] | $Sr^{++}$ added μg/gbody wt. | Deposition %/femur | 95% Confidence range[a] |
|---|---|---|---|---|---|---|
| $Ca^{45}$ | 129 | 0·55 | 0·07 | 50·2 | 2·55 | 0·57 |
| | 416 | 0·58 | 0·10 | 125 | 3·03 | 0·60 |
| | 648 | 0·77 | 0·28 | 200 | 2·92 | 0·25 |
| | 834 | 1·10 | 0·23 | 308 | 3·12 | 0·39 |
| | 1019 | 1·41 | 0·69 | 404 | 3·69 | 0·42 |
| | | | | 510 | 3·80 | 0·32 |
| | | | | 649 | 4·87 | 0·86 |
| | | | | 729 | 4·51 | 0·93 |
| | | | | 800 | 5·27 | 0·87 |
| | | | | 942 | 5·24 | 1·21 |
| $Sr^{89}$ | 0 | 0·54 | 0·24 | 0 | 0·54 | 0·24 |
| | 16·2 | 0·28 | 0·11 | 55·5 | 0·73 | 0·25 |
| | 31·3 | 0·40 | 0·09 | 129 | 0·46 | 0·17 |
| | 63·8 | 0·34 | 0·11 | 202 | 0·52 | 0·02 |
| | 103 | 0·40 | 0·10 | 327 | 0·54 | 0·10 |
| | 160 | 0·58 | 0·19 | 447 | 0·53 | 0·16 |
| | 183 | 0·44 | 0·13 | 563 | 0·55 | 0·18 |
| | 245 | 0·73 | 0·22 | 660 | 0·72 | 0·32 |
| | 259 | 0·94 | 0·27 | 754 | 0·52 | 0·21 |
| | 311 | 0·81 | 0·81 | 1060 | 0·77 | 0·16 |
| | | | | 1263 | 0·81 | 0·55 |

[a] The 95% confidence interval estimates are based on sample range of mean effect.

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values increased toward, but did not exceed, those observed following intraperitoneal injection. In the case of $Ca^{45}$–$Sr^{++}$ the variability which accompanied the high values attained suggested a high degree of uncertainty.

*Discussion.*—The results shown in Table I indicate that intraperitoneal injection of non-radioactive calcium has no perceptible effect on deposition of tracer quantities of $Ca^{45}$ or $Sr^{89}$ in femur. The value of 2·71% for $Ca^{45}$ was not statistically different from the 2·85% obtained with $Sr^{89}$ in the presence of stable calcium, a result which may be related to the chemical similarity of these metals. The very small tracer amount of $Sr^{89}$ may be readily incorporated under these experimental conditions into bone concurrently with, and similarly to, the normal calcium uptake.

Injection of very large amounts of stable strontium did not apparently affect the overall processes associated with calcium uptake enough to be reflected in any alteration of the amounts of tracer $Ca^{45}$ deposited. This is indicative of a close regulation of bone calcium uptake. Although $Ca^{45}$ uptake was not influenced in this way, femur strontium uptake decreased following injection of massive doses of stable strontium by about 30% from the value obtained with carrier-free solutions. This reduction may have some analogy to isotopic dilution effects where $Sr^{89}$ may compete with stable strontium in the processes associated with bone deposition of this metal. The results do not preclude the possibility of similar competition as between stable calcium and $Ca^{45}$ but small effects produced in this way may have been undetectable within the limits of experimental error, especially in consideration of the recent demonstration[4,5] that calcium supplementation of a diet adequate in calcium results in minimal decreases in bone deposition of $Ca^{45}$, even over extended experimental periods.

The amounts of $Ca^{45}$–$Sr^{89}$ deposited in femur following oral administration appeared to reflect some reduced availability of the radioisotopes, for with the exception of the $Ca^{45}$-stable strontium series, the values of Table II were less than those observed after intraperitoneal injection. This exception which attained values as great or greater than after intraperitoneal injection, was also in accord with the general trend of the femur radioisotope deposition to increase with increasing stable metal.

The positive dependency of radioisotope depositions on amount of stable metal orally administered suggests that mixing and dilution with intestinal contents as well as excretion may have had some effects to alter the amounts of isotopic mixtures available for gastrointestinal absorption.

If absorption of the isotopic mixtures from the gastrointestinal tract is related to their concentration gradient across the intestinal barriers as recent considerations of absorption and membrane transfer suggest[6], dosage effects of stable calcium or strontium administered orally might also be reflected in absorption differences. These factors may account indirectly to some extent for the generally lower, but increasing positive proportionality observed between oral dosage and femur deposition of calcium and strontium. Variabilities in quantities of metals absorbed from the gastrointestinal tract would in effect influence their femur deposition more directly than the values of total oral dosage of the isotopic mixtures. Further and more extensive study would be required to establish this possibility.

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*Biology Operation, Hanford Laboratories, Richland (Washington), April 6, 1959.*

*Zusammenfassung*

Nach einmaliger intraperitonealer Verabreichung von stabilem Kalzium oder Strontium in Mengen bis zu 3000 µg/g Körpergewicht war der Bruchteil des gleichzeitig verabreichten $Ca^{45}$, das sich im Rattenschenkel ablagerte, unverändert. Unter denselben Umständen verabreicht, verminderte stabiles Strontium im Schenkel abgelagertes $Sr^{89}$ ein wenig, aber stabiles Kalzium hatte keine solche Wirkung auf $Sr^{89}$.

Während sich die Konzentration des Radioisotops nach einmaliger peroraler Verabreichung im entsprechenden Verhältnis zur Dosis des stabilen Kalzium- oder Strontiumgehalts vermehrte, waren im allgemeinen die Konzentrationen im Schenkel nach einmaliger peroraler Verabreichung kleiner als nach intraperitonealer Verabreichung.

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## Fructose and Fructolysis in Human Semen Determined Chromatographically

The work of DAVIS and MACUNE[1], BIRNBERG, SHERBER, and KURZOK[2], and VAISHWANAR[3] has shown that the rate of fructolysis of human spermatozoa can give an indication of their activity. MANN[4] has shown that fructose is metabolised in bull semen incubated *in vitro* and that the rate of fructolysis as assessed by the colorimetric estimation of disappearing fructose gives an accurate measure of the activity of spermatozoa. Most workers have employed the colorimetric estimation of fructose using the resorcinol method as a simple and convenient procedure for the estimation of the content of fructose and the rate of fructolysis in semen. However, MANN[5] has pointed out that, unlike bull semen, that of man contains a large amount of reducing material other than fructose which can seriously interfere with the conventional methods of sugar determinations. He has further pointed out that these reducing substances are not only present in fresh human semen but that their content increases during incubation of semen *in vitro*. If however fructose could be separated from other reducing substances occurring in human semen and then estimated, such values would be more accurate. With this view in mind, an attempt was made to apply to semen a chromatographic procedure for the estimation of fructose based in principle, on the method of GIRI and NIGAM[6].

A 1% potassium hydroxide solution was prepared in 95% alcohol. The triphenyl tetrazolium chloride solution was prepared by dissolving 2 g of the substance in 100 ml of butanol saturated with water. Both solutions were kept in the cold in brown bottles. The tetrazolium reagent (T.T.C.) was

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