TABLE II. Comparison of Values of CO_2 Content Obtained by the New Method with Those Determined by the Van Slyke Method in 12 Samples of Varying Protein Content.

No.	Total protein, g/100 ml	CO_2 content, vol % — New method	CO_2 content, vol % — Van Slyke
1	6.5	65.7	65.6
2	6.4	63.5	63.3
3	7.2	77.6	76.7
4	6.2	65.4	65.8
5	5.4	55.7	55.5
6	5.5	57.8	56.1
7	6.7	58.9	58.4
8	5.7	70.9	72.0
9	5.1	61.3	62.2
10	5.5	61.1	61.6
11	5.2	55.5	56.1
12	7.0	60.2	62.0

agree quite well with the Van Slyke method in samples of varying protein contents. Of 307 determinations analyzed to date, 74% agreed within 2 volumes per cent of values found by the Van Slyke method. The agreement was always good between 40 and 80 volumes per cent, but there was an appreciable discrepancy in samples in which the carbon dioxide content was outside this range.

Summary. A method is described for the determination of carbon dioxide content of 0.1 and 0.2 ml samples of serum or plasma by adding 0.1 ml of standard acid and 0.1 ml of standard alkali and estimating the pH with a glass electrode. The values of carbon dioxide are then calculated from the amount of alkali found on an average composite curve for plasma or serum between an average initial pH at room temperature of 7.60 and the experimental final pH. Data are presented to show that the results obtained by this method agree quite well with values determined by the Van Slyke manometric method.

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Effect of Parathyroids on Radio-Calcium Uptake and Exchange in Rat Tissues.* (19688)

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These studies were undertaken to determine the uptake of radio calcium (Ca^{45}) by various tissues of the rat in normal and hypoparathyroid animals in the hope that further light might be thrown on the effect of the parathyroids on calcium metabolism. Until recently the Ca^{45} available for biological studies contained varying amounts of additional stable calcium. Since calcium in small amounts has marked effects on mammalian physiology, the carrier accompanying the radio-isotope could conceivably have a major influence on the results obtained. In this regard, L'Heureux *et al.*(1) found that the ratio of excreted urinary Ca^{45} to fecal Ca^{45} could be changed from 1:20 to 1:1 by increasing the amount of stable calcium administered with the radio-isotope. Lansing *et al.*(2) studied the uptake and exchange of Ca^{45} in mouse tissues in which the results obtained could be attributed to the large amount of stable calcium administered with the Ca^{45}. Tweedy *et al.*(3), in studying the excretion and distribution of radio-calcium in normal and thyroparathyroidectomized rats, administered Ca^{45} accompanied by total calcium in amounts ranging from 0.21 mg to 7.0 mg. Because of this apparent problem in the use of this isotope, it was thought best to first study the difference in the uptake by tissues of Ca^{45} when accompanied by widely varying amounts of carrier calcium.

Material and methods. A total of over 150 male rats, weighing between 200 and 250 g,

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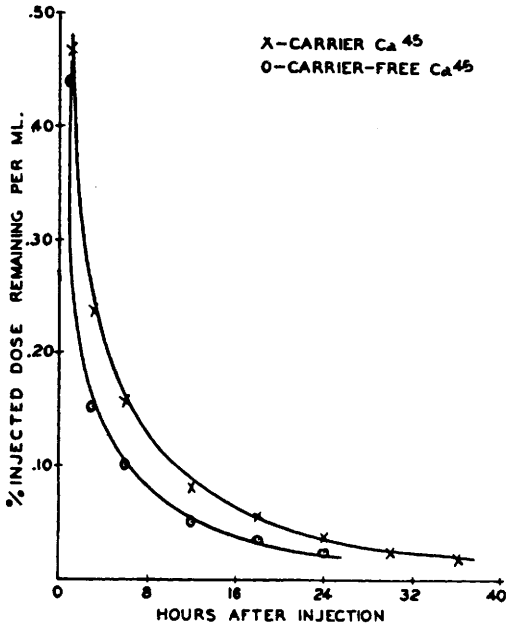

FIG. 1. Disappearance of Ca^{45} from plasma of normal rats after administration of 20 μc carrier and carrier-free Ca^{45}.

were used, and each point on the graphs included represents an average of from 4 to 10 animals. The Ca^{45} was obtained from the Atomic Energy Commission and administered intraperitoneally as $CaCl_2$. Most of the animals were given the radio-isotope accompanied by approximately 0.5 mg of total calcium. Since preliminary work showed the uptake to be similar to that of carrier-free Ca^{45}, this is considered *physiologically* carrier-free; and this term, though not technically correct, is used for the sake of convenience in the remainder of this paper. For comparison, the Ca^{45} was also administered accompanied by 10 mg of stable calcium. The term, carrier-Ca^{45}, will be used in this regard. In all cases, the total radio-activity per animal amounted to approximately 20 μc. Dilutions of the injection solution were used as standards for measurement of radio-activity, and all results are given in percentage of the injected dose. The radio-activity measurements were done by standard procedures and corrected for self-absorption. Total serum calciums were determined by the method of Clark and Collip (4). The parathyroids were removed individually following the procedure described by Greep(5). Since some accessory parathyroid tissue is usually present in the rat, the criterion used for determining hypoparathyroidism was the drop in serum calcium to 7 mg % or lower. As a standard procedure, the animals were allowed 3 days after the operation for recovery and then starved overnight before use in the experiments. The control animals were also starved a similar period. Radioactivity determinations were done on both the tibia and the femur, and radio-autographs of the latter were made after trimming the bone on a freezing microtome to obtain longitudinal sections.

Results. The data summarized in Fig. 1, 2, and 3 show a comparison of the uptake of Ca^{45} administered under the two conditions discussed above. Fig. 1 shows the disappearance of radio-activity from the blood. As might be expected the carrier-Ca^{45} disappeared from the blood somewhat slower than did the carrier-free Ca^{45}. Total serum calciums, which were run concurrently, remained normal in the carrier-free group. However, in the carrier group, these values rose to above 15 mg % within the first hour after injection but returned to normal within 6 hours. It is of interest to note that these changes in serum

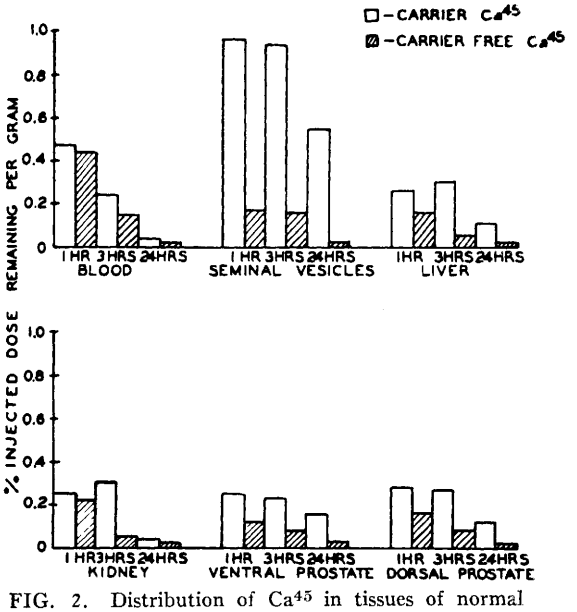

FIG. 2. Distribution of Ca^{45} in tissues of normal rats after administration of 20 μc carrier and carrier-free Ca^{45}.

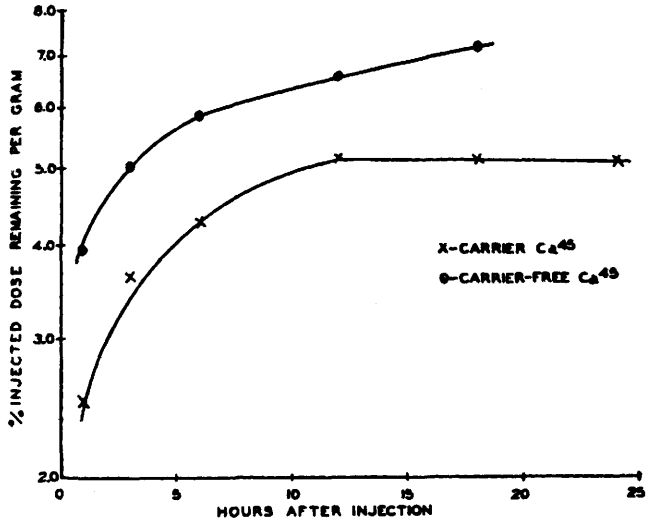

FIG. 3. Uptake of Ca^{45} in bone of normal rats after administration of 20 μc of carrier and carrier-free Ca^{45}.

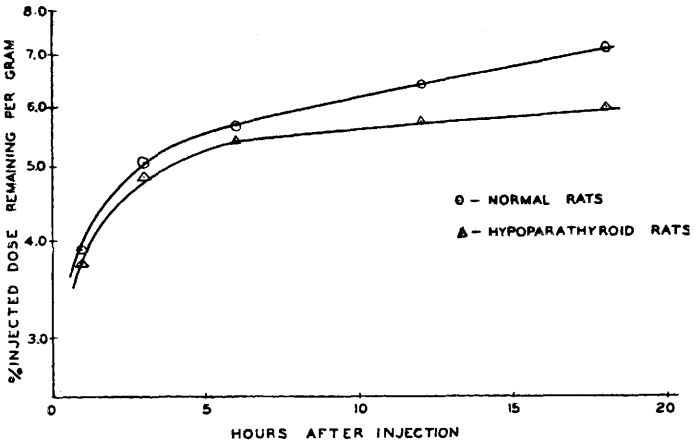

FIG. 4. Uptake of Ca^{45} in bone of normal and hypoparathyroid rats after administration of 20 μc carrier-free Ca^{45}.

calcium were not reflected in the radio-activity disappearance curves.

In Fig. 2 is given the Ca^{45} distribution for a few selected tissues. It can be seen that with the carrier-free Ca^{45} the tissue activity approximates but never exceeds the radio-activity of the blood at any given time. The uptake by the tissues of these animals appears to be a function of the blood supply and indicates very little actual calcium deposition. When carrier-Ca^{45} is administered, the situation is strikingly different. Twenty-four hours after injection, the activity of all the tissues studied surpassed that of blood, indicating deposition of the administered calcium. This was emphasized in the seminal vesicles in which the uptake approached one-fourth of that seen in long bone. The significance of this unusual uptake by the seminal vesicles is under study.

The uptake of radio-calcium by bone (Fig. 3) in the carrier-group is significantly less than that in the carrier-free group. However, due to the low specific activity of the carrier-Ca^{45} and the increase in the serum calcium in those animals receiving it, it is apparent that despite the lower bone activity, considerably more total calcium is taken up by the bones

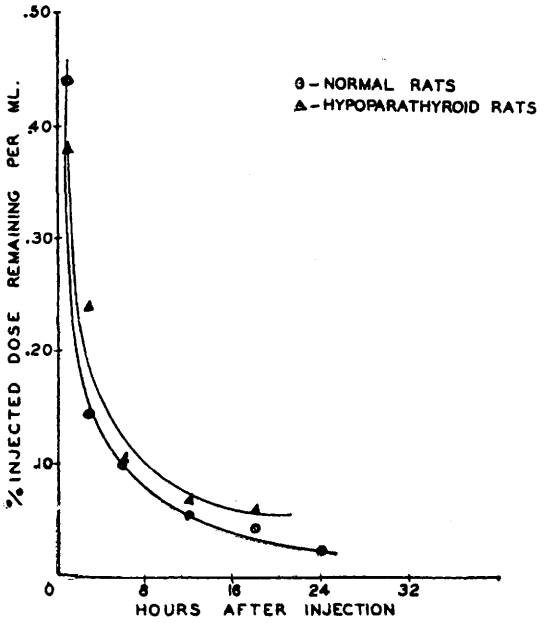

FIG. 5. Disappearance of Ca^{45} from plasma of normal and hypoparathyroid rats after administration of 20 μc carrier-free Ca^{45}.

of the carrier-Ca^{45} group.

The uptake of carrier-free Ca^{45} by bone can be divided into two time periods. The first 6 hours can be considered the "exchange period" in which the radio-calcium is equilibrating with calcium in blood, soft tissues and that portion of the calcium of bone which is either in ionic form or in loose organic union. The straight line portion of the graph following this period is termed the "turnover rate" portion and is considered to indicate the rate at which calcium is being actively laid down by the long bone.

For our comparison of normal and hypoparathyroid rats, only the carrier-free Ca^{45} has been used. Fig. 4, 5, and 6 summarize this work. In Fig. 4, the uptake by long bone is illustrated. It will be noted that while there is little or no difference during the "exchange" phase in the uptake of radio-calcium, the straight-line portion of the graph, indicating turnover rate, in the hypoparathyroid condition markedly falls away from the controls. This lower turnover rate in the bones of the experimental animal is substantiated by the slightly slower rate of disappearance of the radio-calcium from the blood (Fig. 5) and the corresponding higher uptake in those tissues studied (Fig. 6). All radio-autographs made of bones showed a similar deposition of Ca^{45} in the periosteum and the epiphyseal plates, indicating that the differences in uptake noted were in degree only and that metabolism of the bone may not have been affected.

Discussion. The use of the radio-isotope of calcium, Ca^{45}, presents several problems. Due to the effect of calcium on the cardiovascular system, very little carrier is required to markedly alter at least some of the physiological processes of the animal. The work presented here shows the differences in uptake of radio-calcium which can be produced by using two extreme conditions: one, the administration of an amount of calcium sufficient to raise the blood calcium level of the animal; the other, Ca^{45} accompanied by the minimum amount of carrier present in the Oak Ridge material available for our use. Not only could it be shown that the carrier present with the radio-isotope drastically affected the distribution of Ca^{45}, and that the carrier masked some of the uptake indicated by the radio-isotope, but also that changes

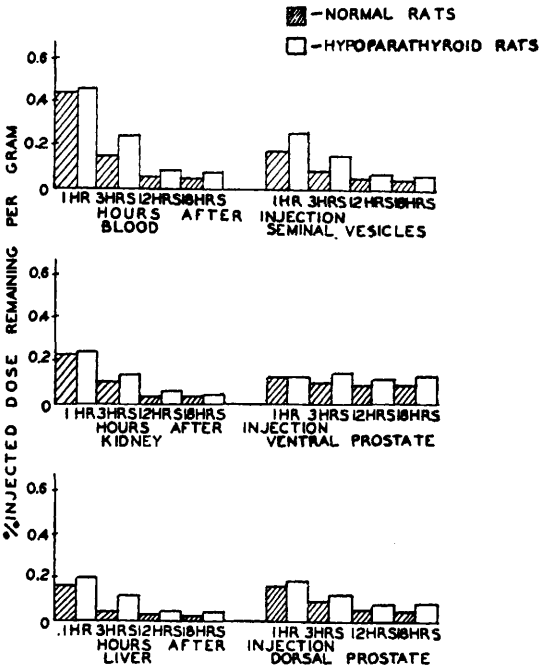

FIG. 6. Distribution of Ca^{45} in tissues of normal and hypoparathyroid rats after administration of 20 μc carrier-free Ca^{45}.

produced by the carrier were not necessarily portrayed by the radio-isotope. These are problems in common with any radio-isotope which is being used in biological experiments, but particularly important with one as physiologically sensitive as calcium. However, though the uptake of carrier-Ca^{45} as presented here is not a physiological situation due to the excessive amount of calcium given, the use of carrier-Ca^{45} may prove to be a useful tool in determining the ability of bone to handle large amounts of calcium.

These preliminary experiments with normal and hypoparathyroid rats did not yield any information having a direct bearing on the physiological functioning of the parathyroid hormone. However, they do illustrate very clearly the difference in bone uptake and turnover of calcium in the two situations. All radio-autographs showed the primary sites of calcium entry into the bone to be at the epiphyses and in the periosteum and indicate that this process is affected only in degree in the various situations tested by these experiments. Whether or not one could conclude from this that the basis of the action of the parathyroids is other than in bone itself is doubtful, but it is hoped that further experiments now in progress will bear on this point.

Summary. This study has presented two factors which can markedly affect the uptake and distribution of Ca^{45} in tissues of the rat. The first of these was concerned with the influence of the carrier calcium accompanying the radio element and emphasizes the need for carefully controlled experiments if even small amounts of carrier are administered with the Ca^{45}. The second factor was concerned with the influence of parathyroidectomy on the uptake of Ca^{45}. The uptake by bone was divided into two time periods, the "exchange period" and the "turnover rate" portion. The exchange period was not markedly affected by the absence of the parathyroids while the turnover of calcium in the bone was at a significantly lower rate.

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Effect of Sympathomimetic Amines upon Amino Acid Nitrogen Content of the Blood. (19689)

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The lowering of blood amino acid nitrogen which follows upon the administration of insulin to man and experimental animals (1-8,11) is also observed when epinephrine is injected(9-11). The insulin effect persists even when the hypoglycemia is prevented by the oral administration of glucose(12) or by the simultaneous administration of epinephrine(9). Insulin hypoaminoacidemia is not observed, however, in the adrenodemedullated rabbit(13) although, in such animals, epinephrine continues to evoke its characteristic lowering of the concentration of blood amino acids(13). It was, therefore, concluded by Luck and associates(10,11,13), that insulin hypoaminoacidemia is, in reality, the result of stimulation of the adrenal medulla, and that the epinephrine thus secreted evokes the observed response or stimulates another gland of internal secretion in a chain of hormonal interactions.

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