for several days requires further study. Acquisition of the ability of male rats to accumulate citrate in the liver after adrenalectomy suggests that adrenal cortical hormones also exert a regulatory influence on citrate synthesis in liver and the similar response obtained after feeding male rats desiccated thyroid(2) suggests that several hormones may be involved in the regulation of citrate synthesis in the liver.

The exact manner in which androgens influence the response of the liver to fluoroacetate requires further investigation. However, the present studies have provided a basis for subsequent experimentation on the influence of androgens on intermediary carbohydrate metabolism. The available data indicate that testosterone inhibits the formation of citrate from pyruvate or acetate in the liver and this effect may be of significance in connection with the basic mechanism underlying the metabolic action of the sex hormones. The alteration in the metabolic pattern of the liver which we previously observed(1) in X-irradiated male rats may be due to a release of the liver from the regulatory action of androgens through suppression of steroid synthesis.

Summary. A study of factors influencing citrate synthesis was conducted using the fluoroacetate technique of Potter(2) to determine the cause of the marked sex difference(1) in citrate accumulation in the liver following administration of fluoroacetate to rats. Castration of female rats did not decrease citrate synthesis in the liver but castrated and adrenalectomized male rats acquired the ability to accumulate citric acid in the liver after fluoroacetate treatment. Administration of testosterone to female rats for several days decreased citrate synthesis in the liver and testosterone inhibited citrate synthesis by rat liver homogenates *in vitro*. The results of these experiments indicated that androgens suppress citrate formation in the liver.

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Effect of Calcium Status, Mass of Calcium Administered and Age on Ca^{45} Metabolism in the Rat. (19102)

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The value of radiocalcium in nutritional studies has been obscured by the lack of agreement between investigators and difficulties of interpretation due to the exchange of Ca^{45} between extracellular fluid and bone (1-5). Observations during the course of nutrition studies with Ca^{45} indicated a surprisingly rapid response of the rat to changes in the calcium content of the diet. It seemed important, therefore, to establish the extent to which conventional experimental procedures were producing a bias in the results obtained by this sensitive method of studying calcium metabolism.

This study shows the effects of the following factors upon the behavior of labeled calcium administered orally to the rat: (a) the actual calcium status of the animal at the time of dosage, (b) the calcium content of the intestinal contents at the time of dosage, and (c) age of the animal. Physiological implications of calcium depletion and the interpretation of Ca^{45} studies are discussed.

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TABLE I. Composition of Experimental Rat Rations (g per 1000 g of Ration).

Ingredients	Diet Ca-1 (Low)	Diet Ca-3 (Normal)
Corn starch	655	643
Casein (vitamin-free)	180	180
Irr. yeast*	10	10
UT-AEC min mix†	20	20
Cod liver oil‡	2	2
Wesson oil	40	40
Cellulose	40	40
Vitab§	40	40
KH_2PO_4	13.2	13.2
$CaCO_3$	0	12.2
% calcium (by analysis)	.013	.5
% phosphorus "	.4	.4

* Irr. yeast contained 360 I.U. vitamin D/g.

† UT-AEC basic mineral mixture No. 1 is a modification of the calcium-phosphorus free mixture used by H. G. Day *et al.* (*J. Nutr.*, 1938, v16, 525) and includes the following minerals: NaCl 39.84, KCl 18.2, $KHCO_3$ 26.2, $MgSO_4$ (anhyd) 12, $CuSO_4 \cdot 5H_2O$ .4, $MnSO_4 \cdot 4H_2O$ .2, $ZnSO_4 \cdot 7H_2O$ .6, $FeCl_3 \cdot 6H_2O$ 2.5, $K_2SO_4 \cdot 2H_2O$ .02, KI .03 and NaF .01 %.

‡ Cod liver oil contained 90 I.U. vit D and 900 I.U. vit A/g.

§ Vitab, available from Nopco Chemical Co., Harrison, N. J.; fortified with .4 mg riboflavin per g immediately preceding mixing.

Methods. These experiments were carried out with highly inbred Wistar rats of both sexes. The young were weaned at 28 days from mothers maintained on commercial rat pellets and, as determined by the experiment, were placed on the normal (Ca-3) and/or low calcium (Ca-1) experimental diets. These diets were identical except for the variation in calcium content (Table I). All rats were fed and watered *ad libitum*. Following the definite pre-experimental periods of preparation on known rations, feed was removed from all cages 4 hours before dosing to insure uniformity of conditions; the animals received a single oral dose of labeled calcium by stomach tube and were placed immediately into metabolism cages equipped for the separation of the urine from the feces for complete balance studies. Unless otherwise indicated, the dosage consisted of 0.5 ml calcium 45 chloride solution containing about 10 microcuries in less than 1 mg calcium with the pH adjusted to 6.0. At sacrifice after 96 hours, fresh tissue samples, feces and urine were taken for routine total calcium and phosphorus determinations and for the measurement of calcium 45 by methods previously reported(6). All values involving labeled calcium have been calculated on an equivalent basis so as to be comparable between animals and experiments.

Effects of changing calcium level of ration. The first study of this series was designed to show the effects of changing the calcium level of the ration at varying times before administration of the labeled calcium. Following the definite pre-experimental period, animals were selected from the low and normal calcium groups and placed on the current intake ration for 48, 96 and 408 hours previous to dosage with Ca^{45} as indicated in Table II. The results show the actual calcium intake and excretion per rat per day during the 96-hour experimental period following oral administration, the percent of labeled calcium excreted, the percent of excreted calcium which was derived from the body stores, and the calcium content and calcium 45 accumulation in the femur shaft. The percent of excreted calcium which was derived from the body stores was calculated without taking into consideration the labeled calcium which reached the body stores and was subsequently excreted during the experimental period. It is considered that this method of expression does not change the overall picture. The important basic effects can be noted from consideration of Lots 1 to 4. As might be expected, Lot 1 rats were in a positive calcium balance, excreting about 50% of the total calcium intake, whereas Lot 4 rats on the low calcium diet throughout, were in negative balance. Lot 2 rats which had been on the low calcium diet for only 48 hours had gone from a positive to a negative balance, whereas Lot 3 rats which had been on a normal diet for only 48 hours, were already in positive balance. In the case of Lot 1, about 30% of the labeled calcium was excreted, whereas in the other three lots, significantly lower amounts were excreted. In line with this it is noted that in Lot 1 rats about 40% of the excreted calcium was derived from the body stores, while with the other groups this value

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TABLE II. Effects of Dietary Calcium Changes Previous to Oral Ca45 Administration on Its Excretion and Bone Accumulation (4 rats in each group; 72 days of age).

Lot No.	Hr on current intake	Previous Ca intake	Current Ca intake	mg Ca per day: Intake	mg Ca per day: Excreted	% excreted: Total Ca	% excreted: Ca45	% excreted: Body* stores	Femur shaft: mg Ca/g	Femur shaft: Ca45†	Femur shaft: Specific‡ activity	Ca45: Frac. Ca45 absorbed
1	48	Normal	Normal	44	22	50	30	40	196	.60	3	.86
2		,,	Low	1.8	1.9	106	6.5	94	170	1	5.8	1.1
3		Low	Normal	45	3.1	6.9	.20	97	85	1.5	17.7	1.5
4		,,	Low	1	1.5	150	.60	99	60	1.5	26.7	1.5
5	96	Normal	,,	1.8	2.1	117	8.3	93	168	.80	4.7	.87
6		Low	Normal	46	.9	2	.24	88	116	1.7	14.2	1.7
7	408	Normal	Low	2.6	.2	7.7	.44	95	140	1.4	10	1.4
8		Low	Normal	49	5.2	10.6	4.8	55	143	1.5	11	1.6

* % of excreted Ca derived from body stores equals $100\left[1-\left(\frac{\%\ Ca^{45}\ \text{excreted}}{100}\right)\left(\frac{\text{mg Ca intake}}{\text{mg Ca excreted}}\right)\right]$.

† μg of labeled Ca per g fresh tissue based on dosage of 10 μg per 100 g body wt.

‡ μg of labeled Ca x 10^3 per g of total Ca based on above dosage.

was from 95 to 100%. In considering the values for the femur shaft it is noted that 48 hours on a low diet had caused an apparently slight decrease in the calcium content (Lot 1 vs. Lot 2), whereas 48 hours on a normal diet had caused an increase (Lot 3 vs. Lot 4). The accumulation of Ca^{45} in the bone was lowest in Lot 1 and significantly higher in the other lots. Greater differences in turnover or specific activity between lots was due to the fact that both the Ca^{45} accumulation and calcium content of the bone entered into the calculation. Lots 5-8 show the same trends accentuated by the longer period on the changed ration. It is of interest in comparing Lots 7 and 8 to note that the calcium balance and bone values were about the same. Nevertheless, the Lot 8 rats, on a current normal intake, were beginning to show an increased excretion rate of Ca^{45} even though the bone calcium content had not yet reached a normal level. The implications of these results are clear: a biased picture of calcium behavior may well be obtained if the dietary calcium is changed radically before administration of the labeled element, or during the balance period.

The physiological aspects are of considerable interest. As will be discussed in more detail later, it can be noted that the mass of calcium in the intestinal contents at the time of absorption of Calcium 45 had little effect on the results obtained. In the case of simple mass effects, one would normally expect a lower excretion value when the mass was lower. Thus Lot 2 rats certainly had a lower intestinal content of calcium than did those of Lot 3, but Lot 2 nevertheless showed a higher excretion: this comparison also holds for Lots 5 and 6. It seems apparent that rats changed from a normal to a low calcium diet rapidly develop an increased capacity for absorption of this element from the tract. In the case of animals maintained on low calcium for longer periods of time (>96 hr) it seemed likely that the calcium status of the bone was reflected in the increased capacity for absorption of this element from the tract. It may be noted from the last column of Table II that the bones of animals on low calcium for longer than 96 hours showed a higher Ca^{45}

TABLE III. Effects of Calcium Depletion on Excretion and Bone Accumulation of Orally Administered Ca^{45} (8 rats in each group; 32 days of age at start of experiment).

Days on low Ca (Ca-1)	% Ca^{45} dose excreted	Femur shaft				Ca^{45}
		Ca^{45}*	mg Ca/g	Specific† activity	% ash	Fraction Ca^{45} absorbed
0	8.8	.71	105	8.7	32	.78
12	.7	1.4	102	13	27	1.4
24	.5	1.6	63.5	25	18	1.6
36	.8	1.7	65	26	17	1.7
48	1.2	1.6	68.5	24	18	1.6
60	.9	1.4	68	21	18	1.6

* μg of labeled Ca per g fresh tissue based on dosage of 10 μg per 100 g body wt.
† μg of labeled Ca $\times$ 10^3 per g of total Ca based on above dosage.

uptake in the femur shaft per unit of dose absorbed. This would indicate that in animals on a depletion regime, increases in the ability to absorb calcium from the tract may precede changes in the bone.

In part, the results thus far discussed have been concerned with short-term depletion effects. The following study is concerned with the course of depletion continued over a longer time period. Six lots of 8 weanling rats each were placed on a low calcium ration (Ca-1) and at intervals designated in Table III they were given an oral dose of Ca^{45} and sacrificed after 96 hours for the usual tissue analyses. Table III shows the values obtained for the excretion and femur shaft. As expected, the 12 days' depletion caused a marked effect: at 24 days the main change was in the mineral content of the bone. After 24 days depletion there was no further demonstrable effect. These values considered in combination with those of Table II emphasize the finding that when a normal rat is placed on a low calcium diet, sometime between 4 and 12 days the bones increase in their capacity to accumulate absorbed Calcium 45 and this increased capacity remains fairly constant even though the bones lose considerable mineral through further depletion. It should be noted in consideration of these data that effects due to age in addition to depletion may have been a contributing factor.

Effects of specific activity at absorption site. The specific activity of the calcium available for a given study will determine the mass of calcium which must be administered to an animal to provide sufficient radioactivity for the measurements. It seemed desirable therefore to determine the mass level below which significant effects due to carrier calcium would not be encountered on both the normal and low dietary intake. This information is also necessary for interpretation of data on the factors governing absorption from the tract. In this study 2 groups of litter mate rats, reared as previously described on low and normal calcium rations were dosed orally with the same amount of Ca^{45} activity to which varying amounts of carrier were added as indicated in Table IV.

The results in Table IV indicated that the mass of calcium in the dose had little effect on the absorption of this element. With the exception of an increase in fecal excretion when 20 and 51 mg of calcium were given to the low calcium animals, there was no demonstrable effect. Analysis of tissue samples from these animals also showed no differences due to the mass of carrier calcium used. This is in agreement with the findings previously discussed. A preliminary report to this

TABLE IV. Effects of Mass of Calcium on Excretion of Ca^{45} Orally Administered to Rats on Low and Normal Dietary Calcium Levels (Rats Were 84 Days of Age).

Ration	No. of rats	Dose, mg of Ca	Fecal excretion of Ca^{45}, % of dose
Low Ca	3	.28	.50
	4	1.3	.60
	3	10	.85
	4	20	4.4
	2	51	3
Normal Ca	3	.28	31
	3	1.3	33
	3	10	40
	3	20	33
	3	51	26

effect was published by Hansard *et al.*(7) and essentially confirmed by Carlson(8).

Effects of age. The following study was designed to show quantitatively the effects of age of the animal on Calcium 45 absorption and accumulation in the bone. Four lots of weanling litter mate rats were placed on a normal (Ca-3) ration and at the intervals designated in Table V were given a single oral dose of Calcium 45 and sacrificed after 96 hours for the usual studies. As may be noted from Table V the younger rats excreted less of the Ca^{45} although there was no significant difference between the 6½ and 16-month-old animals in this respect. The calcium and ash content of the femur showed a progressive increase with age, whereas the Ca^{45} accumulation and specific activity showed a progressive decrease with age. From the Ca^{45} accumulation per unit of Ca^{45} absorbed it can be noted that similar values were obtained for the first 3 groups, whereas the 16-month-old animals showed a significant decrease. This may be interpreted to mean that between 1 and 6½ months the capacity for the Ca^{45} to accumulate in the bone from the extracellular fluid was relatively constant, while at 16 months of age this capacity had decreased.

Discussion. It is generally accepted that Ca^{45} introduced into the extracellular fluid undergoes a very rapid exchange with the calcium in the bone(9-11). Without entering into the controversial issues of the relationships of exchange and growth or the mechanisms of retention of Ca^{45} in bone, it is an expected and an established fact that practically all of the Ca^{45} which was originally present in the serum will eventually be found in the bone, from which it has displaced an approximately equivalent amount of stable calcium. As equilibrium is approached, at about 72 hours after oral administration, the specific activity of the skeleton will approach that of the serum, and due to the difference in the calcium content of the two tissues there will be of the order of hundreds of times as much Ca^{45} in the skeleton as in the serum. In any comparative experiment, then, a higher uptake of orally administered Ca^{45} in the skeleton cannot be interpreted as indicating an increased capacity of the bone for uptake of calcium, but may well reflect simply the fact that more Calcium 45 was absorbed from the tract and was thus available for exchange. It is suggested that the bone concentration of Ca^{45} per unit of Ca^{45} absorbed may be an indication of the exchange potential of the bone.

This exchange reaction must also be taken into consideration in the interpretation of excretion data. Where there has been absorption of Ca^{45} from the tract and the subsequent exchange with stable calcium of the bone, the excreta will contain a higher ratio of stable to radioactive calcium in proportion to the degree of exchange which has occurred. This should not be interpreted as an indication of demineralization.

An illustration of how the results obtained may depend upon the strict standardization of experimental conditions is furnished by consideration of the Ca^{45} fecal excretions of the 1-month rats in Tables III and V which were 8.8 and 23% respectively. The only difference between these two groups was that the Table III rats were placed on a low calcium diet after dosage, whereas the others were continued on the normal diet.

Summary. (1) The fecal excretion of orally administered Ca^{45} is a sensitive indication of the calcium status of the rat; (2) placing normal rats on a low calcium diet for as little as 2 days greatly increased their ability to absorb Ca^{45} from the tract; (3) the specific activity of labeled calcium at the site of absorption had little effect upon its absorption; (4) in general, younger rats absorbed more Ca^{45} than did older ones although there was no significant difference between 6½ and 16-

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TABLE V. Effects of Age of Rat on Excretion and Bone Accumulation of Orally Administered Ca^{45}.

Age, mo	No. of rats	Avg body wt (g)	Fecal excretion of Ca^{45}, % of dose	Femur shaft				Ca45	
				Ca^{45}*	mg Ca/g	Specific† activity	% ash	Fraction	Ca^{45} absorbed
1	9	36	23	.57	90	6.3	25	.74	
3	12	171	33	.45	168	2.7	49	.67	
6½	4	312	56	.30	195	1.5	50	.68	
16	6	310	51	.18	210	.86	56	.37	

* μg of labeled Ca per g fresh tissue based on dosage of 10 μg per 100 g body wt.
† μg of labeled Ca × 10^3 per g of total Ca based on above dosage.

month-old animals; (5) for meaningful studies of this type, interpretations must include consideration of exchange reactions, and care must be given to initial selection of diets and animals, preparation of animals for the experiment, and dietary management during the experimental period.

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Inhibition of Urinary 17-Ketosteroid Excretion Produced by "Benemid".* (19103)

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(Introduced by Walter Fleischmann.)

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Benzoic acid(1,2) and carinamide (4'-carboxyphenylmethanesulfonanilide)(3-6) are known to block the renal tubular secretion of penicillin and thus to enhance plasma penicillin concentrations. A new compound, Benemid,† p-(di-n-propylsulfamyl)-benzoic acid has also been found to increase the plasma concentrations of penicillin and para-aminosalicylic acid (PAS)(7-11). Only 2 g of Benemid per day are necessary to produce consistent elevation of plasma penicillin and PAS levels(7-11), whereas 6 to 24 g per day of carinamide were necessary to produce similar results(3-6).

Bissell and colleagues(12) and Ceresa and

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