Having nicked the tail of a rat so as to expose a small drop of blood, the latter is touched with the end of the tube, which then fills by capillarity. The tube holding the blood is again weighed and dropped into a 100-ml glass-stoppered volumetric flask containing about 20 ml of a mixture of the Fischer reagent and methyl alcohol. This mixture has previously been brought to a light cherry color so as to match a color standard. The capillary tube is cracked open while in the reagent by tapping with a glass rod. The water decolorizes the Fischer reagent, and this is back-titrated with more reagent to the original color. The operation takes about 3 min and the end point is definite.

The preparation of solid material is likewise simple. A small piece of tissue (muscle, liver, brain) weighing from 100 to 250 mg is excised and placed upon a tared slip of filter paper. After rapid weighing the paper and tissue both are dropped into the flask containing the reagent. In order to facilitate the extraction of the water, the tissue mass is crushed with a glass rod. Titration then follows. A blank for the water in the filter paper is secured by extracting and titrating a plain piece equal in weight to that used in the experiment.

In order to test the method we examined both whole blood and tissues of normal adult laboratory rats. Samples of blood were taken from 55 rats and titrated with the Fischer reagent. The values for water showed a range of 77–80%, with a mean of approximately 79%, values which correspond very closely to those found by numerous previous investigators who used the standard drying methods.

For tissues we used muscle, liver, and brain. Three rats were sacrificed. From each we took two samples of the tissues mentioned. The water content of one sample of each pair was determined by the titration method. That of the other sample was determined by placing the tissue in an oven at 90° C and drying to constant weight. For the three rats the results were as shown in Table 1, expressed as percent of water.

TABLE 1

Tissue	Percentage water found by	
	Titration	Drying
Muscle	74.1	74.4
	74.1	74.7
	74.6	74.6
Liver	72.1	72.1
	73.2	74.2
	72.4	72.6
Brain	79.2	78.3
	77.1	77.5
	79.1	76.3

The slight variation in estimate of water content found by the two methods appears to be purely random in character. Their accuracy therefore is substantially equal. The advantage of the titrimetric method lies in the rapidity with which the determinations may be made. With it one may not only secure immediately the data pertaining to an extensive array of samples but he may also follow changes in the water content of blood or tissue almost as soon as they occur. The procedure thus lends itself particularly neatly to periodic blood tests or biopsies performed with laboratory animals or even man.

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Bone Implantation as a Means of Studying Vitamin D Action[1, 2]

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The specific action of vitamin D in the body economy is still to be determined; in fact, there is still no agreement as to the locale of its action. In 1924 Shipley (*1*) demonstrated calcification *in vitro,* using nonrachitic serum as a calcifying medium. Fischmann (*2*) noted that rachitic serum would not calcify osteogenic tissue and failed to compensate for the deficiency by addition of calcium and phosphorus. This suggests the possibility that healing of rickets is dependent on the presence of vitamin D or some derivative thereof. Sobel and co-workers (*3*), however, showed that calcification took place *in vitro* without the presence of vitamin D.

It would appear as a result of these excellent *in vitro* investigations that vitamin D exerts its influence on the environment from which bone receives its mineral. An attempt to test the above observations with *in vivo* studies was carried out by using the intact animal as the culture medium.[3]

Tibiae were taken from 1-day-old chicks, and one of each pair was placed under the skin of a 2-week-old rachitic or nonrachitic chick. The bone was inserted alongside the ribs through a small incision, which was immediately sewn up. The host chicks were fed AOAC rachitogenic diet without vitamin D or with 1 unit vitamin D/g of feed throughout the experiment.

After 4 days the implanted tibia was removed and the Ca content compared with that of its nonimplanted mate. The results are shown in Table 1. In the rachitic host the bone lost calcium, whereas in the nonrachitic host a slight amount was gained, indicating the presence of a pathological change in the composition of the body fluid of a rachitic animal.

It should be noted that the loss or gain of Ca shown in Table 1 is the resultant of the amount of Ca that entered and the amount that left the bone through ex-

[1] Presented at the Congress of the International Union of Pure and Applied Chemistry, New York 1951.

[2] Contribution No. 214 of Division of Chemistry, Science Service.

[3] The assistance of L. J. Carter and P. M. Richard with the care and treatment of the animals is gratefully acknowledged.

TABLE 1

CALCIUM CONTENT OF PAIRED CHICK BONES BEFORE AND AFTER IMPLANTATION

	Rachitic host			Nonrachitic host		
	Left tibia (mg Ca)	Implanted right tibia (mg Ca)	Ratio	Left tibia (mg Ca)	Implanted right tibia (mg Ca)	Ratio
	7.80	6.70	.86	7.85	8.12	1.03
	8.05	7.72	.96	8.90	9.45	1.06
	7.38	6.30	.85	7.35	7.40	1.01
	7.60	6.50	.86	6.80	6.85	1.01
	7.82	7.05	.90	7.52	7.38	.98
	9.05	8.10	.90	7.30	8.20	1.12
Mean	7.95	7.06	.89	7.62	7.90	1.04

change, deposition, and solution. An attempt was made to approximate these separate quantities by implanting a bone made radioactive with Ca^{45}. In this experiment 12-day-old rachitic and nonrachitic chicks were made radioactive by injection of 1 μc Ca^{45}. The chicks were killed 48 hr later, and both tibiae removed. One was kept for reference, and the other was implanted, as above, into a 3-week-old rachitic or nonrachitic chick. Seven days later the implanted tibiae were removed, and their content of Ca and Ca^{45} was compared with their nonimplanted mates. The Ca^{45} analyses were carried out according to the method described by Migicovsky and Emslie (*4*).

The summarized results are shown in Table 2. It is seen that the loss and the gain of Ca in the nonrachitic host were less than in the rachitic host, and the ratio of loss to gain was less in the nonrachitic host.

TABLE 2

EFFECT OF RACHITIC STATE ON MOVEMENT OF CALCIUM IN IMPLANTED CHICK TIBIAE

Mean (8 values)	Implanted normal bone		Implanted rachitic bone	
	Normal host	Rachitic host	Normal host	Rachitic host
Net difference* mg Ca	0.48	−2.80	1.62	−2.76
Total loss† mg Ca	4.51	9.61	3.85	8.64
Total gain† mg Ca	4.99	6.81	5.47	5.88
Ratio§	0.90	1.40	0.67	1.46

* Mg Ca/implanted tibia − mg Ca/nonimplanted tibia.

† $\frac{\text{Cpm of nonimplanted tibia} - \text{cpm of implanted tibia}}{\text{Mean specific activity of implanted and nonimplanted tibia}}$.

‡ Net difference plus total loss.

§ $\frac{\text{Total loss}}{\text{Total gain}}$.

A similar experiment was conducted with rachitic and nonrachitic rats, except that nonrachitic femurs were implanted into the peritoneal cavity. The summarized results are shown in Table 3.

This technique of using the intact animal as the culture medium for a bone from another animal has demonstrated that in rickets the changes in the composition of body fluid could be the cause of the rachitic lesions of bone. These lesions could arise by virtue of an increased rate of Ca solution relative to the rate of Ca deposition. The problem of how vitamin D prevents the changes in the body fluid remains to be resolved, although there is strong evidence favoring the absorption mechanism.

TABLE 3

EFFECT OF RACHITIC STATE ON MOVEMENT OF CALCIUM IN IMPLANTED RAT FEMORA*

Mean (4 values)	Implanted normal bone	
	Normal host	Rachitic host
Net difference mg Ca	1.21	0.46
Total loss mg Ca	1.16	3.65
Total gain mg Ca	2.36	4.31
Ratio	.48	.84

* Calculations as in Table 2.

In addition it has been observed that after 7 days the ends of the implanted bone became encapsulated by a cellular tissue which was partially calcified. A similar observation had been made by Bull (*5*) with rabbit bone fragments implanted into abdominal muscle.

It appears that this cellular tissue and the implantation technique herein described could be advantageously employed in the study of the calcification mechanism. Further study along this line is in progress.

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The Critical Frequency of Taste

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One of the classical experiments in the field of gustation is a study reported in 1925 by Allen and Weinberg (*1*), which presents rather unequivocal evidence for four taste systems and their relative sensitivities, based upon the fusion frequency of electrical stimuli applied to the tongue. In the discussion of gustation in a recent important handbook (*2*) these results have been cited in some detail, and considerable weight has been given them. The present authors, however, encountered considerable difficulty in following Allen and Weinberg's reasoning and statistical procedures, and, in the absence of the raw data.