The distribution of the values of the coefficient in any one specimen was not random, but tends to decrease as the position of measurement passes from the crown toward the apex of the root. In order to establish this difference between coronal and root dentin conclusively, the values in these 2 regions were grouped and averaged for each specimen. The differences between the corresponding means for crown and root were 7.4 ± 1.0, 6.4 ± 1.3, 4.2 ± 1.0, and 2.2 ± 1.2 respectively. Consistent with the occurrence of this gradient, the coronal values were scattered far less than were the root values—using the probable error as a criterion of scatter.

It appears, therefore, that the coefficient approaches an upper limit in the coronal dentin of non-carious teeth—which is in fairly close agreement for all 4 specimens—and that the deviation from this maximum value tends to increase with the distance between the position of measurement and the dentino-enamel junction. Dentin formation is known to start at this junction and to proceed toward the root apices. Thus, the gradient in the X-ray absorption coefficient of the dentin appears to parallel the progress of its formation. Whether the gradient results from the pre- or post-eruptive suspension of the calcification process cannot yet be inferred from these observations.

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Volume Concentration of Rat Muscle Measured by the Phosphate Partition Method.

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A simple method, based on the assumed impermeability of muscle cells to phosphate, has recently been described for finding the volume concentration (relative volumes of interspaces and cells) in frog muscle.[1] This investigation was undertaken with the idea that the method might be applicable to rat muscle.

The method used was essentially that of M. G. Eggleton. Adult rats were decapitated, and paired leg muscles (extensor digitorum longus or tibialis anticus) were quickly dissected out and weighed.

[1] Eggleton, M. G., *J. Physiol.*, 1933, **79**, 31.

One muscle of a pair was placed in mammalian Ringer's solution of a given phosphate content, and the other in a solution of a different phosphate content. The phosphate (4 parts Na_2HPO_4 to 1 part KH_2PO_4) was added in M/15 concentration to insure isotonicity. The muscles were left in the solutions for 1, 2, 3, or 9 hours, and at the end of the period they were again weighed and the P of the solution was determined by the method of Martland and Robison.[2] From the weights of the muscles and from the initial and final phosphate concentrations in the Ringer's solution, one can calculate *c*, the initial concentration of phosphate in the interspaces, and *a*, that fraction of the muscle water contained in the interspaces.[3] The volume concentration is (1-*a*), and Eggleton found a mean value of about 23% for frog muscle.

In many cases results similar to Eggleton's were obtained (Table 1.) In these experiments, *a* has the mean value of 0.35 and varies between 0.20 and 0.53. In other experiments (Table II), however, unreasonable or even impossible results were found for *a*. (The two tables do not represent different series of experiments;

TABLE I.

Original P in Ringer, mg./100 cc.		Duration of experiment,	Final P in Ringer, mg./100 cc.			
low	high	hrs.	low	high	*a*	*c*
10	20	1	11.3	21.0	.43	0.54
10	20	1	11.1	21.0	.31	0.83
30	40	2	30.0	39.7	.41	0.30
20	40	1	22.0	41.3	.20	0.85
20	40	1	22.3	40.8	.53	0.52
10	30	1	11.0	30.5	.51	0.37
20	40	1	20.6	40.3	.26	0.52
10	40	1	11.1	40.3	.36	1.49
30	60	9	32.9	62.2	.25	1.69
30	60	9	31.9	61.2	.30	1.24

TABLE II.

Original P in Ringer, mg./100 cc.		Duration of experiment,	Final P in Ringer, mg./100 cc.			
low	high	hrs.	low	high	*a*	*c*
30	40	2	29.7	39.7	—0.03	1.90
30	40	2	29.9	39.7	—0.14	0.39
10	60	3	11.6	61.8	—0.02	18.80
10	30	1	11.0	31.1	—0.05	—1.93
40	80	1	40.3	80.8	—0.22	—0.16
40	80	1	41.1	80.0	0.08	1.67
40	80	1	40.8	80.8	—0.01	—7.33
30	60	9	30.6	60.8	—0.17	0.60

[2] Martland, M., and Robison, R., *Biochem. J.*, 1926, **20**, 847.

[3] The equations are given in Eggleton's paper. Through what seems to be a misprint, *c* is there expressed in mg. per 100 g., instead of in mg. per g.

we have merely separated the results which are comparable with Eggleton's from those which are not). Indeed, an inspection of the figures in Table 1 raises the question as to whether even these are reliable, for the scatter is very great. A similar scatter in the value of a appears in Eggleton's experiments on frog muscle, and close inspection shows that large variations in a and c are very apt to be inherent in the method and can scarcely be avoided. This is because a small error in the phosphate determination makes such a great error in a and c; for example, in one experiment we found a to be 0.25 and c to be 1.69, but if a 1% error is allowed in the phosphate determination, the true value of a might work out at any figure between —0.12 and 0.63, while that of c might be anything from 2.19 to 0.93. To get reliable values for a and c, it is necessary to have the diffusion of a large amount of phosphate determined with great accuracy, and in the case of rat muscle at least, this large amount does not diffuse; considerations of this kind can easily explain the variations in a both in our experiments and in Eggleton's.

Unlike the behaviour of frog muscle as reported upon by Eggleton, there was always some gain in weight in isotonic Ringer's solution by the muscles in our experiments, irrespective of the fact that they were immersed in the phosphate solutions at about 2°C. This probably indicates either a change in the osmotic pressure within the muscle as a whole, or post-mortem changes in some of the cells only, and it is impossible to be sure that the latter do not occur. Some of our values for a, and some of Eggleton's too, are so high that they are best accounted for on the grounds that some of the cells are permeable to phosphate, perhaps as a result of such post-mortem changes, and the fact that most of our rat muscles gave a feeble response to electrical stimulation even after only one hour immersion points in the same direction.

Conclusion. The phosphate partition method used by Eggleton for finding the volume concentration in frog muscle is not satisfactory when applied to rat muscle (extensor digitorum longus and tibialis anticus). The variability obtained appears to be due partly to the extreme sensitiveness of the results to small errors in phosphate determination, and partly to the fact that complete impermeability of the muscle cells, under the conditions of the experiments, is extremely doubtful.