THE PHOSPHORUS CONTENT OF THE BODY IN RELATION TO AGE, GROWTH, AND FOOD.*

By H. C. SHERMAN and E. J. QUINN.

(*From the Department of Chemistry, Columbia University, New York.*)

(Received for publication, February 3, 1926.)

A previous paper from this laboratory[1] has dealt with the results of an extended series of determinations of calcium in the bodies of experimental animals (rats) and especially with the relation of the calcium content of the body to the age, growth, and food of the animal. The white rat was selected as the experimental animal for this study because of its omnivorous food habits, the close resemblance of the chemical processes of human and rat metabolism, the convenient size of the rat for investigations of this character, and the fact that many features of the nutrition and life history of the rat have been extensively studied by numerous investigators.

The constantly increasing recognition of the importance of phosphorus in nutrition, and especially of the intimate connection between calcium and phosphorus in the development of the growing bone, has led us to extend our studies of the mineral elements in food and nutrition to an investigation of the phosphorus content of the body in relation to age, growth, and food, on a plan similar to that followed in our previous studies of the calcium content of the body. The purpose of the present paper is to summarize the results of our analyses with respect to phosphorus and to point out certain relationships between the calcium and the phosphorus content of the body at different stages of growth and development, and as influenced by differences in the food supply and by the nutritional demands of reproduction and lactation.

As in the case of the corresponding study of calcium,[1] the albino rat was used as experimental animal and each animal analyzed was of definitely known family and nutritional history. In all cases

* Published as Contribution No. 505 from the Department of Chemistry, Columbia University, New York.

[1] Sherman, H. C., and MacLeod, F. L., *J. Biol. Chem.*, 1925, lxiv, 429.

dealt with in this paper the food of the experimental animals and of their mothers had consisted of weighed proportions of food materials so finely ground and so thoroughly mixed as to ensure that the actual food intake of each animal was of accurately known composition at all times. The animals were given food and distilled water *ad libitum;* and to prevent any possible consumption of other material they were kept in all-metal cages without bedding.

From among animals raised in this manner, specimens taken for analysis at the different desired ages were chloroformed and (in all cases except those less than 28 days of age) the contents of the digestive tract removed, weighed, and rejected. The gross weight of the animal minus the weight of the contents of the digestive tract was taken as the net body weight.

For the determination of phosphorus, the body of the rat was placed in a silica dish, charred slowly over a free flame, and burned at a dull red heat in an electric muffle to a white or light gray ash. The ash was dissolved with nitric or hydrochloric acid, the solution filtered and made up to volume. An aliquot portion of this solution was then withdrawn, diluted, heated on a steam bath for several hours and until the solution was evaporated to from 30 to 50 cc. This solution was then employed for the determination of phosphorus by the method of double precipitation, first as ammonium phosphomolybdate and then as ammonium magnesium phosphate, with final weighing of magnesium pyrophosphate, all essentially as described in the Methods of Analysis of the Association of Official Agricultural Chemists.

Phosphorus Content of Normal Rats at Different Ages.

Since previous work in this laboratory had shown that our Diet B (Laboratory No. 13), a dry food mixture consisting of one-third whole milk powder and two-thirds ground whole wheat, with sodium chloride in the proportion of 2 per cent of the weight of the wheat, served for normal nutrition generation after generation (some families in our colony being now in the seventeenth generation upon Diet B and distilled water only) the analyses of rats raised on this diet are regarded as furnishing values for the normal phosphorus content of the body at different ages; and by far the greater number of the animals used for the determination of these

normal values were raised on this diet. As in the case of our similar investigation of the calcium content of the body,[1] we have confirmed the choice of animals from this diet for the establish-

TABLE I.

Average Phosphorus Content of Normal Male Rats.

Age.	No. of cases.	Average net weight.	Phosphorus in body.		Probable error of per cent.	Coefficient of variation of per cent.
days		*gm.*	*gm.*	*per cent*		
At birth.	21	4.3	0.0147	0.34	±0.007	14.34
15	31	21.2	0.1032	0.49	±0.003	5.00
28	24	51	0.268	0.53	±0.005	6.53
61	34	142	0.814	0.57	±0.002	2.47
92	18	226	1.399	0.62	±0.005	5.35
119	8	253	1.633	0.65	±0.004	2.67
241	12	304	2.076	0.68	±0.005	3.90
459	12	301	2.138	0.71	±0.009	6.45

TABLE II.

Average Phosphorus Content of Normal Female Rats.

Age.	No. of cases.	Average net weight.	Phosphorus in body.		Probable error of per cent.	Coefficient of variation of per cent.
days		*gm.*	*gm.*	*per cent*		
At birth.	21	4.3	0.0147	0.34	±0.007	14.34
15	18	19.7	0.0959	0.49	±0.003	4.09
28	30	44	0.248	0.56	±0.005	7.36
61	33	108	0.704	0.65	±0.004	5.75
90	14	160	1.091	0.68	±0.006	5.09
120*	7	188	1.296	0.69	±0.006	3.55
235*	10	213	1.573	0.74	±0.007	4.49
154†	4	192	1.268	0.66	±0.018	8.30
267†	15	211	1.419	0.67	±0.007	6.17
559†	15	226	1.704	0.75	±0.008	6.67

* Adult females which had borne no young.

† Adult females which had raised young.

ment of our norm by analyzing a number of rats which had been fed upon slight modifications of this diet and finding that these gave essentially the same results. Hence we conclude that this diet, while it does not give the greatest rate of growth which we are

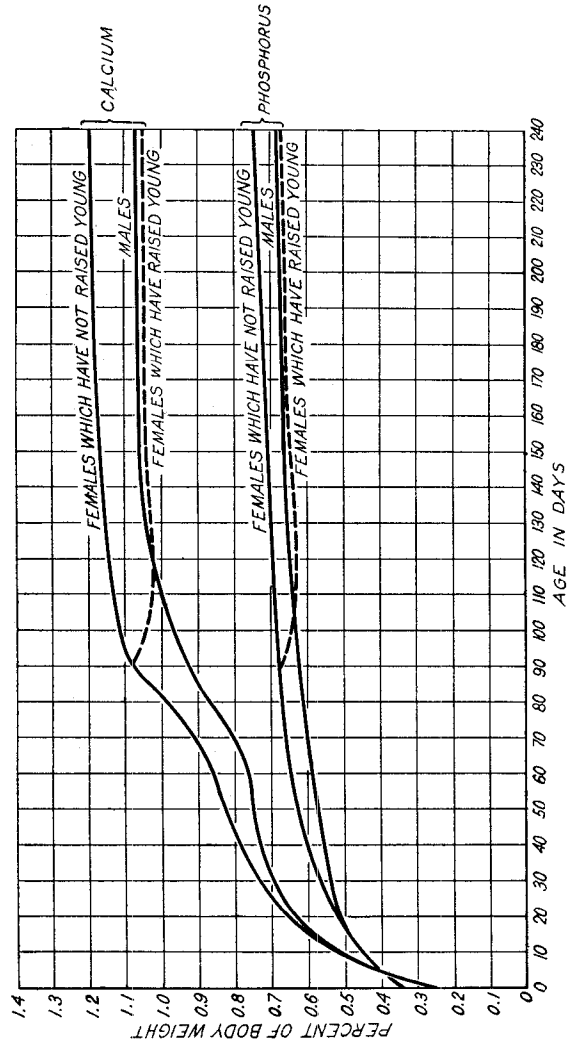

FIG. 1. Changes in the percentages of calcium and phosphorus with age, as shown by analyses of normal albino rats.

able to induce, is well within the range of what should be considered the normal.

Animals were taken for analysis at birth, at 15, and at 28 days, at approximately 60, 90, and 120 days, and at intervals throughout adult life. For purposes of tabulation and charting, all the individuals of the same age, or of a given range of age, are here averaged. The average data are given in Tables I and II and smoothed curves showing the trend of the results in comparison with the corresponding data for calcium are shown in Figs. 1 and 2.

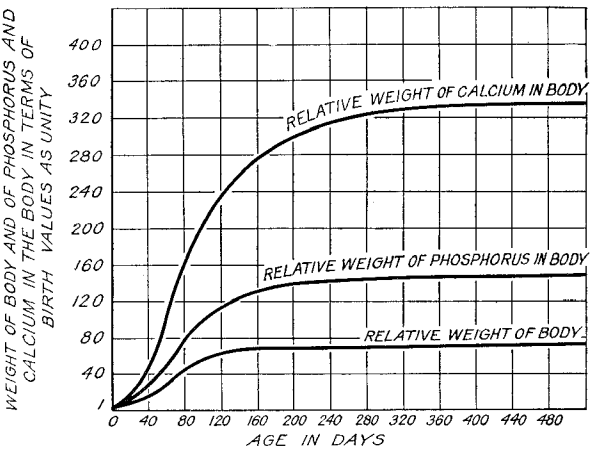

FIG. 2. Relative gains in body weight and weights of calcium and phosphorus in the body, in terms of birth values as unity. (Based upon analyses of normal white rats.)

Taking the data for phosphorus here reported and those for calcium reported in a previous paper[1] it will be seen that at birth the average rat contains 14.7 mg. of phosphorus or 0.34 per cent of its body weight, as compared with 10.8 mg. or 0.25 per cent of calcium. During growth, the body phosphorus increases in greater ratio than the body weight, but not in such great ratio as does the body calcium. Thus the average adult male has about 70 times the body weight with which he was born, whereas this adult body contains about 150 times as much phosphorus and about 340 times as much calcium as did the body at birth (Fig. 2).

During the first 15 days of life the rat increases his body weight

about fivefold, his body phosphorus about sevenfold, his body calcium about twelvefold. This brings the average male rat at the age of 15 days to a body content of 0.49 per cent phosphorus and 0.58 per cent calcium. Thus well within the suckling period the percentage of calcium overtakes and passes the percentage of phosphorus in the body. The percentages of both elements continue to increase steadily throughout growth, the calcium more rapidly than the phosphorus, as is readily seen by a comparison of the corresponding curves for the two elements in Fig. 1.

As was found in the corresponding study of calcium,[1] the females with their less rapid growth and lesser adult size than the males, show smaller amounts but higher percentages of phosphorus; except that the bearing and suckling of young causes a loss of phosphorus as well as of calcium from the body. This results in lowering the average percentages of these elements in the bodies of females which have raised young to a level very slightly below that of males of the same age which have had the same food.

Omitting the females which have raised young, the average percentages of phosphorus found at successive ages beyond the suckling period are: at 28 days, males 0.53, females 0.56 per cent; at 61 days, males 0.57, females 0.65 per cent; at 90 to 92 days, males 0.62, females 0.68 per cent; at 119 to 120 days, males 0.65, females 0.69 per cent; in young adults (about 8 months old), males 0.68, females 0.74 per cent.

It is noteworthy that whether we consider the gains during growth, the comparison of males and females, or the effect upon the females of the bearing and suckling of young, we find that the percentages of phosphorus in the body always show the same trend as those of calcium, though the differences are less pronounced. Undoubtedly this is because the differences in both cases are attributable chiefly to the changing proportion of calcium phosphate in the bones, and to the further fact that this (the calcium phosphate of the bones) constitutes a larger proportion of the total calcium than of the total phosphorus of the body. In the rat the bones contain relatively little calcium phosphate at birth but calcification proceeds rapidly during infancy and continues more gradually throughout the whole period of growth and into early adult life.

It is interesting to consider the changes in the calcium and phos-

phorus contents of the body not only in terms of percentages, but also of atomic ratios.

The new born rat with its undeveloped bones contains, in its body as a whole, about three atoms of calcium to five atoms of phosphorus. During the suckling period, while there is a considerable increase of soft tissue which is presumably of about the same composition as that in the new born, the largest gains of calcium and phosphorus are due to the deposition of tricalcium phosphate in the bones. As tricalcium phosphate contains three atoms of calcium to two of phosphorus the rapid gain of this substance must tend to increase the ratio of calcium to phosphorus in the body as a whole, and correspondingly we find that the ratio which was three atoms of calcium to five of phosphorus in the new born, has before the end of the suckling period become a ratio of approximately one to one. Then for some time the calcification of the bones and the growth of the body as a whole appear to run about parallel so that the body retains about equal numbers of atoms of calcium and phosphorus and the atomic ratio of the two elements remains at about one to one. Thereafter a relatively earlier or more pronounced slackening of the general growth than of the calcification of the skeleton results in another (gradual) increase in the ratio of calcium to phosphorus, up to about six atoms of calcium to five of phosphorus in the body of the fully mature adult. This last ratio corresponds with the general belief that the skeleton contains about 99 per cent of the calcium and nearly four-fifths of the total phosphorus of the adult body.

The evidence, afforded by our analyses, of a more rapid calcification in females than in males coordinates well with the observation recently reported by Hammett[2] of a higher water content in the bones of males than of females of the same age.

The bearing and suckling of young causes a loss of body phosphorus coincident with that of body calcium[1] but less pronounced in degree. The tendency to regain the mineral elements thus lost is also observable about proportionately for the two elements. It seems probable that these losses occur primarily because of the drain of lactation upon the mother's body calcium, causing losses from the bones since there is little other available store of calcium in the body, and that the withdrawal of calcium phosphate from

[2] Hammett, F. S., *J. Biol. Chem.*, 1925, lxiv, 409.

the bones to furnish material for milk production results in simultaneous losses of calcium and phosphorus in about the proportions in which they exist in the calcium phosphate of the bones.

This general conception of the gains and losses of phosphorus, as largely dependent upon deposition or depletion of the calcium phosphate of the bones, is strikingly supported by the data obtained in analyzing for phosphorus the animals which had previously been analyzed for calcium after having been kept upon a diet somewhat low in calcium with or without the addition in some cases of cod liver oil and in other cases of calcium lactate. These are described in the section which follows.

Phosphorus Content of Rats on a Diet Adequate According to Current Standards but of Less Than Optimal Calcium Content, with and without Dietary Supplements.

The experience of this laboratory has shown[3] that a mixture of one-sixth whole milk powder and five-sixths ground whole wheat with table salt (Diet A, Diet 16) is adequate according to current standards since it is able to meet all the nutritional needs not only of growth but also of reproduction and lactation for generation after generation, some families of rats in our colony being now in the fourteenth successive generation on this diet alone. This diet, however, does not induce as good results as are obtained with Diet B which contains a higher proportion of the whole milk powder and which in chemical terms is richer in calcium and in fat-soluble vitamin. On determining the calcium content of animals taken from Diet A at 60 and 90 days of age it was found that this was appreciably below the average for rats taken at the same ages from Diet B[1] and now a similar, though slightly less pronounced, difference is found in the phosphorus content. The results are summarized in Table III. Evidently on Diet A the deposition of calcium phosphate in the growing bones is slightly retarded or at least does not proceed so rapidly as it does on Diet B. Moreover it was found that the data for phosphorus also paralleled those for calcium in the bodies of the rats which had received in addition to Diet A a supplement of fat-soluble vitamin in the form of cod liver oil or of calcium in the form of calcium lactate. The addition of

[3] Sherman, H. C., and Campbell, H. L., *J. Biol. Chem.*, 1924, lx, 5.

the fat-soluble vitamins of cod liver oil to this diet had no appreciable effect upon the storage of calcium or phosphorus in the body, while the addition of calcium resulted in an increased storage of both calcium and phosphorus.

Evidently the "limiting factor" in these cases was the low calcium content of Diet A which retarded the deposition of calcium phosphate in the bones even when extra "calcification vitamin" was supplied by adding cod liver oil to the diet. Because of the shortage of calcium and consequent slowing of the deposition of calcium phosphate, the normal increases both of the calcium and of the phosphorus content of the body were retarded; when extra calcium was supplied, more rapid deposition of calcium and phosphorus was made possible, and, although there had been no in-

TABLE III.

Average Percentages of Phosphorus in Rats Fed on Diet A and Modifications of Diet A.

	Males.		Females.	
Age	60 days.	90 days.	60 days.	90 days.
Diet A	0.51	0.54	0.52	0.57
" " + 1 per cent cod liver oil.	0.49	0.52	0.51	0.57
" " + 1 " " calcium lactate	0.59	0.62	0.59	0.71

crease in the phosphorus content of the food, the phosphorus content of the body was increased.

The converse of this, namely, that a deficiency of phosphorus in food may retard the deposition of calcium phosphate in the bones and thus the storage of both calcium and phosphorus by the body, and that in such case the addition of a phosphate to the diet will induce the increased storage of calcium as well as phosphorus, was demonstrated several years ago in this laboratory[4] and has since been confirmed by many observers in connection with studies of experimental rickets.

It is interesting also to find that notwithstanding the retardation of calcification and therefore of storage of both calcium and phos-

[4] Sherman, H. C., and Pappenheimer, A. M., *Proc. Soc. Exp. Biol. and Med.*, 1921, xviii, 193.

phorus in the rats on Diet A, they were able, when simply continued upon this same diet, to continue their gradual storage until they had finally (after the period of most rapid growth in body weight) attained the same normal percentage of body phosphorus as was found in the rats which had been raised on Diet B and used in establishing the normal averages shown in Table I.

SUMMARY.

Large numbers of normal white rats of known heredity and nutritional history have been analyzed in order to establish the normal phosphorus content for each sex at various stages of growth and development.

The average percentage of phosphorus in the body of the normal white rat is found to increase from 0.34 per cent at birth to about 0.49 per cent at 15 days; 0.53 to 0.56 per cent at 28 days; 0.57 to 0.65 per cent at 61 days; 0.62 to 0.68 per cent at 3 months; 0.65 to 0.69 per cent at 4 months; 0.70 to 0.75 per cent in adult life.

At the age of 15 days and thereafter, the total weight of phosphorus averaged higher in the males than in the females; but only because of their greater average body weights.

Females which had not borne young showed higher percentages of phosphorus than males of the same age and inheritance and of the same dietary history.

Females which had borne and suckled young showed lower percentages of body phosphorus than those which had not raised young. Such animals show a tendency to regain at least a part of the phosphorus lost, apparently somewhat in proportion to the length of time which has elapsed since the last lactation period.

In all of these respects the variations in the phosphorus content of the body show similar relations to the variations in calcium content as reported in a previous paper from this laboratory.

That the gains and losses of calcium and phosphorus, while not arithmetically parallel, are chemically interdependent is further attested by determinations of calcium and phosphorus in animals which had received a diet adequate according to current standards but low in calcium content, with and without supplements of cod liver oil or of calcium lactate. Here it was found that the calcium intake was a limiting factor as regards storage of both calcium and

phosphorus in the body, and that while under the conditions of this investigation cod liver oil did not increase the storage of either of these elements, the storage of both calcium and phosphorus was increased when calcium only was added to the food.

In some respects the results become clearer when considered in terms of the relative proportions of calcium and phosphorus atoms in the body as a whole and in the calcium phosphate of the bones.

In whatever terms expressed, it is striking to find that whereas the body of the rat, in making a normal growth and development from birth to maturity must multiply its original weight by about 70, it must multiply its original phosphorus by about 150, and its original calcium by about 340.

The quantitative relations for all ages from birth to middle age are shown graphically. The data are offered both as a contribution to the fuller understanding of the chemistry of normal growth and development and as a basis for the interpretation of experiments in which the development of the body as a whole, or of the bones specifically, is retarded by dietary deficiency or other abnormality of nutrition.