XV. THE EFFECTS OF CALCIUM GLYCEROPHOSPHATE, SODIUM GLYCEROPHOSPHATE AND SODIUM DIHYDROGEN PHOSPHATE UPON THE SKELETON OF RATS KEPT ON A DIET DEFICIENT ONLY IN FAT-SOLUBLE FACTOR.

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I. HISTORICAL.

MELLANBY [1920, 1921], Korenchevsky [1921, 1922], Goldblatt [1923], Zilva, Golding and Drummond [1924] have shown that a diet deficient in fat-soluble factor produces rickets in puppies, rats and pigs. An increase in the calcium content of such a diet does not cure this type of rickets [Korenchevsky, 1923].

It is also known that human rickets cannot be cured by an increased ingestion of calcium salts. In Orgler's opinion [1911] the capacity to deposit calcium in the bones is lost during the active stage of this disease.

Grosser [1920], however, found that marked calcium retention occurred in rickety children when this element was introduced subcutaneously in the form of a glycerophosphate. As far as we know, Grosser's observations remain unique and this question has not been studied clinically or experimentally since. However, they are not only theoretically important but may be of practical use in the treatment of diseases affecting the calcium metabolism.

II. TECHNIQUE OF THE PRESENT INVESTIGATIONS.

We conducted 19 experiments on 20 litters containing altogether 148 rats. Clearly, for the proposed experiments, only a diet containing an adequate amount of calcium and phosphorus could be used. Therefore, during the experiment, our animals were kept on a rickets-producing diet deficient only in fat-soluble factor, because other similar diets are either deficient in calcium or phosphorus. In order to discover whether the compound calcium glycerophosphate or the glycerophosphate radical alone is the influencing factor, we divided each of our litters into two or more of the following groups.

Group I. Control group of rats kept on a diet deficient in fat-soluble factor.

Group II. Rats on the same deficient diet, a solution of calcium glycerophosphate being injected subcutaneously.

Group III. Rats on the same diet, a solution of sodium glycerophosphate being injected subcutaneously.

Group IV. Rats on the same diet, a solution of sodium dihydrogen phosphate being injected subcutaneously.

Group V. Rats on the same diet, but to which has been added calcium glycerophosphate (to show the influence of the ingestion of calcium glycerophosphate).

Two diets, — A and — A₂, were used, both being deficient only in the fat-soluble factor; the composition of these diets is given in our previous papers [Korenchevsky, 1923; Korenchevsky and Carr, 1924]. These diets contained an abundant amount of phosphorus, *i.e.* 0.51 % in the fresh paste, and differed only in their calcium content. — A diet contained an adequate amount of calcium (0.26 % in fresh paste), — A₂ diet an excessive amount of calcium (0.52 % in fresh paste). These different diets were employed to ascertain if an excessive ingestion of calcium might not affect the results obtained.

In two experiments, Nos. 750 and 751 (Table I), the amount of calcium in the — A diet was increased, not by the addition of CaCO₃ as was done in — A₂ diet, but by the addition of calcium glycerophosphate, so that the fresh paste contained about 1.6 % of this salt. This diet contained 0.47 % Ca and 0.68 % P in the fresh paste.

The technique of the experiments was, on the whole, similar to that used in our previous investigations. The control rats always belonged to the same litter as those on glycerophosphate salts. We had great difficulty to choose a suitable preparation of calcium glycerophosphate. Grosser used Kahlbaum's salt which was soluble to about 5–6 %. But the salts now prepared by this firm as well as by English firms are only about 2.5 % soluble and are of different texture, and, when injected into rats, very often produce indurations followed by ulcerations. We were fortunate enough to obtain some of Kahlbaum's pre-war salt, which was 6 % soluble in water. This specimen of calcium glycerophosphate when injected in a 3 % watery solution was usually supported well by the rats.

As calcium glycerophosphate is more soluble in cold than in hot water it is difficult to sterilise a solution of it. Moreover we found that when dissolved in cold water, it becomes partly precipitated at 37.5° after about 24 hours from a 4 % solution and from a 5 % solution after about 4–5 hours. From this it is to be presumed that some precipitation probably occurs when such concentrations are injected subcutaneously. This probably explains the cause of the indurations and ulcerations produced, especially easily in young and weak rats on deficient diets, when 4 and 5 % solutions of this salt are injected subcutaneously. Rats on a normal diet or those put on deficient diets at an older age support injections of 4 % solutions of Kahlbaum's calcium glycerophosphate much better.

In our experiments a 3 % solution warmed to about 37° proved the most suitable. 1–2 cc. of this solution, according to the size of the animal, were

injected slowly into the subcutaneous tissue four to five times a week, the place of injection being altered each time. Without all these precautions indurations and necrosis were produced at the places of injection. In some of our first experiments on larger animals we began by injecting 3.5–4 % solutions which we finally replaced by 3 %. Kahlbaum's calcium glycerophosphate which we used contained 14.72 % Ca and 11.34 % P.

We also used Kahlbaum's sodium glycerophosphate which contained 10.006 % P. A solution was prepared for injection, 1 cc. of which contained the same amount of phosphorus as did 1 cc. of the calcium glycerophosphate solution used for corresponding animals of the same litter. For example, when animals from one group of the litter were injected with a 3 % solution of calcium glycerophosphate, others from the same litter were injected with a 3.4 % solution of the sodium salt (in the experiments Nos. 833, 845, 1163, 1007, 1041). As sodium glycerophosphate may possibly be excreted by the body more rapidly than the calcium salt, in experiment No. 1204 (Table II) the rats were injected twice daily with a 6 % solution of sodium glycerophosphate. In this way we tried to keep the concentration of the sodium salt in the body as high as possible. The strength of the sodium dihydrogen phosphate (NaH_2PO_4) solution was determined on the same principle as that of the sodium glycerophosphate solution. In experiments Nos. 1226 and 1233, 2.35 % solutions of the sodium phosphate and 6 % of sodium glycerophosphate were injected once daily. Neither of these sodium salts produced any harmful local or general effects upon the rats injected.

Details of the experiments and of the results obtained are clear from Tables I–III. The theoretical calcium requirement during the period of injection, viz. 1 % of the gain in weight during that time, based on a method mentioned elsewhere [Korenchevsky, 1922], was calculated in some experiments so as to show what proportion of the calcium required normally for complete calcification of the skeleton was provided in the form of calcium glycerophosphate.

III. INGESTION OF CALCIUM GLYCEROPHOSPHATE.

The influence of the ingestion of calcium glycerophosphate was studied in two small litters each containing two control rats fed on — A diet and two fed on this same diet with the addition of calcium glycerophosphate (see Table I). No difference was observed between the chemical composition of the skeleton of the animals in these two groups; the ingestion of a nearly doubled amount of calcium in the form of calcium glycerophosphate did not influence calcification at all.

The calcium ingested by the rats on — A diet and more especially by those on the calcium glycerophosphate diet was well in excess of the theoretical requirement. The average total consumption of calcium by a rat on the calcium glycerophosphate diet from litter 750 was 2.5 g. and from litter 751 was 2.4 g.

The ratio of Ca : P (1 : 1.45) in the calcium glycerophosphate diet was a good one for normal calcium metabolism.

Table I. *The influence of the ingestion of calcium glycerophosphate upon the growth and skeleton of rats kept on — A diet.*

No. of experiment	No. of rats	Duration of administration of Ca G.P. in days	Total amount of Ca administered as Ca G.P. g.	Final average weight of rats in g.		In bones			Approximate theoretical calcium requirement in g.
				Males	Females	H ₂ O %	Fresh Ca %	Dry Ca %	
750 controls	2	—	—	102	94	49.9	8.4	16.8	—
„ Ca G.P.	2	46	1.175	116	72	49.6	8.4	16.6	0.48
751 controls	2	—	—	126	97	46.7	8.9	16.8	—
„ Ca G.P.	2	39	1.071	105	98	48.3	8.8	16.9	0.47
<i>Normal rats of the same age:</i>									
—	—	—	—	—	—	43.8	11.5	20.5	—

Note. In this and the other tables the following abbreviations are used:

Ca G.P. Calcium glycerophosphate.
Na G.P. Sodium glycerophosphate.
Na P. Sodium dihydrogen phosphate (NaH₂PO₄).

Therefore (1) this experiment confirms Korenchevsky's [1923] previous conclusions that the ingestion of increased quantities of calcium does not improve the rachitic calcium impoverishment of the skeleton of rats on a diet deficient in fat-soluble factor, provided that this diet already contains an adequate amount of calcium.

(2) This experiment also shows that calcium glycerophosphate is of no more value than other calcium salts for calcification of the skeleton when taken *per os*.

IV. INJECTION OF CALCIUM GLYCEROPHOSPHATE INTO RATS KEPT ON — A DIET.

This series consisted of five experiments conducted on 37 rats, 22 of which were injected and 15 were controls (see Table II).

The experiment was started before the growth of the animals was arrested, because otherwise it was difficult to keep the animals alive for a sufficient length of time to give the necessary number of injections, although with rats on — A₂ diet this was done successfully in four cases (see below). The period of injection lasted from 1–3 months. The injections were begun when the animals commenced to grow more slowly and all gained in weight during the injection period (see Table). Therefore the results obtained might be attributed more to the preventive influence of the injections than to their power of curing skeletal disorders already developed. Theoretically one would expect that if the store of fat-soluble factor was not completely exhausted the injected calcium could be retained more easily by the organism and deposited in the skeleton. However, as will be shown later, there was not any special difference between the results obtained from rats which had ceased to grow and those which continued to grow during the period of injection.

When the total amount of calcium glycerophosphate injected was small, 0.08 g. and less, no influence was produced upon the skeletons of the rats (see litter 803, Table II). But when a larger amount (total of 0.17–0.22 g.) of this salt was injected in litters 787, 800, 809 and 811, calcification of the bones of these animals was much better than that of the control animals from the same litter. Comparing the injected with the control animals there was an average increase in the former of 13.4 % in calcium content of the fresh skeleton (minimal 5.4 % in litter 809 and maximal 20.7 % in litter 811) and of 14.6 % in the dry bones (minimal 8.3 % in litter 809 and maximal 20.9 % in litter 787). The water content of the bones changed very little, the average being an increase of 1.1 %.

Table II. *The influence of injections of calcium and sodium glycerophosphate and sodium dihydrogen phosphate upon the growth and skeleton of rats kept on — A diet (Ca content of fresh diet 0.26 %).*

No. of the experiment and of group	No. of rats	Gain (+) or loss (–) in weight in g. during period of injections		Total amount of Ca and P injected g.		In bones			Approximate theoretical calcium requirement in g.
		Male	Female	Ca	P	H ₂ O %	Fresh Ca %	Dry Ca %	
803 control	4	+29	+1	—	—	45.6	8.8	16.1	—
„ Ca G.P.	7	+29	+9	{0.04– 0.08}	—	48.6	8.4	16.3	0.1–0.3
809 control	4	+33	+26	—	—	45.2	9.3	16.9	—
„ Ca G.P.	4	+33	+29	0.17	0.13	46.3	9.8	18.3	0.3
787 control	2	+23	+19	—	—	45.1	8.2	14.7	—
„ Ca G.P.	4	+35	+11	0.18	0.14	44.9	9.8	17.8	0.25
800 control	3	+50	+41	—	—	39.7	11.1	18.4	—
„ Ca G.P.	3	+71	+50	0.20	0.15	40.7	12.0	20.2	0.6
811 control	2	+19	+30	—	—	41.0	8.2	14.0	—
„ Ca G.P.	4	+27	+19	0.22	0.16	40.6	9.9	16.7	0.23
1204 control	4	+24	+28	—	—	45.1	8.5	15.4	—
„ 3.4 % Na G.P.	3	+49	+27	—	0.11	45.6	8.9	16.4	—
„ 6.0 % Na G.P.	4	+45	+23	—	0.29	46.2	8.7	16.1	—
1226 control	2	+27	+7	—	—	49.8	7.4	14.7	—
„ 2.35 % Na P.	3	+12	+17	—	0.13	47.1	7.7	14.4	—
„ 6 % Na G.P.	2	+10	+5	—	0.13	47.4	7.7	14.7	—
<i>Normal calcification at the ages of litters:</i>									
Nos. 803, 1204 and 1226	—	—	—	—	—	43.8	11.5	20.5	—
Nos. 787, 800, 809 and 811	—	—	—	—	—	36.8	14.2	22.4	—

The calcium content of the skeletons of the injected animals never reached the level found in rats of the same age on a normal diet (see Table II). This was especially clear in one litter (811) in which the amount of calcium injected was nearly as much as the total calcium requirement of the rats during this period. It is probable that some of the injected calcium glycerophosphate is excreted by the body before there has been time for the deposition of the calcium in the skeleton. *A priori*, more satisfactory results would be expected were it possible to inject larger amounts of calcium. Technically it was difficult to inject a larger amount of the salt (1) because the rats on the deficient diet become emaciated rather quickly and die and (2) because it is impossible to inject a saturated solution of the salt.

The daily injection of such large volumes of calcium glycerophosphate solution as 1.5 cc. and 2 cc. (for larger animals) did not impair the general nourishment of the rats. On the contrary, in several cases the weights of the treated animals were sometimes greater than those of the controls. From the weight figures it is difficult to draw any definite conclusion about the influence of these injections upon the growth.

V. INJECTION OF CALCIUM GLYCEROPHOSPHATE INTO RATS
KEPT ON — A_2 DIET.

Sixty-two rats belonging to nine litters were used for this series of experiments, 35 animals were injected and 27 remained as controls (see Table III). The duration of the injection period varied from about 1 to $1\frac{1}{2}$ months, during which time 0.1–0.16 g. Ca and 0.08–0.12 g. P were injected in the form of calcium glycerophosphate solution into each animal. The results obtained were similar to and even more pronounced than those of the previous series in spite of the doubled amount of calcium in the diet. In four (869, 879, 833 and 845) of the nine litters growth practically ceased; sometimes it increased a little during the first half of the injection period and nearly always decreased during the second half. In the other five litters (1213, 1169, 1163, 1007 and 1041) the rats grew during most of the experiment, but towards the end the growth was slower and in several cases stopped. There was no essential difference between the results obtained in these two groups of litters. It is difficult to draw any conclusions concerning the effect of the injections upon the growth of the animals.

The least pronounced influence of the injections upon the skeleton was noticed in litter 1213, to each animal of which was only administered a small amount of calcium glycerophosphate containing 0.1 g. Ca and 0.08 g. P. In litter 1041 the deficient diet produced only a slight calcium impoverishment of the skeleton and the rats grew well owing to the good food given to the mother during pregnancy and lactation. One rat even became pregnant, which is unusual for animals on a diet deficient in fat-soluble factor. These facts suggest that there was a greater storage of fat-soluble factor in these animals than in those of other litters and so a fairly high calcification of the skeleton resulted in spite of the deficient diet. Because of this the injections caused the calcification of the skeleton of the treated animals to be nearly normal. The calcium content of the dry bone was normal, but the water content was greater, being 42.1 % instead of 39 %, making the calcium in the fresh bone lower than that of the normal animal (12.5 % instead of about 13.2 %).

The following figures show the average percentage changes produced by the injections in all the other litters. The calcium content of dry bone was 18.4 % (minimal 10.9 % in litter 1007 and maximal 27.7 % in litter 845) higher in the injected animals than in the controls. Similarly in fresh bone there was an increase of 16.5 % in the calcium content (minimal 7.2 % in litter 869 and maximal 27.5 % in litter 845). In most cases the water content

of the bones was influenced slightly, being higher by about 2 % than that of the controls. Only in one litter, 869, did the skeletons of the injected animals contain much more water (12.1 %) than the controls.

Table III. *The influence of injections of calcium and sodium glycerophosphates and sodium dihydrogen phosphate upon the growth and skeleton of rats kept on — A₂ diet (Ca content of fresh diet 0.52 %).*

No. of experiment and of group	No. of rats	Gain (+) or loss (—) in weight in grams during period of injections		Total amount of Ca and P injected g.		In bones		
		Male	Female	Ca	P	H ₂ O %	Fresh Ca %	Dry Ca %
1213 control ...	2	+22	+27	—	—	48.0	8.6	16.4
„ Ca G.P. ...	3	+26	+25	0.1	0.08	50.2	9.0	18.0
869 control ...	4	0	— 5	—	—	44.6	8.3	14.9
„ Ca G.P. ...	6	— 1	— 3	0.15	0.11	50.0	8.9	17.8
879 control ...	3	— 13	— 6	—	—	44.2	8.3	14.9
„ Ca G.P. ...	4	—	+ 2	0.15	0.11	46.3	9.6	17.9
1169 control ...	3	+21	+22	—	—	45.6	9.2	16.9
„ Ca G.P. ...	4	+33	+29	0.15	0.11	43.7	11.1	19.4
833 control ...	3	— 5	— 9	—	—	47.2	8.0	15.1
„ Na G.P. ...	4	— 8	— 5	—	0.11	47.4	8.4	15.9
„ Ca G.P. ...	4	0	— 6	0.15	0.11	48.7	9.1	17.8
845 control ...	3	— 4	— 9	—	—	51.5	6.9	14.1
„ Na G.P. ...	3	— 1	— 4	—	0.11	49.2	8.0	15.8
„ Ca G.P. ...	3	— 6	+ 1	0.15	0.11	51.2	8.8	18.0
1163 control ...	4	—	+12	—	—	46.6	8.7	16.2
„ Na G.P. ...	4	—	+17	—	0.11	44.6	9.8	17.6
„ Ca G.P. ...	4	+31	—	0.15	0.11	47.1	10.1	19.1
1007 control ...	2	+30	+24	—	—	45.3	10.5	19.2
„ Na G.P. ...	3	+26	+13	—	0.12	42.2	11.4	19.7
„ Ca G.P. ...	3	+28	+12	0.16	0.12	43.9	12.0	21.3
1041 control ...	3	+68	+92*	—	—	43.2	11.5	20.2
„ Na G.P. ...	3	+69	+65	—	0.12	42.9	11.5	20.4
„ Ca G.P. ...	4	+90	+49	0.16	0.12	42.1	12.5	21.5
1233 control ...	2	—	+18	—	—	47.4	7.6	14.5
„ 2.35 % Na P. ...	2	—	+19	—	0.13	48.5	8.0	15.4
„ 6 % Na G.P. ...	2	+27	+20	—	0.13	51.8	7.3	15.2
<i>Normal calcification at the ages of litters:</i>								
Nos. 1213 and 869	—	—	—	—	—	43.8	11.5	20.5
Nos. 833, 845, 879, 1007, 1041, 1163 and 1169 ...	—	—	—	—	—	39.0	13.2	21.5

* This was one female rat which had become pregnant.

Table IV. *Percentage changes above (+) or below (—) the controls in the composition of the skeleton of rats injected with sodium and calcium glycerophosphates.*

No. of litter	Na G.P. group In bones			Ca G.P. group In bones		
	H ₂ O %	Fresh Ca %	Dry Ca %	H ₂ O %	Fresh Ca %	Dry Ca %
833	+0.4	+ 5.0	+ 5.3	+3.2	+13.7	+17.9
845	—4.5	+16.0	+12.1	—0.6	+27.5	+27.7
1163	—4.3	+12.0	+ 8.6	+1.1	+16.1	+17.9
1007	—6.8	+ 8.6	+ 2.6	—3.1	+14.3	+10.9
1041	—0.7	0	+ 1.0	—2.5	+ 8.7	+ 6.4
Average	—3.2	+ 8.4	+ 5.7	—0.4	+16.1	+16.2

It is interesting to note that in most cases the water content of the bones of the rats injected with calcium glycerophosphate is either the same or higher than that of the controls notwithstanding the improvement in the calcification. This shows that, although calcium glycerophosphate caused an increase in the calcium deposited in the skeleton of rats on a diet deficient in fat-soluble factor, it did not produce such a general improvement in the general composition of the bones as is caused, for instance, by the fat-soluble factor.

VI. INJECTION OF SODIUM GLYCEROPHOSPHATE AND OF SODIUM DIHYDROGEN PHOSPHATE INTO RATS KEPT ON — A OR — A₂ DIETS.

This series consisted of eight experiments in which 33 rats were injected. In five of these experiments, Nos. 833, 845, 1163, 1007 and 1041, the same 15 rats were used as controls as were mentioned above in Section V; each of these five litters was divided into three groups: controls, those injected with calcium, and those injected with sodium glycerophosphate (Table III). The sixth experiment, litter No. 1204, was divided into the following three groups: 4 control rats, 3 injected with 1.0 cc. of 3.4 % solution of sodium glycerophosphate once daily, and 4 injected with 1.0 cc. of 6 % solution of the same salt twice daily. This latter series of injections was made in order that the animals might retain as much sodium glycerophosphate as possible. The aim of the last two experiments, Nos. 1226 and 1233, was to compare the influence upon the calcification of the skeleton of injections of sodium glycerophosphate with those of sodium dihydrogen phosphate. Four rats were used as controls, 5 were injected with 1 cc. of 2.35 % solution of sodium dihydrogen phosphate and 4 rats with 1 cc. 6 % solution of sodium glycerophosphate once daily. Two (Nos 1204 and 1226) of these eight experiments were performed on rats kept on — A diet and the remaining six on rats kept on — A₂ diet. No difference was found in the results obtained from the injected rats on whichever of these two diets they were fed. If the influence of the injections of sodium glycerophosphate is examined in all the 28 rats treated, the following figures show the average percentage changes in the chemical composition of the bones above (+) or below (—) that of the controls.

The calcium content of dry bone was 5 % higher in the injected animals than in the controls (minimal 0 in litter 1226 and maximal + 12.1 % in litter 845). Similarly in fresh bone there was an average percentage increase of 5.5 % in the calcium content (minimal 0 in litter 1041 and maximal + 16 % in litter 845). The water content in all cases was slightly influenced, being on an average — 0.9 %. In five litters (Nos. 833, 1041, 1204, 1226 and 1233) the changes were within the usual fluctuations found in the calcification of the skeleton of rats kept on a diet deficient in fat-soluble factor.

Table IV is constructed to show the comparative value of calcium and sodium glycerophosphates in the calcification of the skeleton of rats kept on a deficient diet. In it are summarised the results obtained in those five litters

mentioned above which were divided into three groups: (1) control rats, (2) those injected with calcium glycerophosphate, and (3) those injected with sodium glycerophosphate. These groups were discussed separately above. When these figures are compared (Table IV) and most particularly the average figures, it will be seen quite clearly that the injection of the calcium salt always produced a more striking effect than that of the sodium salt. The effect on calcification of the former is approximately twice as great as that of the latter. In some litters this difference is found to be even greater (litters 833, 1041, and in the calcium content of the dry bone in litter 1007). When the average percentage changes in the composition of the skeleton of all 28 rats injected with sodium glycerophosphate are compared with those of 47 rats injected with about a corresponding amount of calcium glycerophosphate the difference becomes even more striking than that in Table IV. In the first group (sodium glycerophosphate) the average percentage changes in the bones when compared with the controls were as follows: H_2O - 0.9 %, Ca in fresh bone + 5.5 % and Ca in dry bone + 5.0 %, in the second group (calcium glycerophosphate) these changes were + 0.6 %, + 15.3 % and + 17.1 % respectively.

Unfortunately, up to the present time, our experiments with sodium dihydrogen phosphate are not conclusive owing to the few rats used for the injection treatment and to the small changes and contradictory results obtained. On the basis of our previous experiences such results may only be the fluctuations usually obtained in the composition of the skeleton of rats kept on this deficient diet. In experiment 1226 (Table II) sodium phosphate did not produce any influence upon the calcification of the skeleton, whereas in experiment 1233 (Table III) the animals injected showed a slightly higher calcium content of the skeleton than the controls. In neither litter was there any appreciable difference between the effect of sodium dihydrogen phosphate and that of sodium glycerophosphate.

In a paper published simultaneously with this, Robison and Soames have found evidence in one experiment of a slight increase in the calcification of the skeleton of rats kept on — A diet after subcutaneous injections of sodium glycerophosphate, while no such increase was found after the injection of sodium phosphate [1925].

We cannot enter into a theoretical explanation of the mechanism resulting in the increase of the calcification of the skeleton after subcutaneous injections of these glycerophosphates. Robison and his co-workers [1923, 1924], who have made many interesting experiments on this subject at the Lister Institute, put forward the following theory. There is present in ossifying cartilage an enzyme which hydrolyses certain phosphoric esters including glycerophosphoric ester. By this reaction the concentration of inorganic phosphate is increased locally and calcium phosphate deposits in the bone.

VII. HISTOLOGICAL INVESTIGATION.

In all control rats kept on — A or — A₂ diets we found slight rickets with osteoporosis or even only the latter, because the mother rats were well fed during pregnancy and lactation. As we have mentioned several times before, it is difficult to draw any definite conclusions concerning slight histological changes observed in experimental rats, when the controls show only mild rickets and osteoporosis. In rats on — A diet the histological pictures vary sometimes to a large degree principally because of more or less pronounced osteoporosis. Notwithstanding the great difference in the histological pictures, the chemical composition of the skeleton of such rats is very similar in most animals belonging to the same litter. Therefore, when only a slight influence of some agent is applied to rats on this deficient diet, the chemical results are found much more reliable and objective than the histological ones.

Bearing these limitations in mind, we may summarise the histological results of the present experiments shortly as follows. Injections of sodium phosphate and sodium glycerophosphate as well as the ingestion of calcium glycerophosphate did not alter the histological structure of the bone of the rats. When the total amount of calcium glycerophosphate injected was not smaller than 0.15 g., a definite improvement was observed in the pathological changes in the bones of about half of these animals.

Therefore, although in a lesser degree, the histological results corroborated those obtained chemically.

CONCLUSIONS.

1. The ingestion of calcium glycerophosphate, like that of other calcium salts, did not cure the rachitic calcium impoverishment of the skeleton found in rats kept on a diet deficient in fat-soluble factor only.

2. Subcutaneous injections of a watery solution of calcium glycerophosphate considerably increased the degree of calcification of the skeleton of rats kept on the same deficient diet.

3. The water content of the bones of the rats injected with calcium glycerophosphate was the same as, or higher than, that of the controls in spite of the improvement in the calcification.

4. Similar injections of sodium glycerophosphate produced in most cases the same effect as the calcium salt, but in a much less degree.

5. In about 50 % of the rats injected with calcium glycerophosphate a slight improvement of the rachitic changes in the skeleton was found histologically, which was not observed in animals injected with sodium glycerophosphate.

6. Therefore, the presence of calcium in the compound calcium glycerophosphate injected into rats on a diet deficient in fat-soluble factor seems to be of considerable importance for the greater deposition of calcium in the skeleton.

7. Calcium glycerophosphate may probably be considered as a weak therapeutical agent in the treatment of rickets produced by a deficiency of fat-soluble factor, as compared with cod-liver oil, after the ingestion of which the composition and structure of the rachitic bone of such animals returns to the normal.

8. These facts suggest a difference in the mechanisms of calcification induced in the skeleton of rats on a diet deficient in fat-soluble factor by administration of calcium glycerophosphate and of cod-liver oil respectively.

9. Our results agree with those of Grosser, who showed some retention of calcium in rachitic children after subcutaneous injections of calcium glycerophosphate.

10. No change or only a small increase in the calcification of the skeleton compared with that of controls was found in 5 rats injected with sodium dihydrogen phosphate. This increase was within the limits of the usual fluctuations observed in rats kept on the deficient diet.

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