LVI. EXPERIMENTAL RICKETS IN RATS ON A PURIFIED SYNTHETIC DIET DEFICIENT IN PHOSPHORUS AND FAT-SOLUBLE ORGANIC FACTOR¹.

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INTRODUCTION.

SHERMAN and PAPPENHEIMER [1921] and later, Pappenheimer, McCann, Zucker and Hess [1921] induced the development of severe experimental rickets in rats by a diet (No. 84) consisting of 95 % patent flour and 5 % salt mixture. This diet was not only deficient in phosphorus and fat-soluble A, but was also very obviously deficient in many other respects. McCollum, Simmonds, Shipley and Park [1921], by means of diets (No. 3143 and No. 3127) composed of complex natural food substances deficient in phosphorus and the fat-soluble organic factor, also succeeded in inducing the development of severe rickets in rats. Later, McCollum, Simmonds, Becker and Shipley [1922], using diets (No. 3407 and No. 3408) composed essentially of purified food substances and deficient only in phosphorus and the fat-soluble organic factor, again succeeded in producing severe rickets in rats. On the other hand, Korenchevsky [1921] failed to produce rickets in rats by feeding them on a purified synthetic diet, referred to as — A-P diet, deficient in phosphorus and the fat-soluble organic factor. He suggests that the discrepancy between his results and that of the other authors “is no doubt due to the difference in phosphorus content in the basal diets used.” But the following table shows that his — A-P diet had a lower percentage of phosphorus than some of the diets of McCollum and his co-workers. In a later communication, Korenchevsky [1922] recognised this but laid emphasis on the fact that the composition of the diet in his experiments was entirely different from those of McCollum and his co-workers.

¹ In this communication the term “fat-soluble organic factor” is used to denote the deficiency of the diet in both the fat-soluble growth-promoting and anti-rachitic organic factors. During the process of purification of the caseinogen by the heat and oxidation method, presumably both factors were completely destroyed.

Table I.

	Diet	Phosphorus %	Calcium %	Weight ratio calcium : phos- phorus
Sherman and Pappenheimer	84	0.087	0.553	1 : 0.15
McCollum, Simmonds, Shipley and Park	3143	0.301	1.231	1 : 0.25
McCollum, Simmonds, Becker and Shipley	3407	0.217	1.202	1 : 0.18
Do. Do. Do. Do.	3408	0.088	1.202	1 : 0.07
Korenchevsky	-A-P	0.22	0.256	1 : 0.86

Table I shows that in all but the - A-P diet there is a very definite disproportion between the calcium and phosphorus contents, the former being relatively high. Now McCollum and his co-workers [1921] have drawn particular attention to this disproportion in the calcium-phosphorus ratio as an important factor in making their diets severe rickets-producing ones, and have suggested that the "physiological relation in the diet between the calcium and phosphate is of infinitely greater importance in insuring normal calcification than the absolute amount of the salts themselves." Sherman and Pappenheimer [1921] also state that "the quantitative relations of the inorganic ions rather than an absolute deficiency of any one of them may have been the determining factor" in the causation of the rickets which was induced by their diets Nos. 83 and 84.

It seemed probable that the absence of this disproportion between calcium and phosphorus in the - A-P diet of Korenchevsky might account for his failure to produce rickets with it. Korenchevsky, in his monograph on Rickets [1922], admitted the possibility of such an explanation. The following experiments were therefore performed in order to elucidate this point.

Experimental.

Each of two litters of rats was divided into three groups. One group was put on the following diet which is similar¹ in almost every respect to the - A-P diet of Korenchevsky, and will also be referred to as - A-P.

- A-P diet				
Caseinogen (purified)	...	...	...	20 g.
Starch	...	...	...	50 "
Cotton-seed oil (hardened)	...	...	...	15 "
Salt mixture (No. 4 [Korenchevsky, 1922])	...	...	...	5 "
Marmite	...	...	...	5 "
Lemon juice	...	...	...	5 cc.
Distilled water	...	...	...	50 "

Another group was put on the same diet, to which, however, was added sufficient calcium carbonate to raise the calcium-phosphorus ratio from 1 : 0.88 to 1 : 0.20. This will be referred to as the "- A-P + Ca" diet.

A third group was put on a control diet which was the same in all respects as the - A-P diet described above, with the exception that the salt mixture contained sufficient phosphorus to make good the deficiency. In order to supply the necessary amount of the fat-soluble organic factor, five drops of

¹ Korenchevsky's - A-P diet contained only 18 % caseinogen and orange, instead of lemon, juice.

a very potent cod-liver oil were administered daily to the rats. One-fifth of a drop of this oil given daily was sufficient to promote growth in rats on a fat-soluble A deficient diet and to protect rats on McCollum's diet No. 3143 from developing rickets. The percentage of calcium and of phosphorus in these three diets and their ratios by weight are given in Table II.

Table II.

Diet	Calcium %	Phosphorus %	Ratio by weight calcium : phosphorus
Normal	0.25	0.54	1 : 2.16
- A-P	0.25	0.22	1 : 0.88
- A-P + Ca	1.08	0.22	1 : 0.20

The detailed results given in Table III show that in each litter the rats on the normal diet were normal in every respect. The bones of the rats on the - A-P diet showed only a slight degree of osteoporosis histologically, and a deficiency of 12 % in the calcium. This agrees with the results obtained with the same diet by Korenchevsky. But all the rats on the - A-P + Ca diet developed rickets of from moderate to very severe degree. This was evident even grossly and was verified in every case by histological examination of the ribs. In these rats, chemical examination of the bones (femur, tibia and fibula of both legs) showed in every case a very great deficiency in calcium as compared with those on the normal and - A-P diets. As is usual in rickets, the percentage of water in the bones of the rats on diet - A-P + Ca was greatly increased (see Table III).

As part of another investigation, 16 rats from three litters were put on the - A-P + Ca diet. In every rat rickets developed which varied in degree from moderate to very severe. The average percentage of calcium in their bones was 6.36 % per dry and 14.38 % per wet weight of the bones. This is mentioned here merely to show that the - A-P + Ca diet can be depended upon to produce some degree of rickets in practically every young rat fed on it for from six to eight weeks.

Another group of 14 young rats from four litters were put on the - A-P + Ca diet, but with the caseinogen replaced by cooked commercial egg-white. Egg-white contains less phosphorus than caseinogen, so that this diet had only 0.08 g. of phosphorus per 100 g. of the wet paste. On this diet the rats all developed severe rickets in from four to five weeks, more rapidly therefore than on the - A-P + Ca diet which contained caseinogen. The average percentage of calcium in their bones was also lower, being 4.22 % per wet and 10.30 % per dry weight of the bones. But the two groups are not strictly comparable, since they were not from the same litters. Whether the greater rickets-producing power of the egg-albumin diet was due to the lower percentage of phosphorus or to the different protein, or to both, cannot be stated.

Table III.

No. of rat	No. of litter	Sex	Diet	Initial age days	Final age days	Days on diet	Initial weight g.	Final weight g.	Gain in weight g.	Av. amount of food eaten daily g.	In bones				Histological diagnosis
											% H ₂ O	% Ca wet	% Ca dry		
G 906	XXVII	♀	Normal	37	99	62	33	104	71	12.6	41.65	13.13	22.28	Normal	
G 907	"	♀	"	37	99	62	38	111	73	12.6	39.98	12.51	21.52	"	
G 913	XXVIII	♂	"	26	77	51	32	179	147	14.7	41.17	12.46	21.17	"	
G 914	"	♂	"	26	77	51	32	113	81	14.7	40.57	12.08	20.36	"	
Averages											40.84	12.54	21.53		
G 908	XXVII	♀	- A-P	37	99	62	32	99	67	12.4	43.67	12.12	21.52	Nearly normal	
G 909	"	♀	"	37	99	62	31	115	84	12.4	41.06	11.85	20.15	Osteoporosis slight	
G 915	XXVIII	♀	"	26	77	51	34	99	65	—	39.75	11.39	18.89	Nearly normal	
G 916	"	♂	"	26	77	51	36	103	67	—	45.99	9.35	17.32	Osteoporosis moderate	
G 917	"	♂	"	26	77	51	36	88	52	—	41.29	10.39	17.69	"	
Averages											42.35	10.92	19.40		
G 910	XXVII	♀	- A-P + Ca	37	99	62	28	68	40	8.9	52.35	7.15	15.06	Rickets slight (healing)	
G 911	"	♀	"	37	99	62	27	62	35	8.9	53.00	7.84	16.71	" moderate	
G 912	"	♂	"	37	99	62	30	94	64	8.9	52.82	7.67	16.28	" severe	
G 918	XXVIII	♂	"	26	77	51	40	62	22	10.3	57.86	6.26	14.86	" moderate	
G 919	"	♂	"	26	77	51	37	74	37	10.3	58.33	5.30	12.74	" severe	
G 920	"	♀	"	26	77	51	38	79	41	10.3	53.77	6.62	14.35	"	
Averages											54.68	6.80	14.99		

DISCUSSION.

From the results shown in Table III it is quite evident that the mere addition of calcium carbonate to Korenchevsky's — A-P diet was sufficient to transform it from a non-rickets-producing into a very definite rickets-producing diet. The addition of the calcium carbonate raised the proportion of calcium to phosphorus in the diet from 1 : 0.80 to 1 : 0.20. Whether it is the increase in the calcium content, and, consequently, the greater disproportion between the two elements calcium and phosphorus, or merely the increased basicity which is responsible for this change in the character of the diet cannot be stated. The administration to some of the rats of the — A-P diet with sodium carbonate instead of calcium carbonate added to it might have helped to clear up this point. The investigations of McCollum, Simmonds, Shipley and Park [1922] led them to the belief that the reaction of their diets did not affect their rickets-producing power. They found that the substitution of calcium chloride, which contains a non-oxidisable acid radical, for the carbonate or lactate, which are both potential neutralisers of acid in the body, had no effect on the rickets-producing character of their diets. On the other hand, Zucker, Johnson and Barnett [1922] state that the acid-base ratio of their diet was of the utmost importance in determining its rickets-producing quality. The substitution of calcium chloride for the calcium lactate, or the addition of ammonium chloride to the diet, had the effect of changing it from a rickets-producing to a non-rickets-producing one, while the addition of sodium carbonate to their non-rickets-producing diet changed it to a severe rickets-producing one. In view of these divergent results the influence of the acid-base ratio on the rickets-producing quality of a diet must still remain an open question.

CONCLUSIONS.

To a purified synthetic diet low in phosphorus and the fat-soluble organic factor, the addition of calcium carbonate in such an amount that the proportion of calcium to phosphorus was raised from 1 : 0.88 to 1 : 0.20 had the effect of changing it from a non-rickets-producing to a rickets-producing one.

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