

## EXPERIMENTAL RICKETS IN RATS.\*

### I. A DIET PRODUCING RICKETS IN WHITE RATS, AND ITS PREVENTION BY THE ADDITION OF AN INORGANIC SALT.

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PLATES 10 TO 17.

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It is remarkable that, in the experimental study of rickets from the dietary point of view, the rat has been so little used as an experimental animal. With the exception of the recent papers of McCollum and his associates, we find no record of a direct attempt to induce rickets in these animals by modification of the diet.

This is the more surprising in view of the fact that the occurrence of rickets in rats maintained under laboratory conditions has been known to pathologists since the publication of Morpurgo (1) in 1901. The essential identity of the lesions with those of human rickets has been amply demonstrated by Morpurgo (2) himself, by Schmorl (3), Weichselbaum (4), Hohlbaum (5), Iselin (6), Pappenheimer (7), and especially by the detailed studies of Erdheim (8). None of these workers standardized or controlled the diet of the animals which they studied. Morpurgo was interested in the supposed infectious origin of the disease; Iselin, Hohlbaum, and Erdheim, in its relation to the parathyroid gland; Pappenheimer, in the supposed influence of thymus extirpation on the production of rickets.

The recent paper of McCollum, Simmonds, Parsons, Shipley, and Park (9), which appeared while our work was in progress, deals specifically with the influence of deficient diets upon the production of rickets in rats. A number of diets are cited, deficient in various respects, upon which presumably the rats developed rachitic lesions. However, although the particular deficiencies are pointed out, it is not definitely stated which diets led to the development of rickets. Nor is reference made in the legends to the illustrations of rachitic

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lesions, to the particular diets which produced them. Since this paper is a preliminary one, one may assume that these data will be forthcoming in subsequent publications. The authors do not commit themselves to the particular dietary deficiencies concerned, stating<sup>1</sup> that:

“Any suggestion regarding the absence of a specific antirachitic substance or deficiency of either fat-soluble A or calcium as the primary agent in the production of rickets would be ill considered and might be far from the truth.”

In the second paper, by Shipley, Park, McCollum, Simmonds, and Parsons (10), the curative effect of the addition of cod liver oil to the diet is demonstrated. The two rations used in these curative experiments were No. 2638, “low in Ca, Na, and Cl ions, as well as in fat-soluble A;” and Ration 2677, containing an adequate amount of calcium, but “low in the fat-soluble A.” In this paper, a more definite statement is made as to the factors concerned in the production of the rachitic lesions, as follows:<sup>2</sup>

“Previous experience with the rat had taught us that by the use of faulty diets, especially certain diets deficient in the so called fat-soluble A or in both that substance and calcium, the cartilage and adjacent portions of the metaphysis of the long bones of the extremities could be rendered entirely free from calcium deposits and a condition identical with the rickets of human beings be obtained.”

There are obvious advantages in the use of rats for the experimental study of this disease. Aside from the close resemblance of the lesions to those of human rickets, the ease with which controls of the same litters can be obtained, the rapid development of the lesions, the fact that variations in susceptibility, possibly due to differences in breed, can be eliminated, the possibility of working with large numbers of animals, and the economy of space and expense, and finally the ease with which histological examinations of the bones can be carried out—all these considerations make the rat an ideal experimental animal for the study of the disease.

In continuing, by means of feeding experiments upon rats, the study of the mineral elements, which has engaged the attention of one of us for the past 15 years, we have found a relatively simple diet which, as far as our experience goes, leads regularly to the production of marked rickets. It has been further found that the introduction of 0.4 per cent secondary potassium phosphate ( $K_2HPO_4$ ) in place of an equal weight, *i.e.* replacing about one-seventh, of the

<sup>1</sup> McCollum, Simmonds, Parsons, Shipley, and Park (9), p. 340.

<sup>2</sup> Shipley, Park, McCollum, Simmonds, and Parsons (10), p. 344.

calcium lactate contained in the rickets-producing diet, has completely prevented the development of the rachitic lesions, although without influence upon the growth and body weight. Our experiences are summarized in Table I.

TABLE I.

Diet No.	Composition of diet.		Total No. of rats examined.	
			(a) Rachitic.	(b) Non-rachitic.
83	Patent flour,	95.0 per cent.	2	0
	Calcium lactate,	3.0 " "		
	Sodium chloride,	2.0 " "		
84	Patent flour,	95.0 " "	13	0
	Calcium lactate,	2.9 " "		
	Sodium chloride,	2.0 " "		
	Ferric citrate,	0.1 " "		
85	Patent flour,	95.0 " "	0	15*
	Calcium lactate,	2.5 " "		
	Sodium chloride,	2.0 " "		
	<i>Potassium phosphate</i>	0.4 " "		
	Ferric citrate,	0.1 " "		

\* Five of these placed on special diet at age of 60 days; one at 81 days; all remaining animals started at 4 weeks of age.

The diagnosis in each case has been based not only upon the gross changes found in the thorax and long bones at autopsy, but also upon the microscopic examination of several ribs (Figs. 1 to 5), incompletely decalcified (5 to 7 days in Müller's fluid). The histological criteria of rickets were: (1) the great increase in the width of the zone of preparatory cartilage, and its irregular projection towards the diaphysis; (2) the failure of calcium deposition in this zone of preparatory calcification; (3) the pronounced increase in the osteoid tissue, both in the region of the metaphysis and along the shafts of the bones. Most of the rats showed spontaneous infractions, visible especially on the internal surface as callus beads and especially numerous in the lower ribs near the chondrocostal junctions.

It was found that the diagnosis could be readily established during life by x-rays (Figs. 6 to 8). In the rachitic rats there was plainly seen in the head of the tibia a clear zone immediately beneath the

epiphysis, 1 or 2 mm. in width and concave towards the shaft. The normal controls or rats in which the potassium phosphate was included in the diet showed a distinct sharp line in place of this rarefied area.

*Illustrative Protocols.*

*Rat 1750.*—Put on Diet 83 at 29 days of age.

*Record of Body Weight.*

Age, days.....	29	36	43	50	57	64	71	78	85	92	94
Weight, gm.....	59	46	49	50	54	49	52	52	49	52	Died.

*Gross lesions.*—Extreme pallor of eyes and mucous membranes. Slight corneal opacity. A little bloody exudate at inner canthi. *Skeletal system.*—Thorax: On the left side there is an extraordinary deformity. The costal margin is at a higher level than on the right side, and the liver shows a corresponding upward displacement. This is caused by a deep linear infolding of the ribs on the left side, beginning at the seventh and extending to the eleventh. The angulation is at the junction of bone and cartilage, and slightly external to the attachment to the left leaf of the diaphragm. There is no marked swelling of the chondrocostal junctions. Visible on the internal surface of the right wall of the thorax, about 2 to 4 mm. from the junctions of the bone and cartilage, there are pearly bead-like thickenings of the shafts of the ribs, extending from the fifth to the tenth rib inclusive. On the left side, the angulation, seen from within, appears to be just proximal to the cartilage, and there is the same nodular formation, which is more evident from the external surface, but is visible also from within. Long bones: Normal. *Lungs and abdominal viscera.*—Show no noteworthy change. *Spleen.*—Small and flat.

*Microscopic Examination.*—*Rib.*—There is great increase in the width of the zone of preparatory cartilage, which extends towards the shaft in the form of two deep prolongations in which the linear arrangement of the hypertrophic cartilage cells is entirely lost. Calcium deposition in the matrix of the cartilage is imperfect in the proximal portion and completely absent in the distal part. There is a dense spongiosa occupying the region of the metaphysis, and composed wholly of broad trabeculae of osteoid, with only an occasional central remnant of calcified bone. The blood vessels penetrate the cartilage irregularly. The cortex is broad, and the calcified bone surrounded both on its periosteal and endosteal surfaces by a wide osteoid margin. The bone marrow shows no fibrosis. *Femur.*—There is increased width of the proliferating cartilage at both extremities, with deficient calcification of the matrix. The spongiosa is dense, and the trabeculae are surrounded by wide osteoid zones. An excess of osteoid is present along the corticalis, especially about the perforating canals. Viscera show no lesions of interest.

*Diagnosis.*—Rachitis.

*Rat 2605.*—Put on Diet 84 at 28 days of age.

*Record of Body Weight.*

Age, days.....	28	31	38	45	52	59	60
Weight, gm.....	34	31	32	34	36	34	Died (weight 33).

*Gross Lesions.*—*Eyes.*—Cornea steamy at margin. Ingrowth of blood vessels from periphery of left eye. *Thorax.*—No gross deformity. There are definite early rachitic changes at the chondrocostal junctions; namely, increased width of zone of growing cartilage, perforating blood vessels, and irregular calcification in preparatory zone. *Femur.*—Cuts easily. Cartilage at lower epiphysis broader than normal. Spongiosa thickened. *Teeth.*—Opaque; hemorrhages in gums at roots of incisors. *Viscera.*—Normal.

*Microscopic Examination.*—*Ribs.*—Zone of preparatory calcification four to twenty-five cells deep with lateral projections into spongiosa. Latter composed exclusively of dense trabeculae of osteoid, surrounded by well formed osteoblasts; hyperemic marrow vessels. There is great excess of osteoid along the cortex.

*Diagnosis.*—Rachitis, marked.

*Rat 2603.*—Of same litter; maintained on Diet 84; also showed marked rachitic lesions.

*Rat 2611.*—Put on Diet 84 at 29 days of age.

*Record of Body Weight.*

Age, days.....	29	30	37	44	51	58
Weight, gm.....	35	33	33	35	37	Died (weight 34).

*Gross Lesions.*—*Eyes.*—Normal. *Thorax.*—No gross deformity or callus along ribs. Chondrocostal junction slightly beaded and definitely irregular, with increased translucency, and widening of zone of preparatory cartilage. Marked beading at vertebral extremity of ribs. Marrow hyperemic. *Femur.*—Shows broad zone of cartilage and cuts with less than normal resistance. *Teeth.*—Opaque. No deformity. *Viscera.*—Postmortem changes; not taken for section.

*Microscopic Examination.*—*Rib.*—(Müller's fluid for 5 days.) Zone of preparatory cartilage fifteen to twenty-five cells deep, irregular; calcification of matrix imperfect. Dense spongiosa near cartilage, composed almost wholly of osteoid surrounded by active osteoblasts; wide osteoid margin in shaft. Marrow spaces narrow; vessels hyperemic. Periosteum and perichondrium thickened over chondrocostal swelling.

*Diagnosis.*—Rachitis.

*Rats 2615 and 2617.*—Of same litter; maintained on Diet 84; also showed marked rachitic lesions.

*Rat 2606.*—Put on Diet 85 at 28 days of age.

*Record of Body Weight.*

Age, days.....	28	31	38	45	52	59	60
Weight, gm.....	34	33	36	36	38	35	Died (weight 33).

*Gross Lesions.*—*Eyes.*—Clear corneæ. *Thorax.*—No deformity, beading, or callus. Junction sharp and clear. Opaque zone of preparatory calcification visible. *Femur.*—Cuts with greater resistance than that of No. 2605. *Viscera.*—Normal. *Teeth.*—Opaque.

*Microscopic Examination.*—*Rib.*—(Müller's fluid for 5 days.) Zone of preparatory calcification four to eight cells deep. Calcium deposit in matrix normal. Slight irregular projections in one or two places. Ossification about cartilage fairly active; cortex thin, completely calcified—osteoid margin, not wider than normal controls of same age. Spongiosa rarefied, marrow very hyperemic.

*Diagnosis.*—Normal bones, showing inactive osteogenesis.

Rat is from same litter as Nos. 2603 and 2605.

*Rat 2612.*—Put on Diet 85 at 29 days of age.

#### *Record of Body Weight.*

Age, days.....	29	30	37	44	51	58	60
Weight, gm.....	39	37	39	42	42	43	Died (weight 38*).

\* May have lost weight after death.

*Gross Lesions.*—*Eyes.*—Normal. *Thorax.* No swelling of chondrocostal junction; line straight, with opaque transverse band corresponding to zone of provisional calcification. *Femur.*—Firm; normal epiphyseal line. *Viscera.*—Normal, save for congestion and patches of collapse in lungs.

*Microscopic Examination.*—*Rib.*—(Müller's fluid.) Practically normal line. Preparatory zone two to three cells deep. Adequate calcification of ground substance. Osteoid invisible; osteogenesis inactive. Marrow cavity wide and hyperemic. Spongiosa scant.

*Diagnosis.*—Normal bone.

Rat 2616, of same litter, maintained on Diet 85, showed normal bones, whereas Rats 2611, 2615, and 2617, the remaining members of the litter, on Diet 84, developed rickets.

#### *Calcium Content of the Bodies of Rachitic and Non-Rachitic Rats.*

Quantitative determinations of the total calcium contained in the bodies of rats of similar origin, feeding, and age to those described in the protocols, have also been made. Since not less than 99 per cent of the calcium in the body belongs to the bones, the calcium content of the body necessarily runs closely parallel with the growth and ossification of the skeletal tissue. A normal rat of 28 to 30 days of age may be expected to contain about 0.7 to 0.8 per cent of calcium, or about 0.3 gm. in a rat weighing about 40 gm. In rats which have been kept upon the experimental diets here described, the percen-

tages of calcium are somewhat more variable and their interpretation is complicated by the unavoidable differences in size of the animals, and, therefore, presumably in their calcium content at the time of placing upon the experimental diet, and by the fact that the final body weight is sometimes lower than the maximum weight attained by such an experimental animal. Nevertheless, in all comparable cases thus far examined, it has been found that Diet 85, although containing less calcium than Diet 84 has induced a larger assimilation and retention of calcium by the body, evidently as a result of the favorable influence of the potassium phosphate fed. This may be illustrated by the data of Rats 2613 and 2614 which were of the same litter as Rats 2611 and 2612 described in the protocols. Rat 2613, which had remained practically stationary in body weight (32 to 36 gm.) on Diet 84, contained 0.311 gm. of calcium, while Rat 2614, which also remained practically stationary in weight (34 to 38 gm.), but was fed Diet 85, showed 0.475 gm. of calcium. Thus, although growth in body weight was not induced by Diet 85, it did improve the assimilation of calcium to such an extent that the calcium of the body became fully 50 per cent higher than in the parallel case on Diet 84.

#### DISCUSSION.

It would be unprofitable to discuss at this time the specific factors in Diets 83 and 84, which may be responsible for the production of the rachitic lesions. These diets are inadequate for growth in the amount and character of the protein, as well as in their content of fat-soluble A. They contain liberal amounts of calcium lactate and sodium chloride and only a small amount of potassium. Experiments to determine the effect of adding various deficient substances to this basic rachitis-producing diet are now in progress.

The definite protective action of the potassium phosphate, when substituted for a part of the calcium lactate, is also not as yet to be explained; and we would carefully avoid the conclusion that rickets in these cases is due necessarily to a deficiency of potassium or of phosphorus. The quantitative relation of the inorganic ions, rather than an absolute deficiency of any one of them may be a determining factor, and it may well be that under certain conditions of diet in

which there is an unbalanced quantitative relationship of the organic as well as the inorganic foodstuffs, rickets may develop.

However, our experiments seem to demonstrate that rickets may be induced or prevented without change in either the protein or vitamin components of the diet; the presence of an adequate amount of calcium also in itself does not afford protection against the disease. These facts appear to us to be firmly established by the experiments, and lead us to question the importance attributed by some writers to the deficiency of fat-soluble A and calcium in the production of rickets. Further specific evidence bearing upon the rôle of fat-soluble A will be presented in a subsequent paper.

#### CONCLUSIONS.

1. A simple diet is presented which regularly induced rickets in young rats.

2. The substitution of 0.4 per cent secondary potassium phosphate for a small part of calcium lactate in this diet completely inhibited the development of rickets.

3. Quantitative determinations of calcium in the bodies of parallel rats showed a marked increase of calcium content in the rats receiving the added phosphate over those which developed rickets.

4. While it is thus shown by x-rays and by histological examinations and by quantitative chemical analysis that added potassium phosphate increased the assimilation and normal deposition of calcium, it may be the quantitative relationship between the inorganic ions rather than actual deficiency of any one of them which was here the determining factor in the cause or prevention of rickets. Our experiments and conclusions do not exclude the possibility of other causes of rickets than those here discussed.

We are indebted to Dr. J. M. Steiner for assistance in the x-ray examinations and to Miss F. L. MacLeod for the quantitative determinations of calcium.



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## EXPLANATION OF PLATES.

*RC*, resting cartilage; *Pr<sub>1</sub>*, zone of growing cartilage; *pr<sub>2</sub>*, zone of preparatory calcification; *Pr<sub>s</sub>*, prolongation of cartilage into spongiosa; *Sp.*, spongiosa; *Os*, osteoid tissue; *Co*, calcified corticalis; *CB*, calcified bone; *C*, calcified cartilage; *Po*, periosteum; *M*, medullary cavity.

## PLATE 10.

FIG. 1. Rat 1750. Diet 83. Section of rib showing rachitic changes.

## PLATE 11.

FIG. 2. Rat 2605. Diet 84. Section of rib showing rachitic changes.

## PLATE 12.

FIG. 3. Rat 2611. Diet 84. Section of rib showing rachitic changes.

## PLATE 13.

FIG. 4. Rat 2606. Diet 85. Section of rib. Normal chondrocostal junction.

## PLATE 14.

FIG. 5. Rat 2612. Diet 85. Section of rib. Normal chondrocostal junction.

## PLATE 15.

FIG. 6. Rat 2623. Diet 84. Radiograph showing rachitic changes at upper epiphysis of tibia.

## PLATE 16.

FIG. 7. Rat 2624. Diet 85. Radiograph. Normal epiphyses.

## PLATE 17.

FIG. 8. Rat 2625. Maintained on Diet 13, adequate for growth. Same litter as Rats 2623 and 2624. Radiograph. Normal epiphyses.

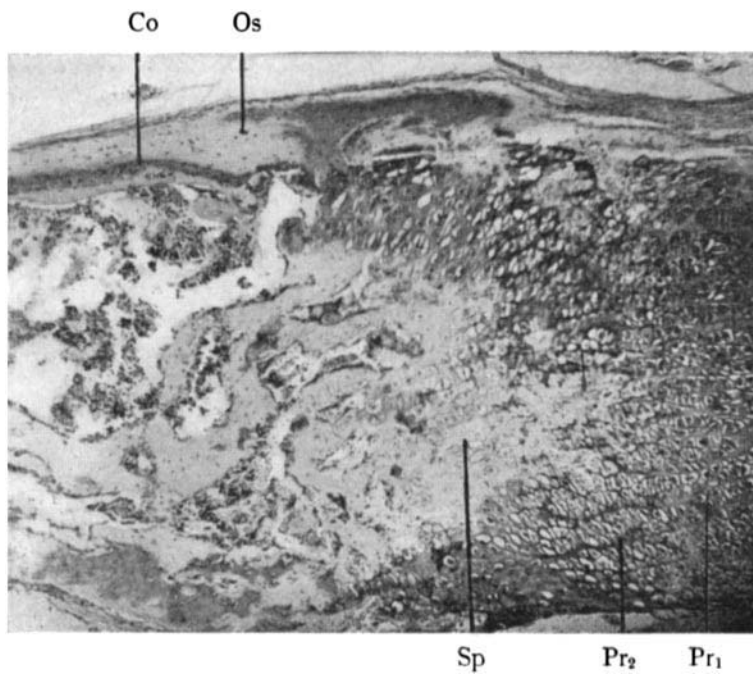


FIG. 1.

(Sherman and Pappenheimer: Experimental rickets. 1.)

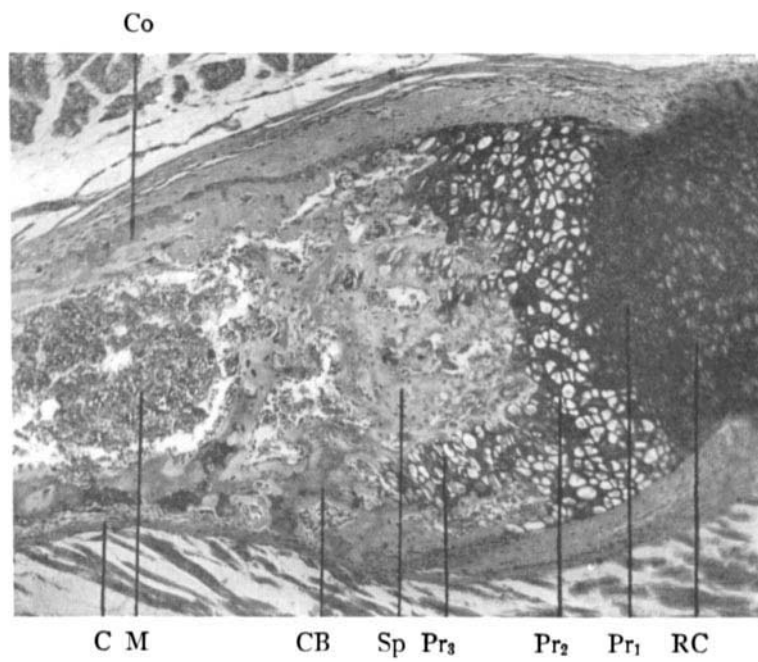


FIG. 2.

(Sherman and Pappenheimer: Experimental rickets. I.)

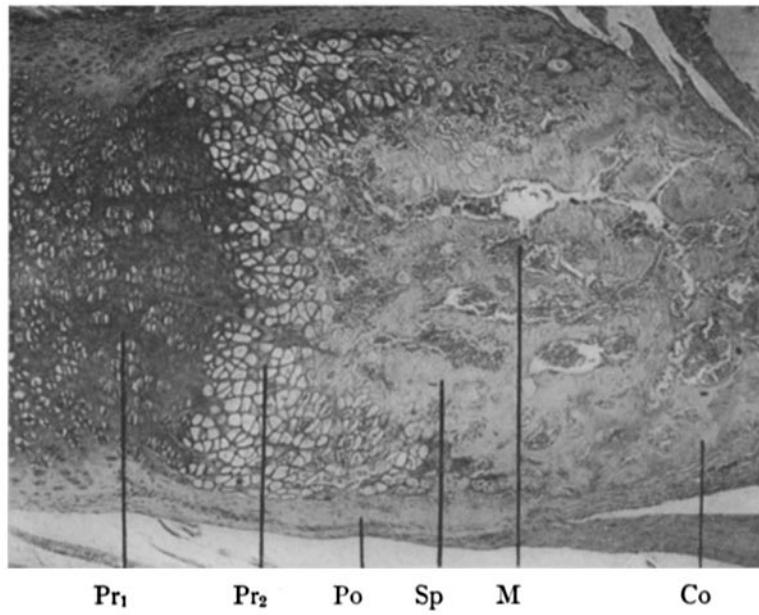


FIG. 3.

(Sherman and Pappenheimer: Experimental rickets. I.)

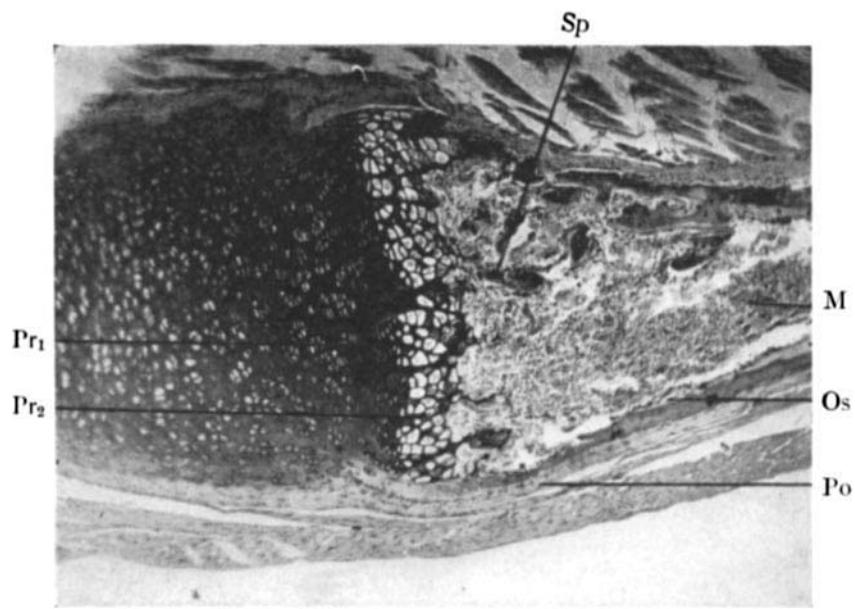


FIG. 4.

(Sherman and Pappenheimer: Experimental rickets. I.)

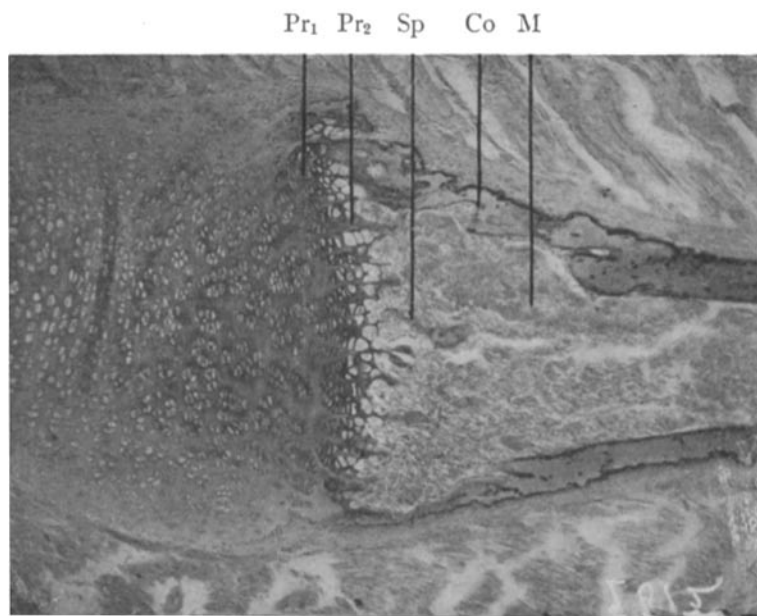


FIG. 5.

(Sherman and Pappenheimer: Experimental rickets. I.)



FIG. 6.

(Sherman and Pappenheimer: Experimental rickets. 1.)





FIG. 7.

(Sherman and Pappenheimer: Experimental rickets. I.)



FIG. 8.

(Sherman and Pappenheimer: Experimental rickets. I.)