Virchows Arch. Abt. A Path. Anat. 346, 330—344 (1969)

The Effect of Strontium Administration on Bones and Teeth of Rats Maintained on Diets with Different Calcium Contents

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Received October 12, 1968

Der Einfluß von Strontium auf Knochen und Zähne von Ratten bei verschiedenen Kostformen mit unterschiedlichem Calciumgehalt

Zusammenfassung. Junge Ratten zeigen bei calciumarmer Kost, selbst wenn Vitamin D in der Nahrung enthalten ist, am Skelet wie an den Zähnen rachitische Veränderungen. Wird der Nahrung noch Strontium beigegeben, so entwickelt sich eine sehr schwere Rachitis. Es kommt zu rachitischen Veränderungen selbst dann, wenn der Calciumgehalt der Diät genügend ist, um eine Rachitis zu verhindern. Bei weiterer Steigerung der Calciumzusätze verwischen sich die rachitischen Veränderungen. Strontiumzusätze zu calciumarmen Kostformen führen zu einer markanten Senkung des Serumcalciumspiegels. Dagegen bleiben die Blutphosphatwerte unverändert. Erhalten die Ratten bei einer calciumarmen Kost mit Strontiumzusätzen während eines dreiwöchigen Versuches mindestens während 9 Tagen Calciumgluconat-Injektionen, so zeigt das Skelet rachitische Veränderungen in Heilung. Bei diesen Versuchstieren ist der Blutcalciumspiegel signifikant erhöht. Die Senkung des Blutcalciumspiegels durch Strontiumzusätze zur Nahrung ist für die Entwicklung von Rachitis von entscheidender Bedeutung. Darüber hinaus muß doch auch mit anderen Einwirkungen des Strontiums gerechnet werden, beispielsweise auf örtliche Faktoren die den Knochenumbau beeinflussen.

Summary. In experiments on young rats with different levels of calcium intake it was found that rachitic changes in the bones and teeth developed when the diet contained very small amounts of calcium even though vitamin D was present in the food. When strontium was added to these diets more pronounced rickets developed. In animals receiving diets with a calcium content sufficient to prevent rickets, and also when the calcium intake was optimal, the addition of strontium resulted in rachitic changes. By increasing the calcium level of the diet with the strontium level kept constant these changes could be mitigated. Addition of strontium to the diet significantly depressed the blood calcium value in rats receiving low calcium diets when compared with controls on the same calcium intake. The blood phosphate remained unchanged.

Animals with a low calcium intake and with strontium added to the diet, and which were given injections of calcium gluconate during the last 9 days of a 3 week period, exhibited healing rickets. In these animals the blood calcium level was significantly increased.

The influence of addition of strontium to the diet on the blood calcium level is a fundamental factor in the development of strontium rickets but other effects have also to be considered, such as the influence of strontium on local factors in bone remodelling.

Introduction

The influence of strontium on different functions of the body has been widely studied during recent years (for ref. see LENIHAN *et al.*, 1967). One aspect of these problems is the effect of administration of strontium on mineralized tissues

(cf. EGER and LAUP, 1953; STOREY, 1961, 1962; ENGFELDT *et al.*, 1962). In a previous paper our group reported on the effect of strontium intake on rats maintained on a very low calcium diet (ENGFELDT *et al.*, 1962). Findings were described to the effect that strontium combined with a low calcium diet resulted in skeletal and dentinal changes in conformity with those of classic rickets. These changes were more advanced in strontium treated animals than in animals given a low calcium diet alone. It was suggested that this could be attributed to an adverse effect of strontium on intestinal absorption of available calcium. In the study reported below these problems were elucidated further by a series of dietary experiments in rats where the morphology of mineralized tissues and the blood chemistry were studied.

Material and Methods

Albino rats 3—4 weeks old were put on diets with different contents of calcium. Two different rat strains were used. Rats of the Sprague-Dawley strain were studied with diets at all calcium levels. In additional experiments at the lowest calcium level Wistar rats were used. These latter rats were offspring from a strain used by the laboratory which supplied them for testing the vitamin D content of food.

The *basic diet* used was that recommended by WRETLIND and ROSE (1950) but modified with respect to calcium carbonate. To this diet various amounts of calcium carbonate were added, starting with no calcium carbonate at all, in which case analyses showed a calcium content of 0.02%. In further experiments calcium carbonate was added so that the diet contained 0.05, 0.10, 0.15 and 0.69% calcium, the latter diet having an optimal calcium content. Groups of rats were fed on diets with each calcium level, each group being subdivided into two where one group received 0.95% Sr as strontium carbonate in addition. One further group of rats received 0.69% Ca and 2.37% Sr and another group of rats 1.6% Ca and 0.95% Sr. The basic diet contained 75 I.U. vitamin D and 1 g PO_4 per 100 g food. To two groups of rats a diet without vitamin D was given. These particular diets contained 0.02 and 0.05% calcium, respectively. The rats were maintained on the diets for approximately 4 weeks. The composition of the diet and the grouping of these animals can be seen in Table 1.

In another series of experiments, rats maintained on a diet containing 0.69% calcium were injected subcutaneously with either 1 ml 3% $SrCl_2$ or 1 ml 6% $SrCl_2$ daily for 3 weeks.

In a further series, rats receiving a diet containing 0.10% Ca plus 0.95% Sr were given 2 ml 5% calcium gluconate per day for 9 days after being fed the above diet for 12 days.

The rats were killed and blood was taken for calcium and strontium analyses. These analyses were performed on serum by flame photometry after precipitation as oxalate. In one group of animals receiving a low calcium diet (0.10%) and in another group receiving the same diet with strontium (0.95%) added, blood was analyzed for inorganic phosphorus, using the method of YOUNGBURG and YOUNGBURG (1930). Such analyses were also made in two other groups of rats put on an optimal calcium diet (0.69%) and on the same diet with addition of strontium (0.95%), respectively.

After killing of the animals the tibiae and jaws were taken for histological examination. The material was decalcified in a mixture of equal parts of 20% sodium citrate and 44% formic acid for 3—4 days. Paraffin sections 5 μ thick were stained with haematoxylin and eosin.

Results

In Table 1 the composition of the diets with respect to calcium, strontium and vitamin D is given together with the number of animals in each group and some blood chemical data.

The animals which had received diet 1a with a calcium content of 0.02% exhibited an irregular widening of the epiphysial plate with an increased amount

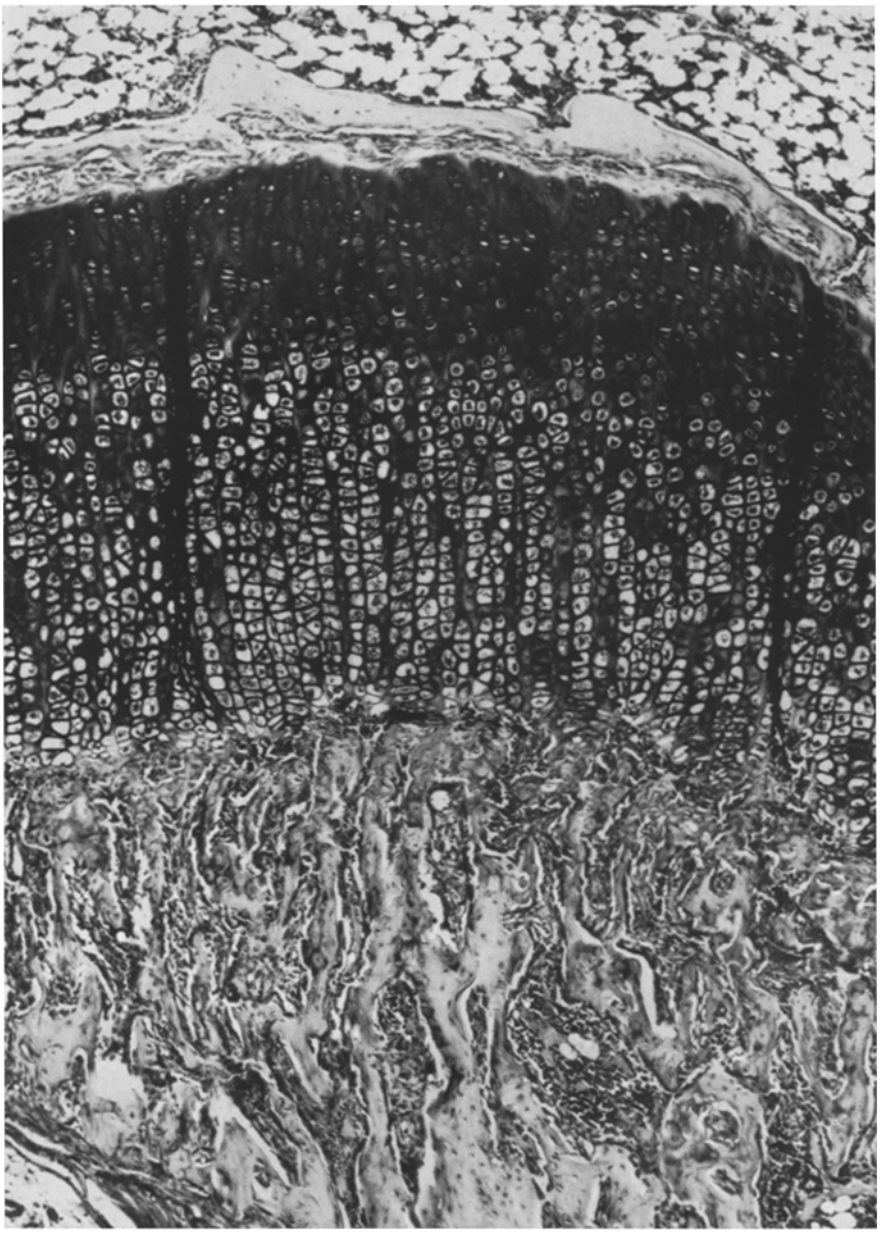


Fig. 1 a. Tibial epiphysial plate of a rat maintained on a low calcium diet (group 1 a, Ca 0.02%) for 4 weeks. Moderate rachitic changes are seen. Haematoxylin-eosin. ×85.

of osteoid in the metaphysis and cortex. The predentin was increased in amount and the border between the predentin and dentin was irregular (Fig. 1). The rachitic changes in the bones were more pronounced in the Wistar than in the Sprague-Dawley rats.

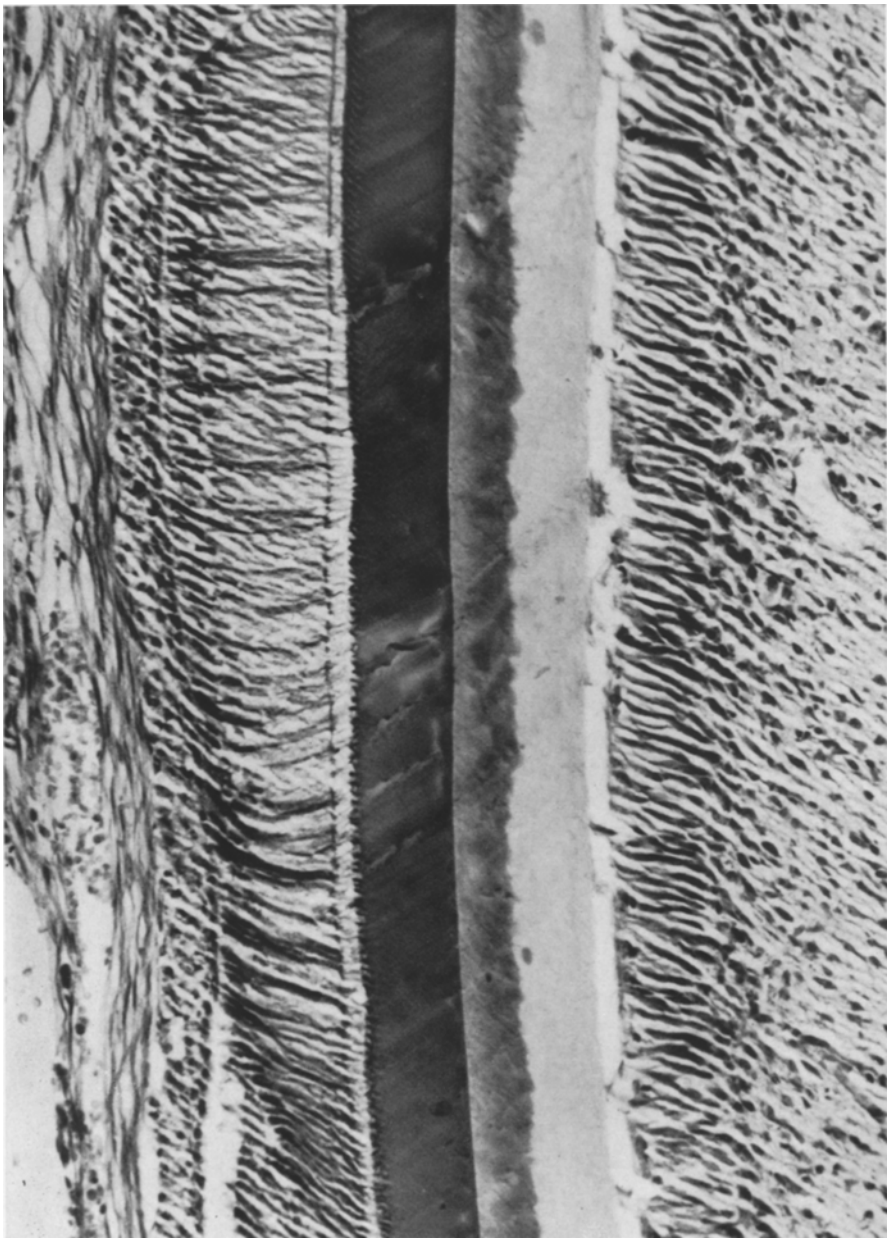


Fig. 1b. Lower incisor of the same animal as 1a, showing irregular, thickened predentin. Haematoxylin-eosin. ×275

In the animals which had received diet 2a with a calcium content of 0.05%, the following histological findings were made. The epiphysial plate showed, as a rule, a normal width, but some slight irregularities of the metaphysial trabeculae were seen, with a suggestion of persistence of hypertrophic cartilage cells. A few

animals in this experimental group exhibited a clearly thickened epiphysial plate. The osteoid seams found in the cortex were somewhat wider than normal. The predentin was increased in amount.

The preparations from animals on diets 3a, 4a and 5a containing 0.10%, 0.15%, and 0.69% calcium, respectively, showed a normal histological appearance.

In group 1b, where the diet contained 0.02% calcium and in which no vitamin D was present, florid rickets was evident in the bones and teeth. Thus, the epiphysial plate was greatly increased in width and large amounts of osteoid were present in the metaphysis. The predentin was increased in amount.

In group 2c where the diet contained 0.05% calcium and no vitamin D the epiphysial plate showed a slight but definite increase in width and the metaphysial border was irregular with an increased amount of osteoid in the metaphysis and cortex. Furthermore, areas of hypertrophic cartilage cells were found in the metaphysis. The border between the predentin and the dentin was irregular, and the predentin was increased in amount.

After the addition of 0.95% strontium to the diets containing low amounts of calcium (experiments 2b, 3b and 4b), severe rachitic changes were found. Thus the epiphysial cartilage was greatly increased, the border between this cartilage and the metaphysis was irregular and large amounts of osteoid were present. The predentin was increased in amount and greatly irregular in thickness (Figs. 2a and 3a).

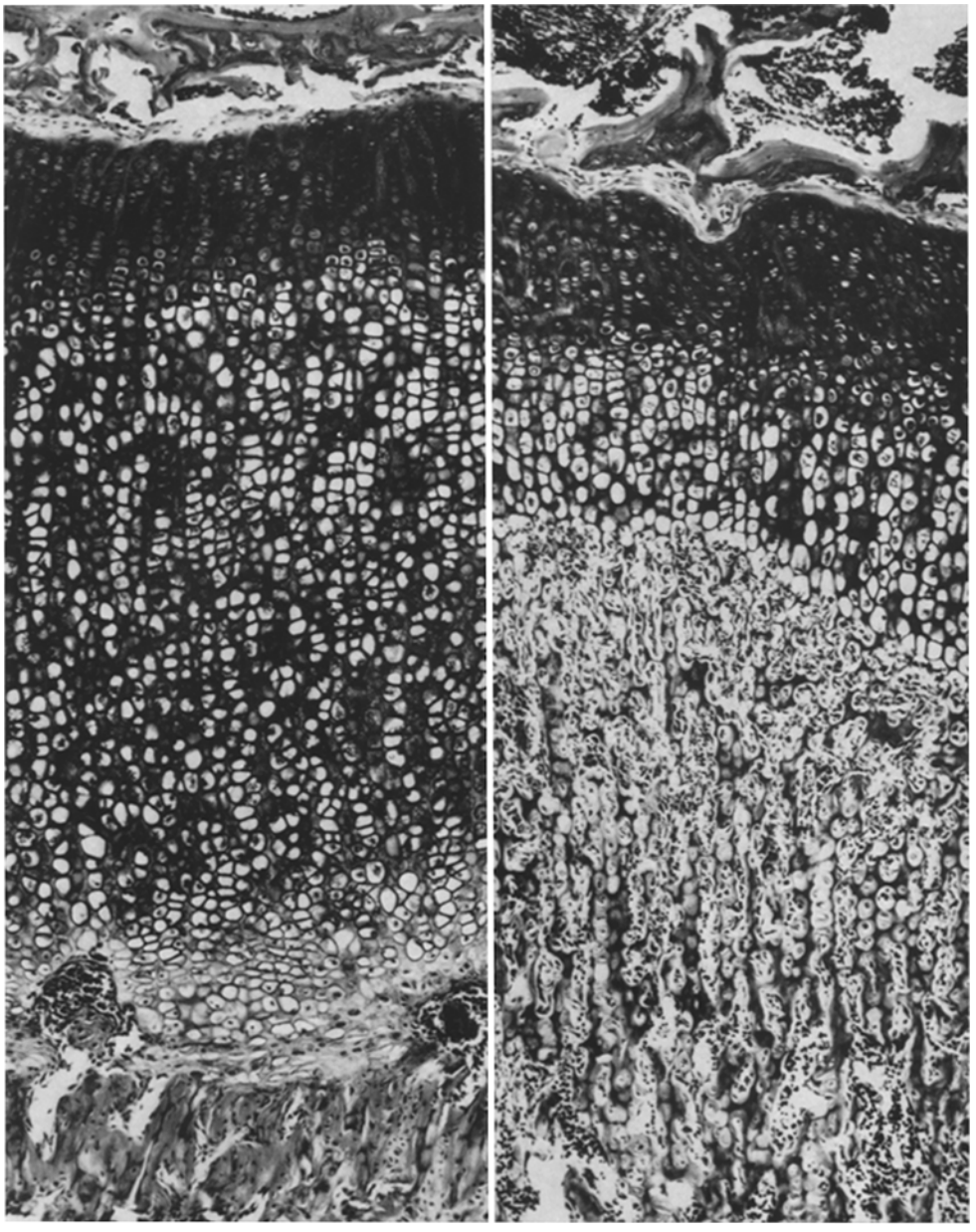
In the animals which received a diet with optimal amounts of calcium (0.69%) and to which 0.95% Sr was added (group 5b), rachitic changes were evident (Figs. 4a and 5a) but these were not so advanced as in the case of low calcium diets with strontium. An increase of the calcium content to 1.6% at the same strontium level (group 5d) resulted in bones and teeth with no rachitic changes and with by and large a normal histological appearance.

When 2.37% Sr was given to rats receiving optimal calcium diets (group 5c, 0.69% Ca), the rachitic alterations (Figs. 4b and 5b) were found to be definitely more advanced than in group 5b (0.69% Ca, 0.95% Sr).

In some of the rats on the strontium diets which exhibited rachitic changes, inclusions of odontoblasts in the predentin and dentin were seen (Fig. 5). These changes were noted at all calcium levels of the diet. In the present investigation no such phenomenon was found in the rats receiving a low calcium diet without added strontium, but it was observed in a previous investigation in rats on an extremely low calcium diet (ENGFELDT *et al.*, 1962).

In rats receiving an optimal calcium diet and given injections of 1 ml 3% $SrCl_2$ daily (group 6, Table 2) the histological findings in the bones were normal. In the dentin, however, alternating basophilic and less basophilic zones were seen running parallel with the odontoblastic border, as described earlier by IRVING and WEINMANN (1948). In the rats receiving 1 ml 6% $SrCl_2$ (group 7, Table 2) there were, in general, no changes in the bones. However, a few rats showed a somewhat increased thickness of the epiphysial plate. The dentin showed similar changes to those in the previous group, and in addition a few animals demonstrated evidence of increased predential zone (Fig. 6).

In the experimental group where 2.37% strontium was added to the diet, as also in the group receiving 1 ml 6% $SrCl_2$ daily subcutaneously (groups 5c



a b

Fig. 2. a Tibial epiphysial plate of a rat maintained on a low calcium diet with strontium added (group 3b, Ca 0.10%, Sr 0.95%) for 3 weeks. Severe rickets is evident. Haematoxylin-eosin. ×85. b Tibial epiphysial plate of rat maintained on a low calcium diet plus strontium as in 2a and receiving calcium injections subcutaneously at the end of the experimental period (group 8). Healing rickets is seen. Haematoxylin-eosin. ×85

and 7), the amount of strontium given was so high that a generalized toxic effect became evident and a certain degree of mortality occurred.

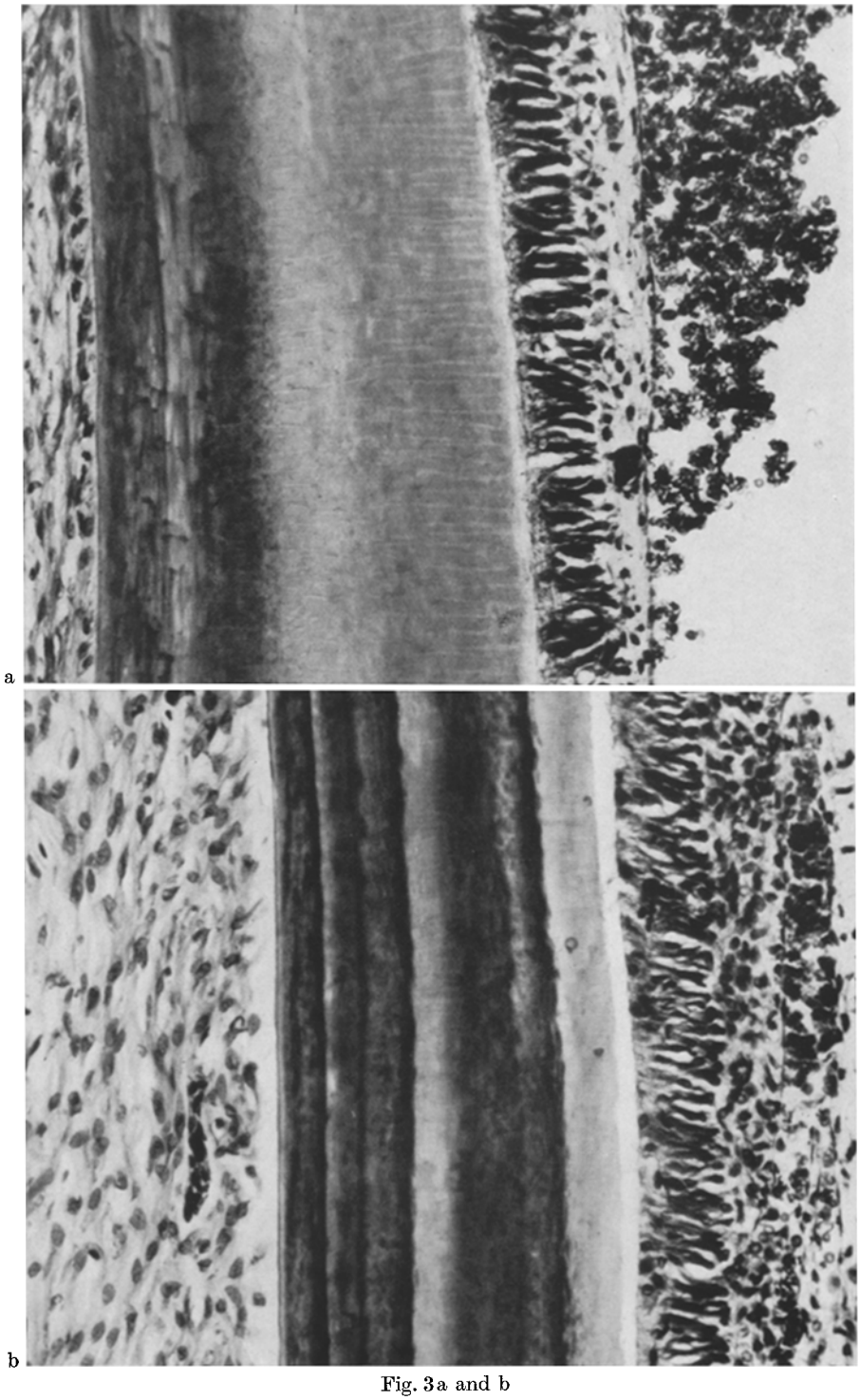


Fig. 3a and b

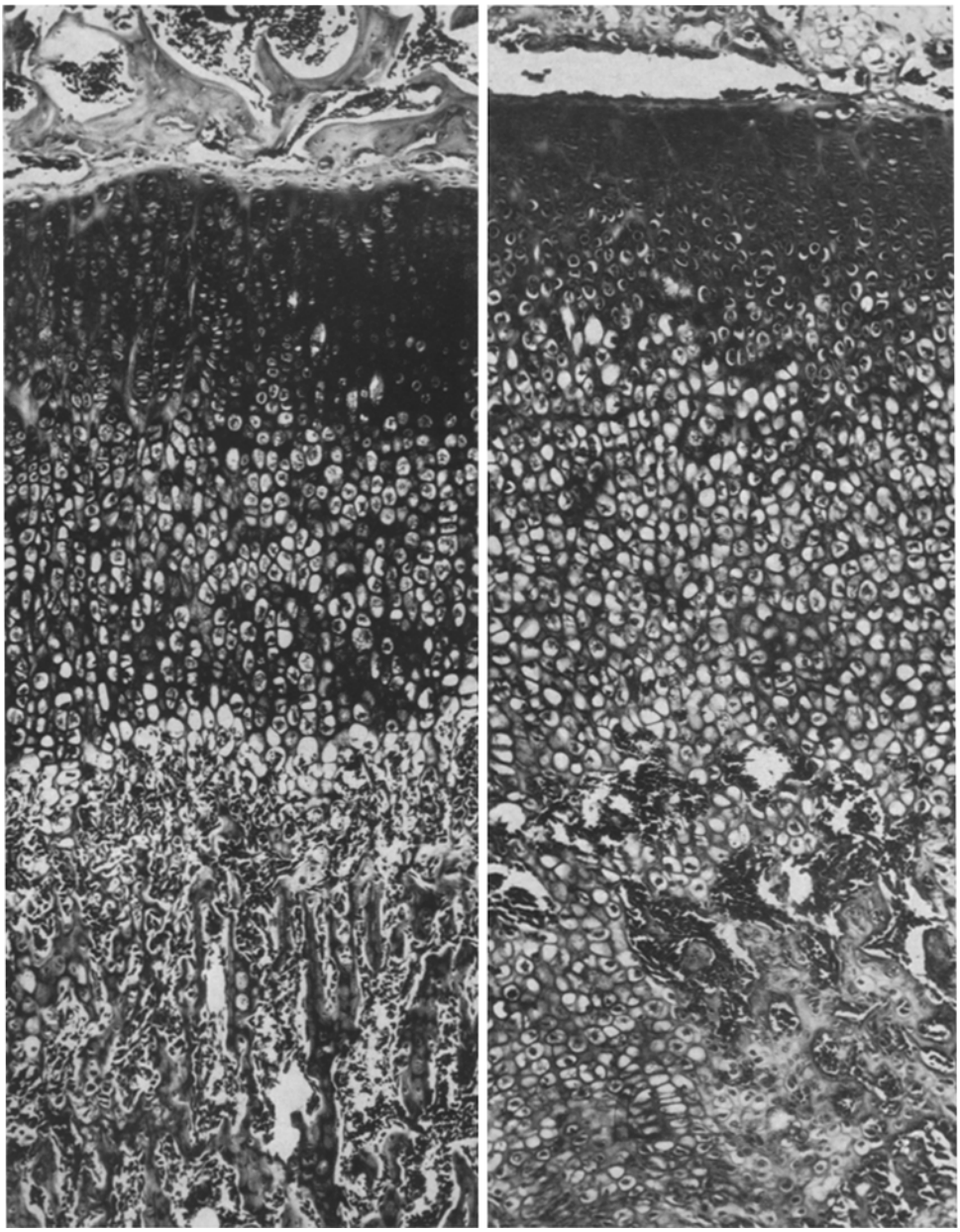


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Fig. 4. a Tibial epiphysial plate of a rat maintained on an optimal calcium diet with strontium added (group 5b, Ca 0.69%, Sr 0.95%). Moderate rickets is seen. Haematoxylin-eosin. ×85. b Tibial epiphysial plate of a rat maintained on an optimal calcium diet with large amounts of strontium added (group 5c, Ca 0.69%, Sr 2.37%). Severe rickets is present

Fig. 3. a Lower incisor of the same animal as in Fig. 2a. Greatly thickened predentin and deficient mineralization is present. Haematoxylin-eosin. ×275. b Lower incisor of the same animal as in Fig. 2b. Evidence of healing is shown. Haematoxylin-eosin. ×275

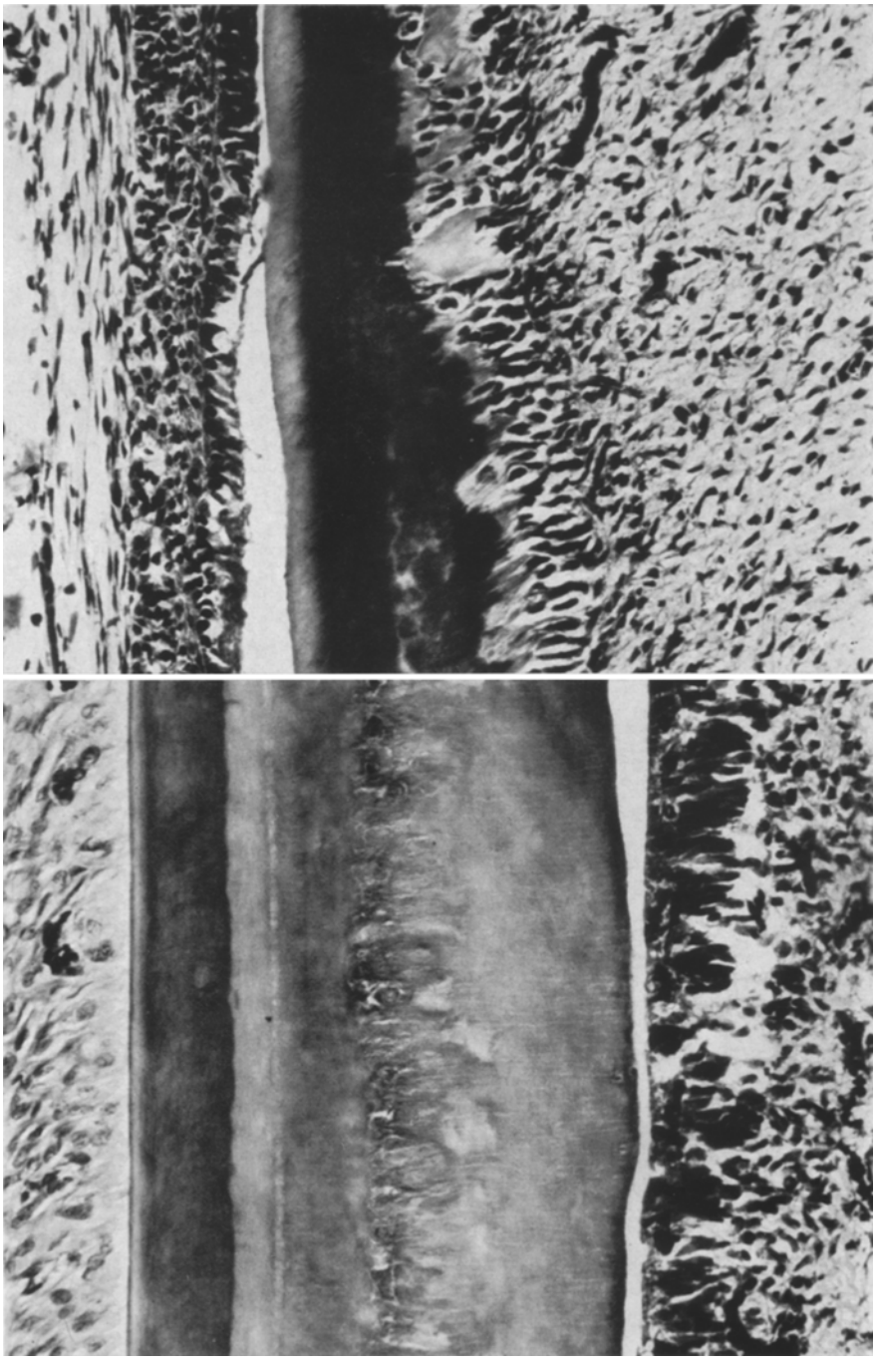

Fig. 5. a Lower incisor of the same animal as in Fig. 4a. Unevenly grouped odontoblasts and inclusions of odontoblasts are seen in the thickened predentin. Further interglobular dentin is present. Haematoxylin-eosin. × 275. b Lower incisor of the same animal as in Fig. 4b. Unevenly grouped odontoblasts and inclusions of odontoblasts in the greatly thickened predentin. Haematoxylin-eosin. ×275

In the group of rats maintained on diets containing 0.10% Ca and 0.95% Sr for 3 weeks and injected with 2 ml 5% calcium gluconate daily for the last 9 days (group 8, Table 3), both the bones and the teeth showed evidence of healing rickets (Figs. 2b and 3b). The control animals (group 3b) showed florid rickets.

The blood chemical studies showed that the animals of group 1a (0.02% Ca) had a calcium value in serum of 6.3 mg-%, while the corresponding value in group 1b (0.02% Ca, no vitamin D) was only 4.5 mg-%. When increasing amounts of Ca were added to the diet, higher blood calcium values were noted and the animals receiving optimal calcium (0.69%) showed a blood calcium value of 8.5 mg-% (Table 1).

Table 1. *Composition of diet with respect to calcium, strontium and vitamin D, and blood chemical data*

Group number	No. of animals studied morphologically	Ca in diet (%)	Sr in diet (%)	Vit. D in diet	Blood Ca[a] (mg/100 ml)	t	Blood Sr[a] (mg/100 ml)
1a (Ca−)	20	0.02	—	+	6.3 ± 0.2 (11)		—
1b (Ca−, D−)	20	0.02	—	—	4.5 ± 0.1 (11)	8.18[d]	—
2a (Ca−)	49	0.05	—	+	7.9 ± 0.2 (12)		—
2b (Ca−,Sr+)	22	0.05	0.95	+	6.3 ± 0.1 (8)	7.28[d]	7.6 ± 0.2 (2)
2c (Ca−,D−)	5	0.05	—	—	4.4 ± 0.3 (3)		—
3a (Ca−)	8	0.10	—	+	8.1 ± 0.1 (8)		—
3b (Ca−, Sr+)	20	0.10	0.95	+	6.7 ± 0.2 (11)	6.36[d]	9.2 ± 0.4 (4)
4a (Ca−)	13	0.15	—	+	8.3 ± 0.1 (9)		—
4b (Ca−, Sr+)	11	0.15	0.95	+	7.3 ± 0.1 (6)	7.14[d]	—
5a	50	0.69	—	+	8.5 ± 0.1 (11)		—
5b (Sr+)	8	0.69	0.95	+	7.9 ± 0.3 (3)	1.88	5.3 ± 0.2 (3)
5c (Sr++)	15	0.69	2.37	+	9.0 ± 0.3 (9)	1.56	9.5 ± 0.5 (7)
5d (Ca+, Sr+)	11	1.60	0.95	+	8.9 ± 0.2 (9)	1.82	3.1 ± 0.1 (10)

[a] Mean ± standard error of the mean. The number of blood analyses made in each group is given in parenthesis.
[b] $0.05 > P > 0.01$.
[c] $0.01 > P > 0.001$.
[d] $P < 0.001$.

When 0.95% Sr was added to the diets of the rats with increasing calcium intake, the blood calcium was found to be highly significant lower in the low calcium groups (groups 2b, 3b and 4b), when compared with control animals on the same calcium intake. The animals on an optimal calcium diet (group 5b) and those on a high calcium diet (group 5d) receiving 0.95% Sr in addition showed no significant decrease of blood calcium, and neither was any blood calcium decrease noted in animals on a diet containing 2.37% Sr (group 5c, Table 1).

The rats on an optimal calcium diet receiving $SrCl_2$ subcutaneously (both the 3% and the 6% dose) showed no significant change of the blood calcium level. Further, the blood strontium level was much lower than in the animals receiving strontium in the diet (Tables 1 and 2).

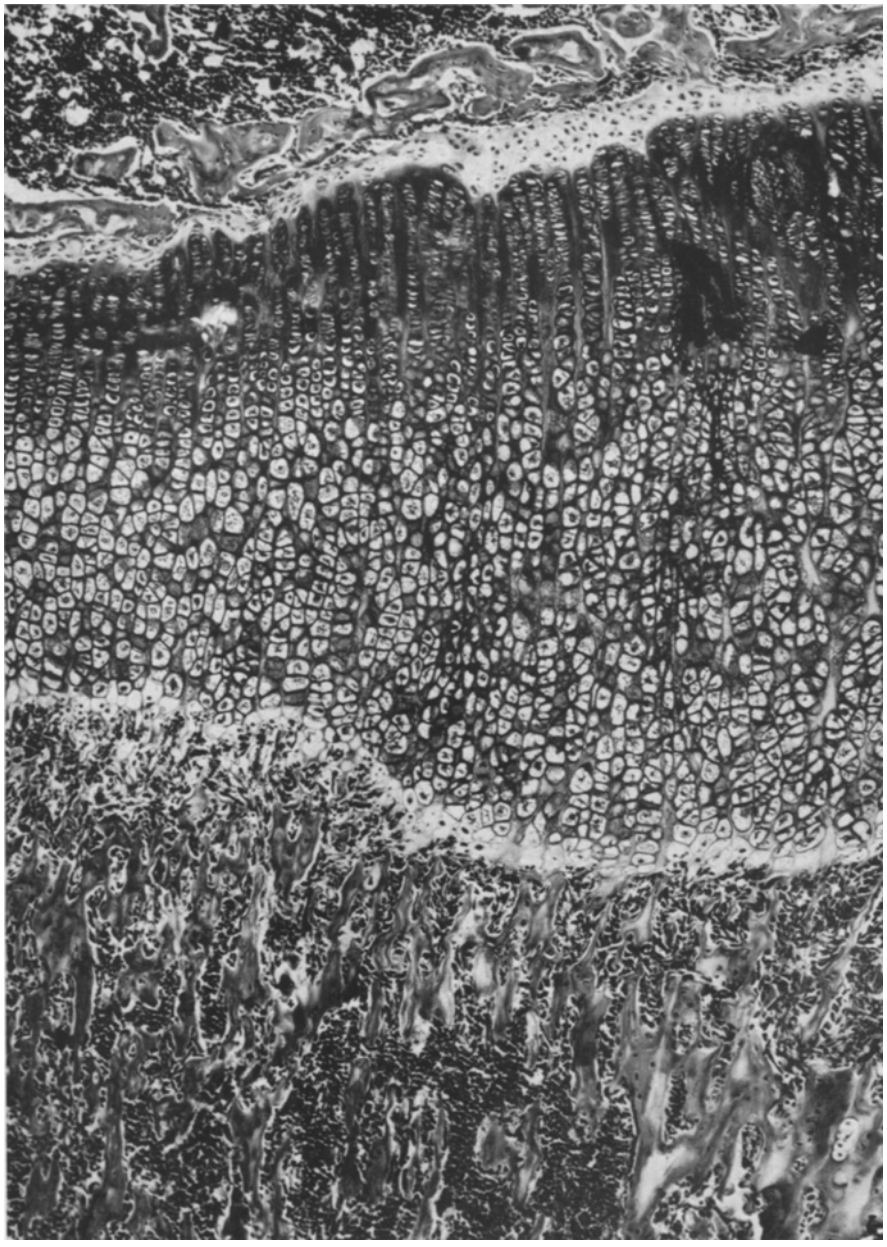

Fig. 6a. Tibial epiphysial plate of a rat maintained on an optimal calcium diet but receiving $SrCl_2$ injections subcutaneously daily for 3 weeks (group 7, Ca 0.69%, 1 ml 6% $SrCl_2$ daily). Moderate rickets. Haematoxylin-eosin. ×85

As stated above, the animals on a low calcium intake (0.10% Ca) with 0.95% Sr added to the diet and which were given injections of Ca gluconate daily during the last 9 days of the 3 week diet period (group 8) showed

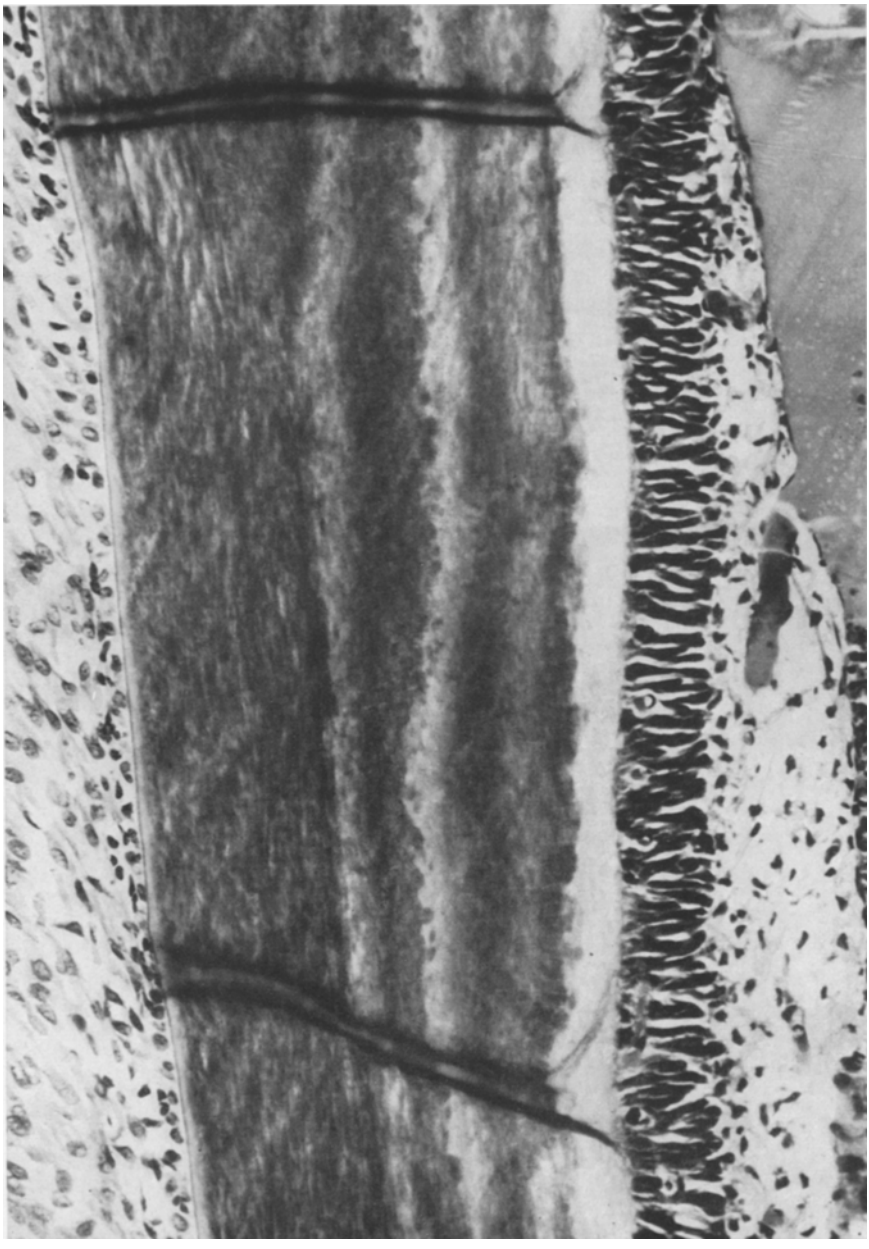

Fig. 6b. Lower incisor of the same animal as in 6a. Alternating basophilic and less basophilic (hypomineralized) zones are seen running parallel to the odontoblastic border. The predentin is probably somewhat increased in thickness and the predentin-dentin border somewhat uneven. Haematoxylin-eosin. ×275

evidence of healing rickets. In these animals the blood calcium values (Table 3) were found to be significantly increased in comparison with the similarly fed

Table 2. *Rats on optimal Ca diet receiving $SrCl_2$ subcutaneously*

Group number	No. of animals studied morphologically	Ca in diet	Vit. D in diet	Treatment subcut. injections $SrCl_2$	Blood Ca[a] (mg/100 ml)	Blood Sr[a] (mg/100 ml)
6 (Sr+)	20	0.69	+	1 ml 3% daily for 3 weeks	8.0 ± 0.3 (11)	0.8 ± 0.3 (11)
7 (Sr++)	20	0.69	+	1 ml 6% daily for 3 weeks	8.6 ± 0.2 (8)	1.7 ± 0.2 (8)

[a] Mean $\pm$ standard error of the mean. The number of blood analyses made in each group is given in parenthesis.

Table 3. *Rats on Sr diet receiving Ca gluconate injections (2 ml 5% daily) subcutaneously, and controls on the same Sr diet but not given injections*

Group number	No. of animals studied morphologically	Ca in diet (%)	Sr in diet (%)	Blood Ca[a] (mg/100 ml)	t	Blood Sr[a] (mg/100 ml)
8 (Ca injected)	13	0.10	0.95	7.4 ± 0.1 (5)		7.0 ± 0.4 (5)
3b (controls)	20	0.10	0.95	6.7 ± 0.2 (11)	3.18[c]	9.2 ± 0.4 (4)

[a] Mean $\pm$ standard error of the mean. The number of blood analyses made in each group is given in parenthesis.
[b] $0.05 > P > 0.01$.
[c] $0.01 > P > 0.001$.
[d] $P < 0.001$.

but noninjected animals. A depression of the blood strontium level was also noted.

In animals on a low calcium intake (0.10% Ca, group 3a) blood phosphorus was also determined. A value of 7.5 ± 0.3 mg-% was found. After the addition of 0.95% Sr to this diet (3b) no significant change in blood phosphorus was noted. In similar experiments with an optimal Ca intake (0.69%) the same negative result was obtained.

Discussion

In a previous study we found that rats maintained on a diet extremely deficient in calcium (1 mg/100 g) but otherwise optimal (including vitamin D) developed severe rickets (ENGFELDT *et al.*, 1962). Rickets also developed when strontium was added to the low calcium diet. The rickets here seemed to be more advanced than in calcium deficiency alone. Hypothetically it was considered that this finding might be due to an inhibitory influence of strontium on the absorption of calcium in the gut, with a consequent decrease in the blood calcium level. In the present study further evidence has been presented to support this assumption.

It has thus been demonstrated that when strontium is added to a calcium deficient diet the blood calcium level is supressed (groups 2b, 3b and 4b) and the rachitic changes are more advanced than in animals with the same calcium level in the diet but with no strontium added (group 2b versus 2a). Furthermore, in animals on diets containing calcium in an amount sufficient to prevent rickets (groups 3a, 4a and 5a), the addition of strontium results in severe to moderate rickets (groups 3b, 4b, 5b and 5c), and the rachitic changes in the mineralized tissues can be mitigated by the addition of more calcium to the diet (group 5d).

In further experiments the importance of the blood calcium level for the development of strontium rickets was *further* elucidated. Morphological changes showing healing were achieved when rats on a calcium deficient diet to which strontium was added received calcium gluconate subcutaneously at the end of the experimental period (group 8). An increase of the calcium level in the blood was also observed in these animals.

However, the calcium level of the blood does not seem to be the only factor of importance in the development of strontium rickets. In our experiments moderate rachitic changes were also found to occur in animals with normal blood calcium values and receiving a diet with a normal calcium content when strontium was added to the diet (groups 5b and 5c). When the amount of added strontium was increased from 0.95 to 2.37% (group 5c), more severe rickets developed. Subcutaneously administered strontium chloride also resulted in evidence of slight rickets.

From the results of experiments with administration of strontium to animals on an optimal calcium diet it seems reasonable to assume that strontium has an effect on local factors which determine the bone growth and mineralization.

The possibility has been considered that strontium rickets may be related to a deficiency of phosphate (Roche, 1932); it has been postulated that the rickets may be brought about by the formation in the intestine of large amounts of insoluble strontium phosphates, with a resultant decrease in the blood phosphorus level. The changes produced by feeding strontium to experimental animals would thus result in phosphate rickets. The theory of a decrease in blood phosphate as a cause of strontium rickets has not been supported by our data. In our experiments no changes in the blood phosphorus level after adding strontium to a diet optimal in phosphate were found either when the calcium content of the diet was low or optimal.

In the present study our earlier finding of the development of rachitic changes in rats with an extremely low calcium intake was verified in animals given diets with more moderate calcium deficiency (20 and 50 mg Ca/100 g food). In 3—4 weeks old rats maintained on these diets for 4 weeks, moderate and slight rickets developed, respectively. It should be pointed out that the diet was optimal in all other respects, including vitamin D. There was no definite osteoporosis, as far as could be ascertained morphologically.

It has been claimed that pure calcium deficiency produces osteoporosis, not rickets, in rats (Harrison and Fraser, 1960; Hartles, 1962, 1964; Ferguson and Hartles, 1963), in cats (Jowsey and Gershon-Cohen, 1964), as well as in dogs (Campbell and Douglas, 1965). The divergent results may depend, however, among other things upon the degree of calcium deficiency, the age of

the animals and the length of the experimental period. As suggested by our results the strain of animal may also be of importance.

In conclusion our results indicate that there are good reasons to believ that the calcium level of the blood is a fundamental factor for the development of strontium rickets. It seems probable, however, as discussed at length by STOREY (1961), that strontium also has some sort of direct influence on the growth and mineralization of hard tissues.

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