XCVII. VARIABILITY IN THE ACTIVITY OF THE CALCIFYING MECHANISM IN THE BONES OF RACHITIC RATS.

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(Received February 26th, 1934.)

It was noted by Robison *et al.* [1930] that the calcifying power attributed to the second mechanism in the hypertrophic cartilage varied considerably in different rachitic rats, even in those of the same age and litter. The variation was manifested by differences in the minimum level of the $\text{Ca} \times \text{P}$ product required for calcification *in vitro* in inorganic solutions and also in the extent of the deposit obtained at higher levels.

Experiments bearing on the relation of such variation to the rate of growth and the condition of the rachitic animals are presented in this paper.

For the first group of experiments litters were selected in which the rats showed unusually varied growth over a 3 to 4-week period on McCollum 3143 diet, and the possible connection of this variation in growth rate with the calcifying power of the cartilage was investigated. The litter-mates were killed on the same day, and tibia slices from each rat were immersed in 20 ml. of the specified calcifying solutions, one slice per flask. The flasks containing the slices were kept at 37° for 20 hours and rotated continuously. The p_{H} in all the flasks remained constant at 7.4 throughout each experiment.

The growth curves of the rats are given in Fig. 1 and a summary of the experiments in Table I. In the marks assigned to the calcification the figures, with maximum 10, show the approximate extent of the new deposits, and the bars, with maximum 5, indicate the density. The depth of the uncalcified zone in the control slices varied widely, and the figures show the proportion of this zone which became calcified and not the absolute amount of deposit.

The results show that the second mechanism activity developed in the hypertrophic cartilage for the most part varied directly with the amount of growth of the rat during the period on rachitogenic diet.

The figures given for the inorganic solutions (Exps. 1–5) show the variation in the amount of calcification due to the second calcifying mechanism alone. Those given for the ester solutions (Exp. 2) illustrate in an equally striking manner the variation in the calcification produced by the combined mechanisms under conditions which probably approached more closely to those existing *in vivo*.

In inorganic solution calcification at the low level² 8:2:0 was obtained in the case of two of the rats showing the most growth over the period on diet, while in the solutions 8:3:0 and 8:4:0 calcification was more general but

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² The experimental solutions are referred to in this way throughout the text. Thus solution 8:5:3 contains 8 mg. Ca, 5 mg. P as inorganic phosphate and 3 mg. P as organic phosphate per 100 ml. basal salt solution.

decreased in amount with decreasing rate of growth. In solution 8:5:0 differences were less pronounced.

Fig. 1.

Table I.

Exp.	Period on diet (days)	Litter	Rat	Increase in wt.		mg. per 100 ml.	{ Ca P inorg. P org.	Inorganic solutions				Ester solutions		
				Wt. at 21 days*	on diet			8	8	8	8	8	8	8
1	23	2384	a ♂	45	29			2	3	4	5	2	2	2
			b ♀	42	21			0	0	0	0	1	2	3
			c ♀	41	21			0	2 =	7 =	7 ≡	.	.	.
			d ♀	43	17			0	<1 -	4 ≡	5 ≡	.	.	.
			e ♀	41	14			0	<1 -	4 ≡	5 ≡	.	.	.
2	25	2661	a ♂	40	38			0	0	3 =	5 ≡	.	.	.
			b ♀	41	34			0	0	2 =	5 ≡	.	.	.
			c ♀	38	19			0	0	3 =	5 ≡	.	.	.
			d ♂	37	14			0	0	3 =	5 ≡	.	.	.
3	21	2869	a ♀	36	29			1 =	7 ≡	8 ≡	.	.	.	.
			b ♀	36	21			0	6 ≡	8 ≡	.	.	.	.
			c ♀	37	16			0	1 =	5 ≡	.	.	.	.
4	27	2720	a ♀	37	26			0	0	1 =	5 ≡	.	.	.
			b ♀	40	13			0	0	0	5 ≡	.	.	.
5	21	2675	a ♂	52	29			0	3 ≡	4 ≡	.	.	.	.
			b ♀	47	26			0	4 ≡	5 ≡	.	.	.	.
			c ♂	45	23			0	3 ≡	5 ≡	.	.	.	.
			d ♂	46	17			0	3 =	5 ≡	.	.	.	.

* All the litters were 21 days old when placed on the rachitogenic diet.

The relation of calcification to growth rate is not apparent in the case of Litter 2675, Exp. 5. It may be noted that these rats were exceptionally well grown at the beginning of the dietary period.

As regularly observed in previous experiments, the amount of new calcification in the slices from any one rat showed a marked gradation in passing from lower to higher levels of the $\text{Ca} \times \text{P}$ product. The use of such material, that is bone slices taken from the same animal, for observing the effect of various factors on the calcifying mechanisms is thus justified.

The favourable effect on calcification of phosphoric ester even in low concentration is again emphasised by the results of Exp. 2.

In another series of experiments quoted in Table II, three or four rats selected from different litters were killed on the same day after different periods of 1 to 6 weeks on McCollum 3143 diet.

Table II.

Exp.	Rat	Days on diet	mg. per 100 ml.	<div> <div>Ca</div> <div>P inorg.</div> <div>P org.</div> </div>	Inorganic solutions				Ester solutions		
					8 3 0	8 4 0	8 5 0	8 6 0	8 3 3	8 4 3	8 3 10
1	1 ♀	7			.	10 ≡	.	.	.	.	10 ≡
	2 ♀	10			.	10 ≡	.	.	.	.	10 ≡
	3 ♀	28			.	<1 —	.	.	.	.	7 ≡
	4 ♀	37			.	1 =	.	.	.	.	2 ≡
2	1 ♂	7			.	10 ≡	10 ≡	10 ≡	10 ≡	10 ≡	.
	2 ♀	17			.	2 =	3 ≡	3 ≡	4 ≡	5 ≡	.
	3 ♀	30			.	0	<1 =	<1 =	4 ≡	5 ≡	.
3	1 ♂	15			.	3 =	6 =	.	5 ≡	.	10 ≡
	2 ♂	17			.	2 =	6 =	.	6 ≡	.	8 ≡
	3 ♂	28			.	<1 —	<1 =	.	6 ≡	.	6 ≡
	4 ♀	40			.	<1 ≡	<1 ≡	.	4 ≡	.	4 ≡
4	1 ♂	28			2 =	7 ≡	8 ≡	.	.	.	10 ≡
	2 ♂	42			<1 —	3 ≡	7 ≡	.	.	.	8 ≡
	3 ♀	56			<1 —	<1 —	6 ≡	.	.	.	7 ≡

The results show that the calcifying power of the cartilage decreases with increasing periods on a rachitogenic diet.

The position of the deposit in these bone slices is a point of considerable interest. In the bones of the young rats which had been less than 2 weeks on the diet (Exp. 1, rats 1 and 2; Exp. 2, rat 1) the whole of the narrow zone of hypertrophic cartilage was apparently in the optimum condition for calcification. Deposition *in vitro* extended from the "line" to the shaft, and the slices calcified *in vitro* resembled bone slices from normal animals of the same age (Plate II, Figs. 2 and 3). In bone slices from the rats which had grown well for 3–4 weeks on a rachitogenic diet, deposition *in vitro* had the appearance familiar in the "line test," that of a band of calcification separated from the diaphysis by a zone of disorganised and less readily calcifiable tissue.

This band of cartilage calcifiable in inorganic solution became narrower as the period on diet increased, until after 4 weeks or more there were large areas of uneroded hypertrophic cartilage which did not become calcified, even in solution 8 : 6 : 0.

It would seem that the calcifying power of the second mechanism is greatest where the cells have just attained full hypertrophy, decreasing after this stage has been passed. A similar conclusion was reached by Niven and Robison in experiments with the cartilage of embryonic rabbit femora [quoted by Robison, 1932]. The same general statement may also be made for the combined calcifying power of the phosphatase and second mechanisms, although the area of cartilage calcified *in vitro* was invariably more extensive in presence of ester than in the corresponding inorganic solutions.

The formation of a calcified line in healing rickets is probably due to the maximum calcifying power of the dual mechanism in this region.

The validity of the argument based on the results shown in Tables I and II may be criticised on the grounds that the observed variations in calcifying power do not in every case run parallel either with the growth rate or with the length of time on the rachitogenic diet. These results do, however, furnish strong evidence that the calcifying power of the cartilage in a rachitic rat varies with the condition of the animal; and this conclusion is supported by the general results of experiments carried out in this laboratory over many years.

Fig. 1.

Fig. 4.

Fig. 2.

Fig. 5.

Fig. 3.

Fig. 6.

Whether this variation is connected with deficiency in some specific factor, and whether it is due to the same cause as the variation in the growth rate, cannot at present be decided. The experimental diet (McCollum 3143) was notably deficient in two nutritional requirements, inorganic phosphate and vitamin D. Coward *et al.* [1932] state that "the reserves of vitamin D in the young rat vary in different individuals and in different litters," basing their conclusion on the variation in the growth of these animals on a vitamin D-deficient, but otherwise adequate, basal diet. The results of the experiments described in this paper are not inconsistent with the view that the observed variations in the calcifying power of the cartilage may, like those in the growth rate, be a result of differences in the vitamin reserves of the animals. Although it is held on good grounds that changes in the composition of the blood are primarily responsible for the deficient calcification in rickets, it is difficult to avoid the conclusion that changes in the calcifying power of the cartilage may also play a part in the story.

SUMMARY.

The calcifying power of the cartilage in the bones of rachitic rats varies with the rate of growth of the animals over a 3 to 4-week period on a rachitogenic diet and decreases as the period on diet is increased.

I should like to thank Prof. R. Robison for much help and valuable criticism.

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DESCRIPTION OF FIGURES IN PLATE II.

Tibia slices stained by v. Kossa's silver nitrate method.

X = calcification *in vitro* in the hypertrophic cartilage zone.
h.c. = hypertrophic cartilage.
s.c.c. = small-celled cartilage.

Figs. 1-3. Table II, Exp. 1, Rat 2. 10 days on a rachitogenic diet.

Figs. 4-6. Table II, Exp. 1, Rat 3. 28 days on a rachitogenic diet.

Fig. 1. Control slice. Note the narrow zone of uncalcified hypertrophic cartilage.

Fig. 2. Slice after immersion for 20 hours in the inorganic solution 8 : 4 : 0 at 37° and p_H 7.4.

Fig. 3. Slice after immersion for 20 hours in the ester solution 8 : 3 : 10. Note the dense band of new calcification extending to the diaphysis in this slice and in that shown in Fig. 2.

Fig. 4. Control slice. Note the breadth of the uncalcified zone.

Fig. 5. Tibia slice after 20 hours in solution 8 : 4 : 0. The very small amount of new calcification (<1) obtained *in vitro* indicates the decreased calcifying power of the hypertrophic cartilage. Note the position of the deposit on the "line."

Fig. 6. Tibia slice from the same rat as (5) after 20 hours in solution 8 : 3 : 10. Calcification is denser and more extensive than in absence of phosphoric ester.