XCI. FACTORS INFLUENCING BONE FORMATION IN THE ALBINO RAT.

II. THE EFFECT OF THE INJECTION OF PARATHYROID EXTRACT.

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GREENWALD AND GROSS [1925] showed that in a dog on a low-calcium diet the administration of parathyroid extract caused an increased excretion of calcium. They concluded that the parathyroids produced a substance which either exercised a direct influence on the balance between the calcium of the blood and of the tissues or stimulated the production of such a substance. They suggested that this hypothetical substance maintained the calcium of the blood in solution, and, if present in excess, might raise the saturation level of the calcium in the blood. Under these circumstances, calcium might be withdrawn from the tissues to increase the calcium content of the blood. In the experiment on parathyroid administration, which they described [1926], nearly 3 g. of calcium were excreted in excess of that contained in the food. This could only have been derived from the bone, but X-ray examination failed to show specific bone changes. If, however, the calcium content of the dog was at all similar to that of a rat, *i.e.* approximately 1 % in the adult [Sherman and MacLeod, 1925], then this loss of 3 g. could not have represented more than 6 % of the total calcium in the bones of the dog, a loss which would hardly be demonstrable radiographically unless the calcium was withdrawn from localised areas.

Since the experiment about to be described was completed, Bauer, Aub and Albright [1929] have reported that the administration of parathyroid extract to rabbits caused a withdrawal of calcium from the trabeculae of the bones. They failed to produce this effect in cats or rats, but Lambie, Kermack and Harvey [1929] reported a thinning of the trabeculae and a decrease in the ash of the bones of rats to which they had administered parathyroid extract. From this conflicting evidence and from the results of Hunter and Aub [1927], Robinson, Summers, Huffman and Burt [1927], Hoag, Rivkin, Weigele and Berliner [1927] and Liu [1928] it would appear that the influence of parathyroid extract administration on the calcium of the bone and serum and on calcium excretion may depend on the diet used, the initial functional

activity of the parathyroids of the subject of the experiment, and on the species of animal.

Rats are known to be very resistant to the over-dosage phenomena which follow the administration of excess of parathyroid extract to other animals and Greenwald and Gross [1926] suggested that this resistance might be due to the efficiency of their excretory mechanism which removed the excess calcium as it was liberated. Continued administration of parathyroid extract to these animals ought therefore to lead to continued excretion of calcium greater than that in control rats on the same diet. If this increase in excretion were great it might lead to gross differences in body growth and calcification of bone, such as are found in animals on low-calcium diets [Korenchevsky, 1922]. If the increase were only slight, the condition of the animals receiving the extract might be more comparable to the rats described by Sherman and MacLeod [1925] which were reared on a diet containing enough calcium to give fair growth and reproduction, but less than optimal calcium storage. (The calcium content per 100 g. of body-weight of these animals at 90 days old was only 0.70 as against 0.93 g. in the group on the ration richer in calcium.)

The following experiment was designed to test whether the injection of doses of parathyroid extract into young growing rats on a diet considered to be fully adequate in calcium and in fat-soluble vitamins (*i.e.* a diet favouring calcium retention) would result in diminished storage of calcium in the injected rats as compared with litter-mate controls.

The determination of the dose of the parathyroid extract used ("parathormone") presented considerable difficulty. Preliminary experiments showed that 100 units (5 cc.) given to an adult rat in the course of 24 hours produced no signs of discomfort. The cost of the extract however was a limiting factor. Greenwald gave to a dog of about 5 kg. from 10 to 20 units per day. The equivalent dose for a rat of 50 g. would be 0.1 to 0.2 units per day. For the first few injections, 0.1 cc., *i.e.* 2 units were used. This was gradually increased to 0.3 cc., *i.e.* to 6 units, and for the last fortnight of the experiment the dose was 10 units. Thus the dose used was about ten times that necessary to produce marked hypercalcaemia in a dog.

EXPERIMENTAL.

A group of twenty-six rats from four litters was divided into three lots, of ten and two eights respectively. The animals were born between the 6th and the 15th of October. They were therefore all autumn rats, which have been found in this laboratory to react more readily to deficiencies which tend to produce bone lesions. They were purchased from Messrs Glaxo who kindly supplied genealogical details showing that they were closely inbred.

The litters were divided in as representative a way as possible between the three groups, and injections were begun on November 23rd and were given on alternate days. The solutions injected were as follows.

(1) Saline solution (0.7 % NaCl in tap water). Dose 0.5 cc. to 2 cc. as the animal grew from 50 to 200 g.

(2) Ammonia in lactic acid (the solution was made neutral to phenol red (p_H about 7.0) by the use of the necessary amount of lactic acid. The strength of the ammonia solution was 0.33 *N*). The same dose was used as for the saline injections.

(3) "Parathormone" (Eli Lilly). The dose used was increased from 0.1 cc. (2 units) to 0.3 cc. (6 units) and finally to 0.5 cc. (10 units) as the animals grew from 50 to 200 g.

The diet used throughout was Steenbock's stock diet [Bethke, Steenbock and Nelson, 1923], but after 6 weeks of the experiment, dried milk was substituted for the fresh milk, and to every 100 g. of the mixture of maize, etc. 15 g. dried milk (roller process) were added, as this seemed to be the equivalent of the fresh milk actually consumed by the rats. The calcium content of this final mixture was 0.52 %. The diet was supplied to the animals *ad libitum*. The growth curves of the animals were noted over an injection period of 14 weeks, when the animals were killed, and their bones and teeth examined.

Examination of the bones. The femora, tibiae and fibulae were dissected out and analysed for ash, water, fat, organic residue, calcium and phosphorus as described by Chick, Korenchevsky and Roscoe [1926].

Examination of teeth. One upper incisor tooth was taken from each rat in a group. The combined teeth of the group were dried and ashed, and the ash, calcium, magnesium and phosphorus were determined on the dry tooth.

The results are shown in Tables I and II.

Table I.

Litter	Body-weight (g.)			Bone weight (g.)			Ash of bone %			A/R		
	Saline	Am- monium lactate	"Para- thor- mone"	Saline	Am- monium lactate	"Para- thor- mone"	Saline	Am- monium lactate	"Para- thor- mone"	Saline	Am- monium lactate	"Para- thor- mone"
R ♂	159	{229 209}	209	1.556	{2.130 1.909}	1.745	41.4	{43.2 43.0}	42.9	1.69	{1.87 1.86}	1.87
B 1 ♂	{169 182}	199	225	{1.580 1.520}	1.821	2.100	{43.1 42.2}	43.1	41.2	{1.93 1.81}	1.87	1.81
B ♂	171	—	—	1.574	—	—	44.0	—	—	1.91	—	—
N ♂	208	219	{209 241}	1.879	2.229	{1.930 1.960}	42.9	41.7	{40.8 42.6}	1.83	1.85	{1.79 1.84}
Mean	178	214	221	1.622	2.022	1.934	42.7	42.7	41.9	1.83	1.86	1.83
R ♀	155	159	151	1.526	1.555	1.429	41.1	42.2	41.8	1.78	1.84	1.79
B 1 ♀	{144 136}	130	—	{1.464 1.336}	1.272	—	{44.0 43.8}	41.3	—	{1.87 1.87}	1.77	—
B ♀	137	—	{152 136}	1.409	—	{1.514 1.264}	41.0	—	{44.3 41.1}	1.72	—	{1.77 1.87}
N ♀	151	{165 155}	171	1.500	{1.607 1.513}	1.608	40.4	{40.9 40.9}	42.1	1.68	{1.81 1.79}	1.79
Mean	144	152	152	1.447	1.487	1.454	42.0	41.3	42.3	1.78	1.80	1.80

From Table I it may be seen that the body-weight of the females in all three groups was closely similar, but that in the case of the males, the ammonium lactate and "parathormone" groups had a mean weight slightly

higher than the mean for the saline group. Examination of the individual weights revealed that this difference was mainly due to the representative of litter R, and to one representative of litter B 1 in the saline group. These rats had made exceptionally poor growth as compared with their litter-mates, not only those in the ammonium lactate and "parathormone" groups, but also others used for different purposes. It would therefore seem that this difference in the body-weights expressed individual peculiarities of these rats rather than any specific influence of the injections.

Table II.

No. of rats	Sex	Litter	Treatment	Ash %	Water %	Organic residue %	Fat %	Ca % (ash)	P % (ash)	Ca % (fresh bone)	A/R	Ca/P
5	♂	R, B 1, B, N	Saline injections	42.7	31.3	23.4	2.6	38.0	18.6	16.2	1.83	2.05
5	♀	R, B 1, B, N	Saline injections	42.0	31.5	23.6	2.8	37.6	18.6	15.8	1.78	2.02
4	♂	R, B 1, N	Ammonium lactate injections	42.7	31.5	23.1	2.7	37.6	18.6	16.05	1.86	2.02
4	♀	R, B 1, N	Ammonium lactate injections	41.3	32.7	22.9	3.0	37.7	18.3	15.55	1.80	2.05
4	♂	R, B 1, N	"Parathormone" injections	41.9	29.9	23.0	5.2	37.9	18.8	15.9	1.82	2.01
4	♀	R, B, N	"	42.3	31.5	23.3	2.9	37.6	18.5	15.9	1.80	2.03

Although there was no significant difference between the different groups in the final body-weights, it was noted that the males receiving "parathormone" showed much greater deposition of fat, and this is being further investigated.

Bone weights. The weight of the bones is seen to follow very closely the body-weight of the individual rats, so that there is no evidence of any change in relative growth of bone and body due to the injections. The average value for the leg bone weights expressed as percentage of the body-weights for the three groups are as follows: saline ♂ 0.92, ♀ 1.00; ammonium lactate ♂ 0.94, ♀ 0.98; "parathormone" ♂ 0.88, ♀ 0.96.

The composition of the bones. Table I shows that the ash contents of the bones of the different groups lie within a very narrow range, and that no consistent effect can be attributed to the different substances injected. This is equally true of males and females.

No consistent change in the A/R ratio is to be noticed, and Table II shows that this is true for the entire composition of the bones, except that the fat of the bone is slightly high in the case of the males of the "parathormone" group. There is no change in the proportion of calcium and phosphorus in the ash, and the proportion of calcium in the fresh bone, although varying from rat to rat, is remarkably constant as from group to group. Hence there is no chemical evidence of bone changes as a result of the injection of "parathormone" in the dosage used in this experiment. But in order to determine whether the "parathormone" injections have had any influence on calcium storage relative to the storage of other components of the food, it is necessary

to consider the proportion of calcium to the total body-weight. Table III shows the calcium of the bones (femora, fibulae and tibiae) expressed as percentage of the total body-weight. (In compiling this table it has been assumed that the calcium of these bones bears a constant relation to the total calcium of the body.)

Table III. *Calcium of bone expressed as % of body-weight.*

Litter	Saline	Ammonium lactate	"Parathormone"
R ♂	0.153	{0.151} {0.147}	0.137
B 1 ♂	{0.154} {0.136}	0.150	0.145
B ♂	0.152	—	—
N ♂	0.149	0.159	{0.143 0.130}
Mean	0.149	0.152	0.139
R ♀	0.153	0.154	0.149
B 1 ♀	{0.170} {0.161}	0.154	—
B ♀	0.155	—	{0.145 0.166}
N ♀	0.152	{0.150} {0.150}	0.149
Mean	0.158	0.152	0.152

It may be seen that the values for the proportion of calcium to body-weight lie within a very small range for all the groups and both sexes, but that the value for the "parathormone" males is slightly but appreciably less than the value for the saline and ammonium lactate group of males. This is doubtless explicable by the slightly higher proportion of fat in the "parathormone" males. From these figures, however, no specific influence of parathyroid extract on calcium storage can be deduced. The influence of the extract on the weight and composition of the dry tooth was also found to be negative.

DISCUSSION.

The primary object of this experiment was to determine whether the injection of "parathormone" into young growing rats would reduce the rate of growth or ossification of bone, when the animals were on a diet which, from its content of calcium and fat-soluble vitamins, was considered to be favourable to optimal calcium retention. Should this have proved to be the case it would have emphasised the necessity of the warning given by Greenwald and Gross [1925] as to the need of care in the use of the extract, because of its tendency to deplete the stores of body-calcium. The results of the experiment, however, gave no indication of failure in bone-formation in the rats receiving the extract.

In correlating this fact with any theory as to the mechanism by which parathyroid extract produces its effects, it is necessary to consider not only the possible influence of diet, but also the difference in the reaction of different animals to the extract. In dogs, administration of the extract has been

generally found to result very readily in hypercalcaemia, which Greenwald and Gross [1926] showed was accompanied by increased excretion of calcium under the conditions of their experiments. In rats, however, there is little evidence of hypercalcaemia following the administration of "parathormone." (Tweedy and Chandler [1929] found a slight increase in the serum-calcium of a rat after the administration of 200 units of the extract in the course of 24 hours.) There is, therefore, no direct evidence that parathyroid extract (prepared from ox parathyroids) has a similar influence on calcium metabolism in rats and dogs. If, however, parathyroid extract does cause the same increase in the serum-calcium content in rats as in dogs, accompanied by such a rapid excretion of calcium that hypercalcaemia is not found, then in the experiment above, on a diet supplied *ad libitum*, and adequate in calcium and vitamins, the animals must have been able to secure from the food enough calcium to meet the extra needs arising from the extra excretion, as there is no evidence of decrease in rate of storage of calcium in the bone. The extra calcium may have been supplied by a slight increase in the total intake of food.

It is, however, possible that the doses of parathyroid extract used were either too small or too infrequent to produce any effect on the calcium storage in the bone; but as 580 units given to a dog [Greenwald and Gross, 1926] caused an increased excretion of 1.8 g. of calcium, it would seem that 200 units given to a rat ought to have produced a measurable effect on the calcium in the rat, as the final calcium content of the latter per animal would only be about 2 g. [Sherman and MacLeod, 1925].

SUMMARY.

1. Twenty-six young rats were fed on Steenbock's stock diet (Ca % 0.5 and ample fat-soluble vitamins). Ten received injections of normal saline (0.7 % NaCl in tap water), eight received injections of ammonium lactate (0.3 N ammonia brought to p_H 7.0 with lactic acid) and eight received injections of parathyroid extract.

2. Neither body nor bone growth was retarded in the group receiving parathyroid extract relative to their litter-mate controls.

3. The deposition of calcium in the bones was not influenced by the injections of parathyroid extract.

4. The four males receiving parathyroid extract showed unusually large deposits of fat. This was not noticed in the females of the group.

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