REGENERATION OF BONE AT VARIOUS METABOLIC RATES

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In several previous papers [4-6] we have shown the dependence of the healing of skin and muscle wounds on the basal metabolic rate. Nevertheless, we must not assume that the importance of metabolism in the regeneration of bone is well enough understood. It has long been known that the hormones of the hypophysis, thyroid, and sex glands are of great importance in regulating different aspects of metabolism. The action of the thyroid hormone on the degeneration of bone has been studied previously [1-4].

However, in previous investigations in which the thyroid gland was transplanted, or an excess of thyroidin injected, only the general outline of the healing process was observed. Less attention was paid to the microscopical changes associated with the fracture and to the accurate determination of the nature and the rate of metabolism under the experimental conditions prevailing.

In the present article we have made an attempt to study the relationship between regeneration of bone and the basal metabolic rate while the amount of thyroid hormone is increased.

METHOD

The experiments were carried out on 60 white rats weighing 120-150 g. The animals were divided into three groups. The first or control group was given no thyroid. In the second group thyroid was injected and increased the metabolic rate. The third group received 6-methylthiouracil in order to reduce the metabolic rate. The amount of both preparations given was 10 mg per 24 hours per animal.

In all the groups, the diaphysis of the tibia was broken 3-5 days after the beginning of the injections. An area on the medial aspect of the hindlimb and facing the body was carefully freed from fur. A linear incision of the skin was then made, the muscles were removed by blunt dissection from the crest of the tibia, and the bone was broken by special forceps at one point. This method produced an accurately standardized lesion without any displacement of the fragments, which was very convenient for a quantitative evaluation. The skin was sutured. The operation was carried out under ether anesthesia, and usually there was only a very small loss of blood. The fibula was the sole supporting bone. At various times after the operation (5, 10, 15, 20, 25, and 30 days) the animals were killed in pairs by ether, and the region of the healed wound was carefully studied.

Parts of the bone were sectioned, fixed in 10% neutral formol, taken through alcohols of increasing strength, decalcified in 7½% nitric acid for 10 days, and the acid was then neutralized with 5% potassium alum. The portion of bone were then embedded in paraffin. Longitudinal sections were stained in hematoxylin-eosin.

The material was evaluated in terms of the extent of the periosteal reaction, the size of the callus, the rate of development of the different phases, and rate of differentiation of various cellular elements. An estimate of the rate of regeneration of the bony tissue was based on x-ray studies, and subsequent x-ray pictures of parts of the newly-formed bone. The x-ray study was always made before the material was used histologically. At various stages of regeneration, the basal metabolic rate was determined by Pashutin's method.

RESULTS

During regeneration of the bonev tissue, the overall rate of gaseous exchange underwent considerable changes (see table).

In the animals of the first (control) group the metabolism remained at the original level, except for the first five days immediately after the infliction of the wound.

Change of the Indices of the Basal Metabolic Rate in White Rats during Regeneration of Bone

Group of experiments		Original level	3 days after administration		First day of regeneration		Fifth day of regeneration		Tenth day of regeneration		Twentieth day of regeneration		Twenty-fifth day of regeneration		Thirtieth day of regeneration	
		Absorption of O_2 (in g)	Absorption of oxygen (in g)	Excretion of CO_2 (in g)	Absorption of O_2 (in g)	Excretion of CO_2 (in g)	Absorption of O_2 (in g)	Excretion of CO_2 (in g)	Absorption of O_2 (in g)	Excretion of CO_2 (in g)	Absorption of O_2 (in g)	Excretion of CO_2 (in g)	Absorption of O_2 (in g)	Excretion of CO_2 (in g)	Absorption of O_2 (in g)	Excretion of CO_2 (in g)
First		0,7	0,7	0,7	0,8	0,9	0,8	0,8	0,7	0,7	0,7	0,7	0,7	0,7	0,7	0,7
	RQ	0,73	0,73	0,73	0,82	0,73	0,73		0,73		0,73		0,73		0,73	
Second		0,7	1,0	1,0	1,2	1,4	0,9	0,9	1,0	1,0	0,8	0,8	1,0	1,2	1,0	1,1
	RQ	0,73	0,73	0,73	1,83	0,73	0,73		0,73		0,73		0,87		0,80	
Third		0,7	0,6	0,5	0,7	0,6	0,6	0,5	0,7	0,7	0,6	0,5	0,6	0,5	0,6	0,6
	RQ	0,73	0,61	0,61	0,72	0,61	0,61		0,72		0,61		0,61		0,61	

In animals of the second group (injected with thyroidin) there was an increase in the metabolic rate as early as three days after the start of the injections. The new wave of enhanced metabolism was associated with the onset of the regenerative process in the bone. The main indices of metabolism were greatest during the formation of the first callus and during its subsequent reorganization into bone.

In animals of the third group (injected with 6-methylthiouracil) metabolism was greatly depressed. The onset of regeneration caused no metabolic increase such as was observed in the group receiving thyroidin. The low metabolic indices were maintained in these animals also during the later regenerative stages.

From a study of the bone of rats of the control group it was shown that the formation of the first callus begins 5-7 days after the operation, and proceeds most rapidly at the 10-15th day. From this time on, reorganization of the connective tissue callus into bone starts, and has been completed by the 30th day from the start of healing. During this period, usually the fragments have grown completely together, and are connected by a small hard bony callus.

After 25 days from the start of regeneration, the fractured region has been filled with newly-formed bone; the cartilagenous structure is retained only in a small area between the bones (Fig. 1).

In the second group, where the animals received thyroidin, the process of healing the fracture was considerably accelerated. The formation of the temporary callus was completed by the 10th day, and reorganization in the bone began immediately. In all the animals of this group, on the 20th day after the beginning of regeneration the line of the fraction was smoothed over, and around it there was a small bony callus. Under these conditions there was a rapid replacement of cartilagenous by osteoid tissue. Even by the 15th day, the regions of fracture had been filled by large islets of bone. There was an intense proliferation of young bony trabeculae, and between them connective tissue and blood vessels could be made out. In some parts of the regenerating region the blood vessels were so numerous that large vascular tufts were formed. The intense formation of osteogenous tissue was brought about by the accumulation in the regenerating area of a large number of osteoblasts distributed along the bony trabeculae. At the same time there was an increased formation of osteoclasts. By the 25th day, the intense formation of osteoid tissue had led to the formation of bone (Figs. 2, 3).

In the animals treated with 6-methylthiouracil, the regeneration proceeded very differently, and the healing of the fracture was delayed. The fracture healed in the same stages as it did in the controls, and the difference was only in the time taken for these stages and in the extent of the periosteal reaction. The formation of the temporary callus and its reorganization into bone was delayed. By the 15-20th day,

the region of the fracture was thickened into a club shape through the late formation of the callus. By the 30th day, when in the control animals the line of the fracture was completely smoothed over, and in the second group a bony callus had formed, in the third group there was still a large cartilagenous callus and edema of the surrounding tissues.

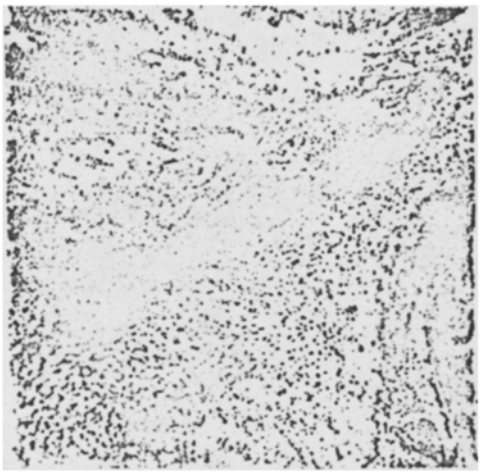

Fig. 1. Regenerating portion of the tibia of a control white rat, on the 25th day after fracture.

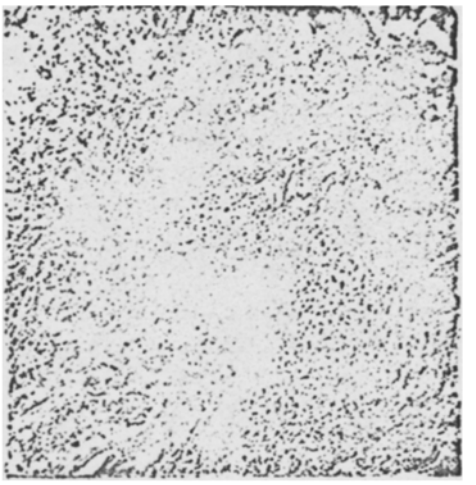

Fig. 2. Regenerating portion of the tibia of a white rat treated with thyroidin. 25th day after fracture.

A microscopical study on the 15th day after the fracture showed the presence of a large callus built up mainly from connective and cartilagenous tissue. At this time the formation of bony trabeculae had barely begun. By the 25th day, at which time in the animals which received thyroidin the boney callus had formed, in those treated with 6-methylthiouracil only the cartilagenous callus had developed (Fig. 4). The formation of bony tissue was delayed through suppression of formation of osteoblasts.

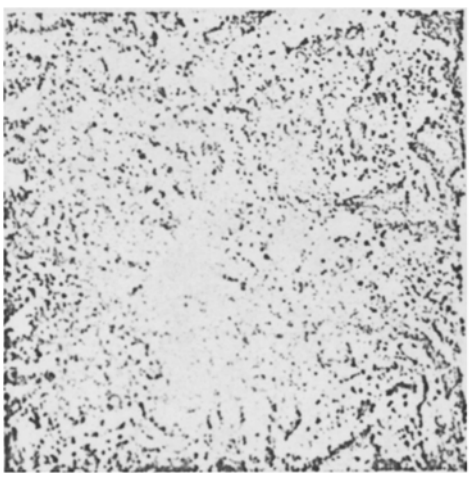

Fig. 3. Regenerating portion of the tibia of a rat treated with thyroidin. 28th day after fracture.

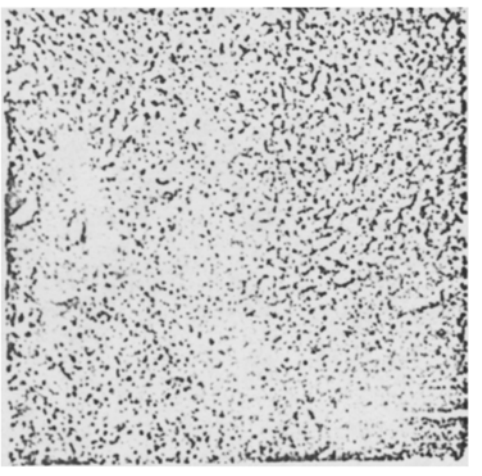

Fig. 4. Formation of a cartilagenous callus in a rat treated with 6-methylthiouracil. 25th day after fracture.

It is known that the injection of thyroidin leads to a high concentration of this hormone in the blood, and depresses the function of the animal's own thyroid, which then shows all the signs of hypofunction. Under these conditions metabolism is strongly increased above the normal value. During this period of increased metabolic activity, regeneration of the bony tissue is accelerated: there is an increased activity of the cellular mesenchymal elements, in the regenerating region osteoblasts accumulate rapidly, and at the same time there is an accelerated formation of osteoclasts and the cartilagenous tissue is replaced more rapidly by osteoid tissue.

Injection of 6-methylthiouracil causes a functional exhaustion of the thyroid with the result that no hormone is contained either in the gland or in the blood. Metabolism is greatly reduced, and regeneration of the bony tissue is slowed: the activity of the cellular elements of the connective tissue is lowered, and but few osteoblasts are formed so that the process of replacement of cartilagenous by osteoid tissue is slowed.

SUMMARY

Bone tissue regeneration after fracture of the tibia was studied in albino rats under conditions of different BMR levels. Thyroidin caused a marked rise of the BMR and accelerated the regeneration of bone: mesenchymal cells exhibited increased activity, large numbers of osteoblasts in the regenerating area accumulated more rapidly, and the replacement of cartilagenous by osteoid tissue was accelerated. A low BMR caused by 6-methylthiouracil retarded bone regeneration.

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