

Effect of Acid Extract of Cattle Ant. Pituitary on Bone Repair in Thyroidectomized Guinea Pigs.

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In former investigations we have shown that the growth-promoting influence of acid extract of anterior pituitary gland of cattle on bone and cartilage of young guinea pigs is exerted without the intervention of the thyroid gland.¹ Correspondingly we wished to determine whether the accelerated healing tendency of fractured bones, caused by the extract,² is also independent of the action of the thyroid.

The experiments were carried out in 26 winter and spring guinea pigs, on the average weighing from 120 to 280 gm. Under deep ether anesthesia the tibia and fibula of the right hind leg were broken about the middle of their shafts and bandaged according to the method described previously. Both lobes of the thyroid were completely removed immediately after fracturing. Injury to the parathyroids was carefully avoided. Sixteen of the animals were injected daily with 1 to 1½ cc. of extract intraperitoneally for periods varying from 6 to 21 days, the first injection being applied immediately after thyroidectomy. Ten guinea pigs were not injected and served as controls. After 6, 10, 14, and 21 days the fractured bones were removed *in toto* and specimens prepared for microscopic examination as previously described.³

As to the gross appearance of the callus in the injected and thyroidectomized animals, it was invariably firmer and harder than that of the non-injected thyroidectomized guinea pigs. This fact was noted as early as 6 days after fracturing, but even more distinctly at subsequent periods. In all cases a complete union by a dense, bony, calcified callus had taken place after 21 days, while in the non-injected animals the callus was looser and softer at that period. There was no marked difference in gross appearance between the callus in injected thyroidectomized guinea pigs and in those with intact thyroid glands, which had been injected.

¹ Silberberg, M., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **33**, 554.

² Silberberg, M., and Silberberg, R., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **33**, 177.

³ Silberberg, M., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1423.

Microscopical Examination. First Series. In thyroidectomized guinea pigs as early as 6 to 7 days after fracturing, the ends of the broken bones have become calcified. They are separated by a newly-formed vascularized granulation tissue representing a loose connective tissue callus. In addition a reaction of the periosteum has taken place, though it is considerably delayed in comparison with that of the normal animals.² In some areas the very beginning of a conversion of the mesenchymal cells into osteocytes and an osteoid tissue is seen. But, on the whole, this new formation of osteoid tissue is inconsiderable. Neither cartilage formation nor any calcification of the osteoid tissue can be observed.

After 10 days a union of the ends of the broken bones by osteoid tissue has occurred, but a strong growth tendency is missing, as indicated by a diminished formation of cartilage and bone and by the very small number of mitotic figures which are seen.

Fourteen days after fracturing a small but definite callus is found; it is apparently less developed than under normal conditions, and the formation of osseous tissue and particularly that of the cartilage is very limited. The vesicular cartilage cells show dense nuclei and a very small amount of light cytoplasm. In comparison with the healing process in normal animals not only is the development of the cartilage considerably diminished but also its calcification is retarded.

Likewise, after 21 days the healing tendency is still very slight, as demonstrated by delayed and by decreased new formation of cartilage, while the osteoid substance is better developed, but evidently less so than normally. In addition, the calcification of the vesicular cartilage is retarded and little osteoclasia is present.

Second Series. In thyroidectomized animals injected daily with extracts of anterior pituitary gland, after 6 to 7 injections a definite union of the broken stumps by osteoid tissue, originating from the periosteum, is seen; the dense callus which thus develops is of considerable size. Large islands of vesicular cartilage distinctly basophilic and tending to become calcified are found. These changes are even somewhat more pronounced than in injected non-thyroidectomized guinea pigs. The cartilage cells show hypertrophic cytoplasm and large nuclei as well as an increased number of mitotic figures. The cartilage appears chiefly in areas that are surrounded by osteoid tissue, in which latter likewise a stimulation of growth processes is noticeable.

As early as after 10 injections the callus is already calcified and ossified, the markedly basophilic cartilage cells exhibiting at this time extreme hypertrophy and hyperplasia.

After 14 injections a considerable amount of osseous and cartilaginous callus is present, together with hypertrophy and hyperplasia of its cells. At that date the process of calcification has further progressed than in the thyroidectomized controls after 21 injections. In addition, a good many mitotic figures and osteoclasts are noticeable. The difference between the injected and non-injected thyroidectomized guinea pigs is very marked. In some cases of this series the process of bone repair seems to be even more accelerated than in the guinea pigs with intact thyroid under the influence of the extract.

After 21 injections there has developed a hard, dense, bony callus connecting the broken fragments. Osteoclasia as well as vascular absorption go hand in hand with the formative processes as described above, thus demonstrating a well-balanced healing tendency.

In contradistinction to the behavior of the callus in injected guinea pigs with intact thyroids, where in about 20% of the cases acceleration of calcification was absent, in the above experiments a uniform acceleration was obtained. The assumption seems, therefore, to be justified that some conditions inherent in the thyroid gland are responsible for the differences between these 2 classes of animals.

Conclusions. In thyroidectomized controls, callus formation is retarded as compared with the findings in normal guinea pigs. In particular, the production of cartilage is delayed and diminished. Osteoid tissue is also less developed. Under the influence of the extract, however, a faster transformation of mesenchymal tissue takes place (1) directly into osteoblasts, osteoid tissue and bone; (2) indirectly into cartilage and afterwards into bone by the way of endochondral ossification.

Thus anterior pituitary extract of cattle counteracts and overcomes the growth-preventing influence on cartilage and bone of young guinea pigs exerted by thyroidectomy and causes an accelerated healing tendency of the fracture and a faster consolidation of the callus. We may, therefore, conclude that the extract exerts its influence independently of the thyroid gland. There is under these conditions a parallelism between the process of endochondral ossification in the epiphyseal line and the healing of the fractures by means of a callus.