

strate the dependence of the autoxidative rate upon glycogenolysis during anesthesia.

Summary. The present findings of decreased autoxidative rates in brains of rats anesthetized with glycogenolytic narcotics or treated with epinephrine are in accordance with the concept of general mediation of many biochemical processes during anesthesia through stimulation of adrenin output, with the possible exception of Dilaudid. In other cases studied, blood sugar curves show an inverse relationship to the rate of autoxidation after administration of narcotics, and no direct correlation with the depth of anesthesia produced by different anesthetics.

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Influence of Acid Extract of Cattle Anterior Pituitary Gland on Bone Repair in Young Guinea Pigs.

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We have shown¹ that a growth-promoting effect is exerted by acid extract of cattle anterior pituitary gland on bone and cartilage in young guinea pigs. The present investigation deals with the influence of this extract on callus formation and healing of fractured bones.

Twenty-four fall and winter guinea pigs averaging from 130 to 300 gm. in weight were used in these experiments. Under deep ether anesthesia both tibia and fibula of the left hind leg as a rule were fractured in these animals. As exposure of the bone with the unavoidable removal of the periosteum was followed by unsatisfactory healing, the following procedure was used: After shaving the skin of the leg the bones were broken at about the middle of their shafts. The broken ends were carefully approximated, the leg fixed with a small wooden splint and tightly bandaged with adhesive plaster. Sixteen animals thus operated on were injected with from 1 to 1½ cc. of extract daily, while 8 others of similar size, weight and age were not injected and kept under the same environmental factors, thus serving as controls. All were fed in the same manner with oats and bran or shorts. In addition, they received some cabbage, hay, lettuce and turnips. The injections were continued for periods

¹ Silberberg, M., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1423.

varying between 6 and 21 days. After 6, 10, 14, and 21 days X-ray pictures were taken in collaboration with Dr. S. R. Johnston in order to observe the position of the fragments and the progress of callus formation.

After 6, 10, 14, and 21 days the animals were sacrificed, and the fractured bones used for microscopical study. In each case the long bones were taken out as a whole, the muscles and tendons removed and *in toto* sections prepared, as described previously.

After the first injections the guinea pigs lost weight, but about 8 days later the original weight had been regained. The control animals gained weight steadily. In each case we compared control and injected guinea pigs in which the position of the fragments and their displacement, if there was any present, were as similar as possible.

The *gross appearance* revealed the fact that the callus formed in the injected animals was invariably harder, denser and more abundant than that of the controls as early as after 6 days, but still more pronouncedly after 10 and 14 days. After 21 days a complete, firm consolidation had taken place in the injected guinea pigs. In the majority of cases the callus had become denser and subsequently smaller in the injected as compared with the control animals.

In addition, the process of decalcification was very much more retarded in the former than in the latter guinea pigs in spite of their similarity in weight, size and age.

The *histological examination* of the callus in the controls 6 days after the fracture shows that the broken ends have become necrotic and completely calcified. They are surrounded by newly formed osteoid tissue originating from the well preserved periosteum. However, a complete union has not yet been obtained. The stumps are still separated by a layer of fresh vascularized loose granulation tissue. There is no evidence of cartilage formation.

The injected animals, at that time, show a complete union of the very well calcified stumps by means of an extensive layer of well developed, newly formed, though not yet calcified osteoid tissue. In addition, a large number of osteoblasts and of islands of vesicular cartilage has been produced. These cartilage cells show a basophilic hypertrophic cytoplasm and very frequent mitotic figures.

Ten days after the fracture the callus of the control animals consists of a large and dense layer of homogeneous vesicular cartilage cells with a very light, somewhat acidophilic cytoplasm. Complete union of the bony fragments by cartilagenous and osteoid tissue is definite at this period. In the animals which had received 10 in-

jections of acid extract the organization of the callus is still further advanced. The callus is very much ossified. Pronounced calcification of the hypertrophic and hyperplastic vesicular cartilage cells has taken place; also the osteoid tissue has become calcified. In the control animals, as a rule, this calcification is not observed until 16 to 18 days following the fracture.

The source of the calcium in these processes, whether from the food or from parts of the skeleton, remains as yet uncertain.

After 3 weeks the callus of the control guinea pigs consists of true bone with remnants of not yet calcified osteoid tissue with the admixture of more or less cartilage. Absorption of bone by osteoclasts as well as by the blood runs parallel with the formative processes.

Within 2 to 3 weeks the absorption of the abundant callus by osteoclasts as well as by blood has become more active in the injected than in the control guinea pigs. After 3 weeks only a densely calcified small bony callus is found.

It may be mentioned that these effects are observed independently of the position of the fragments; furthermore they are the same irrespective of the sex of the animals.

However, in about 20% of the cases the process of calcification is retarded in the injected guinea pigs. Under this condition only an increased growth of hypertrophic vesicular or basophilic cartilage cells with a large number of mitotic figures is noticeable. This is observed without exception in those animals where the closure of the epiphyseal line was not found after the average number of injections.

Conclusions. Acid extract of cattle anterior pituitary gland has a growth-promoting effect on the callus formation of fractured bones in young guinea pigs. The healing tendency of fractures is accelerated. This is due to a faster transformation of mesenchymal granulation tissue which is converted

- (1) directly into osteoblasts, osteoid tissue and bony substance or
- (2) indirectly into cartilage and afterwards into bone by way of endochondral ossification.

Associated with the increase in formative processes there takes place also a more active absorption of the newly formed tissue.

There is noted a parallelism between the formation of cartilage and the rapidity of calcification of the callus on the one hand and the rapidity of endochondral ossification in the epiphyseal line on the other hand.