cause well-formed Nissl bodies can be stained in sections thus prepared, that the said bodies must exist in living nerve cells despite the fact that they have never been seen therein. Thus far we have not compared the homogeneous looking bacilli of the new technique and the more broken up organisms observed in preparations made by standard methods with bacilli of rat leprosy in living cells. We have, however, made a parallel study of human tubercle bacilli in visceral lesions (for which we are indebted to Dr. Ralph S. Muckenfuss). In this case also, the bacilli were distinctly less granular after the freezing and drying technique than in tissues preserved by immersion in 10% formalin in absolute alcohol, straight 10% formalin, formalin-Zenker or Regaud's fluid. These observations suggest, either that there is some unsuspected flaw in the freezing and drying method, or that the bacillus of rat leprosy and the human tubercle bacillus as they exist in the tissues are less granular than is commonly supposed.

8118 P

Effects of Extract of Cattle Anterior Pituitary Gland on Endochondral Ossification in Young Guinea Pigs.

MARTIN SILBERBERG. (Introduced by Leo Loeb.)

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It has been established that the anterior pituitary gland enhances bodily growth as a whole, but it is not yet known whether it has a specific effect on the growth of cartilage and bone and, if this should be so, wherein the effect consists. We wished, therefore, to study the action of anterior pituitary gland extract on the epiphyseal cartilage and bone formation in young not yet fully grown guinea pigs.

About 30 guinea pigs (fall and winter animals) on the average weighing 130 to 220 gm. were injected with from 1 to 1½ cc. extract[1] daily. Additional guinea pigs, of similar weight, were not injected and served as controls. The injections were continued up to 21 days. After 4, 6, 11, 14, and 21 days, the animals were killed and tibia and fibula were used for the study of the epiphyseal line. In each case the specimen, as a whole, was fixed in 10% formalin; then put into absolute alcohol and subsequently trans-

[1] Loeb, Leo, and Basset, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 860. Silberberg, M., *Virchow's Arch.*, 1933, **289**, 201.

ferred into ether for 2 days, in order to remove the fat from the bone-marrow. The bones were then washed in running water and decalcified incompletely in order to preserve and demonstate the calcium salts. In accordance with Pommer's method, they were kept about 3 to 8 weeks in Mueller's solution, until they had become sufficiently soft for cutting; subsequently they were embedded in celloidin and stained with hematoxylin-eosin or hematoxylin-Van Gieson.

After 4 injections, a stimulation of the growth of cartilage and bone can be seen at the epiphyseal line. The undifferentiated mesenchymal cells have become mobilized and transformed into chondrocytes and osteocytes. The columnar resting cartilage cells are increased in size and number, mitotic figures are found everywhere and are more numerous than under normal conditions, the stroma between the cells correspondingly is much diminished in amount. Likewise in the layer of the vesicular cartilage, hyperplasia and hypertrophy are noted. After 7 to 10 injections, the epiphysis as a whole, has become more narrow in comparison with that of the control animals. However, in addition, we notice now pronounced disturbances of endochondral ossification. The epiphyseal line has become completely calcified and is therefore in the majority of cases closed in contradistinction to the normal epiphyseal line in the controls. Furthermore, the layer of the indifferent mesenchymal cells and of the columnar cartilage cells has become irregular, the cytoplasm of the latter having taken up calcium salts. The vesicular cartilage cells are hypertrophic and in some cases may show giant cell formation; the number of mitotic figures is here increased. On the other hand, changes on the part of the osteoclasts were not noted. After the growth of the cartilage has thus been stimulated, this tissue undergoes calcification and is quickly replaced by bone tissue. This process takes place in the cartilage equally on the side of the epiphysis and of the diaphysis. Corresponding growth changes as well as disturbances of the endochondral ossification are seen also in the other long bones.

After 14 injections the epiphyseal line was not yet completely calcified in about 20% of the animals, in which latter the effects observed at this time were the same as those usually seen after 4 injections. After 21 injections, increase in size and proliferation of the various kinds of cartilage cells are still more pronounced, and the epiphyseal line is now completely closed.

Conclusions. Injections of acid extract of anterior pituitary of cattle have a stimulating effect on the growth of cartilage and bone

of growing guinea pigs. The various layers of the cartilage cells become hypertrophic and hyperplastic, and are subsequently quickly calcified and replaced by bone. The epiphyseal line undergoes, therefor, premature closure under the influence of the extract.

8119 C

Effect of Various Anterior Pituitary Gland Preparations on Thyroidectomized Guinea Pigs.

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It has been shown that following repeated injections of cattle anterior pituitary extract a very extensive atresia of follicles takes place in the ovary of the guinea pig, which is in contrast to the growth and maturation of follicles that occurs after implantation of anterior pituitary glands of rabbit, rat and guinea pig.[1] A similar, though less pronounced atresia of follicles may be produced through undernourishment of guinea pigs (development of hypotypical ovaries).[2] It was of interest to determine whether the thyroid gland which is rendered strongly active by these extracts is concerned in this effect on the ovarian follicles. This question was of interest, because it has been shown that some of the effects of anterior pituitary extracts are mediated by the thyroid gland; and furthermore, that thyroid hormone increases basal metabolism and may lead to a loss of weight in the animal in which it is present in excess.

We first carried out control experiments in which we thyroidectomized 21 guinea pigs with an original weight varying between 175 and 205 gm. (except one guinea pig which weighed 260 gm.). Autopsies were performed at intervals between 6 and 38 days following thyroidectomy. The thyroidectomy was complete in 13 animals, while in the remaining 8 some remnants of the thyroid were found. The operation caused a loss in weight or prevented a normal gain, especially in guinea pigs with an original weight less than 190 gm. The heavier animals had an initial loss of weight for

1 Loeb, Leo, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 642, 1128; *Endocrinology*, 1932, **16**, 129.

2 Loeb, Leo, *Biol. Bull.*, 1917, **32**, 91.