

the proteins themselves although this agent appears to be associated with the pseudoglobulin.³

It has been found that by the addition of adequate quantities of alkali or acid to serum (normal or immune), exposure of the mixture to heat and subsequent neutralization, the serum-sickness-causing activity may be destroyed so that rabbits injected with the customary amounts of serum will not develop manifestations of the disease.

In order to minimize or prevent damage to antibody incident to this type of treatment of serum, it is necessary to adjust the various factors concerned in the treatment of the serum. Six factors must be considered: 1, The amount of alkali used (between 0.2 gm. and 0.1 gm. for 100 cc. of serum when using NaOH); 2, the dilution of the mixture (from 1:2 to 1:10); 3, the temperature to which the mixture is exposed (from 45°C. to 55°C.); 4, the time of exposure (from 30 min. to 18 hr.); 5, the type of antibody in the serum; 6, the particular sample of serum being treated. The first 4 factors must be balanced against each other in relation to the last 2 factors.

By this method the serum-sickness-causing factor may be destroyed so that rabbits receiving treated serum (10 cc. per kg.) do not develop serum-sickness while rabbits injected with the same untreated serum develop manifestations of serum-sickness in 6 of 10 animals. In sera which are no longer active in causing serum-sickness, from 50% to 100% of the original protective antibody is retained.

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Changes in Ribs of Guinea Pigs Following Administration of Cattle Anterior Pituitary Extract. (*Acromegalic Rosary*).

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In previous investigations we could show that after 10 to 21 injections of acid extract of cattle anterior pituitary a growth effect becomes manifest in cartilage and bone of young guinea pigs. The

³ Jones, L., and Fleisher, M. S., *J. Immun.*, 1934, **26**, 455.

* We are indebted to Dr. Leo Loeb, who made it possible for us to carry out these investigations.

principal changes observed are as follows: (1) The epiphyseal cartilage in a first stage undergoes hypertrophy and hyperplasia; this is followed in the majority of cases by an accelerated calcification and ossification of the epiphyseal line.¹ (2) In certain cases hypertrophy and hyperplasia of the cartilage take place without acceleration of ossification. (3) Arthropathic lesions of an acromegalic type may occur in the joints.

These changes are accomplished without the mediation of the thyroid gland.²

In these investigations we wished to study the effects of the extract if administered over longer periods of time. The present report deals only with its action on the chondro-osseous junction of the ribs of normal as well as of thyroidectomized guinea pigs.

Twelve young guinea pigs of both sexes weighing on the average 140 gm. received daily intraperitoneal injections of 1 to 1½ cc. of acid extract of anterior pituitary of cattle for periods varying between one and 5 months, while 10 additional animals were not injected and served as controls. In 16 other guinea pigs of approximately the same weight the thyroids were completely removed under ether anesthesia. Eight of these animals received daily injections of acid extract for various periods, up to 5 months. The remaining 8 were not injected and served as controls. All the guinea pigs were kept under the same environmental conditions and their weights were taken daily. At the end of the experimental term various parts of the skeleton were fixed, incompletely decalcified, and stained, as described previously.

Gross appearances: In normal guinea pigs injected for longer than 3 months a thickening of the ribs in their entire length became noticeable. In addition a bulbous enlargement of the chondro-osseous junction of the ribs, particularly of the lower ones, was seen. In some instances small nodular newgrowths with a hard consistency and having a bluish white color were detected. These lesions were still more pronounced in the thyroidectomized injected animals. After 5 months' treatment a typical rosary-like condition of the chondro-osseous junction was found. The largest of these new formations were about the size of lentils.

Microscopic examination: The line of ossification is, on the whole, regular: The bonemarrow is sharply demarcated from the preëxisting cartilaginous parts. The changes occur in the zone of the resting and columnar cartilage as well as at the side of the inner layer

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

² Silberberg, M., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 66.

of the perichondrium and the neighboring periosteum: The resting cartilage cells are more numerous than normally. Their nuclei are generally well preserved and their cytoplasm is somewhat enlarged; it takes on a light stain and contains small vacuoles here and there. The cells of the columnar cartilage are definitely hypertrophic, showing a markedly enlarged vacuolar cytoplasm. In some places the nuclei have assumed an intensely red eosin stain; distinct structural differentiations in the nuclei are lacking. A single cartilage-capsule may enclose as many as 4 or 5 cells and these cell groups may occasionally undergo degeneration, as is indicated by the liquefaction of their cytoplasm. The interstitial connective tissue is diminished in amount. Toward the periphery of the rib the cartilage is distinctly stained bluish by hematoxylin. The normally vertical arrangement of the columnar cartilage assumes more and more an oblique direction, until the position of the columns has become almost radial extending in a bow into the zone between periosteum and bone. In addition to the cartilage cells, the actively stimulated perichondrium and periosteum participate in the new formation by means of hypertrophy and hyperplasia of the spindle cells, and their growth activity may even exceed that of the cartilage proper. These spindle cells become more numerous and then assume a more oval and rounded shape; they are characterized by a large amount of cytoplasm, which encloses nuclei stained deeply blue with the hematoxylin; thus they resemble typical chondroblasts. In advanced stages the most centrally situated cells of the new growth appear polyhedral and are converted into chondrocytes. Here and there calcification of these cartilaginous cells is noticeable; the intercellular stroma is developed only to a very limited extent. In the most advanced cases the newly formed cartilaginous mass represents a callus-like structure situated between the periosteum and perichondrium on the one side and the original rib on the other side.

Associated with these changes are plastic processes within the bone-marrow, in particular in areas adjoining the cartilaginous growth. In the marrow, fibrillar connective tissue has replaced the lymphoid structure. Furthermore a conversion of these fibrous elements into cartilage cells could be observed.

In thyroidectomized animals, all these changes are even more marked than in the guinea pigs with intact thyroid glands. There exists, however, no essential difference between these two groups of animals.

Summary. Administration of acid extract of cattle anterior pituitary, when continued over long periods of time, leads to the

following changes at the chondro-osseous junction of the ribs of the growing guinea pig: (1) The preëxisting resting and columnar cartilage cells undergo hyperplastic and hypertrophic changes which are associated with certain retrogressive processes in the cells. (2) In the cells of the perichondrium and periosteum a pronounced hyperplasia takes place followed by an accelerated conversion of these cells into cartilaginous tissue with subsequent calcification. (3) The bone marrow becomes transformed into fibrous connective tissue in which cartilaginous islands develop in places adjoining the hyperplastic cartilage. Through the coöperation of both these growth processes an abundance of cartilage may develop.

We find various transitions between ordinary hypertrophy and hyperplasia of the preëxisting cartilage cells to the formation of a rosary-like newgrowth. In advanced cases, at a time when usually the growth of the skeleton is terminated, the formation of tissue may become so very extensive that it leads to the production of a parosteal "rosary". These changes are very similar to those described by Erdheim³ in cases of human acromegaly. They also correspond to the alterations observed by us in the cartilage of the epiphyseal line and the joint following injections of the extract over shorter periods of time. These changes may be even more marked in thyroidectomized than in non-thyroidectomized animals, which provides further proof for the conclusion that, in guinea pigs, cattle anterior pituitary extract acts on the growth of bone and cartilage without the mediation of the thyroid gland.

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The Fibro-Muscular Scrotal Sac Under Normal and Experimental Conditions in an Annual Breeder (*Citellus*).*

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What causes the testis of an adult ground squirrel to ascend into the peritoneal cavity during the non-breeding period? Is decrease in testis-size a primary factor, or does the scrotum regress and force the testis to ascend? The scrota of 89 ground squirrels (*Citellus*

³ Erdheim, J., *Veröffentlichungen a. d. Gebiete d. Pathol. in Einzeldarstellungen*, Julius Springer, Berlin, 1931.

* Aided by a grant from the Committee for Research in Problems of Sex, National Research Council.