possess no corpora lutea, or which exhibit corpora of less than 3 or 4 days' maturity. The administration of large doses of oestrone does not appear to interfere with post-coital ovulation and early corpus luteum development in the rabbit, though it may alter in marked manner the anatomic pattern of the coincidental progestational mucosa of the uterus. The undoubted stimulating effect of oestrone upon the anterior pituitary, as shown by the work of Wolfe,[5] Lane,[6] Hohlweg,[1] Selye, *et al.*,[2] and others,[7-10] might therefore be interpreted—as Selye has in fact already suggested—as the calling forth of pituitary factors which have functions other than that of initiating the luteinization of follicular granulosa.

8447 C

Effects of Extract of Cattle Ant. Pituitary on Endochondral Ossification in Thyroidectomized Young Guinea Pigs.

MARTIN SILBERBERG. (Introduced by Leo Loeb.)

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In former investigations we[1] studied the influence of acid extracts of anterior pituitary gland of cattle on the growth of bone and cartilage in young guinea pigs. We analyzed the endochondral ossification in normal animals as well as the callus formation in guinea pigs in which a bone had been fractured; we could establish under these conditions a growth-promoting effect of the anterior pituitary extract. In about 20% of the cases the growth of both cartilage and bone was stimulated to the same extent, with consequent hypertrophy and hyperplasia of their various cells. However, in the majority of the animals the process of ossification predominated over the formation of cartilage, thus causing an earlier closure of the epiphyseal line than would have taken place otherwise.

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[5] Wolfe, J. M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 757, 1192.

[6] Lane, E. C., *Am. J. Physiol.*, 1935, **110**, 681.

[7] Burch, J. C., and Cunningham, R. S., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 331.

[8] Halpern, S. R., and d'Amour, F. E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 108.

[9] Hisaw, F. L., *Am. J. Obs. and Gyn.*, 1935, **29**, 638.

[10] Nelson, W. O., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 452.

[1] Silberberg, M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1423. Silberberg, M., and Silberberg, R., *Ibid.*, 1935, **33**, 177.

The question arises now as to the mechanism by which the extract exerts its effect on bone and cartilage, in particular, whether the pituitary hormone acts directly on bony and cartilaginous tissues or whether it exerts its effects indirectly by way of the thyroid.

Loeb and his collaborators[2] had tested the effects of the anterior pituitary extracts in thyroidectomized guinea pigs in order to determine whether certain actions of the extract depend on the changes produced in the thyroid gland or whether the extract affects the various organs directly.

In these investigations we wished, therefore, to study the influence of the extract on the growth of bone and cartilage in thyroidectomized young guinea pigs.

Thirty-six winter and spring guinea pigs, averaging from 120 to 280 gm. in weight were used. Under ether anesthesia both lobes of the thyroid gland were completely removed in 26 animals. Injury of the parathyroids was carefully avoided. Sixteen of these guinea pigs were injected daily with 1 to 1.5 cc. extract for 7, 10, 14, and 21 days. As in the former experiments, we used freshly prepared extracts. Ten additional thyroidectomized guinea pigs were not injected and served as controls. A series of 10 guinea pigs of approximately the same weight and size which had intact thyroids served as further controls, their growth being daily observed.

The animals were kept in the same kind of cages and received the same kind of food. After 7, 10, 14, and 21 days some animals of each series were killed; the long bones were taken out as a whole and sections were prepared in accordance with the method previously described.

The *normal* guinea pigs gained weight steadily; there was an average rise in 3 weeks from 160 gm. to 295 gm.

The *thyroidectomized* animals lost at first some weight following the operation; after 5 to 7 days they had regained their original weight, and this was followed by a continuous increase of weight up to 220 gm. The endweight in this group was, therefore, lower than that reached by the control animals.

The average endweight of the *thyroidectomized and subsequently injected* guinea pigs was much lower, namely 185 gm. These differences are clearly shown in Chart I, in which the number of days of the experiments represents the abscissae and the weight the ordinate.

There was a parallelism between the difference in weight and the

[2] Loeb, Leo, *Endocrinology*, 1932, **16**, 129; PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 642; *Ann. Int. Med.*, 1935, **9**, 12.

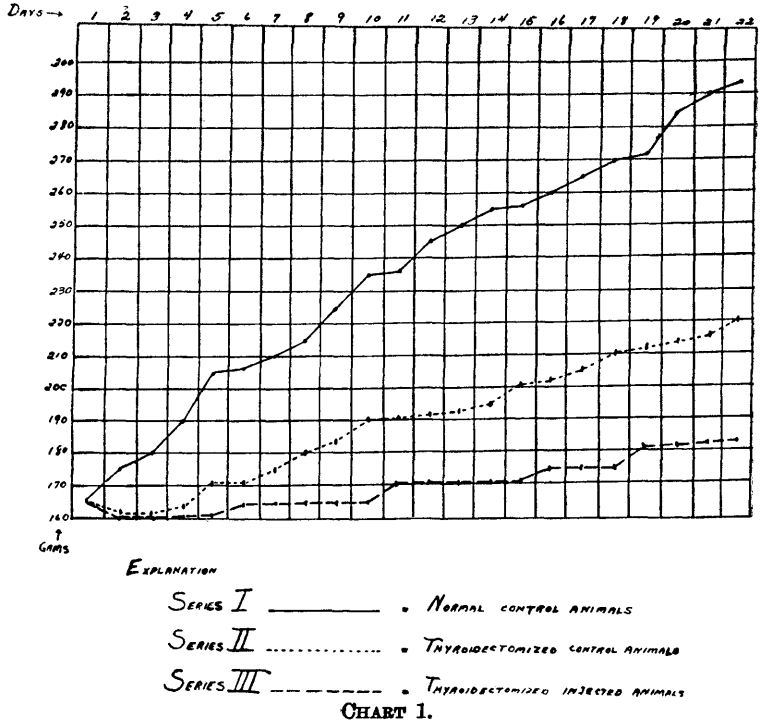

CHART 1.

growth of the animals in these 3 groups. The growth of the thyroidectomized animals was retarded as compared with the normal controls, the whole skeleton remaining considerably smaller; this condition was especially indicated by the length and width of the bony part of skull, thorax and limbs. Still more marked was the retardation in growth of the bones in thyroidectomized, injected animals.

As to the *gross appearance* of the long bones in the thyroidectomized animals, the epiphysis became somewhat clumsy and flatter than in normal guinea pigs and the long bones were shorter.

The decalcification of the bone for the purpose of cutting was accomplished with greater rapidity in the non-injected thyroidectomized animals than in the injected ones, a condition which corresponds to our observations in the experiments on non-thyroidectomized animals.

First Series. In the normal animals the epiphyseal line was always open, the zones of the column-cartilage cells, of the vesicular cartilage and of the calcifying cartilage were well formed and sharply demarcated from one another; the mesenchymal cells of

the chondrophyte were resting and without signs of proliferation or hypertrophy.

Second Series. In thyroidectomized animals, as a rule, the epiphyseal line was open as late as 3 weeks after operation. In comparison with the controls the zone of endochondral ossification was somewhat narrower, while the zone of calcifying cartilage was advancing. In a few cases the amount of calcium within the bony and cartilaginous cells was increased, and in some instances a closure of the epiphyseal line occurred at a later period following thyroidectomy. As to the chondrophyte, from within one to 2 weeks after thyroidectomy the conversion of the indifferent mesenchymal cells into cartilage was more pronounced than in normal animals; the cytoplasm of the cartilage cells was moderately increased and their nuclei were flattened. There was no evidence of hyperplasia or of calcification.

Third Series. In thyroidectomized and injected guinea pigs as early as after 7 injections the growth of the different cell layers of the epiphyseal line was distinctly stimulated. The column-cartilage cells were increased in number and size, mitotic figures being present everywhere and more numerous than normally; the stroma between the cells was distinctly diminished. Likewise, hyperplasia and hypertrophy were noticeable in the vesicular cartilage cells. In addition, disturbances of nutrition seemed to occur, as indicated by the large amounts of calcium taken up by the cells. These finally underwent complete calcification so that the epiphyseal line became considerably narrower and was prematurely closed, an effect more marked and taking place faster than in normal guinea pigs injected with anterior pituitary extracts. Furthermore, an increased growth of the cells adjoining the chondrophyte was seen.

After 10 to 21 injections these phenomena were still more marked; the epiphyseal line was completely calcified; the hyperplasia of the cells of the chondrophyte was more distinct; they had a basophilic appearance and exhibited a strong tendency to calcification.

In contradistinction to the results obtained in normal injected guinea pigs, only in a single case, namely in that one after 14 injections, was the epiphyseal line still open.

Conclusions. In thyroidectomized young guinea pigs injections of acid extract of anterior pituitary gland of cattle cause a hypertrophy and hyperplasia of the different cell layers of the chondrophyte and of the epiphyseal line, with subsequent rapid calcification and premature closure of the latter. We may, therefore, conclude that the extract exerts its growth-promoting effect on cartilage and

bone also in thyroidectomized young guinea pigs. Lack of thyroid gland does not, therefore, prevent the effect of the extract in this respect; on the contrary the phenomena are even more distinct than in normal animals injected with anterior pituitary extract. We may furthermore conclude that anterior pituitary extract exerts its influence on bone and cartilage without the mediation of the thyroid gland.

8448 P

A Comparison of Lipid Composition with Differential Count of the White Blood Cells.

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During the course of a previous investigation, Boyd and Wilson[1] demonstrated that leucocytes from the blood of the umbilical vein and artery contained only about one-half the concentration of certain lipids contained in the white blood cells of adults. It was suggested that the relative lymphocytosis of fetal blood was responsible for the differences observed. The inference was made that in all probability the polymorphonuclear cell contained a higher percentage of lipids than the lymphocyte. The present study was instituted with the purpose of further investigation of this suggestion.

Samples of blood were taken by venapuncture from a group of 25 patients of the Strong Memorial and Rochester Municipal Hospitals. Subjects were selected only with a view to obtaining a wide range of variation in the differential count. The leucocyte fraction ("buffy layer") was isolated and analyzed for its lipid content by a method previously described.[2] Platelets, the lipid content of which is not known, were included in the leucocyte fraction, but comprised only a small part of the total volume of the buffy layer. The following lipid values were determined: total lipid, neutral fat, total fatty acids, total cholesterol, ester cholesterol, free cholesterol and phospholipid. Lipid values were expressed in milligrams per 100 gm. of the leucocyte fraction, rather than in terms of a given number

[1] Boyd, Eldon M., and Wilson, Karl M., *J. Clin. Invest.*, 1935, **14**, 7.

[2] Boyd, Eldon M., *J. Biol. Chem.*, 1935, **110**, 61.