numbers or distribution of synapses in these ganglia. Section of nerves extending from the celiac ganglia to the stomach and intestine resulted in extensive degeneration of the fibers in the distal portions of the nerves and degeneration of some fibers in the proximal portions. The latter observation supports the assumption that fibers which arise in enteric ganglia join the celiac plexus. The synaptic connections which persist in the celiac ganglia following degeneration of the splanchnic and vagus nerves probably are effected by these fibers. They probably constitute connections through which reflex responses in one segment of the gastro-intestinal canal may be elicited by impulses arising in another segment.

9602

Influence of Cattle Anterior Pituitary Extract on Endochondral Ossification in Young Ovariectomized Guinea Pigs.*

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Continuing our investigations[1] on the growth of cartilage and bone of the young guinea pig it seemed of interest to analyze the rôle of the gonads, to determine whether or not the effect of anterior pituitary is exerted by intermediation of the ovaries, which organs undergo considerable changes after the administration of anterior pituitary preparations (Loeb[2]).

Observations extended over periods of 1, 2, 3, and 4 weeks. In each of the 4 series, 6 animals averaging 140 gm. in weight were used: (a) One served as normal control; (b) one received daily injections of acid extract of cattle anterior pituitary; (c) 2 were ovariectomized; (d) 2 were ovariectomized and subsequently received daily injections of extract.

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1 Silberberg, M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1423; Silberberg, M., and Silberberg, R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 622.

2 Loeb, L., *Endocrinology*, 1932, **16**, 129; PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1335; Loeb, L., and Hayward, S. J., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 250; Loeb, L., Festschrift dedicated to Robert Tilden Frank, June, 1937, p. 2, press of The C. V. Mosby Company, St. Louis.

Six more animals which died at intermediate stages served as additional material. Altogether 30 guinea pigs were used.

Under ether anesthesia, both ovaries were removed in 16 animals. To 8 of the latter, starting 2 to 3 days after the operation, intraperitoneal injections of 1 cc. freshly prepared acid extract of cattle anterior pituitary were given. The weights of the guinea pigs, which all were kept under the same environmental and nutritional conditions, were taken daily. At the end of the experimental term, *i. e.*, after 9, 16, 23, and 30 days, femur, knee joints, tibiae, ribs, vertebrae and jaws were fixed, incompletely decalcified, and stained, as previously described.

Gross Changes. The ovariectomized animals grew rapidly and gained much weight (Group III), distinctly more than the normal control animals (Group I). Both groups of injected guinea pigs remained smaller and lighter in weight (Groups II and IV).

TABLE I.

Days of Observation	Group I Normal. 4 animals	Group II Injected. 4 animals	Group III Ovariectomized. 10 animals	Group IV Ovariectomized and Injected. 12 animals
1	135	136	140	144
5	155	146	166	149
7	162	148	177	152
9	165	146	179	150
11	170	150	182	152
13	175	152	186	158
15	190	147	200	163
17	190	155	207	163
19	190	155	223	163
21	185	160	237	160
23	205	155	235	167
25	210	165	247	167
27	215	172	262	170
29	225	174	270	167

The table demonstrates the mean weights of the animals of the 4 groups, the vertical columns representing the weights in grams.

We studied microscopically the changes which took place in the upper epiphysis of the tibia.

(a) *Normal Guinea Pigs* (4 animals): The epiphyseal line (Illustration a) is regular and patent; the cartilage cell layers are sharply demarcated, the intercellular tissue being well developed. The cells of the chondrophyte, *i. e.*, of the lateral cartilaginous protuberances, are resting. In completely healthy spring guinea pigs, the first signs of closure of the epiphyseal line were not detected before the animals had reached a weight of about 440 gm.

(b) *Injected Control Guinea Pigs* (4 animals): The results obtained in this group (Illustration b) agree with those previously communicated.[1]

(c) *Ovariectomized Guinea Pigs* (10 animals): One week following ovariectomy, a marked hyperplasia and hypertrophy, in particular of the columnar and vesicular cartilage cells, becomes manifest; the intercellular tissue is diminished in amount. Four weeks after ovariectomy, (Illustration c), the epiphyseal line of the large animals is very wide, wider than at any period of normal growth, and still completely open. The newly grown cartilage cells, then, do

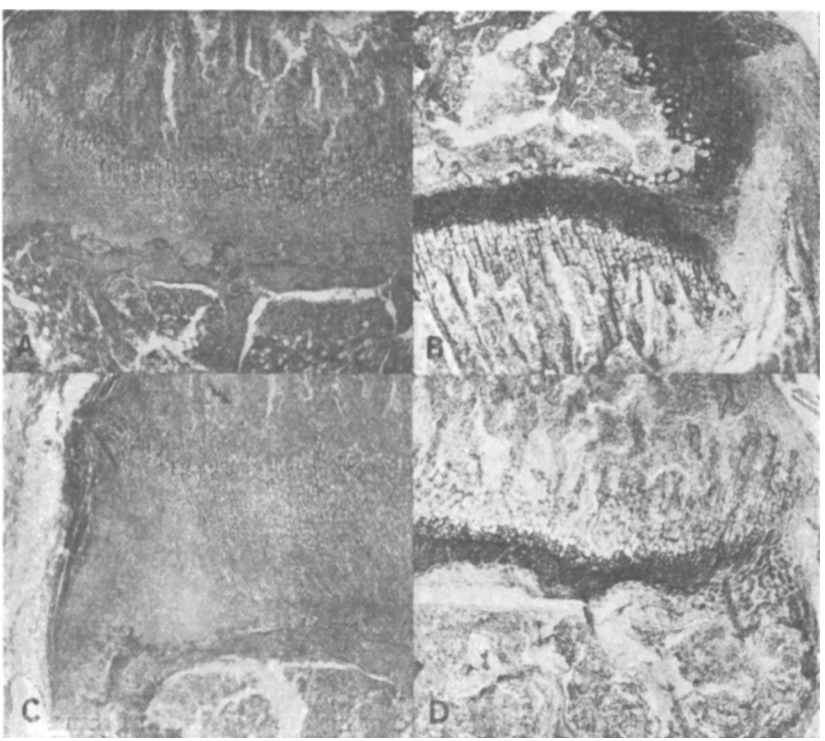

FIG. 1.

Photomicrographs (medium magnification):

The specimens were incompletely decalcified (Pommer's method) and stained with hematoxylin and eosin. The calcified areas appear intensely black.

(a) Open epiphyseal line and chondrophyte of a normal control guinea pig (225 gm.).

(b) Calcified epiphyseal line and hyperplastic chondrophyte after 7 injections of acid extract (weight of guinea pig 148 gm.).

(c) Enlarged, not calcified epiphyseal line of a guinea pig 4 weeks after ovariectomy (weight 280 gm.).

(d) Partly calcified epiphyseal line, hyperplastic and hypertrophic chondrophyte 4 weeks after ovariectomy and 28 injections of acid extract (weight of guinea pig 172 gm.).

not reveal any tendency to calcification, but only slight amounts of calcium deposit are seen in the periphery of the cartilage cells and within the cartilaginous interstitial ground substance, in places where normally much calcification would occur. Also within the chondrophyte, calcification within the growing cartilage is lacking.

(d) *Ovariectomized and Injected Guinea Pigs* (12 animals): The epiphyseal line is somewhat wider than that of the animals which received merely injections of extract, but it is narrower than that of the corresponding normal animals. As early as after 7 injections, the resting, but in particular the columnar and vesicular cartilage becomes hyperplastic. In addition, a marked hypertrophy and subsequent calcification in the cartilaginous cells and the interstitial stroma becomes noticeable; the latter processes representing lines of calcification in the cartilaginous ground substance, however, are evidently less pronounced than in the injected control animals. They differ in degree in accordance with the responsiveness of the animals to the calcifying effect of the extract which may be very accentuated even at earlier stages, but usually becomes gradually more marked and is most pronounced at later stages. After 4 weeks' treatment (Illustration d), in the most advanced cases, strands of hypertrophic blue, more or less calcified cartilage may be observed protruding bud-like into the epiphyseal bone; the epiphyseal line is not yet closed at this period which indicates that the alterations were never so advanced as in the cases of injected non-ovariectomized guinea pigs. In general, at the earlier stages, hyperplastic processes predominate, while later hypertrophy and calcification are more pronounced. The chondrophyte reveals likewise much blue, growing cartilage. In addition, one may detect an ingrowth of the cartilage of the chondrophyte toward the center of the bone and between the zone of the resting cartilage and the bony demarcation of the epiphysis. The histological appearances vary; they depend upon the predominating effective principle and on the duration of the experiment: thus, sometimes the action of ovariectomy, sometimes that of the extract is more accentuated.

Summary. In growing guinea pigs, ovariectomy causes hyperplasia and hypertrophy of the epiphyseal cartilage, whereas its calcification and ossification are not accelerated in the same ratio. Consequently, the closure of the epiphyseal line is delayed, and a relative increase in the size of the individual bone may occur. Whether these changes may lead to gigantism-like, permanent conditions or whether they are only of a temporary nature, which may become compensated at a later stage, is not as yet decided. The

above effect of ovariectomy is interfered with by the interaction of acid extract of cattle anterior pituitary, which has the tendency to exert its specific growth-promoting and calcifying influence on the cartilage. If the two factors act simultaneously, a competition between both principles takes place and a combination effect results, the outcome of which determines the course of the endochondral ossification.

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Enzymes in Ontogenesis (Orthoptera). III. Activation of Naturally Occurring Enzymes (Tyrosinase).*

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Interest in the enzyme tyrosinase seems largely to have centered around its distribution as well as the nature of its action on various natural and artificial substrates. Its rather wide occurrence in plants seems well established while for animals data are available which would lead one to assume a rather limited or restricted distribution (Graubard and Nelson,[1] v. Euler,[2] Graubard,[3] Raper[4]). Insects appear to be rich in the enzyme (Bodine and Boell[5]).

The present paper treats of results of a study on the growth, activation and substrate relations of tyrosinase as found in the developing egg of the grasshopper, *Melanoplus differentialis*. The advantages in using such biological material have been previously indicated and mention will only be made where departures from usual procedures occur.[5] Enzyme preparations were obtained from centrifuged fractions of egg brei after the following manner. For each set of experiments, 200 eggs of known age were removed from pods and carefully selected for morphological stage of development. They were thoroughly washed in tap water, then sterilized in 70% alcohol, washed three times with sterile distilled water and finally

* Aided by a grant from the Rockefeller Foundation for research in cellular physiology.

1 Graubard, Mark, and Nelson, J. M., *J. Biol. Chem.*, 1935, **112**, 135.

2 v. Euler, H., 1934, *Chemie der Enzyme*, J. F. Bergmann, München.

3 Graubard, Mark, *J. Genetics*, 1933, **27**, 199.

4 Raper, H. S., *Physiol. Reviews*, 1928, **8**, 245.

5 Bodine, J. H., and Boell, E. J., *J. Cell. and Comp. Physiol.*, 1935, **6**, 263.