

ited material make definite conclusion of an inhibition of ovulation unwarranted.

Without considering further the limits of technique, it appears that with these dosages and methods, there is no evidence of a response of the female genital system of the fowl to avian pituitary substance. It is recognized that such factors as absorption and elimination perhaps play a rôle in the explanation of these results.

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Influence of Bladder Extracts and Viosterol on Healing of Fractures and Bone Defects.

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Huggins¹ has shown that if a piece of the mucous membrane of the urinary bladder of a dog is transplanted into the abdominal wall of the same animal the transplant forms a cyst lined by bladder epithelium and true bone tends to form in and about the wall of the cyst. We² have confirmed Huggins' observation and have also shown that an autogenous transplant of bladder epithelium may stimulate the repair of defects in normal bone. This was demonstrated by a series of experiments in which comparatively large defects were created in both ulnas of dogs and at the original operation the defect on one side was bridged by a strip of bladder mucous membrane while the similar defect in the other ulna was left as a control. The defect bridged by the bladder transplant tended to heal by deposition of bone while non-union occurred on the control side. Similar results were obtained when scrapings from the mucous membrane of the dog's bladder were placed in fresh ulnar defects in the same animal.

The results of these experiments were so striking that we have performed other experiments in a search for an explanation of the phenomena. It seemed possible to us that the effect of the mucous membrane might be due to a hormone. Accordingly, extracts were made from bladders of dogs and swine and were injected at the site of fractures and bone defects.

¹ Huggins, C. G., *Arch. Surgery*, 1931, **22**, 377.

² Copher, Glover H., and Key, J. Albert, unpublished article.

Fresh mucous membrane, obtained from dog's bladder by stripping it from the muscularis, was titrated by grinding it in a mortar containing sand and normal saline. After standing for awhile to let the sand settle to the bottom of the container, the supernatant fluid containing the macerated cells of the mucous membrane was decanted off and was preserved in an icebox. Two cubic centimeters of this saline extract were injected into the site of experimental fractures of the right ulnae of 5 dogs. The fractures had been made by subcutaneous osteotomy 2 days previously. The fractures made similarly in the left ulna were kept as controls. One week after the first injection another 2 cc. of the extract were injected into the site of each fracture on the right side. The 5 dogs were sacrificed at intervals of 2 to 7 weeks after the first injection had been made. In no instance was union of the fracture influenced by injection of the extract as determined by gross, and microscopic appearance and by X-ray films. Likewise, an alcoholic extract of dogs' bladders was ineffective in hastening union.

In the next series of experiments, defects approximately 1 cm. in length were made in the ulnae of dogs. Fresh pig bladders with fat and connective tissue removed were finely ground and extracted with 70% alcohol (2 cc. per gm.) for 24 hours at room temperature. The mixture was strained through a thoroughly washed chamois and divided into portions representing 12 gm. of bladder tissue each. Alcohol was removed under vacuum over sulphuric acid at room temperature and the residues kept in the desiccator. The residues were taken up in 9 cc. of normal saline solution and 1.5 cc. of the solution was injected into the site of the right ulnar defect in each of 6 dogs. The left ulnar defects were kept as controls. The injections were made at 3 and 4 day intervals until the animals were sacrificed at intervals of from 4 to 8 weeks.

Aqueous chloroform extracts were prepared by treating pig's bladder hash (150 gm.) with 300 cc. of water and 25 cc. of chloroform for 24 hours at room temperature and giving the material an occasional vigorous shaking. The mixture was filtered as in the alcoholic preparation and the extract was preserved in a cold room. Shortly before use, extract representing 12 gm. of bladder was evaporated under vacuum over sulphuric acid at room temperature. The residue was taken up in 6 cc. of normal saline solution and 1½ cc. of this was injected into short ulnar defects as were the alcoholic extracts. The 4 dogs were sacrificed at the end of 8 weeks. Gross, microscopic and X-ray examination of the specimens revealed no difference in the healing of the fractures on the 2 sides.

In another series of dogs 1 cc. of irradiated ergosterol (Viosterol) was injected daily into the tissues around experimental fractures in the same manner as were the bladder extracts. The results of these experiments were also negative. That is, there was no definite difference in the healing on the control and injected sides.

Conclusions. Neither extracts of bladder prepared as described above nor irradiated ergosterol influenced osteogenesis when injected at the site of experimental fractures and bone defects. It is possible that the stimulation of osteogenesis noted when bladder epithelium is transplanted into bone defects is due to a local excess of calcium which is produced by the living bladder epithelial cells. It is also possible that phosphatase is a factor in the production of bone around the bladder transplants.

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Exophthalmos Produced by Injections of Acid Extract of Anterior Pituitary Gland of Cattle.

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In former investigations¹ we observed that by injections of acid and alkali extracts of anterior pituitary of cattle, it was possible to produce in the guinea pig changes in the thyroid gland identical with those in very pronounced cases of Graves' disease in man, namely, a marked hypertrophy and hyperplasia of the acinus cells, softening and absorption of colloid and irregular shape of the acini, associated often with an almost complete disappearance of the lumen of the acinus. There was also a loss of weight induced by these changes which indicated an hyperactivity of the gland. Silberberg found that administration of KI to a certain extent inhibited these changes. This corresponds to the action of this substance in Graves' disease. Siebert noted a marked rise of the basal metabolism in guinea pigs under the influence of acid or alkali extracts and more recently found that simultaneous administration of KI tends to counteract the effect on basal metabolism. Closs, Loeb and MacKay¹ found the changes in the distribution of organic and inorganic

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¹ Closs, Carl, Loeb, Leo, and MacKay, Eaton, *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **29**, 170.