

be kept isolated and 24 hours after injection should be tested (gently stripping) to determine whether eggs have reached the uteri. A sperm suspension (males need not be injected³) is made by teasing apart two pairs of testes in 10 cc of spring or pond water. It is very important to use only water in which sperm are known to survive. After about 30 minutes' standing, this sperm suspension is ready and eggs may be stripped directly into it. It is best to divide the suspension between two finger bowls and to spread the eggs out thinly in the sperm suspension. After half an hour the eggs are flooded with the same water used for the sperm suspension. When the jelly has swollen (about an hour more) the eggs should be distributed so that there are about 25 to 50 eggs per finger bowl full of water. In this manner 100 per cent. fertilization and development can be achieved under controlled conditions.

If a female is injected with the anterior pituitary hormone and is kept at 22°–25° C. it can be used in 14–16 hours to demonstrate all of the reproductive processes from follicle rupture to entrance of the egg into the uterus.⁴ Such a frog should be anesthetized, opened, and the entire body submerged in 10 per cent. Holtfreter's solution. Ovarian contractions will be clearly seen. Numerous follicles will be observed to rupture and the eggs emerge, a process which takes from 4 to 10 minutes for a single egg. Free eggs will be picked up and carried toward the ostia by peritoneal, pericardial and liver cilia. Eggs enter the ostium singly, entirely as a result of ciliary action. They are carried through the oviducts (about 2 hours) in a spiral manner, by ciliary currents. Eggs can be fertilized from any point within the oviduct but not from the body cavity. This situation is a challenge to further research on the mechanism of fertilization.

It has recently been demonstrated⁵ that the dose of the anterior pituitary required to induce ovulation decreases appreciably in the period between November and February. This is explained on a three-fold basis: The potency of the donor's gland increases as the breeding season approaches; the gland of the recipient may begin the elaboration of the hormone toward the end of hibernation; and the ovaries may be differentially susceptible to stimulation at different periods.

The anterior pituitary is readily soluble in water and alkaline solutions. It can be kept in aqueous solutions for several days in the refrigerator. If kept in 70 per cent. alcohol the potency will remain practically unaltered for several weeks, and if kept in 100 per cent. alcohol, where none of the hormone is dissolved, the potency remains indefinitely. Recent tests have indi-

cated normal potency after one year in absolute alcohol. In this latter case, however, the alcohol must be diluted with distilled water until a 35 per cent. solution (or less) is achieved before injection.

The frog's anterior pituitary is so small (0.6 to 1.5 mgm) that extraction of the hormone would entail great loss. Preservation of the entire gland is indicated. In most laboratories many frogs are sacrificed for a single muscle or nerve experiment and the anterior pituitary glands of such frogs may be excised and saved for ovulation induction during non-breeding seasons. There is evidence that this technique, with modifications, may eventually be used on a variety of animal forms which will yield valuable embryological material.

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HOMOTRANSPLANTATION OF ADRENAL CORTICAL TISSUE

IN 1932¹ we reported successful intramuscular homotransplantation of adrenal cortical tissue between albino rat litter mates (100 per cent. in four male rats). We also showed that both autoplasmic and homoplasmic transplants would grow in the same animal at the same time, there being no "preference" for either type of tissue (40 per cent. successful homotransplants in 10 male litter mates. Martin (1932)² also reported three successful homoplasmic intra-ovarian transplants. Nilson and Ingle (1936)³ reported that intra-ovarian homotransplants in sisters were successful, but that "direct homoplasmic transplants of the adrenal glands of adult rats" and cross-strain transplants degenerated.

In connection with other problems it became necessary for us to attempt intramuscular homotransplantation of adrenal glands between non-siblings of our inbred strain of rats. The adrenal glands were exchanged between five pairs of females, three pairs of males and four pairs of a male and female each (24 rats). The members of each pair were not only non-litter mates but were from different parents. Twelve animals died of suprarenal insufficiency. The twelve survivors were killed and the grafts examined histologically from two to four months after operation. The homotransplants had regenerated and were functioning (as testified by the good health of the animals) in eight of the ten females which had received tissue from other females, in two of the four females which had received tissue from males, and in only two of

¹ L. C. Wyman and C. tum Suden, *Am. Jour. Physiol.*, 101: 662–667, September, 1932.

² S. J. Martin, *Am. Jour. Physiol.*, 100: 180–191, March, 1932.

³ H. W. Nilson and D. J. Ingle, *SCIENCE*, 84: 424, November, 1936.

³ R. Rugh, *Proc. Soc. Exp. Biol. and Med.*, 36: 418, 1937.

⁴ R. Rugh, *Jour. Exp. Zool.*, 71: 149, 163, 1935.

⁵ R. Rugh, *Physiol. Zool.*, 10: 84, 1937.

the males. One of these had received tissue from another male and the other from a female. The sex of the donor, therefore, has no particular bearing on the success of non-sibling homotransplants. The sex of the recipient seems to be significant, since over 71 per cent. of the females regenerated homotransplants, whereas only 20 per cent. of the males did so.

Successful homotransplantation of adrenal cortical tissue between non-siblings of the same strain is possible. Obviously, if a large number of "takes" is desired in such experiments females should be used. We have evidence, which will be published elsewhere, that the growth of transplanted cortical tissue in rats is determined and limited by the available adrenotropic hormone from the anterior lobe of the hypophysis. The larger percentage of "takes" in females reported here may depend on a greater amount or greater availability of adrenotropic hormone in females. Such an explanation is consonant with the well-known facts that female rats have larger adrenal cortices than males, and that females regenerate more cortical tissue in transplants or "accessories" than do males.

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IMMUNITY OF CERTAIN INSECTS TO SELENIUM POISONING

A LOW concentration of selenium in foodstuffs is a quick-acting lethal poison for mammals and birds, and small quantities of this element absorbed from the soil are responsible for toxicity of grains and forage plants to live stock.¹ Insects also are regarded as very sensitive to selenium. Aphids are killed by concentrations in wheat plants too low to injure the plants themselves,² and red spiders are quickly destroyed by commercial insecticides containing selenium.³

We were surprised, therefore, to find weevils and seed-chalcids completing their life cycles in the seeds of one of the most poisonous of the range plants, *Astragalus bisulcatus* (collected near Laramie, Wyoming). Analysis showed that the seeds contained 1,475 parts per million of selenium. The weevils were identified by Mr. H. S. Barber as *Acanthoscelides fraterculus* (originally reported from Kansas, Nebraska and Colorado) and the seed-chalcids—small wasp-like insects—were identified by Mr. A. B. Gahan as *Bruchophagus mexicanus* or a closely related species. A second hymenopterous insect, *Amblymerus bruchophagi*, less numerous than the first, was present as a parasite of the seed-chalcid.

The high toxicity of the seeds to mammals was shown in an experiment in which five white rats were fed on a mixture containing ground pods and seeds of a similar *Astragalus* plant. Although the selenium content of the food was reduced by dilution with ground wheat to only 65 ppm, the rats were killed within from 4 to 11 days. Even 22 ppm of selenium in the diet is lethal to young, developing rats;⁴ and grains and fodder containing less than 50 ppm of selenium absorbed from the soil have been reported to cause the death of hogs, cattle and horses.

The *Astragalus* plants, though rooted in soil with a selenium content of only about 3 ppm, are able to accumulate from 1,000 to 9,000 ppm.⁵ The developing weevil larvae present a striking contrast: Although their food contained 1,475 ppm of selenium, the larvae either did not absorb it readily or they eliminated it effectively, perhaps through their respiration. Analysis of their bodies showed the presence of only 65 ppm of selenium.

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⁴ A. L. Martin, *Amer. Jour. Bot.*, 23: 471-483, 1936.

⁵ O. A. Beath, H. F. Eppson and C. S. Gilbert, *Wyo. Agric. Exp. Sta. Bull.*, 206, 1935.

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¹ For literature review, see S. F. Trelease and A. L. Martin, *Bot. Rev.*, 2: 373-396, 1936.

² A. M. Hurd-Karrer and F. W. Poos, *SCIENCE*, 84: 252, 1936.

³ C. B. Gnadinger, *Indust. Eng. Chem.*, 25: 633-637, 1933.