From the data in Table 1 it is concluded:

(1) The composition of the dust is a factor in the amount of crushing under mechanical impingement.

(2) The velocity of impact as well as the surface on which and the medium in which the impact occurs has a bearing on the amount of crushing.

(3) With the exception of impingement on a wetted surface, the smallest particles noted were on the order of a micron. It appears, therefore, there is a limit to the fineness that a particle will shatter at definite velocities and conditions.

(4) In the case of impingement on a wetted surface considerable material below 0.5 micron was noted. It may be that this was formed by attrition of the water-borne particles by other particles in the incoming air stream.

(5) In all cases of dry impingement a variable amount of scattering of particles outside of the field of impingement was seen. This indicates incomplete retention of these dusts on the impingement surfaces.

(6) With two of the three dusts any estimation of particle size distribution in the air from the resultant particles is erroneous.

(7) With each of the three dusts examined an estimation under the above conditions of the number of particles in the air sampled is erroneous.

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OVULATION INDUCED OUT OF SEASON

OVULATION has been induced in a variety of Amphibia during the non-breeding season by the injection of the anterior pituitary hormone. Since the original description of the technique of inducing ovulation and of artificially inseminating eggs of the frog, *Rana pipiens*,[1] there have been a number of refinements so that now one can count on securing fertilized eggs and developing tadpoles at any time of the year from early September until the normal breeding season, in March. From March until July, *Rana clamitans* can be used, and *Rana catesbeiana* (the bullfrog) will respond to pituitary-induced ovulation until late in August. *Acris gryllus* normally breeds from February to October, and *Rana sphenocephala* from February to December. With inhibition of normal ovulation by refrigeration, amphibian eggs may be available for twelve months of the year. The technique, as now used, will be briefly described.

With few exceptions, *Rana pipiens* can not be considered sexually mature unless it measures 74 mm from snout to cloaca. Frogs are secured from one of the frog farms and are placed immediately in a copper-lined tank in the refrigerator, through which runs a slow stream of water. Frogs may be kept at this temperature (about 4 to 6° C.) for several weeks without showing gonadal deterioration. Twenty-four hours before eggs are desired an obviously mature female is injected with whole anterior pituitaries from two adult female or four adult male frogs. Mammalian or fish pituitaries have not been successfully used with frogs in inducing sexual reactions, but such pituitaries will induce ovulation and amplexus in toads and breeding reactions in salamanders. Amphibian pituitaries will, in general, induce such reactions in *Rana pipiens*.

It has been found that the average male anterior pituitary (*Rana pipiens*) is 16 per cent. heavier and 60 per cent. as potent as the average female gland in respect to inducing ovulation. The glands must be quickly excised, as they rapidly lose their potency in dead frogs. If the head is cut off; the lower jaw removed; the base of the cranium cut along each side of the brain; the parasphenoidal bone deflected forward, the anterior pituitary gland will be seen as a pink organ lying just posterior to the optic chiasma. Occasionally it will adhere to the base of the cranium and will be surrounded by white endolymphatic tissue, which has no apparent sex hormone value. The pituitary is placed in 1 cc of distilled water, 35 per cent. alcohol or Ringer's solution. Generally 1 cc of fluid is used per gland, partly as a check on the number of glands used. When the proper number of glands has been secured, they are sucked up into the barrel of a hypodermic syringe, with no attempt to macerate the pituitaries. It has been found that the fresh gland will easily pass through a No. 20 hypodermic needle and that if the gland is previously macerated, some of the hormone is lost by adhesion to the inner sides of the syringe. The needle is applied to the syringe and injected through a lower quadrant of the abdomen, avoiding deep penetration and consequent danger of internal injury. Immediately following injection the frog is placed in a container with enough water to partly immerse the body. If amplexus and normal fertilization are desired, a male may be similarly injected and amplexus will be achieved in about 9 to 12 hours at ordinary laboratory temperatures of 22–25° C. In this case only pond or spring water, or 10 per cent. Holtfreter's[2] modification of amphibian Ringer's can be used, since tap water is generally lethal to sperm.

If insemination is to be controlled, the female should

the impingement orifice. The actual velocities at the surface where impingement takes place are of necessity lower, due to mechanical design.

[5] In all cases using a dry glass plate, the dust-laden air was previously humidified by passage through a tube containing moisture.

[1] R. Rugh, *Biol. Bull.*, 66: 22, 1934.

[2] J. Holtfreter, *Arch. f. Ent. Mech. der Org.*, Bd. 124, S. 404, 1931.

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 injected[3]) 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 ostiae 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 demonstrated[5] 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 indicated 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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[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.

HOMOTRANSPLANTATION OF ADRENAL CORTICAL TISSUE

IN 1932[1] 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 autoplastic and homoplastic 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 homoplastic intra-ovarial transplants. Nilson and Ingle (1936)[3] reported that intra-ovarial homotransplants in sisters were successful, but that "direct homoplastic 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.