INTRODUCTION

The results of hypophysectomy and of injection of extracts show that the gonads of Amphibia are under the control of the anterior lobe of the pituitary (Wolfe, 1929; Hogben, 1930). *Xenopus laevis* does not exhibit any sexual activity in the laboratory. When appropriate nutritive and other conditions are fulfilled (Landgrebe, 1939), the ovaries are close to the threshold of ovulation at all seasons. Injection of gonadotropic extract then leads to ovulation and oviposition. If males in the same container receive the same treatment, clasping occurs and fertilized eggs result (Shapiro, 1936).

In nature *Xenopus* does not shed simultaneously all the eggs which ripen in a season. Its habits of ovulation and oviposition are essentially similar to those of Urodeles. *Rana*, on the other hand, sheds simultaneously at the breeding season all the eggs which have ripened during the preceding year, and there is a rapid growth of oocytes which have not previously attained visible dimensions about the time when ovulation begins. Injection of gonadotropic extracts causes hypertrophy of the ovaries and ovulation but rarely oviposition (Bellerby, 1933).

There are other differences in oviposition between *Xenopus* and *Rana*. The eggs of *Rana* are shed into the body cavity. They pass into and down the upper part of the oviduct by ciliary action (Rugh, 1935). Then they descend to the lower third of the duct (“uterus”) and are retained there for days or weeks. Eventually they are expelled *en masse*. The oviduct is hypertrophied in the sexual season and from its wall the gelatinous egg envelope is formed (Rugh, 1938; Marsh, 1938). Marsh has shown that oviducts become hypertrophied by injection of extracts of the anterior lobe of the pituitary (A.L.P.) combined with oestrin. This does not necessarily mean that the normal enlargement of the ducts is due to the combined effect of these two autacoids, since there is as yet no evidence for the production of oestrin in Amphibia.

In South African ponds *Xenopus* lays its eggs singly with a large amount of jelly. Although the eggs which *Xenopus* can be induced to lay by administration of A.L.P. are fertile, such treatment always results in the extrusion of eggs with little jelly. This is probably because the oviducts are permanently small in the laboratory. That ciliary action is sufficient to bring about oviposition after ovulation in *Xenopus* can be demonstrated by inserting eggs into the body cavity through an incision which
is then stitched up (sealed). Such eggs are passed out into the surrounding medium in a few hours. The same procedure does not result in complete extrusion of the eggs of *Rana*; these pass down the upper part of the oviduct for some distance and then stop.

Cilia can be detected down the whole length of the oviduct of *Xenopus*. We have been unable to detect them in the lower third of the oviduct in *Rana*, even when examination is made shortly after oviposition. Observations were made at this time because it is not intrinsically improbable that there is a seasonal variation in the development of cilia under the influence of sex hormones, e.g. the development of cilia in the mammalian vas deferens after injection of androgens (Vatna, 1930). In any case it is unlikely that ciliary action could move the large mass of eggs which accumulate in the greatly distended lower part of the oviduct of *Rana* prior to oviposition.

Thus ovulation in *Xenopus* is always followed by oviposition. In *Rana* ovulation and oviposition are two entirely separate problems. The agencies responsible for ovulation and for passage of the eggs down the top part of the oviduct are known. Rugh (1938) does not describe their passage further in the normal animal. Hitherto the ultimate extrusion of the egg mass from the lower part of the oviduct (other than by "stripping") has not been investigated.

In elasmobranchs, reptiles, birds and monotremes the complete sequence of female reproductive activity includes the following physiologically distinct events each of which presupposes its appropriate antecedent stimulus:

1. ripening of the ovarian egg;
2. liberation of ovarian egg;
3. the migration of liberated egg from ovary to the oviduct;
4. movement of the egg down the upper part of the oviduct;
5. secretion of an albuminous coat;
6. secretion of shell membrane and shell;
7. extrusion of the completed egg.

In Anura the mechanism of stages 1–3 is already known (Rugh, 1938). It is well established that in the intact animal both 1 and 2 are controlled by the anterior lobe of the pituitary gland. We have made experiments to test the action of other gonadotropic substances on the ovary of *Xenopus* in view of recent work in this field.

Stage 4 is, as has been already pointed out, due to the action of the ciliated lining of the oviduct. In *Xenopus*, but not in *Rana*, no distinction need be made between 4 and 7. Stage 6 is clearly lacking in Amphibia.

On the assumption that stage 5 is represented by the secretion of the egg jelly in Amphibia, there remain therefore, two outstanding problems of the Anuran reproductive cycle in regard to which information is lacking, i.e. the nature of the effective stimulus to, and co-ordinating mechanism which controls (a) the secretion of egg jelly, (b) the expulsion of the ripe egg from the lower part of the oviduct in the Phaneroglossa (e.g. *Rana*).
I. THE EFFECT OF VARIOUS GONADOTROPIC SUBSTANCES ON THE OVARY OF XENOPUS

Removal of the anterior pituitary leads to genital atrophy in Rana and in Xenopus. Injection of anterior lobe extracts evokes enlargement of the ovary and ovulation. There is therefore complete justification for the statement (p. 11) that the anterior pituitary controls the cyclical variations of the ovary.

The gonadotropin present in pregnancy urine also evokes ovulation in Xenopus (Bellerby, 1934) and has been made the basis of a pregnancy diagnosis method (Landgrebe, 1939; Crew, 1939). Recent work in the field of pregnancy diagnosis, in which specificity is of paramount importance, has focused attention on the fact that substances other than the generally recognized gonadotropins—A.L.P. and P.U.—will evoke ovulation.

In view of current controversy on the existence or otherwise of two classes of gonadotropins with different properties—one follicle stimulating, the other luteinizing—experiments were made to test the effect of various substances on the ovary of Xenopus.

Progesterone and other steroids (Shapiro & Zwarenstein, 1937) induce ovulation and oviposition in Xenopus. Other workers have not recorded whether these substances have a direct effect on the ovary. Certainly Shapiro & Zwarenstein (1937) recorded ovulation in the excised ovary of Xenopus immersed in frog Ringer after addition of progesterone and other substances: in our experience, however, heart perfusion experiments have shown that frog Ringer is an unsuitable medium for Xenopus tissues. We therefore tested the effect on the intact animal.

Table 1 which records observations on Xenopus after anterior lobe removal shows that the immediate stimulus to ovulation is direct. The doses given were: 2 c.c. pregnancy urine, 2 mg. progesterone, 1 g. anterior lobe tissue. We have not made experiments to find whether the reduced ovary of a hypophysectomized animal is affected by these substances.

<table>
<thead>
<tr>
<th>No: in each group</th>
<th>No. of days between operation and injection</th>
<th>No. ovulated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A.L.P.</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

Zondek (1930) observed that urine of castrate women (or menopausal urine) when injected into intact mice produced a predominantly follicle stimulating effect. Injections of pregnancy urine had a predominantly luteinizing effect. At that time Zondek considered that the gonadotropic substances of both these fluids originated in the pituitary and he called that from menopausal urine Prolan A (follicle stimulating) and the luteinizing substance Prolan B. He believed that pregnancy urine contained some A as well as B. There is now good reason to believe that Prolan B is chorionic in origin.
Fevold (1939) prepared two fractions from ox anterior lobe which he called follicle stimulating hormone and luteal hormone. In their effect on intact animals the former (F.S.H.) is roughly equivalent to Prolan A and the latter (L.H.) to Prolan B. Maxwell (1934) and Saunders & Cole (1936) have shown that certain gonadotropically inert substances such as casein and zinc sulphate when injected at the same site as pituitary extract augment its effect. Such substances do not have this effect when injected at a different site from the pituitary extract. Zinc sulphate is believed to act on the process of absorption of the gonadotropin. Its effect is not to be confused with that of substances such as picric salts (Marshall et al. 1939) which when injected alone into intact rabbits induce ovulation by stimulating the intact pituitary.

Some attempt has been made to align the pituitary fraction L.H. with inert substances such as zinc sulphate and to explain its action as merely increasing the effect of one potent gonadotropin (F.S.H.). It has been pointed out that a definite criterion of a true L.H., as distinct from an inert substance which locally facilitates absorption of F.S.H., is to prove its efficacy when injected at a separate site from the F.S.H. A more satisfying test, however, is to demonstrate that the alleged F.S.H. will produce enlarged follicles but no lutea when injected in excess into hypophysectomized rats and that L.H. is without effect on the reduced ovary of hypophysectomized rats but causes luteinizing when injected after the follicles have been enlarged by previous injections of F.S.H. This has recently been accomplished (Fevold, 1939). Quite apart from this convincing demonstration it is now generally agreed (Summary, Liu & Noble, 1939) that pregnancy urine, although having a generalized gonadotropic effect on intact animals produces no follicular maturation in the reduced ovary of hypophysectomized rats.

The following experiments were performed to determine whether pregnancy urine evoked any response in the reduced ovaries of hypophysectomized Xenopus. The ovaries of six hypophysectomized Xenopus were examined under anaesthesia. They were all atrophied. The animals were placed in separate containers and two injections of 3 c.c. pregnancy urine were administered each week for several weeks. All the animals ovulated.

If we are prepared to believe that there are two separate gonadotropins which produce distinct effects on the mammalian ovary, such a mechanism is clearly a late evolutionary development.

II. SECRETION OF EGG JELLY

In Rana egg jelly is secreted by the epithelium of the oviduct which is enlarged and glandular at the breeding season. Enlargement of the oviduct may be elicited out of season by the injection of oestrin and A.L.P. (Marsh, 1938). Xenopus eggs produced after injection of gonadotropin in the laboratory are covered with much less jelly than are those eggs which occur naturally in South African ponds. This may be due to the fact that in nature Xenopus sheds its eggs slowly, one at a time, while after injection up to 5000 eggs are shed in a few hours. Such eggs are fertile, develop, and the larvae metamorphose apparently quite
Ovulation and oviposition in Anura

normally. Naturally occurring enlargement of the oviduct of wild Xenopus during the breeding season is not familiar to us, but the oviducts after injection never reach a size comparable to that well known in Rana at the breeding season.

An attempt was made to obtain a larger gelatinous envelope in Xenopus by giving serial injections of A.L.P. on the working assumption that the oviduct has a lower threshold to stimulation than the ovary and that gradually increasing pituitary levels might allow time for the oviducts to enlarge before the threshold of ovulation was reached. Twelve Xenopus were selected from a batch which consistently responded to 0·5 g. of fresh anterior lobe tissue. They were injected daily with the equivalent of 0·1 g. of fresh anterior lobe tissue. On the fifth day seven of the twelve had shed a few eggs and all twelve had ovulated by the seventh day. While demonstrating the additive effect of continued low doses this experiment did not produce more jelly than is usual after one injection. It remains a possibility that injections spread over a longer period might have the desired effect.

Extracts of the posterior lobe of the pituitary injected together with an extract of the anterior lobe had no effect on the number of Xenopus which ovulated (Table 2) and had no effect on the amount of jelly secreted.

Table 2

<table>
<thead>
<tr>
<th>No. of animals injected</th>
<th>Dose in g.</th>
<th>No. ovulated in 18 hr.</th>
<th>No. ovulated in 24 hr.</th>
<th>No. ovulated in 48 hr.</th>
<th>No. of animals injected</th>
<th>Dose in g.</th>
<th>No. ovulated in 18 hr.</th>
<th>No. ovulated in 24 hr.</th>
<th>No. ovulated in 48 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Ant.</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>Ant.</td>
<td>12</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>11</td>
<td>Muscle</td>
<td>10</td>
<td>10</td>
<td>12</td>
<td>11</td>
<td>Post.</td>
<td>6</td>
<td>9 10</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>5</td>
<td>9</td>
<td>12</td>
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</tbody>
</table>

Single injections of either pilocarpine, adrenalin, pitocin, oestrin or posterior lobe extract, made at the same time as injection of anterior lobe extract, had no effect either on the number of animals responding or on the gelatinous envelope.

Six Xenopus were injected with 0·01 mg. of oestrone twice daily for 3 days. Thereafter the animals received 0·2 g. fresh anterior lobe tissue daily. All the animals ovulated by the seventh day but with no increase in the amount of jelly secreted.

Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Av. weight of 3 animals g.</th>
<th>Av. weight of ovary g.</th>
<th>Av. weight of oviduct g.</th>
<th>Oviduct body weight g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>40·0</td>
<td>4·5</td>
<td>0·83</td>
<td>0·21</td>
</tr>
<tr>
<td>3 months after hypophysectomy</td>
<td>36·3</td>
<td>1·6</td>
<td>0·46</td>
<td>0·13</td>
</tr>
<tr>
<td>3 months after ovariectomy</td>
<td>34·6</td>
<td>—</td>
<td>0·48</td>
<td>0·14</td>
</tr>
</tbody>
</table>
Failure to obtain an increase in the secretion of egg jelly led the authors to investigate gravimetrically induced changes in the oviduct. Removal of the pituitary in *Xenopus* results in atrophy of the ovary and of oviduct within a few months. Ovariectomy also results in atrophy of the oviduct (Table 3). So far we have been unsuccessful in obtaining a *Xenopus* ovarian extract which will prevent atrophy of the oviduct after hypophysectomy.

### III. THE MECHANISM OF EXTRUSION OF RIPE EGGS IN RANA

It was first determined whether the presence of a clasping male is necessary for oviposition. Thirty-two couples were separated. Oviposition occurred in twenty-eight of the females: four died with eggs *in utero*. During the same period fifty-one (100%) clasped females oviposited. The male is therefore not essential to oviposition. Two observations suggest that his presence is advantageous. In a number of the separated females extrusion was incomplete and in most of them oviposition was considerably delayed, as Rostand (1934) also found. Prima facie the male would appear at least to aid oviposition by direct mechanical pressure of the female body wall. In this connexion Smith’s finding may be noted that while at coupling time there is no evidence for rhythmic movement of the pectoral muscles of the male, there is a great increase in the sensitivity of these muscles to faradic stimulation of the c.n.s. reaching its peak at the normal time of oviposition (Smith, 1938). Further, there is the possibility that older females may become conditioned to oviposit when clasped.

The following agencies are potentially involved in the extrusion of ripe eggs in *Rana*:

1. The male, by direct mechanical pressure on the female body or otherwise. In a small proportion of cases gentle pressure applied to the body of gravid frogs results in egg extrusion.
2. Ciliary action of the oviducal lining generally.
3. Swelling of the ova and/or jelly coat due to imbibition of water, thus forcing the passage out through the utero-cloacal apertures.
4. Muscular activity (a) of the uterus, analogous to mammalian parturition, (b) of the belly wall, (c) possibly in association with 4 (a) and/or 4 (b)—of other muscles of the posterior body region.

No. (1) will be discussed subsequently (p. 23).

It has been pointed out already that ciliary action (2) is inadequate to bring about extrusion of ova in *Rana*.

No. (3) becomes a real possibility when the size of the jelly envelope of eggs in the uterus is compared with that of eggs which have been deposited. To test this gravid females were kept in minimal humidity conditions. They laid eggs of the same size as those found *in utero*.
Ovulation and oviposition in Anura

(4) Muscular activity

The relations of the oviducts to the cloaca and associated parts in Xenopus are such as to allow easy egress of ova transported singly down the oviducts by cilia and ejected possibly with the additional aid of contraction of the abdominal muscles into an appropriate medium (water), irrespective of the time of ovulation. In Rana, on the other hand, an Amphibian whose habits are largely terrestrial, the presence of water may or may not coincide with ovulation and passage of eggs into the oviducts. The development of a collecting portion in the oviduct, i.e. "uterus," is consistent with a lag between ovulation and actual oviposition. In these circumstances enhanced control of the actual emission of the ova at the cloacal end of the uterus is clearly to be expected.

(i) Endocrine control.

Mechanically two quite distinct events may be distinguished at parturition in some mammals. The uterus undergoes muscular contractions which tend to force the contents out, and evacuation is facilitated in some forms by the distension of the distal passageway (vaginal region) as, for example, in the relaxation of the pelvic ligaments that occurs in guinea-pigs during pregnancy (Hisaw, 1929), the resorption of the pubic bones at the approach of puberty in the pocket gopher (Hisaw, 1925) and the loosening of the sacroiliac junction, and separation of the pubes at the symphysis which occasionally occurs during pregnancy in women (Abramson et al. 1934; Thoms, 1936).

Resorption of the pubic bones in the gopher is dependent on oestrin and the relaxation of the pelvic ligaments in the guinea-pig on a synergistic action of relaxin¹ and oestrin (Hisaw, 1925, 1929). In view of these facts and since labour in mammals is generally believed to be initiated by a synergistic action of oestrin and oxytocin, our first efforts were directed to removal of the ovary and pituitary and the injection of appropriate extracts. In this connexion it may be noted that the frog pituitary yields extracts with oxytocic properties (Hogben, 1923). The pituitaries were removed from fourteen gravid female frogs, the ovaries from fourteen others. Following the operation the male was allowed to reclasp the female. Removal of both ovary and pituitary was always fatal. The results are tabulated (p. 18).

These findings show that expulsion of eggs can take place in the absence of ovarian and pituitary secretions. It is improbable that effective quantities of these substances remain in circulation 2 or 3 weeks after extirpation of the glands. There is no index of the level of ovarian hormones in the blood, but in mammals it is certainly excreted (or destroyed) fairly rapidly. Some idea of the amount of posterior lobe hormones in the blood can be obtained by melanophore readings. The stimulation of the pituitary which immediately accompanies total hypophysectomy does not raise the melanophore index significantly. Injections of the oxytocic fraction of

¹ De Fremery et al. (1931) and Tapfer & Haslhofer (1935) have observed widening of the guinea-pig symphysis pubis after injection of oestrin and express doubt as to the existence of a water soluble luteal hormone relaxin.
posterior pituitary extract either alone or following previous injections of oestrin were without effect on expulsion.

<table>
<thead>
<tr>
<th></th>
<th>Died without oviposition</th>
<th>Oviposited</th>
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</thead>
<tbody>
<tr>
<td>(a) Ovary removed</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>(b) Complete hypophysectomy</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>(c) Anterior lobe extirpation</td>
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<thead>
<tr>
<th></th>
<th>Died without oviposition</th>
<th>Oviposited</th>
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</thead>
<tbody>
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<td>8</td>
<td>6</td>
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<td>23</td>
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<td></td>
<td>17</td>
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</table>

That extrusion of eggs can take place in the absence of ovarian and pituitary secretions provides a sharp contrast to the conception of mammalian parturition held until recently. This conception was based on the known fact that oestrogenic treatment lowers the threshold of uterine stimulation to oxytocin and progesterone inhibits this effect. The essence of the theory was that towards "term" luteal secretion is reduced and the relative (or absolute) increase of oestrin raises the sensitivity of the uterine muscle until it is activated by the oxytocin always circulating in the blood. An increase in the level of oxytocin, as occasionally recorded by some workers, would form an additional safeguard to the safe working of this scheme. Doubt was first thrown on this scheme by Smith's (1932) observation that hypophysectomized rats delivered their litters successfully. Smith aimed at removing the posterior pituitary alone while leaving the anterior lobe intact. Fisher et al. (1938) have pointed out that if the anterior lobe is left in situ the forward extension of the posterior lobe must be left intact also, so that Smith's results do not provide unequivocal evidence for the non-participation of oxytocin in parturition. Fisher found that completely hypophysectomized cats did not deliver their litters normally and in some cases not at all. He concluded from this that the non-essential nature of oxytocin had not been established. Robson (1936) and others have shown that when pregnant rabbits are completely hypophysectomized they can deliver their litters. Selye et al. (1934) had found the same in rats. The conclusion seems clear that either there is an extra pituitary source of oxytocin or that pituitrin may be retained in the circulation for long periods. Clearly the data available do not justify far reaching comparisons, but at a time when doubt is being thrown on the role of pituitary oxytocin in the process of mammalian parturition,
it is doubly interesting to note that the pituitary is not essential for oviposition in *Rana*.

While the above experiments do not rule out the participation of humoral agencies in oviposition they do eliminate the more likely excitant substances.

(ii) Nervous control.

Our next efforts were directed to a consideration of nervous control. Preliminary experiments showed that electrical stimulation of either the general body surface of intact gravid animals or the upper end of the cord of decapitate preparations elicited strong convulsions of the body wall and especially of the dorsal musculature as a whole, sufficient in intensity to evacuate the uterus in the absence of any check at the lower (cloacal) end. No eggs, however, were passed out through the cloaca, though in one preparation in which incision was made in the ventral body wall it was noted that the convulsive contractions induced brought about bursting of the uterus wall and escape of its contents into the surrounding body cavity.

A test to determine whether the extrusion mechanism was under nervous control was made as follows:

Twenty-four pairs of coupling frogs were used immediately they were brought into the laboratory. The spinal cord of the female of each of twenty-one pairs was destroyed by pithing, care being taken not to dislodge the male. The remaining three pairs were kept as controls. Each pair was then segregated in a flat-bottomed tin containing water to a depth of half a centimetre. The three control females all extruded their eggs within 3 days of segregation. Of the experimental frogs twelve males were still coupling after 7 days and eight males continued to couple for 14 days. None of the twelve females of these pairs extruded their eggs. With one exception none of the other pithed females extruded their eggs, though four of their number survived for 3 weeks after pithing. This one exception was found to be incompletely pithed. While these results suggested nervous control, all attempts to elicit extrusion by electrical stimulation by the procedure followed in the preliminary tests gave the same negative result.

Study of relevant literature disclosed little beyond a suggestion of Wiedenbaum (1894) that expulsion is brought about by contraction of muscle fibres of the uterine wall. Microscopic examination of sections and whole mounts showed little evidence for this when the enormous mass of eggs carried in the distended membraneous sac is borne in mind. Apart from its sac-like expansion into a "uterus" and the scarcity of cilia in the lower oviduct in *Rana* previously referred to, there appeared to be no significant difference in the musculature of the oviducal tube generally between *Rana* and *Xenopus*.

Accordingly a re-examination was made of the whole pelvic region with the aid of serial sections, dissections and experimentally. The results were as follows:

1. In *Rana* as in *Xenopus* the ultimate outlet of the cloaca is controlled by a sphincter band of smooth muscle.

2. In *Rana* the urostyle is extended backwards so that its terminal portion extends over nearly the whole length of the cloacal tube in close proximity to the
dorsal surface of the latter. A section through this region (Fig. 1) just anterior to the level of the uterine apertures shows ureters and uterine exits lying closely beneath the urostyle between the latter and the rectal tube. Beneath the cloaca lies the pelvic symphysis. Very slight downward pressure from the urostyle, therefore, brings the cloacal tube into contact with the pelvis in the mid line, and immediately compresses the cloaca in the region of the openings of the three types of duct which enter it. The passageway between urostyle dorsally and pelvic groove ventrally is at best a meagre one, and its dilation to allow of the expulsion of either a faecal mass from the rectum or ova from the uterine apertures clearly implies a lifting of the urostyle and/or appropriate rotation in an anti-clockwise direction of the pelvis which in *Rana* articulates freely at the iliac sacral junction.

(3) Examination of the musculature (Fig. 1) showed that the cloaca is embraced by a symmetrically paired muscle, extending between the hinder part of the urostyle dorsally and the bases of the ilia ventrally. This muscle, the compressor cloacae (Ecker & Wiedersheim, 1904), supports the suspension of the cloaca from the urostyle. Bundles of fibres pass from the urostyle around the whole cloacal tube and a strong band passes around the bases of the uterine openings just above the entry of these into the cloaca and may be readily seen embedded in the thickened wall of the cloaca at this point, clear of and dorsal to the wall of the rectum.

(4) At the tip of the extended urostyle a pair of muscles extend outwards and are inserted along the femur on each side. These muscles are termed, by Ecker &
Wiedersheim (1904), pyriform muscles. They obviously play a considerable role in bracing the thighs firmly against the pelvic region prior to the act of leaping.

(5) Faradic stimulation applied at the tip of the urostyle of a pithed animal showed that the combined effect of the pyriform muscles and the compressor cloacae (that of the former far exceeding the latter) was to pull the urostyle in a ventral direction hard down on the pelvis, clamping down the cloaca and completely occluding it. In this movement the coccygeo-iliac muscles (two thin sheets of muscle extending from urostyle to sides of the pelvis) also shared in a lesser degree.

A common position of the female frog during the act of oviposition is with the thighs extended backward, implying relaxation of the pyriform muscles. In this position general stimulation in the intact animal and/or local stimulation of the pelvic region of a pithed animal immediately resulted in complete occlusion of the cloacal passage. The negative results of attempts to elicit extrusion by general electrical stimulation are thus at once explained.

(6) A powerful opposition to the action of the foregoing muscles exists in the group of muscles controlling the urostyle from the sacral region (Fig. 2). These are the longissimus dorsi and the coccygeo-sacral muscles, reinforced by the paired ileo-lumbar muscles. Stimulation of these, following section of the pyriform muscles and the compressor cloacae, jerks the urostyle strongly upwards high above the pelvis.

![Diagram of principal muscles controlling urostyle in Rana from above.](image_url)
In animals in which the pyriform and compressor cloacae muscles have been sectioned slight stimulation of the ventral body musculature—in particular the pectoralis abdominalis—readily results in the hinder part of the pelvis being drawn ventralwise and slightly forward and, though in a smaller degree, also enlarges the gap between urostyle and pelvis.

It appears, therefore, that there exists a constellation of opposed skeletal muscles whereby the passage between urostyle and pelvis can be enlarged or vice versa, in that the action of the pyriform muscles and the compressor cloacae in occluding the passageway is balanced by the action of dorsal and ventral muscle groups in widening it in a dorso-ventral plane. It is clear from observation that the usual position of the female in oviposition at least facilitates the action of such a mechanism.

A series of experimental tests was then made on spinal *Rana* and repeated on spinal *Bufo* where the length and position of the urostyle are approximately the same as in *Rana*, but where pelvic movements are somewhat less free owing to the slightly dilated processes of the sacrum overlapping the ileo-sacral junctions. The results may be tabulated as follows:

<table>
<thead>
<tr>
<th>Position of electrodes A and B</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td><strong>B</strong></td>
</tr>
<tr>
<td>1. Base of urostyle on spinal column</td>
<td>As A</td>
</tr>
<tr>
<td>2. Front boundary of sacrum</td>
<td>As A</td>
</tr>
<tr>
<td>3. Midway between sacrum and skull</td>
<td>As A</td>
</tr>
<tr>
<td>4. <em>Rana</em>: Front boundary of sacrum</td>
<td>Right axilla</td>
</tr>
<tr>
<td><em>Bufo</em>: Midway between sacrum and skull</td>
<td></td>
</tr>
<tr>
<td>5. Do.</td>
<td>Slightly posterior to axilla</td>
</tr>
<tr>
<td>6. Do.</td>
<td>Posterior to axilla on dorso-lateral abdominal wall approx. at the level of vertebrae VI–VII</td>
</tr>
<tr>
<td>7. Do.</td>
<td>Further back behind level of sacrum</td>
</tr>
</tbody>
</table>

These tests establish that appropriate stimulation will (a) dilate the urostyle-pelvis passageway to an extent far greater in degree than suffices for ordinary defaecation and simultaneously bring about spasmodic contraction of the abdominal musculature. The complexity of the ultimate distribution of the nerve fibres of the
Ovulation and oviposition in Anura

sacral plexus is such that elucidation of the precise part played by separate muscles involved is difficult. Their combined action is, however, clear. When the urostyle is raised to its maximum extent the action of part of the compressor cloacae muscle formerly suggested (Ecker & Wiedersheim, 1904) seems to be well founded, i.e. that the dorsal wall of the cloaca is pulled down towards the external orifice, thus bringing the region which receives the oviducal and urinary apertures closer to the exterior and slightly shunting the anal opening of the rectum in a ventral and anterior direction.

Repetition of the foregoing tests on intact female Rana resulted, when the electrodes were applied as in experiment no. 6 in the above table, in the cloaca being opened widely and the immediate extrusion of paired masses of the characteristic jelly-like material which is normally present in the lower oviducal tract of breeding females. Dissection confirmed that the animals were approaching the gravid condition, with enlarged oviducts which along with the uteri contained copious secretion.

Position of the male

In the light of these findings the possibility previously mentioned that pressure from the male on the body of the female may aid in oviposition—since gentle pressure applied to gravid frogs occasionally results in extrusion—may be further considered.

(1) In Rana the male's clasp is axillary. His body pressure is therefore on the dorsal surface of the female from the sacrum forwards. His arms clasp the body wall of the female anterior to the level of the sacrum. In the normal coupling position in water a tendency for the male to slip backwards slightly owing to gravity may be observed.

The remarkable increase in sensitiveness to faradic stimulation of the pectoral region of the breeding male (Smith, 1938) appears very significant in the maintenance of a position which does not interfere mechanically with the lifting movement of the female urostyle and associated pelvic movements necessary for extrusion of uterine ova, and may be, indeed, of great significance in normal oviposition.

In Xenopus the male's position is posterior to the sacral region. In this position his pectoral region rests on the female's urostyle and his arms clasp the anterior pelvic region. Compared with the condition in Rana the whole sacral region in Xenopus is relatively immobile; urostyle, sacrum and anterior pelvis are firmly bound together and their rigidity is reinforced by large paired wing-like outgrowths of the sacral region, which carry far back between the extended ilia the rigidity of the sacral bridge (Fig. 3). The condition in Xenopus approaches that of Pipa. In the latter the urostyle is fused with the sacrum as in Breviceps, Phyllomedusa and Pelobates (Ridewood, 1897). In Pipa the transverse processes of the sacral region (including the first post-sacral vertebra (Ridewood)) are greatly dilated. In Xenopus the urostyle is fused to the sacrum and the broad distal ends of the transverse processes of the latter meet the inner faces of the extended ilia, contributing greatly to the firmness of the pelvis, e.g. when clasped by the male. The firmness of the
forward pelvic region is in marked contrast to the condition in *Rana* where iliac-sacral articulation is remarkably free and in *Bufo* where the sacral processes are somewhat dilated to cover the tips of the ilia, but do not extend backward between them.

The massiveness of the musculature associated with the urostyle and lumbar region in *Xenopus* is such as readily to support dorsal pressure by the male. Thus he exercises no pressure on the cloaca itself. The urostyle presents a striking contrast to that of *Rana* and *Bufo* in that it ends well forward of the body of the pelvis, and does not overlap the cloacal tube (Fig. 3). Beneath the gap thus left between urostyle and pelvic symphysis the body wall is reinforced ventrally by an ypsiloid cartilage.

![Fig. 3. A, Rana; B, Bufo; C, Xenopus; from above. Y, ypsiloid cartilage (ventral).](image)

Consequently in *Xenopus* the cloacal tube lies on the pelvis and the products of rectum, oviducts and ureters pass freely through without interference from the urostyle. A thin suspensory band of fibres runs well forward under the skin from the dorsal lip of the cloaca to the urostyle tip, but we have not so far identified in *Xenopus* the very prominent pair of pyriform muscles which in *Rana* and *Bufo* run from the extended tip of the urostyle outwards to the femora.

**GENERAL SUMMARY**

1. The normal rhythm of sexual activity of both *Xenopus* and *Rana* is controlled by secretions of the anterior lobe pituitary. Progesterone and testosterone, as well as extracts of A.L.P., evoke ovulation in normal and hypophysectomized *Xenopus*. Injection of pregnancy urine into hypophysectomized *Xenopus* evokes enlargement of the reduced ovary and ovulation.
Ovulation and oviposition in Anura

2. Serial injections of pituitary extracts and oestrin were without effect on the amount of egg jelly secreted.

3. Ovulation in *Xenopus* is immediately followed by oviposition. The eggs are carried by continuous ciliary tracts from the body cavity via the oviduct to the exterior. In *Rana* ovulation is not immediately followed by oviposition. The eggs accumulate in the lower third of the oviduct and are expelled *en masse*.

4. Experiments on extirpation of endocrines and the injection of appropriate extracts, together with the responses to pithing and electrical stimulation, lead to the conclusion that oviposition in *Rana* is probably under nervous not humoral control. The muscular and skeletal mechanism involved in the retention and extrusion of eggs in *Rana* is described.

Acknowledgement is made to the Medical Research Council for their support of this investigation, and to Organon Ltd. for gifts of oestrogens.

REFERENCES


