PHYSIOLOGY

Endocrine Control of Sexual Reproduction in *Opalina ranarum* Parasitic in *Rana temporaria*

It is well known that opalinids parasitizing the recta of Amphibia multiply asexually by binary fission during most of the year. During the breeding season, however, the reproductive pattern changes to a sexual one, the preliminary stages of which are marked by an increase in the multiplication-rate and the production of small forms and minute individuals. The latter eventually encyst and are passed out with the fæces of the frog. If ingested by tadpoles, they excyst, form micro- and macro-gametes, and the resulting zygotes give rise to the multinucleated opalinids in the newly metamorphosed frogs.

It has long been suspected that the stimulus for inducing the change in the reproductive pattern of the opalinids was related to the endocrine behaviour of the host. Bieniarz[1] found that when male *Rana esculenta* were given injections of human pregnancy urine, the opalinids changed to a sexual pattern of reproduction resulting in the production of cysts. He suggested that the change might be initiated by the human chorionic gonadotrophin present. Cehovic[2] injected *R. esculenta* and *R. temporaria* with pregnancy urine, chorionic gonadotrophin or frog pituitaries, and obtained cyst formation. He concluded that the correlation between host and parasite life-cycles was due to pituitary activity and probably due to the production of gonadotrophin.

We have recently carried out a series of experiments on normal, hypophysectomized and gonadectomized *R. temporaria* with the following results.

(*a*) Injection of œstrone or gonadotrophic substances (concentrated or untreated pregnancy urine, chorionic gonadotrophin, serum gonadotrophin, suspension of frog hypophyses) to male and female frogs induced the sexual reproduction pattern of development in the opalinids—as evidenced by cyst production—but only in the period just prior to the breeding season (that is, during November–February in Europe) and not at other times of the year.

(*b*) Testosterone propionate and adrenaline injected into male and female frogs induced the sexual cycle of reproduction in the parasites during any time of the year.

(*c*) In gonadectomized animals, only injections of testosterone propionate were effective in inducing the sexual reproductive cycle in the parasites; adrenalin was ineffective.

(*d*) *In vitro*, gonadotrophic hormones gave negative results in all cases; but some positive results were obtained with untreated pregnancy urine. Some cultures treated with œstrone or hydrosoluble testis extract produced dividing stages and small forms, but cysts were never produced.

(*e*) In frogs prevented from copulating, the sexual reproductive cycle of the opalinids occurred in female frogs 77 days later than those which had copulated, and in the males, 82 days later.

These results clearly suggest that the induction of a sexual reproductive pattern in the opalinids is related, not to the levels of gonadotrophic hormones, but to the levels of gonadal hormones present in the host. In the male frog, the evidence shows that testosterone is implicated. In the female frog, in which the origin and nature of the gonadal hormone are obscure[3], the situation is not clear, for œstrone has been found only to induce cyst formation during the period just prior to the breeding season.

There is no evidence, as yet, to indicate whether the gonadal hormones exert their effect on these Protozoa directly or whether it is their breakdown products which are involved, or whether either of these exert their effect indirectly through some other aspect of the host's metabolism.

The effects of adrenalin injections in Amphibia are complex[4,5], and its action in inducing sexual reproduction in opalinids during any time of the year cannot be simply explained at the present phase of this work. The fact that it failed to induce cyst formation in gonadectomized frogs suggests that its action in some way induces the release of sex hormones, even when, in the male outside the breeding season, interstitial cells are absent from the testes.

Just prior to the breeding season, keeping the frogs at 8–12° C. induced cyst formation. That this result is attributable to the induction of early sexual maturity in the host and the subsequent release of the gonadal hormones may be shown by the fact that, during the summer, maintaining frogs at 20° C. failed to induce cyst formation, although injections of testosterone propionate induced the sexual cycle in the Protozoa, as at other times of the year.

Dr. Elspeth McConnachie[6] has kindly sent us the text of manuscript (in the press) in which she describes the induction of cyst production in *Opalina* by injection of frogs with chorionic gonadotrophin and frog pituitary extract. Although no experiments were carried out with the gonadal hormones, she concludes on theoretical grounds that the factors provoking encystation are probably the gonadal hormones.

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Dipeptidase Activity of Brain

Available knowledge on the dipeptidase activity of brain is limited to the enzymatic hydrolysis of DL-alanyl-glycine[1–3]. Detailed studies by Pope *et al.* have dealt with the intralaminar distribution of dipeptidase activity towards DL-alanyl-glycine in the cerebral cortex of rat[1,2] and man[3]. Our own studies on proteolytic activity of brain led to a systematic inquiry into the existence of any selective or preferential dipeptidase activity of brain towards any particular dipeptidase structure. The present preliminary report deals with the systematic quantitative differences observed in the hydrolysis of the series of N-glycyl-dipeptides by mouse brain.

Freshly removed brains of adult Swiss albino mice were frozen on a glass slide placed on dry-ice, and immediately trimmed with a razor to remove the