Effect of glucagon administration on serum calcium and inorganic phosphorus in the freshwater mud eel, *Amphipnous cuchia*

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Summary. Glucagon injection in *Amphipnous cuchia* evokes a decreased level of serum calcium on day 1 which progresses till day 3. This response declines after day 5. The serum inorganic phosphorus level does not show any significant change.

It has become apparent as a result of many studies that glucagon (a hyperglycemic-glycolytic polypeptide) has marked effects on calcium metabolism in mammals - mongoose[1], house shrew[2], rat[3-5], rabbit[6], dog[7,8], monkey[9] and man[10-13]. To our knowledge, there is no previous report concerning the effects of this drug on calcium and phosphorus metabolism in fishes. This investigation was, therefore, undertaken to determine whether administration of glucagon affects the serum calcium and phosphorus levels in the freshwater mud eel, *Amphipnous cuchia.*

Material and methods. 48 adult fish *(Amphipnous cuchia),* weighing 350–450 g, were maintained under laboratory conditions in glass aquaria for 2 weeks prior to use. They were divided into 2 numerically equal groups a) diluent-injected (control); and b) glucagon-injected (experimental). The experimental fish were daily injected i.p. with crystalline glucagon[14] in a dosage of 1 mg/ml/kg b.wt for 10 days. The drug was dissolved in 0.005 N HCl (pH 2.6) and diluted with 0.6% sodium chloride solution containing 0.1% gelatin (diluent). The control fish were daily injected i.p. with 1 ml/kg b.wt of diluent. Blood samples from both the groups were collected from the caudal artery 2 h after the last injection at 1, 3, 5 and 10 days following the treatment. The analysis of calcium and inorganic phosphorus was performed with the serum by Trinder's[15] and Fiske and Subbarow's[16] method, respectively. The fish were not fed during the experimental period. To avoid the effects of circadian rhythm, the injections were administered at the same hour of the day and the blood samples were collected at approximately the same time of the day throughout the experiment.

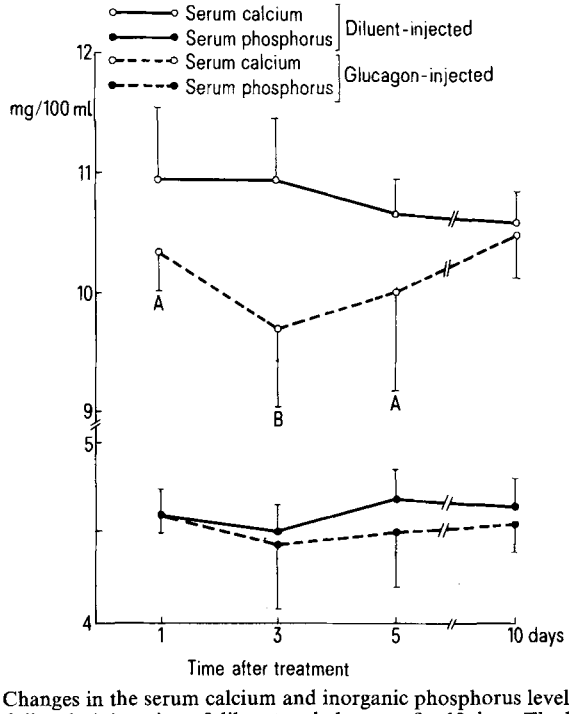
Differences between diluent- and glucagon-injected fish were evaluated using Student's t-test.

Results. In glucagon-injected fish the hormone evokes a decreased level of serum calcium on day 1 ($p < 0.05$) which continues until day 3 ($p < 0.01$). Thereafter, the hypocalcemic response decreases, as is evident from a gradual revival of the serum calcium level (Fig.). The serum inorganic phosphorus level in glucagon-injected fish is not significantly different from that of the diluent-injected fish at any experimental period (fig.).

Discussion. The results obtained in the present study clearly indicate that glucagon evokes hypocalcemia in the fish *A. cuchia.* This is in agreement with the previous reports which describe the hypocalcemic action of glucagon in animals[1-9] and man[10-13]. The augmented urinary excretion of calcium and other electrolytes in dog[17-20] and in man[21] after glucagon administration strengthens this observation.

In mammals, evidence has been produced regarding the stimulation of calcitonin release after glucagon administration[1,2,7,22]. Thus, the hypocalcemia observed in the present study may be attributed to the possible release of calcitonin from the ultimobranchial body (known to be homologous with the calcitonin cells of mammals[23], and like these cells in mammals a rich source of calcitonin[24]).

The present report describes that glucagon has no effect on the level of serum inorganic phosphorus. This is of interest, as the hormone (glucagon) has been reported to cause hypophosphatemia in mammals[1,2,22,25].



Changes in the serum calcium and inorganic phosphorus level after daily administration of diluent and glucagon for 10 days. The blood samples were collected 2 h after the last injection at 1, 3, 5 and 10 days following the treatment. Each point indicates mean ± SD of 6 determinations. A and B represent significant responses, $p < 0.05$ and < 0.01 respectively.

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Bromocryptine treatment and puberty attainment in the female rat

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Summary. The influence of bromocryptine treatment on the timing of vaginal patency and 1st oestrus in female rats was studied. No significant influence of bromocryptine on these parameters was noted, and it is concluded that suppression of prolactin secretion has no effect on puberty attainment.

Exposure of prepubertal rats or mice to males or male odours is known to advance the onset of 1st oestrus[2,3], indicating that this 'male effect' is mediated by a primer pheromone. However, it is still unclear whether the pheromone acts initially via an elevation of gonadotrophin secretion, or a suppression of prolactin secretion, both of which are known to occur[4,5]. Since various reports suggest that prolactin may be implicated in the timing of the onset of puberty in the rat[6-8], it was decided to further investigate its influence by selectively inhibiting its release by administration of bromocryptine to prepubertal female rats.

Materials and method. 40 prepubertal female rats, weaned at 20 days of age, were randomly allocated to one of 2 groups. From an age of 25 days these groups were subjected to one of 2 treatments. The treatments were either daily s.c. injections of 0.5 mg bromocryptine mesylate in 0.1 ml of vehicle consisting of propylene glycol:ethanol:0.9% saline (1:1:2 v/v) or daily injections of 0.1 ml of vehicle alone.

Rats were housed in groups of 4 per cage in a 14:10 h light:dark cycle, and from weaning were fed ad libitum on a standard rat diet. All rats were checked daily for vaginal opening, whereupon the injection schedule ceased and smears were taken daily by flushing the vagina with a small drop of water. The interval between vaginal patency and the 1st oestrus smear was recorded. All rats were weighed at oestrus.

Results and discussion. The results presented in the table indicate that bromocryptine treatment had no significant effect on ages at vaginal patency or 1st oestrus, or on the interval between these events. The weight at 1st oestrus was not significantly affected by treatment. Although determinations of serum prolactin levels were not made, the dose of bromocryptine used is known to effectively inhibit the release of prolactin[9,10].

It has been suggested that the involvement of prolactin in puberty onset may be via an inhibition of LH release[11,12]. Thus, if prolactin is involved in the onset of puberty its removal might be expected to cause an advancement of the 1st oestrus. The results from the present trial do not substantiate this expectation. Thus, it may be concluded that if prolactin is involved in puberty attainment in the rat, it is not indispensible in this role, although final conclusions must await prolactin assay data.

The effect of bromocryptine on puberty attainment in the rat

Treatment	Age at vaginal opening (days)	Age at 1st oestrus (days)	Vaginal opening – oestrus, interval (days)	Weight at oestrus (g)
Bromocryptine	36.0±0.6	38.0±0.5	2.0±0.3	125.2±2.9
Control	34.9±0.5	37.0±0.5	2.1±0.3	118.0±7.7

Values are mean±SEM.

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