the LD_{50} values for 3- and 24-h-old L_5 induced for diapause were but slightly higher than in L_5 induced for pupation, the LD_{50} values in the former increased only by the factors 6.4 and 2000 in the 48- and 72-h-old larvae respectively. These results indicate that the tolerance to the virus increases in diapause-induced L_5 about 24 h later than in L_5 induced for pupation. The decrease of the slopes of the dose-mortality regression-lines with larval age indicate that the populations become less homogeneous with respect to granulosis infection.

Time-mortality data were recorded for the standard dose 3×10^3 capsules per larva (table 2). This dose is close to the minimal dose necessary to cause 100% mortality in a population of freshly molted L5. The results reveal that an increased tolerance to the *Baculovirus* occurs already in 24-h-old L_5, since the larvae survive longer after infection at 24 h than at 3 h. In older experimental groups average time till death as well as the minimal time requested for the appearance of mortality increased generally and mortality decreased. Whereas the standard dose caused still 39% mortality in 72-h-old L_5 induced for diapause, no mortality was caused by this dose in 72-h-old L_5 induced for pupation. Only at the age of 96 h the diapause-induced L_5 were also fully resistant to the standard dose.

Table 2. Effect of age of last instar larvae – induced for pupation (IP) or diapause (ID) – on mortality, and time-parameters after infection with a single dose of 3×10^3 capsules/larva. Larval weight is given as $\bar{x} \pm SD$

Age of L_5 at infection	Larval weight (mg)	Mortality %	First observed mortality at Day	%	Average time till death (days)
IP 3 h	39.1 ± 6.2	100	5	15	6.0
24 h	68.5 ± 9.8	89	6	4	8.8
48 h	88.5 ± 12.0	18	10	9	10.8
72 h	85.0 ± 14.6	0	–	–	–
96 h	73.0 ± 11.7	0	–	–	–
ID 3 h	45.5 ± 7.9	97	6	7	7.5
24 h	75.5 ± 10.5	97	7	23	9.8
48 h	95.0 ± 14.0	67	8	4	10.6
72 h	105.5 ± 16.7	39	10	18	12.2
96 h	93.5 ± 15.8	0	–	–	–

The weight gain of the larvae cannot explain the rapid increase of tolerance to the virus. Tolerance is not correlated with weight (table 2) and is still increasing when larval weight decreases after 72 h.

Our data suggest that the physiological changes in the last instar larva connected with the change of the cellular commitment to pupal differentiation are responsible for the development and rapid increase of virus tolerance. The delay by 24 h of this increase in larvae induced for diapause fits well with this interpretation. The physiological changes that prepare the insect for pupation take also place in diapause-induced larvae, but 24 h later than in L_5 programmed for immediate pupation[9,10].

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In vivo effects of epinephrine in a freshwater fish

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Summary. In vivo effects of epinephrine were investigated in a freshwater teleost, *Barbus conchonius* Hamilton. Fish given 2 mg/kg epinephrine in a single i.m. dose showed significant hypocholesterolemia and elevated liver and kidney cholesterol levels 1–8 h postinjection. Plasma amino nitrogen evinced a transient yet significant fall at 2 h followed by a significant increase after 24 h. A marked reduction occurred in the plasma FFA and organic PO_4 levels after 1–8 h. The results offer little evidence for a lipolytic effect of epinephrine in this species, and the changes in metabolite levels are attributable, in part, to the catecholamine-induced modification of insulin secretion.

Key words. Freshwater fish; *Barbus conchonius;* epinephrine, hypocholesterolemia, insulin secretion; lipolytic effect.

The adipokinetic effects of catecholamines in mammals are well known and elevated levels of plasma and tissue free fatty acids (FFA) have been demonstrated following epinephrine administration[2]. Evidence for a possible lipolytic effect of catecholamines in fishes is still fragmentary and inconsistent. Norepinephrine in the bream, *Abramis brama* and epinephrine in the pike perch, *Lucioperca lucius,* did not stimulate lipolysis[3]. By contrast, epinephrine treatment raised the FFA content[4] in the blood of the eel, *Anguilla anguilla.* A significant norepinephrine-induced decrease occurred in the total uptake of ^{14}C-acetate in the liver lipids of the nurse shark, *Ginglymostoma cirratum* indicating stimulation of lipolysis[5]. The object of the present investigation was to evaluate epinephrine-induced changes in blood cholesterol, FFA, organic PO_4 and amino nitrogen, and liver and kidney cholesterol in *Barbus conchonius* Hamilton.

Material and methods. Adults of *Barbus conchonius* Ham., weighing 4.5–5 g, were procured from the local lake and accli-

matized to laboratory conditions for 2 weeks prior to use. Experimental groups consisted of (a) fish receiving 2 mg/kg b.wt. epinephrine in a single i.m. injection, and (b) fish receiving plain buffer to serve as control. Since the fish used in this study was quite small (total b.wt $\leq$ 5 g) it was found not possible to inject the hormone i.a., and therefore, the i.m. route had to be used. A 0.01% solution of epinephrine (crystalline L-adrenaline, lot No. 148262-21K, Ciba Research Centre, Switzerland), prepared fresh in citrate-phosphate buffer at pH 4.0 was used. From each group, 8–10 individuals were removed and sacrificed ½, 1, 2, 5, 8, 18 and 24 h post injection. Blood samples were drawn into heparinized tuberculin syringes and stored in a freezer until further processing. Samples of liver and kidneys were immediately removed and frozen. Blood samples, 20 µl for each parameter, were analyzed for cholesterol[6], FFA[7], organic PO_4[8], and amino nitrogen[9]. Tissue cholesterol was also assayed[10]. Food provided ad libitum was withheld 24 h prior to and during the experiment. Significance of difference between control and experimental values was evaluated by the Student's t-test.

Results and discussion. Epinephrine treatment in *Barbus conchonius* induced significant hypocholesterolemia ($p < 0.05$) between 2–18 h with a concomitant rise in liver ($p < 0.01$ at 5 and 8 h) and kidney ($p < 0.01$ between 1–8 h) cholesterol content. The response was most pronounced 5 h after the injection. In mammals, large and prolonged doses of epinephrine and norepinephrine caused an increase in plasma FFA followed by a delayed rise in plasma cholesterol and triglycerides[2]. Results of the present study suggest that, in contrast to its action in mammals, epinephrine has a blood cholesterol-lowering influence in this species. The northern pike, *Esox lucius* also responded similarly following infusion of 5 mg/kg epinephrine[11]. On the other hand, epinephrine treatment in the eel, *Anguilla anguilla* elicited a marked and sustained hypercholesterolemia[4]. Physiologically, the diminished blood cholesterol levels in *B. conchonius* might have resulted partly from utilization of cholesterol in corticosteroidogenesis, because catecholamines cause enhanced secretion of glucocorticoids. The observed rise in tissue cholesterol may consequently be associated with accelerated de novo synthesis of this precursor to sustain enhanced corticoid production.

In mammals, epinephrine causes lowering of plasma amino acids and increases their uptake by tissues[12]. In the northern pike, *Esox lucius,* epinephrine infusion failed to alter amino acid levels[11]. In *B. conchonius,* however, a transient yet significant fall in plasma amino nitrogen at 2 h ($p < 0.05$), was followed by a significant increase ($p < 0.01$) after 24 h. Such a response could be related to the influence of endogenously secreted glucocorticoids which apparently mask the action of catecholamines. From mammalian studies it is known the glucocorticoids are protein catabolic and that they are released in response to catecholamine stimulation of the pituitary-adrenal cortex axis[11].

In fishes, while the effects of catecholamines on carbohydrate metabolism are well established[4, 11], their role in lipid metabolism is unclear. Norepinephrine injection into bream, *Abramis brama,* and in vitro addition of epinephrine to the adipose tissue of pike perch, *Lucioperca lucioperca,* did not stimulate lipolysis[3]. Similarly, epinephrine did not promote FFA and glycerol release in the lateral line muscle from the rainbow trout, *Salmo gairdnerii*[13] although the presence in fish muscle of lipases capable of hydrolyzing triglycerides has been convincingly demonstrated[14]. In *B. conchonius,* blood levels of FFA and the organic PO_4, representing mainly phospholipids and phosphoproteins, were distinctly altered in response to epinephrine treatment. These parameters showed a reduction over 1–8 h and the differences between control and epinephrine-treated values were significant ($p < 0.01$) 2,5 and 8 h postinjection. Therefore, an unequivocal lipolytic effect of epinephrine is not demonstrable in this fish. This agrees well with studies on a closely related species. *Cyprinus carpio,* in which i.m. injection of adrenaline and noradrenaline caused a reduction in blood FFA[15]. Similarly, the pike, *Esox lucius* evinced a significant decrease in FFA between 1 and 3 h when injected intraarterially with adrenaline at 1 mg/kg, and the depressed values persisted for up to 24 h[16]. In contrast, epinephrine raised the FFA content in the blood of eel, *Anguilla anguilla*[4] and lamprey, *Lampetra fluviatilis* and scorpion fish, *Scorpaena procus*[17].

In higher vertebrates, the lipolytic effect of catecholamines is mediated via β-adrenergic receptors with resultant formation of cAMP, which in turn, activates the hormone-sensitive lipase. However, both in vivo and in vitro studies failed to demonstrate the lipolytic action of catecholamines in fishes and amphibians[3, 13]. This was attributed to a decrease in the activity of the hormone-sensitive lipase or a lack of the requisite enzyme in the poikilotherms. Similar reasons might explain the absence of adipokinetic influence of epinephrine in the fish under report.

There is evidence to suggest that catecholamines inhibit insulin secretion in man and mammals, an effect which is mediated by their interaction with the α-adrenergic receptors on the islet β-cells[18, 19]. Studies on the hormonal influences on insulin secretion in the lamprey and the scorpion fish have shown that plasma insulin levels are initially depressed and subsequently increased by the catecholamines[20]. A similar biphasic response of plasma insulin to an intraarterial infusion of 25 and 50 µg/kg adrenaline has been demonstrated in the pike, *Esox lucius,* in which an initial significant depression over 30 min to 1 h was followed by a significant elevation after 3 h[21]. A prompt rise in the plasma insulin levels, upon cessation of catecholamine infusion, has been described as a 'rebound effect'[22]. In *B. conchonius* the reduction in blood cholesterol (1-24 h), FFA and organic PO_4 (1–8 h) and amino nitrogen (1–2 h) seem to indicate an insulin-assisted response. Likewise, in the goldfish, *Carassius auratus,* adrenaline or noradrenaline reduced serum FFA in normal fish with functional islet β-cells but not in alloxan-diabetic ones[23]. Manifestation of hyperinsulinaemia, following an initial suppression, are possibly related to the epinephrine-induced hyperglycemia together with the release of

Effect of epinephrine (2 mg/kg in single i.m. injection) on *Barbus conchonius*

Time (h) post injection	Plasma cholesterol ± SE (mg%)	Liver cholesterol ± SE (mg%)	Kidney cholesterol ± SE (mg%)	Amino nitrogen ± SE (mg%)	Free fatty acids ± SE (µEq/ml)	Organic PO_4 ± SE (mg%)
Control[a]	328.6 ± 27.9	284.6 ± 23.5	446.5 ± 34.5	14.9 ± 0.46	4.93 ± 0.13	41.4 ± 1.16
½	308.8 ± 27.5	325.2 ± 37.3	615.8 ± 33.0	14.0 ± 1.90	4.70 ± 0.69	44.2 ± 8.26
1	274.1 ± 16.2	341.8 ± 43.9	634.2 ± 11.3[c]	12.9 ± 2.73	3.97 ± 0.38	40.0 ± 5.00
2	224.4 ± 27.5[b]	355.5 ± 37.9	680.3 ± 17.4[c]	12.2 ± 1.07[b]	2.66 ± 0.37[c]	28.4 ± 2.79[c]
5	56.6 ± 23.7[c]	665.6 ± 12.6[c]	682.2 ± 32.9[c]	14.1 ± 2.94	2.54 ± 0.17[c]	25.0 ± 3.77[c]
8	241.9 ± 20.4[b]	449.0 ± 20.1[c]	651.0 ± 18.5[c]	16.4 ± 1.98	2.39 ± 0.15[c]	13.4 ± 3.39[c]
18	248.9 ± 15.2[b]	387.4 ± 20.9	633.0 ± 99.7	16.7 ± 0.82	4.09 ± 0.13	39.0 ± 2.52
24	267.8 ± 13.0	297.4 ± 19.5	627.0 ± 29.2	23.2 ± 0.73[c]	4.17 ± 0.18	36.6 ± 2.91

[a] Pooled values of all observation times; [b] $p < 0.05$; [c] $p < 0.01$.

β-cells from inhibition by catecholamine inactivation. It is worth mentioning that epinephrine elicited a significant hyperglycemic response in *B. conchonius* ½–1 h post injection (blood glucose: 83.5 ± 3.6 mg% control; 193.8 ± 17.6 mg% and 223.5 ± 2.4 mg% after ½ and 1 h, respectively)[24], which might have triggered an enhanced insulin secretion. Collateral histological examination of the pancreatic islets in the fish[24] revealed degranulation and vacuolation in the β-cells suggesting an enhanced secretory activity.

Compared to the warm blooded animals, bony fishes, like other poikilothermic animals, seem to have a low sensitivity to the catecholamines, which may be related to a lower metabolic rate. In addition, a slow degradation of the administered amine in these animals cannot be overlooked. These considerations, together with the i.m. route of administration and consequently the slower distribution of the amine, might be responsible for a delayed action of epinephrine in *B. conchonius*.

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The effect of lactose and iron on strontium absorption[1]

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Summary. In rats fed on a milk diet with or without the addition of lactose and/or iron the transileal strontium-85 transfer was higher by 14–38% and the intestinal strontium retention lower by 6–23% than in control rats fed on standard laboratory food.
Key words. Rat ileum; milk diet; strontium absorption; lactose, dietary; iron, dietary.

The stimulatory effect of a milk diet on the absorption of some ions from the intestinal tract[2–6] could be explained by the high lactose and low iron content of milk. Bearing in mind the role of radiostrontium in internal contamination, we studied the effect of lactose and iron on strontium absorption from the rat's ileum.

Materials and methods. Strontium transport was determined on ileal segments taken from female 5-week-old rats by the in vitro method of the 'everted intestinal sac'[7]. There were altogether 60 animals in the experiment and two, 4 cm long, ileal segments were cut out from each rat. Before the experiment all the animals were on a standard diet with 1.2% calcium and 0.8% phosphorus. They were divided into 6 equal groups according to the diet they were fed on for 3 consecutive days: 1. Standard laboratory food (SF) + drinking water (control); 2. SF + 15 g lactose (L) added to 100 ml of drinking water; 3. milk (M) (pasteurized cow's milk containing 140 mg Ca and 95 mg P/100 ml); 4. M + L; 5. M + Fe (10 mg Fe was added – as $FeSO_4 \cdot 7\ H_2O$ – to 100 ml of milk); 6. M + L + Fe. On the 4th day all animals were killed by decapitation and ileal everted sacs prepared by the experimental procedure described before[7]. The composition of the medium was as follows (mM/l): 135 NaCl, 11 KCl, 0.05 $SrCl_2$, and 10 mM sodium phosphate buffer, pH 7.4. Strontium-54 (Radiochemical Centre, Amersham, England) in an almost carrier-free form was added to the mucosal solution in the form of chloride. The activity was adjusted to about 300 KBq strontium-85 per 100 ml of the solution. After a 45-min incubation of the samples, the amount of radiostrontium retained in the solution inside (S) and outside (M) the ileal sac and in the intestinal wall was determined in an automatic well-type scintillation counter (Nuclear Chicago, USA).

Results and discussion. The results were calculated as S/M (serosal over mucosal) activity ratios for strontium transport, and as percentages of the initial mucosal solution activity for its intestinal retention. To make comparison between the groups easier the results for experimental groups are presented in the figure, as percentages of the control values. In all groups fed on the experimental diets for 3 days, the mean values for strontium transfer were higher than in the stock diet-fed controls (fig., left part). They were significantly higher (by 14, 24 and 38%) for the following diets: milk, milk + Fe and milk + L + Fe. The addition of lactose alone to either food or milk did not produce significant changes. This is rather surprising since, according to Armbrecht and Wasserman[9] a dose of lactose even 2.5 times lower than ours increased the permeability (for calcium) of the absorptive intestinal cell by over 60%. This difference may be attributed to alteration of several items of the experimental set-up, such as animal species, segment of the intestine studied, dose of lactose, duration of pre-