

# Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout\*

Bruce A. Barton\*\*, Carl B. Schreck & Lesley D. Barton\*\*

Oregon Cooperative Fishery Research Unit\*\*\*, Oregon State University, Corvallis, Oregon 97331, USA

**ABSTRACT:** Juvenile rainbow trout *Salmo gairdneri* were either fed cortisol or subjected to an acute stress daily for 10 wk to determine the long-term effects of these factors on growth, physiological conditions, and stress responses. In fish fed cortisol, growth and condition factor, liver glycogen, and circulating lymphocytes were reduced and resting plasma glucose and hematocrit were increased. In fish stressed daily, all conditions were similar to those in controls except that lymphocyte numbers were lower. Continuous feeding of cortisol to fish for 10 wk completely eliminated the plasma cortisol elevation after acute handling, but the magnitude of the stress-induced glucose increase was unchanged. In fish stressed daily for 10 wk, a reduction in post-stress levels of both plasma cortisol and glucose after handling demonstrated the effect of habituation on these stress responses. We concluded that continuously elevated plasma cortisol from exogenous feeding had a profound long-term effect on juvenile rainbow trout, but that daily stress-induced acute elevations of endogenous cortisol did not, except for a suppression of circulating lymphocytes. The occurrence of changes commonly observed in chronically stressed fish that are similar to those in the cortisol-fed trout in our experiment supports the view that long-term detrimental effects of stress in fish are largely mediated by cortisol. The results also indicated that continuous negative feedback of cortisol on the hypothalamic-pituitary axis may compromise the ability of fish to cope with additional stress factors by limiting their capacity to elicit an interrenal response to a stimulus.

## INTRODUCTION

Biologists are familiar with the physiological responses of fish to stress (Pickering 1981), but mechanisms of action and the resultant consequences of stress on overall performance capacity are not yet well understood. Plasma corticosteroids, which affect both metabolic and immunologic pathways, rise dramatically in fish in response to stressful stimuli, particularly those that have a sensory component (reviewed by Donaldson 1981 and Schreck 1981). To attain our main

objective – determination of the consequences of continually elevated circulating cortisol in fish that would possibly result from long-term chronic stress – we examined the effects of chronic cortisol elevations in fish in the absence of other stressful stimuli. We fed cortisol to juvenile rainbow trout *Salmo gairdneri* and measured growth and condition, plasma cortisol and glucose, liver glycogen, hematocrit, and circulating leucocyte ratios. In addition, we determined the effect of continued cortisol treatment on the ability of the trout to elicit characteristic interrenal and glycemic responses to an acute stress. Our second objective – to assess the long-term effects on fish of being subjected to continual acute stresses, and the later effects of this treatment on characteristic stress responses – was approached by determining the effect of repeatedly elevating endogenous cortisol on these same physiological features by physically disturbing the fish daily.

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\*\* Present address: Utah Division of Wildlife Resources, Fisheries Experiment Station, 1465 West 200 North, Logan, Utah 84321, USA

\*\*\* Cooperators are Oregon State University, U.S. Fish and Wildlife Service, and Oregon Department of Fish and Wildlife

## MATERIALS AND METHODS

Juvenile rainbow trout *Salmo gairdneri* (weight 4 to 5 g) of the Willamette River stock were transferred from the Western Fish Toxicology Station, Corvallis, Oregon, USA, to the Oregon State University Smith Farm research facility 10 d before the study, for acclimation. Fish were held in circular tanks, 0.9 m in diameter, containing 327 l of aerated flow-through well water and having an inflow rate of  $2.8 \text{ l min}^{-1}$ . Flows were directed into the tanks perpendicular to the surface of the water to reduce rotational current; all tanks were cleaned weekly but at least 5 d before any sampling. During acclimation, fish were fed at 2% of body weight per day with Oregon Moist Pellets. For the experiment, we fed duplicate tanks of 185 fish for each treatment once daily at 3% of body weight per day with either control or cortisol-treated Oregon Moist Pellets between 0900 and 1000 h for 10 wk. Extra care was taken to feed the fish slowly, to ensure that all feed pellets were consumed. The experimental diet was prepared by dissolving crystalline cortisol (Sigma Chemical Co., St. Louis, Missouri, USA) in 100% ethanol and then spraying the resultant solution onto the surface of the pellets (Pickering 1984) to produce a concentration of  $100 \text{ mg cortisol (kg feed)}^{-1}$ . The control diet was prepared by using an equivalent amount of ethanol only. Feed pellets were air-dried for 1 h with occasional stirring to evaporate the ethanol, and then refrozen. A third group of fish in duplicate tanks was fed the control diet, but was also subjected to an acute stress daily, 5 to 6 h after feeding, by one of the following 3 methods: (1) after capture, fish were held in the air for 30 s in a perforated bucket, (2) water was completely drained from the tanks and then allowed to fill at the normal inflow rate, or (3) fish were continually chased in the tanks with nets for 15 min. The disturbance used was varied so that the fish would not become accustomed to a particular routine. During the 10 wk experimental period (October to December 1984), fish were maintained under a natural photoperiod and at water temperatures ranging from a high of  $13.0^\circ\text{C}$  at the beginning of the study to a low of  $11.5^\circ\text{C}$  at the end.

At the beginning of the experiment and every 2 wk thereafter, we removed 15 fish per tank to determine length, weight, and condition factor ( $100 \times \text{g cm}^{-3}$ ), and to sample blood, according to the following protocols: (1) from the control and cortisol-fed groups, 5 fish per tank were removed before feeding, and at 1 and 3 h after being fed; (2) from the stressed daily group, 5 fish per tank were removed before (6 h after feeding), and at 1 and 3 h after application of the stress. Feed rations were adjusted at each 2 wk interval in

relation to the numbers and weights of fish remaining in the tanks, to keep the relative ration constant.

Blood samples from the beginning of the study (0 wk), and at 4 and 8 wk were obtained for cortisol and glucose analyses. Cortisol was determined by radioimmunoassay (Foster & Dunn 1974), as modified by Redding et al. (1984b) for use with salmonid plasma and verified for *Salmo gairdneri* by Barton & Schreck (in press a). We measured glucose by the orthotoluidine method (Wedemeyer & Yasutake 1977), using premixed reagent (Sigma Chemical Co.). Blood samples taken 2 wk after the onset of treatment were used for determinations of hematocrit and leucocyte number. Hematocrit, also measured after 6 wk, was determined as percent packed cell volume after centrifugation. We determined leucocyte ratios from Giesma-stained blood smears by counting erythrocytes (average 1386 per slide, range 738 to 2024) and lymphocytes and other leucocytes in 15 random fields per slide (i.e. per fish), using Yasutake & Wales (1983) as a reference. Liver samples for determinations of hepatosomatic index ( $100 \times \text{liver wet weight/body wet weight}$ ) and glycogen, and stomach tissues for histological examination, were obtained after 10 wk. Whole livers were immediately placed in preweighed vials containing chilled 30% KOH, and hepatic glycogen was later analyzed by the method of Montgomery (1957). Cardiac portions of stomachs from control and cortisol-fed fish were preserved in buffered 10% formalin. After histological preparation, we qualitatively evaluated slide-mounted sections,  $6 \mu\text{m}$  thick and stained with hematoxylin and eosin, for evidence of tissue degradation or other abnormalities.

At Week 10, the fish in control and cortisol-fed groups were each separated into 2 subgroups, one of which remained in the 'home' tank. The fish remaining in all 'home' tanks were then subjected to a handling stress (held in the air in a perforated bucket for 30 s, as described earlier). Blood samples for cortisol and glucose analyses were taken before any disturbance (initial) and at 1, 3, 6, 12 and 24 h after handling.

The second subgroups of fish from the control and cortisol-fed groups were placed in 100 l tanks and feeding was continued with their respective diets. After 2 d, both groups were fed with a single meal of cortisol-treated feed at 3% of body weight, to characterize the profile of cortisol in the blood over 24 h post-feeding and the resultant effect on plasma glucose. Blood samples were obtained just before the fish were fed and at 1, 3, 6, 12 and 24 h after feeding.

Cumulative percent increases in mean weight of fish were compared by linear regression of 2 wk growth increments in each tank; slope of regression represents the percent change in body weight per day for the growth period. Differences from controls were assessed

by comparing variances of slopes using Student's *t*-tests ( $p < 0.05$  and  $< 0.01$ ). Specific (instantaneous) growth rates were calculated as:

$$[(\ln \text{weight}_2 - \ln \text{weight}_1) / \text{days fed}] \times 100$$

(Ricker 1979). Multiple-point comparisons were made by using 1-way analyses of variance followed by Duncan's new multiple-range tests at the 5% level (Steel & Torrie 1980); 2-point comparisons were made with Student's *t*-tests ( $p < 0.05$ ). Because of variance heterogeneity (Bartlett's test: Snedecor & Cochran 1967), data for cortisol and glucose were transformed to logarithmic values for analysis.

## RESULTS AND DISCUSSION

### Growth and resting physiological conditions

Relative growth in the cortisol-fed trout was reduced, but growth in the fish stressed daily was not different from that in controls (Fig. 1). Specific growth rate for the 10 wk period was lowest in the fish fed cortisol (Table 1). All 3 groups showed initial reductions in specific growth rates, followed by increases mid-way through the growth period (Table 1). This change may reflect a normal cyclical variation in growth rates in rainbow trout of this size (Wagner & McKeown 1985). Davis et al. (1985), using the same experimental protocol that we used, observed a reduction in absolute growth and condition factor in cortisol-fed channel catfish *Ictalurus punctatus*. Although they attributed the reduced growth to the presence of cortisol in the diet, they were unable to demonstrate a concomitant increase in blood corticosteroids after cortisol had been fed. However, Davis et al. (1985) did find an increase in activity of hepatic tyrosine aminotransferase in cortisol-fed fish and concluded that increased corticosteroid-induced gluconeogenesis resulted in decreased growth. In contrast to our finding for fish stressed daily, Peters & Schwarzer (1985) reported significantly reduced growth of juvenile rainbow trout subjected to a daily, brief handling stress over 4 wk, but did not indicate whether they considered this to be a cortisol-mediated phenomenon.

The continual application of dietary cortisol to the juvenile rainbow trout also reduced the condition factor over the 10 wk period (Table 2). Robertson et al. (1963) found that pharmacological doses of cortisol produced a rapid weight loss in juvenile rainbow trout, and Pickering & Duston (1983) observed that both orally administered and implanted cortisol at physiological levels significantly reduced the condition factor of brown trout *Salmo trutta*.

As Davis et al. (1985) pointed out, continuous stress

may result in reduced growth by chronically elevating plasma cortisol and thereby shifting metabolism toward protein catabolism. Pickering & Stewart (1984)

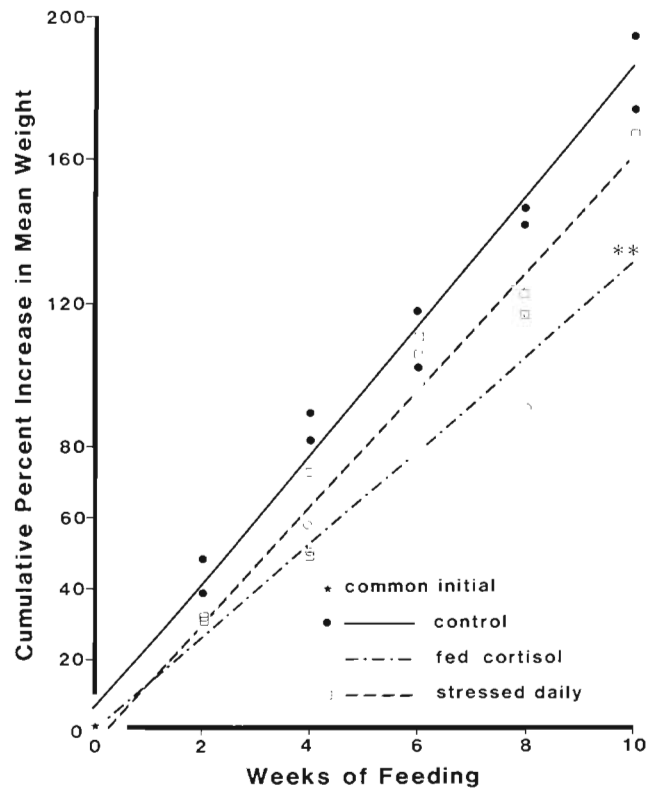


Fig. 1. *Salmo gairdneri*. Cumulative percent increase in mean weight ( $n = 15$  per replicate, 2 replicates per sample week) of juvenile rainbow trout fed either cortisol-treated or control feed, or fed control feed and subjected to an acute stress daily, for 10 wk. Relative growth as  $\% \text{ increase } d^{-1}$  for each treatment is represented by the slope of the line. \*\* Significant difference ( $p < 0.01$ ) in slope from control

Table 1. *Salmo gairdneri*. Specific growth rates at 2 wk intervals and mean initial and final weights ( $g \pm SE$ ,  $n=30$ ) of juvenile rainbow trout fed either cortisol-treated or control feed, or fed control feed and subjected to an acute stress daily, for 10 wk. Data represent pooled values from duplicate tanks for each treatment

Growth characteristic and week	Treatment		
	Control	Fed cortisol	Stressed daily
Specific growth rate			
Week 2	2.52	1.36	1.59
Week 4	2.59	2.02	2.32
Week 6	1.59	1.98	2.63
Week 8	2.20	0.49	0.89
Week 10	2.40	2.31	2.60
Mean, 10wk	2.26	1.65	2.02
Weight (g)			
Week 0	$4.5 \pm 0.2$	$4.3 \pm 0.2$	$5.2 \pm 0.2$
Week 10	$21.4 \pm 0.7$	$13.4 \pm 0.5$	$21.0 \pm 1.0$

Table 2. *Salmo gairdneri*. Mean liver glycogen ( $\text{mg g}^{-1} \pm \text{SE}$ ,  $n=9$  to 12), hepatosomatic index ( $\pm \text{SE}$ ,  $n=12$ ) and condition factor ( $\pm \text{SE}$ ,  $n=12$ ) in juvenile rainbow trout fed either cortisol-treated or control feed, or fed control feed and subjected to an acute stress daily, after 10 wk of treatment. Values represent pooled data from duplicate tanks for each treatment

Treatment	Liver glycogen	Hepatosomatic index	Condition factor
Control	$35 \pm 2.6$	$1.38 \pm 0.07$	$1.29 \pm 0.02$
Fed cortisol	$11 \pm 1.6^*$	$0.88 \pm 0.04^*$	$1.11 \pm 0.01^*$
Stressed daily	$31 \pm 2.8$	$1.19 \pm 0.05^*$	$1.26 \pm 0.02$

\* Significant difference from control ( $p < 0.05$ )

found that chronically crowded brown trout grew less than uncrowded controls fed the same ration; however, plasma cortisol initially rose but had returned to the level of the control group by 39 d. They concluded that long-term growth suppression was not mediated by corticosteroids, but may have been caused by reduced

food intake or decreased efficiency in food utilization relating to social interactions from crowding. For example, Strange et al. (1978) noted that juvenile chinook salmon *Oncorhynchus tshawytscha* stressed by high density confinement refused food, and subsequently grew less.

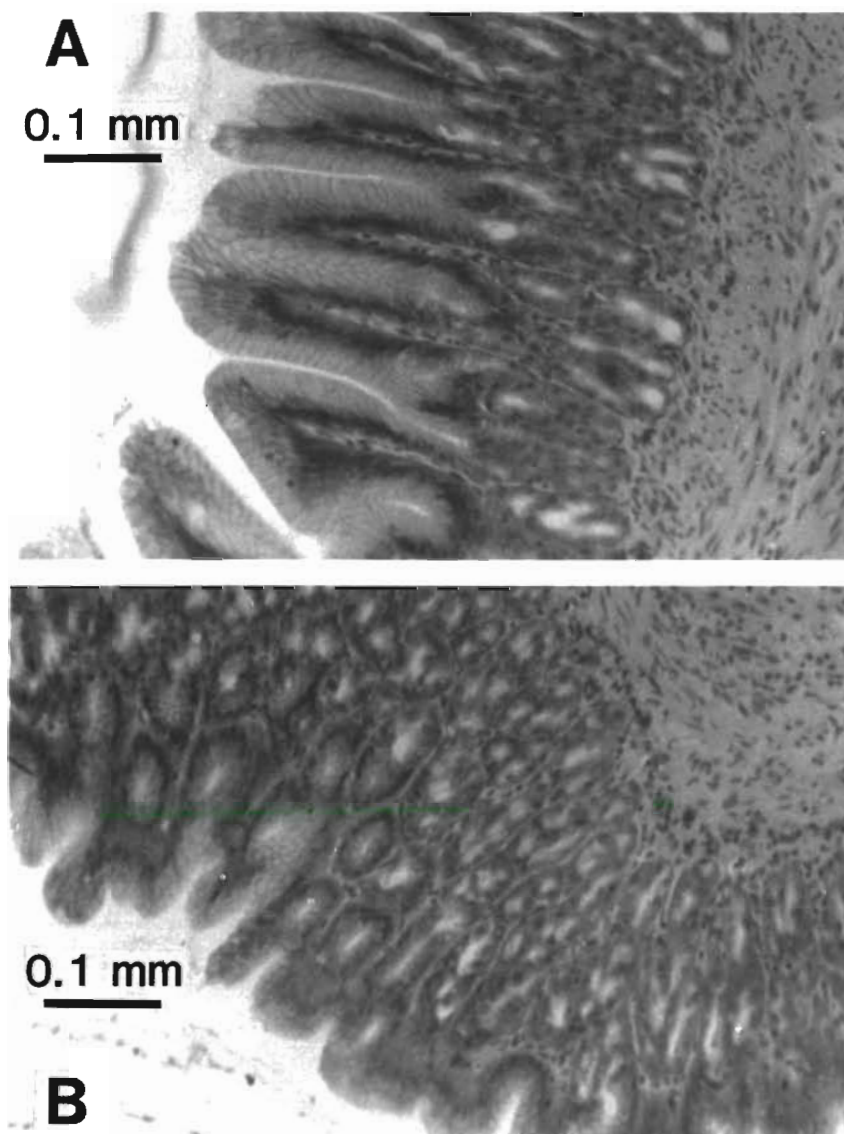


Fig. 2. *Salmo gairdneri*. Cardiac stomach tissue of similar-sized (A) control and (B) cortisol-fed juvenile rainbow trout after 10 wk of treatment, showing apparent reduction of mucosal folds and increases in gastric gland cell numbers and vacuolar spaces. Scale bar = 0.1 mm



As an alternative explanation for the reduced growth in our experimental fish, chronic cortisol administration possibly affected digestion efficiency at the gut wall. We observed a loss of both appetite and aggressive feeding behavior in fish fed cortisol, even though all feed pellets presented to the fish were eaten. There was a noticeably higher accumulation of fecal matter at the bottom of the tanks of the cortisol-fed fish than in those of control fish, suggesting poorer absorption of the food material in the gut. We also noticed qualitative differences in the cardiac stomach tissue of control and cortisol-fed fish (Fig. 2), characterized in the fish fed cortisol by apparent increases in gastric gland cell numbers and vacuolar spaces and reductions in the height of mucosal folds. However, the small size of our sample precluded a conclusion that these changes were representative of the treated population, and we were unable to resolve differences at the cellular level. Our observations of morphological alterations to cardiac stomach tissues in cortisol-fed trout are consistent with those of other investigators in fish with chronically elevated cortisol. In rainbow trout implanted with cortisol pellets, Robertson et al. (1963) reported necrosis of gastric glands and submucosa as well as a reduction in the number and height of mucosal folds. McBride & van Overbeeke (1971) also noticed atrophy of the mucosa and a reduction in epithelial cell height in stomachs of sockeye salmon *Oncorhynchus nerka* injected repeatedly with cortisol for 4 wk. Similar cellular and tissue degeneration was found by Peters (1982) and Willemse et al. (1984) in stomachs of European eels *Anguilla anguilla* under conditions of chronic stress. Peters (1982) further speculated that the resultant alterations in enzyme and acid secretion in the mucosa could interfere with normal protein digestion, and thus lead to reduced growth.

There was a noticeable increase in the post-feeding elevation of plasma cortisol in fish fed with cortisol for 4 and 8 wk, as compared to fish from Week 0 (Fig. 3). Fish stressed daily had lower 1 h post-stress plasma cortisol levels after 4 and 8 wk of treatment than at 0 wk, whereas plasma cortisol in control fish remained relatively unchanged after 1 h as a result of feeding (Fig. 3). However, our sampling regime may have missed a transitory post-feeding increase in plasma cortisol within the first hour such as that demonstrated by Pickering & Pottinger (1983) in brown trout. The trend to higher cortisol concentrations at 1 and 3 h after feeding in cortisol-treated fish was also evident at Week 10 when compared to corresponding plasma levels in fish that had not been previously fed cortisol (Fig. 4). We speculate that chronic cortisol treatment resulted in an increased rate of absorption after feeding by altering physical absorption characteristics across the gut wall, perhaps through a pharmacological action

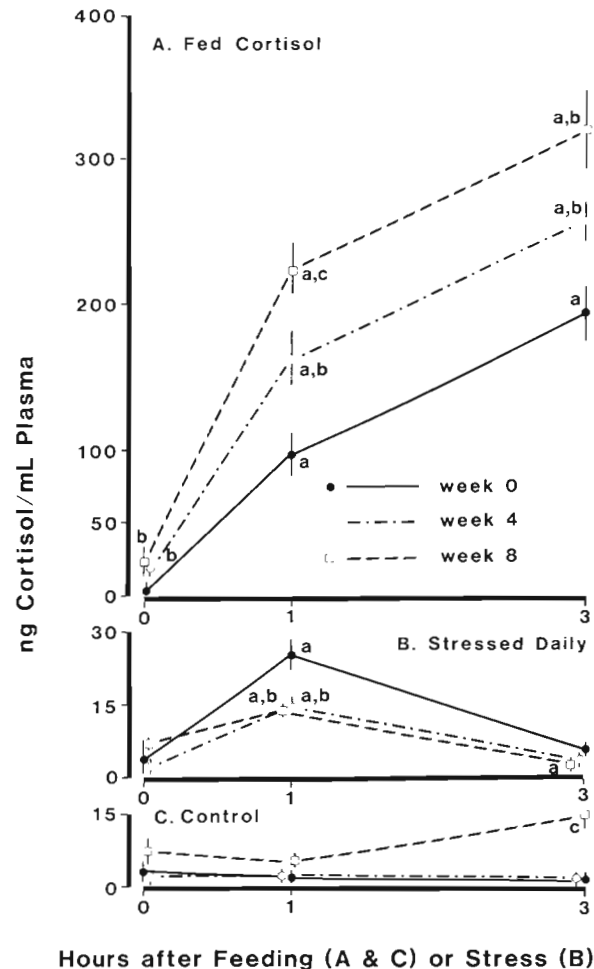


Fig. 3. *Salmo gairdneri*. Mean plasma cortisol levels ( $\text{ng ml}^{-1} \pm \text{SE}$ ,  $n = 10$ ) in juvenile rainbow trout at 0, 4 and 8 wk of treatment. (A) Trout given cortisol-treated feed; levels before (0 h) and 1 and 3 h after being fed. (B) Trout given control (untreated) feed and stressed daily; levels before and after a 30 s handling stress. (C) Trout given control feed; levels before (0 h) and 1 and 3 h after being fed. Values represent pooled data from duplicate tanks for each treatment. Super-scripts indicate significant differences ( $p < 0.05$ ) as follows: <sup>a</sup>different from 0 h (initial) after treatment for that week; <sup>b</sup>different from Week 0 for that hour; <sup>c</sup>different from Weeks 0 and 4 for that hour

such as cellular degradation. Cortisol levels were the same, however, at 6 and 12 h after feeding and were lower in the fish fed cortisol for 10 wk than in the control group after 24 h (Fig. 4), suggesting more rapid clearance in the treated fish. Chronically elevated plasma cortisol, resulting from cortisol administered in implanted capsules, has been shown previously to increase clearance rate of corticosteroids in coho salmon *Oncorhynchus kisutch* (Redding et al. 1984a). Of interest was the straight line formed by declining post-feeding cortisol concentrations in the fish that were fed cortisol for 10 wk (Fig. 4) since clearance rate is non-

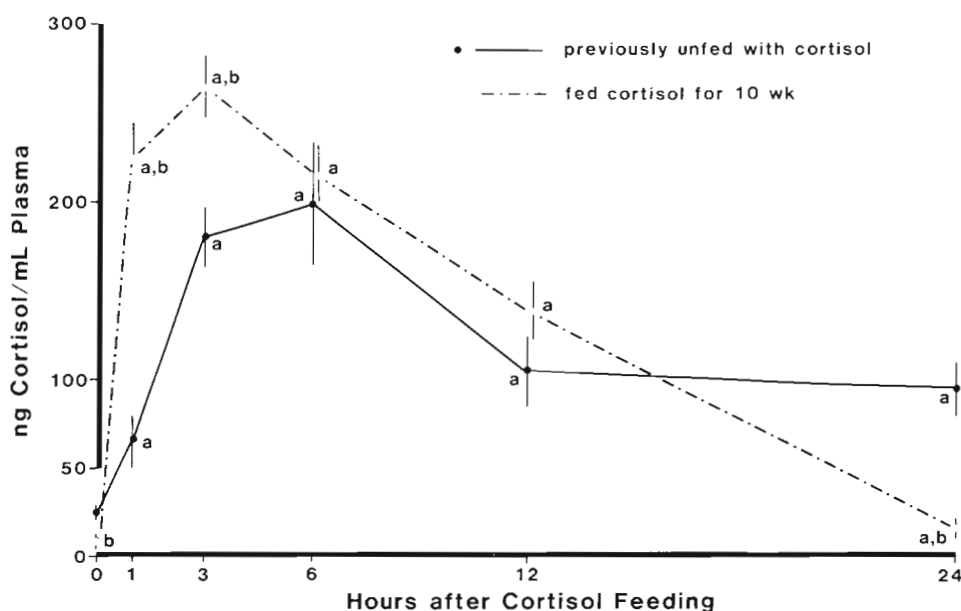


Fig. 4. *Salmo gairdneri*. Mean plasma cortisol ( $\text{ng ml}^{-1} \pm \text{SE}$ ,  $n = 10$ ) after a single meal of cortisol-treated feed in juvenile rainbow trout, previously fed either cortisol-treated or control feed for 10 wk. Values represent pooled data from duplicate tanks for each treatment. Superscripts indicate significant differences ( $p < 0.05$ ) as follows: <sup>a</sup>different from 0 h (initial) within that treatment; <sup>b</sup>different from value for fish previously untreated with cortisol for that hour

linear under normal conditions (e.g. Nichols & Weisbart 1985, Nichols et al. 1985) and decreases with concentration.

Plasma glucose was elevated in fish after 4 and 8 wk of cortisol treatment both before (i.e. 24 h after last feeding) and after feeding (Fig. 5). In the fish stressed daily, glucose levels at Weeks 0, 4, and 8 were similar to each other and showed the same post-stress increase after 1 h (Fig. 5). Post-feeding plasma glucose in control fish remained relatively stable at 4 and 8 wk, but showed an increase in the Week 0 group (Fig. 5). Plasma glucose in fish fed cortisol for 10 wk was characterized by a more rapid increase, followed by a more rapid decline in concentration after a cortisol-treated ration than that found in control fish given the same treatment (Fig. 6). Judging from post-feeding levels at 1 and 3 h (Fig. 5C) and at 6 h (prestress values in Fig. 5B), feeding alone did not appear to alter plasma glucose appreciably.

Increased plasma glucose in fish after 2 wk of feeding with cortisol is consistent with findings in previous investigations (Butler 1968, Patent 1970, Inui & Yokote 1975, Lidman et al. 1979, Leach & Taylor 1982). In many of these studies, high doses of cortisol were applied by injection, thereby introducing the possibility that hepatic glycogenolysis, stimulated by stress-induced catecholamine secretion (Nakano & Tomlinson 1967, Mazeaud et al. 1977), also modified plasma glucose. Davis et al. (1985) observed no increase in plasma glucose after they fed exogenous cortisol to fish. Our

findings of higher resting plasma glucose with chronic cortisol feeding and the apparently cortisol-related transient change in glucose after a single cortisol meal both support the glucocorticoid role of cortisol in teleosts and corroborate the earlier conclusion by Davis et al. (1985) that increased gluconeogenesis resulted in reduced growth. Moreover, our results clearly showed that dietary cortisol appeared in circulation at physiological levels soon after feeding. The concentrations of plasma cortisol we observed at 1 to 6 h after feeding were similar to those found in juvenile salmonids after either a severe acute stress such as handling or chronic stress from confinement (Strange et al. 1977, 1978, Barton et al. 1980, 1985b, 1986, Strange & Schreck 1980, Pickering et al. 1982, 1986, Redding & Schreck 1983).

After 10 wk, liver glycogen and hepatosomatic index were reduced in the fish fed cortisol (Table 2); the hepatosomatic index was also lower in fish stressed daily than in control fish. The large reduction in liver glycogen resulting from the feeding of cortisol is unusual in light of earlier investigations that showed increases in liver glycogen in fish after cortisol treatment (Butler 1968, Hill & Fromm 1968, Swallow & Fleming 1970, Inui & Yokote 1975, Lidman et al. 1979) or in fish with elevated endogenous plasma cortisol (Schmidt & Idler 1962). Other studies have indicated, however, that cortisol treatment can reduce liver glycogen in fish (Storer 1967, Ball & Hawkins 1976), or that endogenous plasma cortisol and liver glycogen are

inversely related (Peters et al. 1980, Paxton et al. 1984). These conflicting reports suggest that there are differences in cortisol-treatment effects on carbohydrate metabolism that are due to the physiological status of the fish, dosages of cortisol used, or interactions of cortisol with other glucoregulatory hormones. The low liver glycogen observed in the fish fed cortisol may also have resulted from a chronically increased metabolic rate. Cortisol injections have been found to increase metabolic rate in fish (Chan & Woo 1978) and there is a significant correlation between standard metabolic rate and blood sugar levels in vertebrates generally (Umminger 1977). An increased metabolic rate could also have contributed to the reduced growth in our cortisol-fed fish by shifting energy away from growth pathways (Brett & Groves 1979).

### Hematological characteristics

After 2 and 6 wk, hematocrit was higher in fish fed with cortisol but was not appreciably altered in the fish stressed daily, compared with that in the respective controls (Table 3). After 2 wk, lymphocyte ratios were 84% lower than control values in cortisol-fed fish and 31% lower in fish stressed daily (Table 3). Combined neutrophil and thrombocyte ratios were not different among treatments (Table 3).

The higher hematocrit in the fish fed cortisol than in untreated fish after 2 wk is interesting in that this phenomenon has not been observed in fish in which plasma cortisol was chronically elevated by using implants (J. M. Redding & C. B. Schreck unpubl.) or that were treated with single or multiple cortisol injections (Johansson-Sjoberg et al. 1978, Leatherland 1985). An increase in hematocrit may be caused by an alteration in intracellular or extracellular fluid or by proliferation in erythrocytes. Pickford et al. (1970) found that repeated injections of cortisol significantly increased the erythrocyte count in the mummichog *Fundulus heteroclitus*, although reasons were not given. We observed no evidence of increased hemopoietic activity in blood smears from cortisol-treated fish, which would be apparent as increased numbers of immature erythrocyte stages. This lack of increase suggests that the increased hematocrit we observed possibly resulted from a cortisol-mediated decrease in extracellular fluid relative to blood cell volume. In adrenalectomized European eels, Chan et al. (1969) found that cortisol treatment increased the glomerular filtration rate and urine production. Such a response from cortisol feeding could account for a reduced blood fluid volume and a resultant higher hematocrit.

A number of authors have also found that physical stress increases hematocrit in fish (Soivio & Oikari

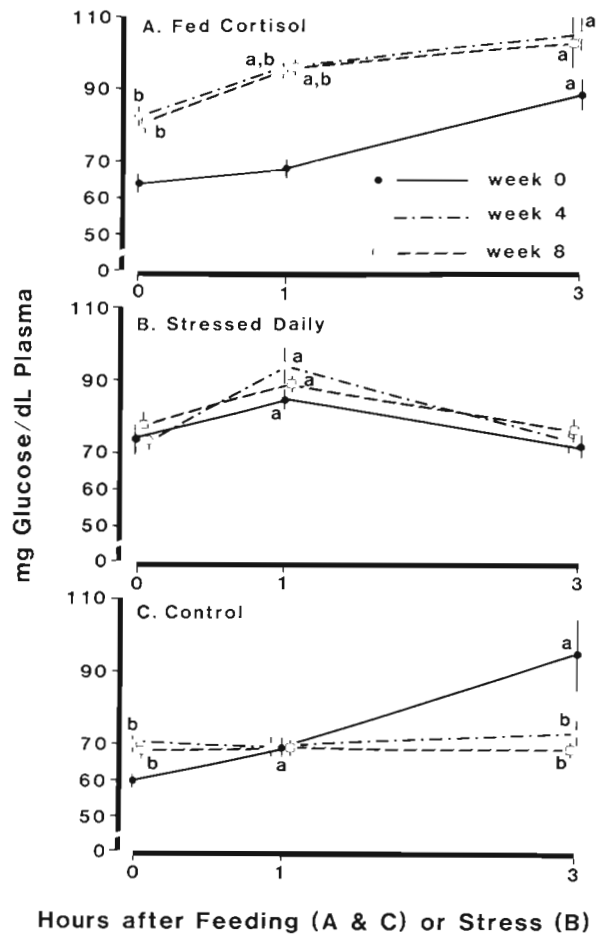


Fig. 5. *Salmo gairdneri*. Mean plasma glucose levels ( $\text{mg dl}^{-1} \pm \text{SE}$ ,  $n = 8$  to 10) in juvenile rainbow trout at 0, 4 and 8 wk of treatment. (A) Trout given cortisol-treated feed; levels before (0 h) and 1 and 3 h after being fed. (B) Trout given control (untreated) feed and stressed daily; levels before and after a 30 s handling stress. (C) Trout given control feed; levels before (0 h) and 1 and 3 h after being fed. Values represent pooled data from duplicate tanks for each treatment. (See Fig. 3 for explanation of significance)

1976, Wells et al. 1984, Barton et al. 1985b), and that this increase may be due to swelling of the erythrocytes, accompanied by a redistribution of body fluids (Milligan & Wood 1982). However, there is no evidence to indicate that this effect is mediated by cortisol. Moreover, this phenomenon appears to be a transient response to acute disturbances, since we saw no difference in hematocrit between the controls and the fish stressed daily. Similarly, Pickering & Pottinger (1985) found that a daily acute stress of exposure to malachite green had no long-term effect on hematocrit in brown trout.

The depression in numbers of circulating lymphocytes (but no change in neutrophils or thrombocytes) was caused by the dietary cortisol, and confirms a

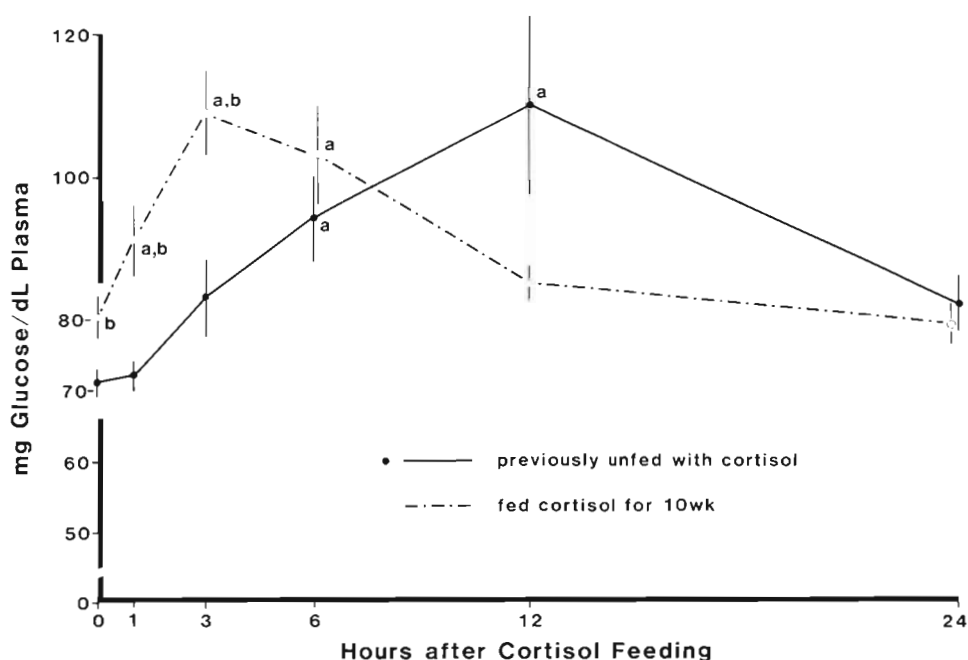


Fig. 6. *Salmo gairdneri*. Mean plasma glucose ( $\text{mg dl}^{-1} \pm \text{SE}$ ,  $n = 10$ ) after a single meal of cortisol-treated feed in juvenile rainbow trout, previously fed either cortisol-treated or control feed for 10 wk. Values represent pooled data from duplicate tanks for each treatment. (See Fig. 4 for explanation of significance)

Table 3. *Salmo gairdneri*. Mean hematocrit (% packed cell volume  $\pm \text{SE}$ ,  $n=18$  to 20) after 2 and 6 wk of treatment, and mean lymphocyte ratio and combined neutrophil and thrombocyte ratio (no. cells per  $10^3$  red blood cells  $\pm \text{SE}$ ,  $n=12$ ) after 2 wk of treatment, in juvenile rainbow trout fed either cortisol-treated or control feed, or fed control feed and subjected to an acute stress daily. Values represent pooled data from duplicate tanks for each treatment

Blood characteristic	Control	Treatment Fed cortisol	Stressed daily
After 2 wk of treatment			
Hematocrit	45 $\pm$ 0.9	53 $\pm$ 0.8*	45 $\pm$ 0.7
Lymphocyte ratio	25 $\pm$ 3.4	4.1 $\pm$ 1.1*	18 $\pm$ 1.5*
Neutrophil and thrombocyte ratio	1.0 $\pm$ 0.3	1.1 $\pm$ 0.2	1.1 $\pm$ 0.3
After 6 wk of treatment			
Hematocrit	43 $\pm$ 0.7	54 $\pm$ 0.8*	42 $\pm$ 1.0

\* Significant difference from control ( $p < 0.05$ )

similar observation by Pickering (1984). Although there was a 22% increase in hematocrit, this increase alone (if due to hemopoiesis) cannot account for the reduction in lymphocyte number to one-fifth of that in control fish. Pickering (1984) found that when brown trout were fed a single meal containing an amount of cortisol equivalent to that we used, lymphocyte numbers were reduced by about 65% but returned to normal in 72 h. Our results suggested that lymphocyte numbers do not compensate, but will probably remain low as long as the cortisol concentration is continually elevated. This observation contradicts that of Pickford et al. (1970) who, after finding an increased white blood cell count in mummichog, concluded that the fish showed an adap-

tive response to chronic cortisol treatment. It has been previously shown that an injection of cortisol into fish also reduces circulating lymphocyte numbers (McLeay 1973, Ball & Hawkins 1976) and that exogenous cortisol treatment increases the susceptibility of fish to various diseases (Robertson et al. 1963, Roth 1972, Pickering & Duston 1983). Grimm (1985) recently found that physiological levels of cortisol suppressed the mitogen-induced proliferation of leucocytes from plaice *Pleuronectes platessa* cultured *in vitro*, suggesting that this may be an important part of the mechanism by which stress reduces disease resistance. Moreover, Maule et al. (1987) found that as coho salmon underwent smoltification, their ability to generate *in vivo* antibody-



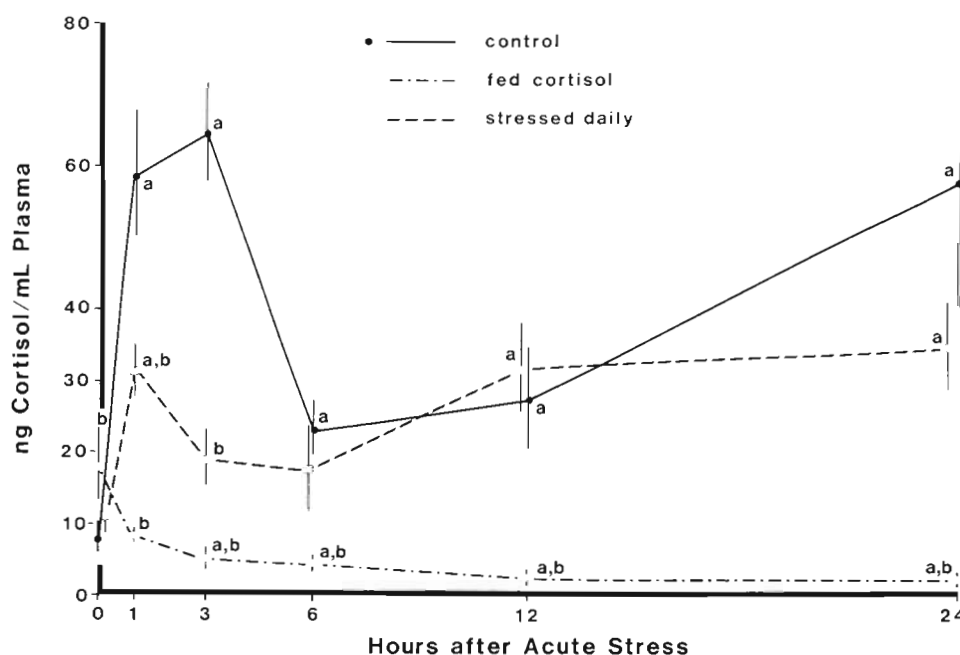


Fig. 7. *Salmo gairdneri*. Mean plasma cortisol ( $\text{ng ml}^{-1} \pm \text{SE}$ ,  $n = 12$ ) after a 30 s handling stress in juvenile rainbow trout previously fed either cortisol-treated or control feed, or fed control feed and subjected to an acute stress daily, for 10 wk. Values represent pooled data from duplicate tanks for each treatment. Superscripts indicate significant differences ( $p < 0.05$ ) as follows: <sup>a</sup>different from 0 h (initial) within that treatment; <sup>b</sup>different from control treatment for that hour

producing cells in the spleen declined and the relative numbers of spleen cells decreased. These changes noted by Maule et al. (1987) were accompanied by increased cortisol concentrations in the fish.

The reduced number of blood lymphocytes in the fish stressed daily after 2 wk of treatment was of particular interest. Various forms of stress have been shown to reduce circulating lymphocyte or leucocyte numbers (Esch & Hazen 1980, Hlavek & Bulkley 1980, Peters et al. 1980, Pickering et al. 1982, Klinger et al. 1983), leucocrit (McLeay & Gordon 1977, Tomasso et al. 1983, Wedemeyer et al. 1983) and possibly lymphocyte function (Ellsaesser & Clem 1986); see review by Fries (1986). Pickering (1984) concluded that this stress-induced response is mediated by cortisol. A positive association between environmental stress and the outbreak of disease in fish is also well established (Wedemeyer 1970, Snieszko 1974, Walters & Plumb 1980, Wedemeyer & Goodyear 1984). Our finding implies that the routinely handled fish may still have been less resistant to disease, even though their growth and other physiological conditions appeared normal.

### Stress responses

When fish fed cortisol for 10 wk were subjected to a 30 s handling stress, plasma cortisol did not increase, as it did in the control group. From 3 h onward, post-stress

cortisol levels were lower than the initial prestress level (Fig. 7) in the cortisol-treated fish. Plasma cortisol 1 and 3 h after handling was noticeably lower in the fish stressed daily than in controls (Fig. 7). Although both control fish and those stressed daily evoked a stress response characterized by a rapid rise in plasma cortisol followed by a decline, concentrations in both groups rose again at 12 h and were still elevated after 24 h (Fig. 7).

The chronic treatment with cortisol eliminated the ability of the fish to elicit an elevation in cortisol after handling. In fact, plasma cortisol declined after handling, suggesting that residual cortisol in circulation was rapidly used peripherally or cleared as a result of the stress. Cortisol secretion from interrenal tissue in fish is regulated by negative feedback on the hypothalamus to inhibit synthesis or release of corticotropin-releasing factor (CRF) that, in turn, suppresses adrenocorticotrophic hormone (ACTH) secretion from the pituitary (Fryer & Peter 1977). Although interrenal cells were not measured, continual negative feedback in the fish treated with cortisol may have caused an atrophy of these steroidogenic cells as a result of the continued absence of stimulation by ACTH. Basu et al. (1965), who found that a dose- and time-dependent atrophy of interrenal tissue was induced by cortisol injections in the cichlid *Tilapia mossambica*, concluded that continued presence of the cortisol probably inhibited ACTH secretion.

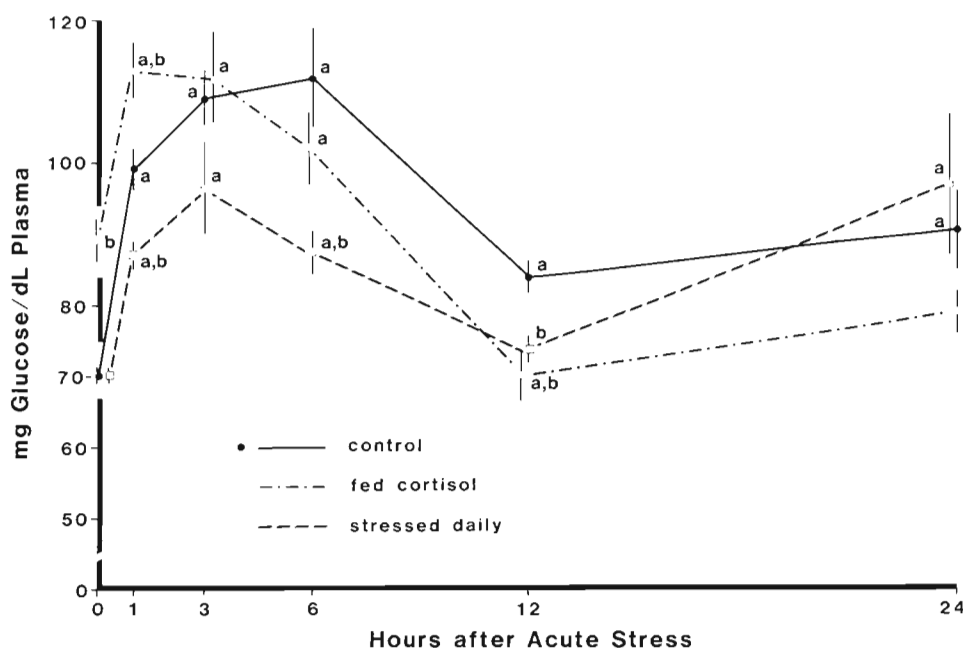


Fig. 8. *Salmo gairdneri*. Mean plasma glucose ( $\text{mg dl}^{-1} \pm \text{SE}$ ,  $n = 11$  or  $12$ ) after a 30 s handling stress in juvenile rainbow trout previously fed either cortisol-treated or control feed, or fed control feed and subjected to an acute stress daily, for 10 wk. Values represent pooled data from duplicate tanks for each treatment. (See Fig. 7 for explanation of significance)

Plasma glucose was elevated in response to handling in all 3 treatment groups (Fig. 8) but reached peak levels sooner in the fish fed cortisol. However, since initial plasma glucose was highest in the cortisol-fed fish, the relative increases in glucose were similar in both the cortisol-fed and control groups (Fig. 8). Plasma glucose 12 h after handling in the group fed cortisol was lower than the initial level and lower than in control fish at that time (Fig. 8). The response patterns of plasma glucose to handling were similar in control fish and those stressed daily, though the magnitude of the elevation was less in the stressed fish (Fig. 8).

Although resting plasma glucose was elevated, cortisol treatment did not appear to affect the hyperglycemic response to handling. Acute stress-induced plasma glucose elevations are mediated by catecholamines (Mazeaud et al. 1977, Mazeaud & Mazeaud 1981) and our finding supports the view that corticosteroids are probably not involved directly in this initial response (Leach & Taylor 1980, Carmichael et al. 1984, Barton et al. 1986).

Except for the secondary increases at 24 h, the responses of cortisol and glucose to short-term handling in control fish were typical of those seen in juvenile salmonids (Strange et al. 1977, Barton et al. 1980, 1985b, 1986, Pickering et al. 1982, Barton & Schreck in press b). However, the reduced plasma cortisol and glucose responses observed in the fish stressed daily clearly indicated the effect of preconditioning. A significant proportion of the normal stress response is

believed to be due to the novelty of the stimulus (Levine 1985). We are unaware of other investigations on the effect of habituation on acute responses of cortisol to handling in fish, but Pickering & Pottinger (1985) showed that daily exposure to malachite green eliminated the cortisol stress response in brown trout after 4 wk. Studies with rats conditioned to exercise indicated that the reduced corticosteroid response in conditioned specimens was not due to adrenal exhaustion, but rather to adaptation of both the hypothalamic-pituitary axis (Frenkl et al. 1968) and the adrenal gland (Tharp & Buuck 1974). Similarly, Rees et al. (1983, 1985) found that the corticosterone response was reduced in ducks after they were habituated to daily exercise stress and exercise plus handling. The investigators concluded that this reduction was caused by reduced endogenous ACTH secretion in response to the stimulus, since the cortisol response to exogenous ACTH was unchanged (Rees et al. 1985).

Using a daily habituation protocol similar to ours, Rush & Umminger (1978) abolished the stress-induced hyperglycemic response in goldfish *Carassius auratus* in 3 wk. Woodward & Smith (1985) found that trained rainbow trout elicited significant hyperglycemia to a disturbance, whereas untrained fish did not; they attributed this difference to a post-stress increase in epinephrine in the trained fish that was not apparent in untrained fish. Their observation appears to contradict that observed by Rush & Umminger (1978) and by us. In Woodward & Smith's (1985) experiment, however,

trout were preconditioned by exercise training and not by similar disturbances, suggesting the possible importance of the novelty factor in elicitation of a stress response in fish.

The maximum post-stress cortisol response was much lower in the 5 g fish at Week 0 (Fig. 3) than it was in 21 g control fish handled for the first time at Week 10 (Fig. 7). An increase over time in the stress response was previously demonstrated in smolting coho salmon (Barton et al. 1985a) and may reflect an increased interrenal responsiveness to stress associated with juvenile development in fish. Similarly, 1 h post-stress plasma glucose was lower in fish from Week 0 (Fig. 5) than in control fish stressed at Week 10 (Fig. 8), suggesting that the change in sensitivity or responsiveness of fish to stress may be a general developmental phenomenon.

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