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The effect of various stresses, corticosteroids and adrenergic agents on phagocytosis in the rainbow trout *Oncorhynchus mykiss*

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Abstract

The effect of acute and chronic stress on the phagocytic activity of putative macrophages from the rainbow trout. *Oncorhynchus mykiss* has been assessed, using an *in vitro* phagocytic index, in which the average number of engulfed yeast cells in a population of phagocytes is determined. An injection stress given under light anaesthesia, or a longer noise stress combined with confinement, both significantly reduced, within 3 h, the level of phagocytic activity of macrophages from the spleen and pronephros. Daily injection stress over six days had a lesser effect on the proportion of phagocytically active cells even though plasma cortisol levels were equally raised. Daily dexamethasone injection depressed the proportion of phagocytically active cells more than saline injection. In these *in vivo* experiments, it was not possible to determine whether stress and steroids depressed the phagocytic activity of individual macrophages or caused the active macrophages to migrate out of the spleen and pronephros. Administration of cortisol (200 nM) to trout macrophages *in vitro* failed to depress phagocytic activity within a 3h period but both α- and β-adrenergic agonists (10 μM) were usually depressive. It is proposed that the autonomic nervous system may be an early regulator of macrophage phagocytosis following stress and that corticosteroids only exert their suppressive effect on macrophage activity in the longer term.

Introduction

It is well established that environmental stress can trigger the outbreak of infectious diseases in fish (Sniezko 1974; Fries 1986; Maule *et al.* 1989). Fish grown in intensive culture systems are frequently subjected to stressors such as handling, confinement, sorting, transportation, and poor water quality including low oxygen or high ammonia concentrations. All these treatments present stressful conditions to the fish, with subsequent physiological changes which often include immunosuppression and an increased susceptibility to infection. (Wedemeyer 1976; Barton *et al.* 1980; Walters and Plumb 1980; Weydemeyer and McLay 1981; Maule *et al.* 1989).

Macrophages play a critical role in the induction and expression of many innate and acquired immune responses. Their primary functions are phagocytosis and destruction of pathogens, the processing and presentation of antigens to the lymphocytes, and mediating immune mechanisms by their secretion of various cytokines such as interleukin-1 and interferons which activate lymphocytes and induce cytolysis of tumor and virally-infected cells. As part of their function in innate

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immunity, macrophages also secrete lysosomal enzymes, complement components and prostaglandins (Dale and Foreman 1989). They therefore play a key role in inflammation, immunity, and pathogenesis.

There is evidence that the macrophages in fish perform similar roles to those described above for mammals. MacArthur and Fletcher (1985) suggested that there is difference between the teleost and mammalian immune systems in that macrophages seem to be of greater importance in fish. Antibody production is slow at the normal environmental temperature of cool-water fish (Avtalion *et al.* 1973; Cottrell 1977; O'Neill 1980) and thus, innate immunity, including the activity of the macrophages, probably provides a particularly important initial defence against potential pathogens in these animals.

The aim of the present study was two-fold: to investigate (1) the extent to which acute and chronic stresses modulate macrophage function; and (2) the influence of the stress-related mediators, corticosteroids and adrenergic agonists, on macrophage activity.

Materials and methods

Fish

Rainbow trout (*Oncorhynchus mykiss*) weighing 220–325 g were obtained from a fish farm (Alderly Trout Ltd, Wotton under Edge, Glos.). They were kept in white 250 l tanks with running water at 11°C, and a photoperiod of 18h light: 6h dark. The fish were fed daily on commercial pellets and left to acclimate to aquarium conditions for at least two weeks before the experiments, unless otherwise stated.

Administration of acute stress

To deliver a mild stress, fish were anaesthetised in phenoxyethanol (6:10000 v/v) and injected intraperitoneally (ip) with 0.5 ml of 0.8% NaCl solution. They were then returned to their home tank and killed 3h later. A more intense stress was delivered by placing the fish in a black plastic dustbin, half filled with 80 l of water, and switching on a small electric aerator motor which vibrated against the side wall of the tank. This noise vibration stress was given for 1h and the fish were killed after a further 2h confinement in the dustbin.

Administration of chronic stress and dexamethasone

Fish were collected from the fish farm and distributed to four tanks in our aquarium. Some fish were killed within 3h of capture at the fish farm (acute stress). Control fish were untreated and undisturbed during a 6 day period. Other fish were given a daily injection, without anaesthesia, of either 1 ml of saline or 1 ml of dexamethasone (0.2 mg/ml) over 6 days, and killed 2h after the last injection. This concentration of dexamethasone is sufficient to suppress endogenous plasma cortisol to control levels. In the absence of an assay for dexamethasone, its plasma concentration could not be measured and the dose given here may give unphysiologically high levels of corticosteroid.

Macrophage phagocytosis assay

At the time of autopsy, fish were caught with one sweep of the net and anaesthetised deeply. The spleen and pronephric tissues were removed and forced individually through a nylon tea strainer into 4 ml of Lebovitz medium (L-15, Sigma Chemical Co., Poole, Dorset) containing 0.1% fetal bovine serum and 4U/ml of sodium heparin (grade II, Sigma). The pronephric cell suspension was diluted a further 4-fold. One or 2 ml of this pronephric or spleen suspension were flooded onto individual, pre-washed microscope slides and the phagocytic cells were allowed to adhere for 90 min at room temperature (approx 22°C). Non-adherent cells were washed off in 0.15M phosphate buffered saline (PBS, pH 7.8) and the attached macrophages were flooded with yeast cells in L-15 medium containing 4% fetal bovine serum. Phagocytosis was

allowed to proceed for 90 min, after which the slides were rinsed in 0.02M PBS. pH 7.8, fixed for 10 min in methanol and stained with May-Grunwald/Geimsa stain (Sigma, UK). The cells were examined under oil immersion.

Two methods of determining phagocytic activity were employed. The average number of yeast cells engulfed per macrophage was determined by inspecting 200 macrophages from each fish (Phagocytic Index). From these counts was also determined the percentage of macrophages which engulfed one or more yeast cells (% Phagocytosis).

Preparation of yeast cell suspension

The yeast cells were prepared by mixing 0.1 g of commercial bakers' yeast (*Saccharomyces cerevisiae*) in 20 ml 0.15M PBS and heating the suspension at 80°C for 15 min. The cells were washed three times in PBS and finally suspended in L-15 medium supplemented with 4% fetal bovine serum to give a concentration of approximately 2.4×10^8 cells/ml.

Effect of cortisol or adrenergic agonists on phagocytic activity

Cells from pronephric or spleen suspensions were distributed onto 1–3 slides/fish. Some slides with their adherent macrophages were incubated for 3h in medium containing either cortisol (80 ng/ml) or an adrenergic agonist (10^{-5}M) before the addition of yeast cells. Control phagocytic cells were incubated in medium only, without further additions. Subsequent presentation of yeast cells and staining was done as described above.

Determination of plasma cortisol

Blood was collected from the severed caudal peduncle of deeply anaesthetised fish, before the removal of the spleen and pronephros. About 2–3 ml of blood was collected into cold polypropylene tubes containing 50 µl 6% EDTA disodium salt (Sigma, UK) as anticoagulant. After centrifugation at 3000 × g for 15 min, aliquots of plasma were stored at −20°C until assay. Cortisol concentration was determined by radioimmunoassay (Rance and Baker 1981); dexamethasone does not interfere with the recognition of cortisol but shows 1% cross-reactivity in the assay. Tritiated cortisol was obtained from Amersham International (Amersham, Bucks). Synthetic cortisol (Sigma, UK) was used as standard.

Statistics

Results are expressed as means ± standard errors. The data were compared using one way analysis of variance (ANOVA) after checking for normal distribution. Logarithmic conversion was used when distribution was not normal.

Results

Effect of time and temperature on phagocytosis

Preliminary experiments, using spleen macrophages, assessed the effect of different temperatures (13 and 23.5°C) on the rate of phagocytosis. The percentage of phagocytes which engulfed yeast cells was initially significantly suppressed at the lower temperature ($p < 0.01$, Fig. 1) but after 90 min the effect of temperature was no longer significant, amounting to only a 6% difference. In all further experiments, therefore, yeast cells were applied to the macrophages at room temperature (approximately 22°C) and left in contact with them for 90 min.

After staining with the May-Grunwald/Giemsa, two types of phagocytic cells were observed. One cell type, believed to be macrophages, had darkly staining cytoplasm and a rounded nucleus. A second type, with chromophobic cytoplasm and a more irregularly-shaped nucleus, may have been neutrophils. In several experiments described below, the phagocytic index of both cell types was determined. The paler, putative neutrophils engulfed fewer yeast cells and did not respond to stress.

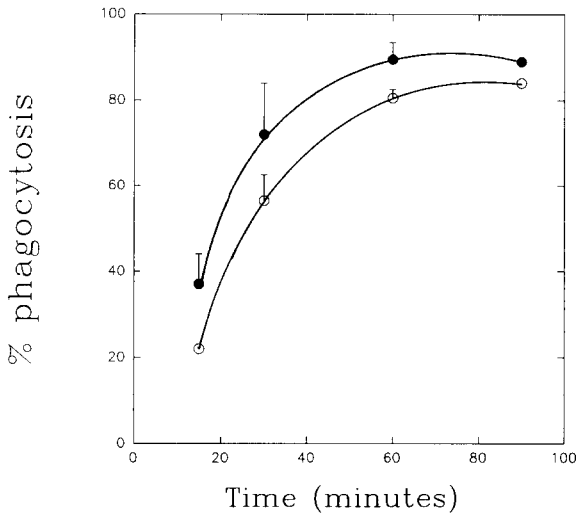

Fig. 1. The percentage of spleen macrophages which phagocytose yeast cells at two different temperatures, 13.5°C (open circles) and 23°C (close circles), n = 3. Analysis by paired Student's t test shows an overall difference between 23.5 and 13°C of 17.7% (p = 0.008).

Thus, throughout this work, only the responses of the darkly stained, putative macrophages are reported.

Effect of acute stress on phagocytosis

A 3h stress period, consisting of 1h noise vibration and a further 2h confinement in the test tank, reduced the percentage of phagocytically active macrophages, whether they were obtained from the pronephros or spleen (Table 1A, 1B); in other words, stress rapidly increased the number of inert macrophages. The average number of yeast cells engulfed by a population of macrophages (phagocytic index) was also significantly depressed (Table 1A, 1B). Ignoring the inert macrophages which engulf nothing, the average number of yeast cells taken up by the active macrophages was similarly depressed by 3h of stress (Fig. 2). In both experiments, stress raised significantly the plasma cortisol levels.

The effect of a very brief stress caused by ip injection of saline to lightly anaesthetised fish was assessed in a third experiment (Table 1C). This brief stress depressed phagocytic activity, within 3h, to an extent equal to that caused by 3h continuous stress, whether the results are expressed as percentage phagocytosis or as phagocytic index of the whole macrophage population. (Table 1C: Fig. 3).

Comparison of acute and chronic stress and dexamethasone injection on phagocytosis

To assess the effect of repeated (chronic) stress on phagocytic activity, fish were injected daily, over 6 days, and without anaesthesia, with either saline or dexamethasone as described in Material and Methods. The percentage of phagocytic cells from the pronephros (but not from the spleen) and the phagocytic index in cells from both tissue sites were

Table 1. Effect of acute stress on macrophage phagocytosis

	Pronephros		Spleen		Cortisol (ng/ml)
	% Phago	Phagocytic index	% Phago	Phagocytic index	
A. Control	88 ± 2.7	3.1 ± 0.11	92 ± 3.5	3.2 ± 0.26	< 1.0
Noise stress	72 ± 5.4*	2.2 ± 0.17**	78 ± 3.3*	2.3 ± 0.18*	34 ± 13**
B. Control	97 ± 1.3	3.3 ± 0.13	95 ± 3.3	3.3 ± 0.2	3 ± 1.3
Noise stress	72 ± 3.9**	1.8 ± 0.13**	82 ± 4.4*	2.0 ± 0.19**	30 ± 13.0**
C. Control	93 ± 0.9	3.6 ± 0.08	97 ± 1.0	4.1 ± 0.22	6 ± 0.9
Injection stress	78 ± 3.2**	2.3 ± 0.11**	82 ± 2.7**	2.3 ± 0.09**	20 ± 3.0**

A and B – replicate experiments in which fish were stressed for 1h by noise vibration and confinement and a further 2h confinement only; C – fish were stressed by a saline injection given under mild anaesthesia, after which they were returned to their home tank and killed 3 h later; * $p < 0.05$ ** $p < 0.01$ compared with controls (ANOVA) (n = 5 or 6).

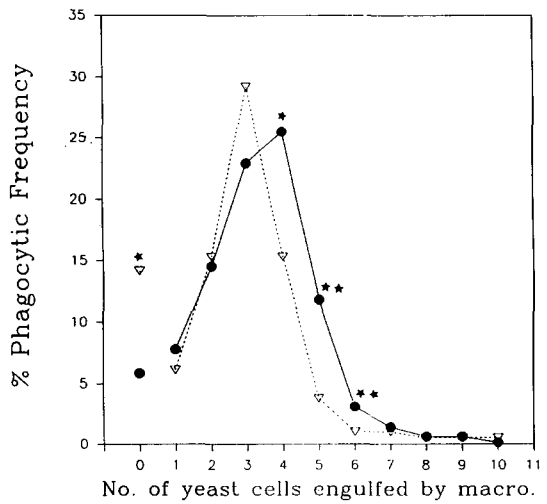

Fig. 2. The percentage frequency distribution of yeast cells phagocytosed by pronephric macrophages from control fish (circle and solid lines) or acutely stressed fish (triangles and dotted line). Two hundred macrophages were assessed from each fish, n = 5; * $p<0.05$; ** $p<0.01$ (Student's t test).

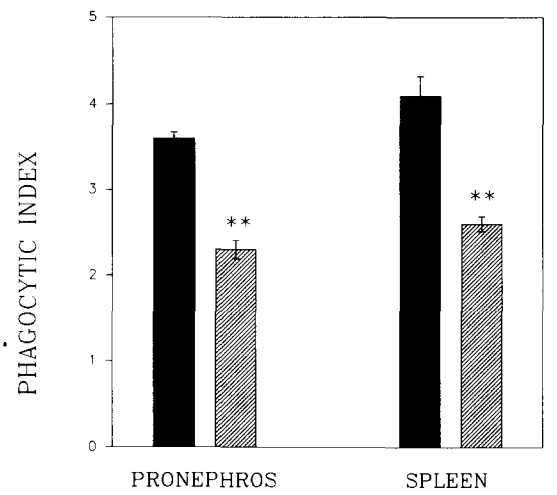

Fig. 3. The influence of a brief acute stress on the phagocytic index of pronephric and spleen macrophages, measured 3h after the stress. Control values shown by solid bars: stressed values shown by hatched bars, (n = 5); ** $p<0.001$ compared to control values.

most suppressed immediately after collection from the fish farm (acute stress) (Table 2). Macrophages from the control fish showed a significant recovery from the stress of transport, which was partially prevented by the stress of a daily saline injection. The corticosteroid analogue, dexamethasone, further suppressed recovery of phagocytic activity, such that macrophages from these fish had a phagocytic index lower than chronically stressed fish. The ranking order of phagocytic index for kidney macrophages was: control > saline injection > dexamethasone injection > acute stress. This ranking order was less evident for splenic macrophages. Acute and chronic stress resulted in similar plasma cortisol values at the time of autopsy (Table 2).

To avoid the potential complicating factor, seen in the previous experiment, of an acute stress before subjecting fish to daily, repeated stress, a second experiment was done using fish which had been reared in our aquarium facilities. As before, some fish were injected daily over a period of 6 days while

Table 2. Effect of acute and chronic stress and steroid on macrophage phagocytosis

	Pronephros		Spleen		Cortisol (ng/ml)
	% Phago	Phagocytic index	% Phago	Phagocytic index	
Control	96 ± 0.4	5.9 ± 0.26^{b}	78 ± 4.1	4.9 ± 0.29^{b}	1.6 ± 0.4
Acute	75 ± 4.4*	2.6 ± 0.16**^{a}	85 ± 2.0	3.0 ± 0.18**^{a}	44.0 ± 3.0**
Chronic	94 ± 2.8	4.8 ± 0.22*^{c}	84 ± 6.6	4.2 ± 0.20^{b}	51.0 ± 6.0**
Dexa	91 ± 1.8*	3.8 ± 0.25**^{d}	79 ± 3.2	3.0 ± 0.32**^{a,c}	1.0 ± 0.1

Fish were collected from the fish farm and some were killed 3h later (acute stress); the remainder were kept in the aquarium for a further 6 days. Of these, five were chronically stressed by a daily injection of 1 ml saline (chronic stress); steroid-treated fish were injected daily for 6 days with 0.2 mg/ml dexamethasone in 1 ml saline (Dexa); control fish were left undisturbed. Fish were killed 2h after the last injection; * $p<0.02$. ** $p<0.001$ compared with controls (n = 5); Values in the same column with different superscript letters are significantly different from each other.

Table 3. Effect of chronic stress on macrophage phagocytosis

	Pronephros		Spleen		Cortisol (ng/ml)
	% Phago	Phagocytic index	% Phago	Phagocytic index	
Control	94 ± 1.2	3.3 ± 0.14	76 ± 6.9	3.7 ± 0.21	1.2 ± 0.07
Stressed	88 ± 2.2	3.0 ± 0.16	64 ± 11.0	2.4 ± 0.19**	54.0 ± 21.0**

Fish reared in the aquarium were stressed by a daily intraperitoneal saline injection for 6 days and killed 2h after the last injection; Controls were undisturbed; * $p < 0.05$; ** $p < 0.01$ compared with control value ($n = 5$).

the controls were undisturbed. The percentage of active macrophages was slightly but not significantly suppressed by the daily injections (Table 3), while the phagocytic index was reduced in the splenic but not the pronephric macrophages. Despite the restrained response of the macrophages to stress, the rise in plasma cortisol was as great as that seen in other experiments.

In vitro effects of cortisol and adrenergic agonists on phagocytosis

In two experiments, macrophages from the same pronephric preparations were incubated for three hours in either medium alone, or medium containing cortisol (80 ng/ml), prior to the addition of yeast cells. Cortisol did not significantly modify the phagocytic index in any case (data not shown).

To test the effect of catecholamines on macrophage phagocytosis, the α or β adrenergic agonists, phenylephrine and isoprenaline were added to the medium (10^{-5}M) for 3h prior to the yeast cells. In two separate experiments, phenylephrine caused a significant depression in phagocytic index (Fig. 4) although the percentage phagocytosis was virtually unaffected by the treatment (results not shown). Isoprenaline also depressed the phagocytic activity of pronephric macrophages in both experiments but spleen macrophages responded to the β agonist in only one case (Fig. 4).

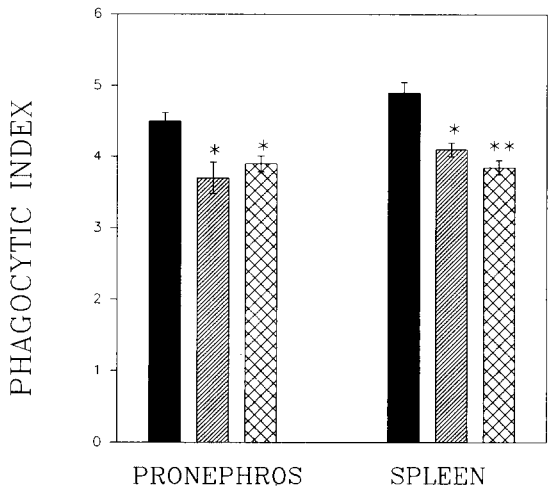

Fig. 4. The effect of 3h *in vitro* treatment with the α- and β-adrenergic agents, phenylephrine and isoprenaline, both at 10^{-5}M, on pronephric and spleen macrophages. Controls – solid bars: isoprenaline – single hatched bars; phenylephrine – crosshatched bars ($n = 5$); * $p < 0.02$; ** $p < 0.002$.

Comparison of pronephric and splenic macrophage responses

Since the pronephros is the major haemopoietic tissue in fish, it seemed possible that its macrophages might be in a different stage of maturation and exhibit a different functional activity from those in the spleen. To investigate this, the phagocytic indices of all experimental fish were expressed as a percentage of their controls (Table 4). This comparison suggests that in most cases, macrophages from both sites respond to stress, dexamethasone or adrenergic agents in the same way and to the same extent.

Table 4. Percent suppression of phagocytic index by different treatments

Treatment	% Suppression of phagocytic index*	
	Pronephros	Spleen
3h noise stress (Table 1A)	69	72
3h noise stress (Table 1B)	55	61
Injection stress (Table 1C)	64	63
3h transport stress (Table 2)	44	61
Chronic stress (Table 2)	81	86
Chronic stress (Table 3)	92	65
Dexamethasone (Table 2)	64	61
Phenylephrine (Figure 4)	87	80

* Values are the phagocytic indices of experimental macrophages expressed as a percentage of their controls.

Discussion

The activity of macrophages can be assessed by a number of methods, based on the different facets of their physiological repetoire. Many methods that have been applied to fish studies have monitored some aspect of the microbicidal capacity of the cells, for instance the production of reactive oxygen species such as O_2^- and H_2O_2 (Chung and Secombes 1987), the associated reduction of cytochrome C (Bayne and Levy 1991a) or enhanced chemiluminescence (Flory and Bayne 1991; Bayne and Levy 1991b, Ellsaesser and Clem 1986; Whiskovsky *et al.* 1987; Angelidis *et al.* 1987) or else the level of the lysozomal enzyme, acid phosphatase (Secombes 1986; Chung and Secombes 1987). The present work has utilized one of the most easily observed events – the phagocytic activity of the cells. Other studies using this criterion have expressed phagocytosis simply as the percentage of cells which show any phagocytic activity. This is satisfactory in cases involving a very marked depression of macrophage activity, as in the study by Weeks and Warinner (1986) which reported a 3-4 fold decrease in the percentage of phagocytically active cells in fish from polluted water, but it may be rather insensitive in other situations. We have therefore expressed macrophage activity in terms of a phagocytic index, defined as the mean number of yeast cells engulfed per macrophage in a population of 200 macrophages. This method of assessment seems to be more sensitive, and can reveal a significant change in phagocytic activity even when the percentage phagocytosis is not significantly altered (*e.g.*, after the addition of adrenergic agonists, and data in Tables 2 and 3).

A depression of the phagocytic index was observed within 3 h of an acute stress even if it was brief, as in the case of a saline injection to anaesthetized fish. Such stresses reduced the average number of phagocytic events to about two-thirds to one half of that seen in the controls. The time course of macrophage responsiveness following stress has not been investigated previously but a similar rapid response to stress has been noted for other types of immune cells. The activity of natural killer cells, for instance, is significantly reduced 2h after footshock (Jain *et al.* 1991). One could, in fact, anticipate a rapid response from the macrophages since it has been shown that adrenergic agents have an immediate effect *in vitro* on other aspects of macrophage activity, such as their luminol-induced chemiluminescence or the reduction of cytochrome C (Nielson 1987; Bayne and Levy 1991a,b; Flory and Bayne 1991). Similarly, contact of lymphocytes with adrenalin for only 15 min can impair their subsequent response to mitogenic agents (Crary *et al.* 1983). When stress was applied repeatedly to fish, the depressive effect on the macrophages was less marked and in some experiments was undetectable (Tables 2 and 3) even though plasma cortisol levels at autopsy were as high as those seen after acute stress. Whether this reflects a loss of macrophage receptors to the suppressive agent(s), reduced second messenger response or reduced release of suppressive agents (other than cortisol), is not known at present. Whatever the explanation, the results in

the present study suggest that the depression of phagocytosis is not closely correlated with plasma cortisol titres.

In fish, as in mammals, there are many examples that raised plasma corticosteroids suppress the immune system. Thus, implants of cortisol increase the susceptibility of trout to infection (Pickering and Duston 1983; Pickering and Pottinger 1985, 1989) and reduce the production of antibody secreting cells (Kaattari and Tripp 1987; Tripp *et al.* 1987; Maule *et al.* 1987, 1989). Physiological concentrations of cortisol are also effective *in vitro* in depressing the production of plaque forming cells during a 7-day incubation of kidney leukocytes (Slater and Schreck 1993). Although cortisol has not consistently been found to depress all aspects of immunological activity – for instance, plaice (*Pleuronectes platessa*) injected with 3 mg/300 g body weight for 3 days showed no change in the rate of *in vivo* antigen clearance or organ uptake of antigen (MacArthur and Fletcher 1985), we have shown here that corticosteroids are effective in depressing macrophage phagocytic activity when administered over several days.

In spite of what has been reported, our failure to depress phagocytic activity by incubating macrophages with cortisol for 3h makes it questionable that the steroid is responsible for the reduction in phagocytosis seen after acute stress. In contrast to our work, which used physiological concentrations (80 ng/ml; 200 nM) of cortisol, most previous studies which have been able to demonstrate an immediate and direct effect of corticosteroids on one or another aspect of macrophage activity have usually used supraphysiological concentrations of steroid, in the range of 1–100 μg/ml (van Zwet *et al.* 1975; Balow and Rosenthal 1973; Masur *et al.* 1982). Although Werb (1978) showed that lower concentrations (1–100 nM dexamethasone) can influence mouse macrophages, the response was dose and time related and affected only certain aspects of macrophage activity. Thus, a depression in plasminogen activator secretion was just detectable 2h after the addition of 100 nM but not 1 nM dexamethasone and the effect became progressively more pronounced over 20h culture. Other macrophage enzymes, such as elastase, were less sensitive to dexamethasone suppression while lysozyme secretion was completely insensitive even to high (1 μM) concentrations of the steroid. Thus, while cortisol may exert a direct action on trout macrophage phagocytosis, its effect is likely to be a gradual, progressive one, taking more than 3h to become evident at the 200 nM dose.

The different response of macrophages observed in the present work may thus be due to the extended period over which dexamethasone was administered or to the possibility that the plasma concentration of dexamethasone was higher than the concentration of cortisol used *in vitro*. Yet another interpretation is that stress or corticosteroids cause a redistribution of leukocytes within the body. Thus, the reduced proportion of active macrophages in the spleen and kidney may reflect, not their inactivation, but their migration away for these sites and into the blood or into other lymphoid tissue or the general stroma. If inert macrophages remained within the pronephros and spleen, the percentage of inactive cells would appear increased.

Other immunosuppressive factors secreted during stress include the catecholamines, adrenaline and nor-adrenaline, released from the chromaffin cells in the pronephros of fish, and from sympathetic nerve endings within the spleen. In mammals, both adrenaline and nor-adrenaline block the activation of macrophages to a tumoricidal and antiviral state (Koff and Dunegan 1985, 1986), while studies using specific adrenergic agonists or antagonists show that activation of the α- and β-adrenoceptors can have opposite effects on neutrophil/macrophage activity. Whereas α-adrenergic agonists are stimulatory, enhancing myelopoiesis (Maestroni *et al.* 1992) and cytokine secretion (Spengler *et al.* 1990), β-adrenoreceptor agonists depress many features of macrophage activity, such as superoxide production (Nielson 1987; Ogunbiji *et al.* 1988) or cytokine secretion (Chelmickaschorr *et al.* 1992). As far as fish are concerned, the α- and β-adrenergic agonists, phenylephrine and isoprenaline, also appear to exert opposite effects on the respiratory burst of trout macrophages (Flory and Bayne 1991; Bayne and Levy 1991a,b). Bayne and co-workers have shown that α-adrenoceptor

stimulation enhances the production of reactive oxygen radicals as assessed by the chemiluminescent response, while β-adrenoceptor stimulation depressed it, as for mammals. The apparently opposite response seen when the respiratory burst was monitored by the reduction in cytochrome C, was explained by an effect of the β-adrenergic agents on the activity of the enzyme superoxide dismutase, suggesting that measurements of reduced cytochrome-C alone may be unsatisfactory for monitoring superoxide production.

Since other workers have shown that α-adrenergic agonist are stimulatory to various aspects of macrophage activity, our finding that phenylephrine depressed the phagocytic index, when used at the same high concentration as by Bayne and coworkers, was unexpected. The response was consistent in two experiments, however, and showed that the α-receptor stimulation was as depressive, if not more so, as the β-adrenergic agonist. These results seem to suggest that adrenergic stimulation does not necessarily alter phagocytosis and superoxide production in the same direction.

The present results show, therefore, that acute stress can significantly depress the proportion of phagocytically active macrophages in the spleen and kidney, and that this could be attributable, at least in part, to a depression of phagocytic activity caused by catecholamines. Corticosteroids similarly depress the proportion of phagocytically active cells in these tissues but, if they have any influence on the level of phagocytosis, it may be apparent only in the longer term. The plasma cortisol concentration is a poor indicator of the extent to which phagocytic activity is depressed in stressed fish.

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