Influence of the Adrenal Glucocorticoids on the Stimulation of Synthesis of Hepatic Ribonucleic Acid and Plasma Acute-Phase Globulins by Leucocytic Endogenous Mediator

By WILLIAM L. THOMPSON, FREDERICK B. ABELES, FRANCIS A. BEALL,
RICHARD E. DINTERMAN and ROBERT W. WANNEMACHER, JR.
U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701,
U.S.A.

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An injection of unpurified leucocytic endogenous mediator into rats results in an increased incorporation of [6-¹⁴C]orotate into hepatic RNA, an increase in the concentration of RNA associated with the bound ribosomal fraction of liver, and increases in the concentrations in serum of acute-phase proteins such as α_2 -macrofoetoprotein and haptoglobin. If given 3 days after adrenalectomy or 7 days after hypophysectomy, leucocyte factor did not induce the increase in RNA synthesis or α_2 -macrofoetoprotein concentrations but did stimulate an increase in serum haptoglobin. When hypophysectomized or adrenalectomized rats received daily subcutaneous injections of 0.5 mg of cortisol, leucocyte factor again induced a significant increase in the synthesis of hepatic RNA and an increase in the concentration of serum α_2 -macrofoetoprotein. These observations suggest that leucocyte factor can regulate acute-phase-protein synthesis at several different sites, one or more of which requires permissive action of the glucocorticoid hormones. Further, leucocyte factor will stimulate an increased rate of incorporation of orotate into hepatic ribosomes when added *in vitro* in the presence of cortisol to a liver-perfusion system. Thus the stimulatory effect of leucocyte factor may be directly on liver but may require the presence of other hormones to stimulate the incorporation of orotate into RNA.

The acute phase of various inflammatory states, including physical injury, surgery, burns, infection and other forms of stress, is associated with a marked increase in a number of plasma proteins that have been termed 'acute-phase globulins' (Weimer & Benjamin, 1965; Owen, 1967). This inflammatory state is also characterized by an increased flux of amino acids to liver and an increased rate of incorporation of labelled precursors into hepatic RNA (Chandler & Neuhaus, 1968; Shihabi *et al.*, 1970; Wannemacher *et al.*, 1971; Thompson & Wannemacher, 1973). A protein mediator (or mediators) released from activated polymorphonuclear leucocytes, termed 'leucocytic endogenous mediator', was shown to stimulate a flux of amino acids from serum to liver, increase the rate of incorporation of labelled nucleotides into hepatic RNA and increase the concentration in plasma of a number of acute-phase globulins (Kampschmidt *et al.*, 1973; Pekarek *et al.*, 1974; Wannemacher *et al.*, 1975b). It was therefore postulated that this leucocyte factor might be the mediator of alterations in hepatic amino acid and protein metabolism during acute inflammation (Wannemacher *et al.*, 1972; Kampschmidt & Upchurch, 1974).

The concentrations of the circulating adrenal glucocorticoids are markedly increased during inflammatory states (Szafarczyk *et al.*, 1974). In turpentine-induced inflammation in rats, a plasma protein, namely α_2 -macrofoetoprotein (formerly termed ' α_2 -acute-phase globulin') showed an increase from undetectable to highly detectable concentrations in 48 h. Adrenalectomy prevents the turpentine-induced increases in α_2 -macrofoetoprotein concentration, and an injection of 0.3 mg of cortisol/100 g body weight returned the induced response back to that observed in the intact rat (Weimer & Benjamin, 1966; Heim & Ellenson, 1967). However, adrenalectomy lessened, but did not prevent, the inflammation-induced increase in concentration of other acute-phase globulins such as plasma haptoglobin, fibrinogen or α_2 -acid glycoprotein (Henriques *et al.*, 1950; Krauss, 1963; Darcy, 1965; Gordon & Koj, 1968). These investigators postulated that adrenal glucocorticoids play a permissive role in the inflammatory stimulation of acute-phase-globulin synthesis. The present experiments were therefore designed to determine the influence of adrenal glucocorticoids on stimulation of synthesis of hepatic RNA and plasma acute-phase globulins by leucocytic endogenous mediator.

Materials and Methods

Materials

[6-¹⁴C]Orotate hydrate (57.7mCi/mmol) was obtained from New England Nuclear Corp., Boston, MA, U.S.A.) Cortisol sodium succinate was from the Upjohn Co., Kalamazoo, MI, U.S.A. Ox zinc-insulin and pig glucagon were obtained from Eli Lilly and Co., Indianapolis, IN, U.S.A. Bovine growth hormone and amino acids were from Calbiochem, San Diego, CA, U.S.A. Shellfish glycogen was obtained from Schwartz Mann, Orangeburg, NY, U.S.A. Sodium chloride for injection was from Lincoln Laboratory, Decatur, IL, U.S.A.).

Animals

Male rats, weighing 150–200g, which were either intact or hypophysectomized, were obtained from Charles River Laboratories, Wilmington, MA, U.S.A. The rats were maintained on a basal diet that contained 18% casein (Allison *et al.*, 1964) and on a 12h light (06.00–18.00h)/12h dark schedule in a room at a temperature of 25–26°C with 40% relative humidity. Bilateral adrenalectomy was performed in intact rats under anaesthesia by using the dorsal approach. Two incisions were made and the wounds were each closed with two sutures. Sham-operated rats were subjected to the same surgical procedure with the exception that the adrenals were left intact. All adrenalectomized rats were maintained on 1% NaCl in their drinking water and were injected subcutaneously with various doses of cortisol diluted in 0.9% 'injection' NaCl, or in control studies with 0.9% NaCl alone. In conducting the research described in the present paper we adhered to the Guide for the Care and Use of Laboratory Animals, as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association of Accreditation of Laboratory Animal Care.

Preparation of rabbit leucocytic endogenous mediator

Leucocytic endogenous mediator was prepared from peritoneal polymorphonuclear leucocytes of rabbits infused with shellfish glycogen as described previously (Pekarek *et al.*, 1974; Wannemacher *et al.*, 1975b). All preparations of the leucocyte factor met the criteria previously described (Wannemacher *et al.*, 1975b). The preparations for control studies were inactivated by heating at 90°C for 30min. This control will detect pyrogen contamination in the preparations and is similar to an injection of 0.9% NaCl or extracts from unstimulated leucocytes (Pekarek *et al.*, 1974).

Incorporation of orotate into various hepatic cellular RNA fractions

Rats were injected intraperitoneally with [6-¹⁴C]-orotate (5μCi/100g body wt.) and were killed 4h after injection. The livers were separated into nuclear, free and bound ribosomal, and soluble RNA fractions by the procedure of Venkatesan & Steele (1972). This method separates the free and bound polyribosomal ribosomes by initial centrifugation at 135000g for 12min into two fractions, one of which contains the bulk of the free polyribosomes (supernatant) and the other the rough membranes plus larger cellular organelles (pellet). By this technique it was possible to recover 75–80% of the cellular RNA in the form of ribosomes, with very little cross-contamination in the free and bound fractions. The radioactive content and concentration of RNA of total liver homogenate and cellular fractions, as well as total DNA concentration, were determined by previously described procedures (Wannemacher *et al.*, 1965). RNA activities were expressed on a per cell basis by using whole liver DNA values as a basis for activity in various subfractions.

Incorporation of labelled orotate into perfused liver

Isolated liver perfusions were performed by a modification of the procedure of John & Miller (1969) as previously described by Zenser *et al.* (1974). An amino acid mixture was prepared by pulverizing the following weights (in mg) of L-amino acids: alanine (884), arginine (107), asparagine (256), aspartic acid (218), cysteine hydrochloride (156), glutamine (1689), glutamic acid (644), glycine (433), histidine hydrochloride (264), isoleucine (424), leucine (604), lysine hydrochloride (1012), methionine (147), ornithine hydrochloride (256), phenylalanine (244), proline (424), serine (631), threonine (689), tryptophan (327), tyrosine (231) and valine (378). This mixture reflects the free amino acid composition found in rat serum (Wannemacher *et al.*, 1971). The perfusate consisted of 30ml of packed sheep erythrocytes, Krebs–Ringer bicarbonate adjusted to pH7.4 (Krebs & Henseleit, 1932), 60ml; bovine albumin, 3g; glucose, 150mg; heparin, 10000 units; penicillin, 3000 units; streptomycin, 3.0mg; insulin, 5.1 units; bovine growth hormone, 0.5mg; cortisol, 5.0mg; glucagon, 10.0μg; amino acid mixture, 45mg; leucocyte factor, 3ml; and enough Krebs–Ringer bicarbonate to bring the the volume to 100ml. In addition, at the beginning of the perfusion a solution (which contained: Krebs–Ringer bicarbonate, 7.5ml; leucocyte factor, 7.5ml; bovine albumin, 450mg; glucose, 420mg; penicillin, 2500 units; streptomycin, 2.5mg; insulin, 5.6 units; bovine growth hormone, 820μg; cortisol, 4.16mg; glucagon, 12.4μg; and amino acid mixture, 260mg) was continuously added to replace substrates at a rate of 1.5ml/h. At the end of a 60min equilibration period, 1μCi of [6-¹⁴C]orotate was added to the

perfusate. Then, 3h after the addition of [6-¹⁴C]-orotate, the liver was removed and nuclear, free and bound ribosomal, and soluble fractions were isolated, by using the procedure described above.

Serum protein determinations

The relative concentration of α_2 -macrofoetoprotein in each sample with respect to a standard reference serum was determined by a single radial immunodiffusion procedure as described by Eddington *et al.* (1971). One unit of α_2 -macrofoetoprotein is equal to the diameter of the circle produced by 10 μ l of serum from a rat 24h after a subcutaneous injection of 1 ml of turpentine. Serum haptoglobin was determined by a colorimetric procedure which was based on the peroxidase activity of haptoglobin-methaemoglobin complexes (Owen *et al.*, 1960).

Statistics

Group means were compared by Student's *t* test and difference between two means was considered significant at $P < 0.01$ under the null hypothesis.

Results

After a single injection of unpurified leucocytic endogenous mediator from 1×10^8 polymorphonuclear leucocytes, the incorporation of [6-¹⁴C]-

orotate into total liver RNA was significantly increased, and the change was very similar to those reported previously (Wannemacher *et al.*, 1975b). As illustrated in Table 1, almost all of the induced increase in the incorporation of orotate into RNA was observed in the RNA of the bound ribosomal fraction when compared with the heat-inactivated control. By contrast, no significant differences were observed in the incorporation of orotate into the RNA of the free ribosomal, nuclear or soluble fractions. Since little change was noted in the other cellular fractions or in the HClO₄-soluble filtrates, it was assumed that the increased incorporation of orotate into the RNA by the bound ribosomes of the livers of the rats treated with the leucocyte factor was not due to alteration in RNA precursor pool size. Further, the cellular concentration of RNA in the bound ribosomal fraction of the liver from the rat treated with the leucocyte factor was significantly increased compared with that found in the controls. Little change was noted in RNA from the nuclear or soluble fractions, whereas the free RNA from the ribosomes was significantly decreased in the liver of the rat receiving the leucocyte factor (Table 1).

Interactions of leucocyte endogenous mediator and cortisol

In the normal and sham-adrenalectomized rats, and injection of the leucocyte factor significantly increased the incorporation of orotate into total hepatic RNA and an injection of 0.5mg of cortisol per day had no effect on this response (Table 2). By contrast, after an injection of leucocyte factor, adrenalectomy or hypophysectomy prevented the expected increased incorporation of orotate into hepatic RNA. The daily injection of 0.5mg of cortisol to the adrenalectomized or hypophysectomized rat restored the ability of this factor to stimulate incorporation of labelled precursor into total liver RNA (Table 2). In normal rats, serum α_2 -macrofoetoprotein was increased, from almost undetectable concentrations to 40 units/ml, 24h after an injection of the leucocyte factor, and cortisol had no effect on this response (Table 2). At 3 days after surgery, sham-adrenalectomized rats had significantly greater concentrations of serum α_2 -macrofoetoprotein compared with the intact rats. However, leucocytic endogenous mediator stimulated further increases in this acute-phase protein in the absence or presence of cortisol. After an injection of the leucocyte factor, adrenalectomy or hypophysectomy prevented the expected increase in serum α_2 -macrofoetoprotein, but the daily injection of 0.5mg of cortisol allowed for the injury-induced increase in the control rats to occur, and the factor stimulated additional increase in the amount of this acute-phase protein (Table 2).

A definite dose response was obtained for the cortisol stimulation of the increase in serum α_2 -macro-

Table 1. Effects of leucocyte factor on cellular incorporation of orotate and content of RNA in nuclear, free and bound ribosomal and soluble fractions of liver

At 4h after an intraperitoneal injection of either 1 ml of leucocyte factor or heat-inactivated leucocyte factor, six rats in each group were injected intraperitoneally with 5 μ Ci of [6-¹⁴C]orotate/100g body wt. The rats were killed 4h later and the livers were separated into various fractions by the procedure of Venkatesan & Steele (1972). The RNA from these fractions and total liver DNA were extracted and analysed as described previously (Wannemacher *et al.*, 1965). Results are means $\pm$ S.E.M. of six rats.

Fraction	Incorporation (d.p.m./ μ g of DNA)	Concentration (mg of RNA/mg of DNA)
Ribosomal bound		
Control	40 $\pm$ 4	1.03 $\pm$ 0.04
Leucocyte factor	70 $\pm$ 6*	1.44 $\pm$ 0.10*
Ribosomal free		
Control	40 $\pm$ 3	0.79 $\pm$ 0.03
Leucocyte factor	36 $\pm$ 2	0.56 $\pm$ 0.02*
Nuclear		
Control	71 $\pm$ 21	0.26 $\pm$ 0.04
Leucocyte factor	82 $\pm$ 10	0.31 $\pm$ 0.05
Soluble		
Control	44 $\pm$ 4	0.71 $\pm$ 0.05
Leucocyte factor	42 $\pm$ 2	0.63 $\pm$ 0.03

* $P < 0.01$ when compared with heat-inactivated control.

Table 2. *Interaction of leucocyte factor and cortisol on incorporation of orotate into RNA of liver and serum α_2 -macrofoetoprotein and haptoglobin from normal, hypophysectomized, sham-adrenalectomized, or adrenalectomized rats*

Normal, hypophysectomized, sham-adrenalectomized, or adrenalectomized male rats were injected daily (subcutaneously) with either 0.5 mg of cortisol or saline. After 7 days for normal and hypophysectomized rats and 3 days for adrenalectomized and sham-adrenalectomized groups, 12 rats from each were injected intraperitoneally with 1 ml of leucocyte factor and 12 with heat-inactivated leucocyte factor. Then 4 h later six rats in each group were injected intraperitoneally with 5 μ Ci of [6- 14 C]orotate/100 g body wt. The rats were killed 4 h later and total hepatic RNA and DNA was extracted and analysed for concentration and radioactive content as described previously (Wannemacher *et al.*, 1965). The remaining six rats in each group were killed 24 h after the leucocyte-factor injection and blood was obtained for serum α_2 -macrofoetoprotein determination by the procedure of Eddington *et al.* (1971) and serum haptoglobin by the technique of Owen *et al.* (1960). Results are means $\pm$ S.E.M. for six rats.

Treatment	Incorporation (d.p.m./mg of DNA)	Concentration of	
		Serum α_2 -macrofoetoprotein (units/ml)	Serum haptoglobin (mg/100ml)
Normal rats			
Control	332 $\pm$ 18	0.4 $\pm$ 0.2	82 $\pm$ 4
Leucocyte factor	449 $\pm$ 20*	37.7 $\pm$ 2.1*	155 $\pm$ 14*
Cortisol	361 $\pm$ 19	0.6 $\pm$ 0.5	84 $\pm$ 7
Cortisol + leucocyte factor	484 $\pm$ 23*	41.1 $\pm$ 2.8*	159 $\pm$ 7*
Hypophysectomized rats			
Control	280 $\pm$ 30	1.4 $\pm$ 0.2	113 $\pm$ 12
Leucocyte factor	240 $\pm$ 27	1.6 $\pm$ 0.3	153 $\pm$ 6*
Cortisol	270 $\pm$ 20	2.0 $\pm$ 1.8	121 $\pm$ 9
Cortisol + leucocyte factor	440 $\pm$ 18*	62.3 $\pm$ 3.4*	162 $\pm$ 15*
Sham-adrenalectomized rats			
Control	274 $\pm$ 20	25.8 $\pm$ 5.9	122 $\pm$ 6
Leucocyte factor	411 $\pm$ 25*	59.8 $\pm$ 3.3*	204 $\pm$ 18*
Cortisol	287 $\pm$ 10	25.5 $\pm$ 4.7	128 $\pm$ 9
Cortisol + leucocyte factor	415 $\pm$ 20*	64.6 $\pm$ 4.9*	215 $\pm$ 20*
Adrenalectomized rats			
Control	282 $\pm$ 26	1.3 $\pm$ 0.3	120 $\pm$ 9
Leucocyte factor	246 $\pm$ 13	1.4 $\pm$ 0.3	199 $\pm$ 15*
Cortisol	230 $\pm$ 14	27.4 $\pm$ 2.5	125 $\pm$ 10
Cortisol + leucocyte factor	381 $\pm$ 26*	60.5 $\pm$ 3.5*	209 $\pm$ 19*

* $P < 0.01$ when compared with heat-inactivated control.

foetoprotein and incorporation of precursors into hepatic RNA of adrenalectomized or hypophysectomized rats which were injected with leucocytic endogenous mediator (Fig. 1). This stimulation was linear when plotted against the log of the dose of cortisol and was maximal at 1 mg/day in adrenalectomized and 0.5 mg/day in hypophysectomized rats. Preliminary results indicated that, at the administered daily doses of cortisol that allowed for the stimulation in the adrenalectomized rat, the serum concentration of glucocorticoids was less than 0.5 μ g/100 ml, which was the limit of detection for assay of glucocorticoids.

A single injection of leucocytic endogenous mediator in the absence or presence of cortisol significantly increased the amount of serum haptoglobin in normal, hypophysectomized, sham-adrenalectomized or adrenalectomized rats compared with that of the controls (Table 2). This stimulation of serum haptoglobin may have been slightly impaired in the hypo-

physectomized or adrenalectomized rats, but cortisol did not alter this response. The control concentration of serum haptoglobin was slightly higher in the hypophysectomized, sham-adrenalectomized, or adrenalectomized rats compared with intact controls, and cortisol had no significant effect on this difference.

The daily injection of 0.25–5 mg of cortisol in normal rats had no significant effect on the subsequent concentrations of serum α_2 -macrofoetoprotein or serum haptoglobin. In addition, none of these administered doses of cortisol had any stimulatory or inhibitory effects on the ability of leucocyte factor to increase the serum concentration of these two acute-phase proteins (Table 3).

Interactions of leucocytic endogenous inhibitor and actinomycin D or cycloheximide

In rats treated with 1 mg of actinomycin D/kg body wt., leucocytic endogenous mediator did not

Fig. 1. Effect of various concentrations of cortisol on leucocyte-factor-induced increase in serum α_2 -macrofoetoprotein and the incorporation of orotate into liver RNA of adrenalectomized or hypophysectomized rats

Adrenalectomized (a and c) or hypophysectomized (b and d) rats were injected subcutaneously with various doses of cortisol for 3 and 7 days respectively, after which 12 rats from each group were injected with 1 ml of leucocyte factor and 12 with heat-inactivated leucocyte factor. Then 4 h later, six rats in each group were injected with $5 \mu\text{Ci}$ of $[6\text{-}^{14}\text{C}]$ orotate/100 g body wt. The rats were killed at the end of an additional 4 h; total hepatic RNA and DNA was extracted and analysed for concentration and radioactivity as described previously (Wannemacher *et al.*, 1965). The remaining six rats in each group were killed 24 h after the leucocyte-factor injection and blood was obtained for serum α_2 -macrofoetoprotein determination by the procedure of Eddington *et al.* (1971). The daily dose of cortisol is plotted on a log scale against the leucocyte-factor-stimulated increase in either serum α_2 -macrofoetoprotein or incorporation of label into hepatic RNA. Results are means $\pm$ S.E.M. for six rats. The line is drawn from slope of the regression analyses; values for r , the correlation coefficient, were (a) 0.990, (b) 0.999, (c) 0.995, and (d) 0.998.

stimulate an increase in serum α_2 -macrofoetoprotein concentration (Table 4). In the rats treated with actinomycin D, however, the leucocyte factor did stimulate a significant increase in serum haptoglobin concentration but the magnitude of the response was much less than that observed in the controls that were injected with 0.9% NaCl. At 24 h after an injection of the leucocyte factor in the rats without actinomycin D, the concentration of total serum protein was significantly higher than that in the controls. Actinomycin D significantly decreased total serum proteins at 24 h, and the leucocyte factor was not stimulatory in these rats.

An injection of 2 mg of cycloheximide/kg body wt. completely inhibited the expected increase in serum α_2 -macrofoetoprotein or haptoglobin, after an injection

of leucocytic endogenous mediator. This concentration of cycloheximide caused more than 90% inhibition of hepatic protein synthesis.

Studies in perfused rat liver

In the presence of cortisol, leucocytic endogenous mediator stimulated a significant increase in the incorporation of $[6\text{-}^{14}\text{C}]$ orotate into the RNA of the bound ribosomes of perfused liver (Table 5). When compared with the daily saline control, the leucocyte factor stimulated increased incorporation in each liver that was studied. If cortisol was removed from the perfusate, this factor did not stimulate an increased incorporation of this precursor into RNA of the bound ribosomes. The leucocyte factor in the absence or presence of cortisol had no effect on incor-

Table 3. *Effect of daily injections of cortisol in normal rats on leucocyte-factor-induced increases in serum α_2 -macrofoetoprotein and haptoglobin*

Normal male rats (150–200g) were injected daily (subcutaneously) with various concentrations of cortisol. After 7 days, the rats were injected with 1 ml of either leucocyte factor or heat-inactivated leucocyte factor. Then 24 h later all rats were killed and blood was obtained for determination of serum α_2 -macrofoetoprotein by the procedure of Eddington *et al.* (1971) and haptoglobin by the method of Owen *et al.* (1960). Results are the means $\pm$ S.E.M. for five rats in each group.

Dose of cortisol (mg/day)	Treatment	Concentration of	
		Serum α_2 -macrofoetoprotein (units/ml)	Serum haptoglobin (mg/100ml)
0	Control	0.4 $\pm$ 0.4	74 $\pm$ 7
	Leucocyte factor	39.0 $\pm$ 3.1*	153 $\pm$ 13*
0.25	Control	0.9 $\pm$ 0.5	81 $\pm$ 11
	Leucocyte factor	38.2 $\pm$ 2.9*	156 $\pm$ 14*
0.5	Control	0.6 $\pm$ 0.3	82 $\pm$ 12
	Leucocyte factor	38.3 $\pm$ 3.1*	168 $\pm$ 15*
1.0	Control	0.9 $\pm$ 0.4	84 $\pm$ 7
	Leucocyte factor	44.4 $\pm$ 4.2*	175 $\pm$ 6*
5.0	Control	6.2 $\pm$ 4.1	94 $\pm$ 15
	Leucocyte factor	42.3 $\pm$ 4.8*	156 $\pm$ 13*

* $P < 0.01$ when treatment is compared with heat-inactivated control.

Table 4. *Effect of actinomycin D on leucocyte-factor-induced increases in serum α_2 -macrofoetoprotein, haptoglobin and total protein*

Normal male rats (150–200g) were injected intraperitoneally with either 0.9% NaCl (1 ml/kg body wt.) as a control or actinomycin D (1 mg/kg body wt.) in an equal volume. Then 1 h later, the rats were injected with 1 ml of either leucocyte factor or heat-inactivated leucocyte factor; 24 h later all rats were killed and blood was obtained for determination of serum α_2 -macrofoetoprotein by the procedure of Eddington *et al.* (1971), haptoglobin by the method of Owen *et al.* (1960) and total protein by refractometric techniques (Barry *et al.*, 1960). Results are means $\pm$ S.E.M. for six rats.

Treatment	Concentration of:		
	Serum α_2 -macrofoetoprotein (units/ml)	Serum haptoglobin (mg/100ml)	Total serum protein (mg/100ml)
0.9% NaCl			
Control	7 $\pm$ 1	74 $\pm$ 2	5.9 $\pm$ 0.1
Leucocyte factor	44 $\pm$ 1*	101 $\pm$ 3*	6.4 $\pm$ 0.1*
Actinomycin D			
Control	0	55 $\pm$ 2	4.5 $\pm$ 0.2
Leucocyte factor	0	68 $\pm$ 2*	4.1 $\pm$ 0.1

* $P < 0.01$ when treatment is compared with heat-inactivated control.

poration of orotate into the free ribosomal, nuclear, or soluble RNA fractions. During the 3 h perfusion, 85% of the pulse dose of labelled orotate was taken up by the liver in both the presence and absence of the factor. Thus, it would appear that the increased incorporation of orotate into the RNA of bound ribosomes of rats treated with the leucocyte factor was not the result of alteration in precursor pool size.

Discussion

A single injection of leucocytic endogenous mediator resulted in a significant increase *in vivo* in both

the concentration and synthesis of RNA in the bound ribosomal fraction of rat liver. This increase in RNA synthesis was associated with a significant increase in the concentration of serum acute-phase proteins (serum α_2 -macrofoetoprotein and haptoglobin). In adrenalectomized or hypophysectomized rats, the daily administration of cortisol was necessary for the increase in the synthesis of hepatic RNA and α_2 -macrofoetoprotein in rats treated with leucocyte factor. Similar requirements for the glucocorticoid hormones in adrenalectomized rats have been reported for injury-induced increases in α_2 -macrofoeto-

Table 5. *Interaction of leucocyte factor and cortisol on the rate of incorporation of labelled orotate into ribosomal RNA of perfused liver*

Isolated liver perfusions were performed by using a modification of the procedure of John & Miller (1969) as previously described by Zenser *et al.* (1974). Control experiments were run each day exactly as described in the Materials and Methods section except that 0.9% NaCl was substituted for leucocyte factor and cortisol was present or absent in both perfusate and infusions. At the end of a 60-min period of equilibration, 1 μ Ci of [6- 14 C]orotate was added to the perfusate. At 3 h after the addition of orotate, the liver was removed and the nuclear, free and bound ribosomal and soluble RNA fractions were isolated by the procedure of Venkatesan & Steele (1972). The RNA from these fractions and total liver DNA were extracted and analysed for concentration and radioactivity content as described previously (Wannemacher *et al.*, 1965). Results are means $\pm$ S.E.M. for six rats.

Fraction	Incorporation (d.p.m./ μ g of DNA)			
	Cortisol		Minus cortisol	
	Control	Leucocyte factor	Control	Leucocyte factor
Ribosomal				
Bound	17.6 18.6 21.8 18.8 15.3 17.0	27.4 28.4 23.9 29.4 27.3 29.9	18.5 17.5 19.9 14.6 18.4 19.2	18.2 11.6 18.9 18.4 19.5 21.5
(averages)	18.2 $\pm$ 0.9	27.7 $\pm$ 0.9*	18.0 $\pm$ 0.7	18.0 $\pm$ 1.4
Free	12.8 $\pm$ 1.4	13.4 $\pm$ 2.0	18.0 $\pm$ 2.1	18.3 $\pm$ 1.6
Nuclear	58.2 $\pm$ 8.4	59.2 $\pm$ 6.9	83.0 $\pm$ 4.0	76.4 $\pm$ 6.8
Soluble	5.1 $\pm$ 0.5	4.9 $\pm$ 0.6	7.5 $\pm$ 1.6	6.5 $\pm$ 0.8

* $P < 0.01$ when compared with mean of the control or by paired analyses.

protein (Weimer & Benjamin, 1966; Heim & Ellensen, 1967).

In the adrenalectomized or hypophysectomized rat, a linear glucocorticoid dose response was observed for the leucocytic endogenous mediator-stimulated increase in both the synthesis of hepatic RNA and the concentration of serum α_2 -macrofoetoprotein. The presence of cortisol was required also in the perfusate for the leucocyte factor to stimulate an increased incorporation of labelled orotate *in vitro* into the RNA of the bound ribosomes of the perfused liver. These observations constitute a classic example of the permissive effect of the glucocorticoids.

In sham-adrenalectomized rats, serum haptoglobin and α_2 -macrofoetoprotein were increased after surgery. Injection of the leucocyte factor resulted in a further increase in the concentration of these two acute-phase proteins. No significant difference was noted in the rate of incorporation of orotate into hepatic RNA of the sham-adrenalectomized rats; however, they were studied 3 days after surgery. It was reported that increased synthesis of hepatic RNA is maximum 8 h after injury and returns to base-line values by 24 h (Chandler & Neuhaus, 1968).

Serum haptoglobin is another acute-phase protein that increases in amount after an injection of leucocytic endogenous mediator. However, by contrast with α_2 -macrofoetoprotein, this acute-phase protein

increased in amount after an injection of this factor in adrenalectomized or hypophysectomized rats, and daily doses of cortisol did not modify this response. When rats were treated with cycloheximide, leucocytic endogenous mediator did not induce increases in either serum haptoglobin or α_2 -macrofoetoprotein concentrations, but when rats were treated with actinomycin D at a concentration that blocks synthesis of both mRNA and rRNA (Wilson & Hoagland, 1967), the leucocyte factor increased the concentration of haptoglobin but did not increase the concentration of α_2 -macrofoetoprotein. Thus it appears that the increases in serum concentration of α_2 -macrofoetoprotein requires the transcription of an mRNA species that is specific for this protein, whereas the increases in serum haptoglobin concentration may, in part, represent an increase in utilization of pre-existing messengers for this protein.

The injection of glucocorticoids into intact rats markedly decreased the injury-induced increases in serum haptoglobin and α_2 -macrofoetoprotein concentrations (Krauss, 1963; Weimer & Benjamin, 1965). In the present study, daily injections of pharmacological doses of cortisol had no effect on the stimulation of increases in serum haptoglobin and α_2 -macrofoetoprotein by leucocytic endogenous mediator. It has been postulated that the injury-induced increase in concentration of acute-phase

plasma proteins is the result of the release of humoral factors into circulating blood, which then act on liver (Darcy, 1964; Weimer & Benjamin, 1965; Neuhaus *et al.*, 1966). Thus high doses of glucocorticoids could inhibit the release of this humoral factor. By contrast, leucocytic endogenous mediator, which may be the humoral factor, acts directly on liver, and is not effected by high doses of the hormone. It is also possible that the anti-inflammatory effects of glucocorticoids could act as a feedback control mechanism to suppress the release of the leucocyte factor.

Leucocytic endogenous mediator has been suggested as the humoral factor that mediates the biological alterations produced during inflammation or infections (Wannemacher *et al.*, 1972; Kampschmidt *et al.*, 1973). It was possible to demonstrate the presence of a similar mediator in the serum from infected humans and rats (Wannemacher *et al.*, 1975a; Powanda *et al.*, 1975) and turpentine-injured rats (F. B. Abeles, personal communication). When livers were isolated from normal rats and perfused with blood from acutely injured rats, several investigators observed increases in synthesis of acute-phase plasma globulins but others have not been able to confirm these conclusions (Gordon & Darcy, 1967; Sarcione *et al.*, 1965; Schumer *et al.*, 1967). However, John & Miller (1969) reported that livers from normal rats synthesized significant amounts of α_2 -macrofoetoprotein, and the addition of glucocorticoids significantly increased the rate of synthesis at 10 and 12 h of perfusion. The normal liver *in vivo* produces barely detectable amounts of this acute-phase protein. Therefore during the perfusion the hepatocytes must have been stimulated by a factor, perhaps from other circulating granulocytes or Kupffer cells, which induced the synthesis of α_2 -macrofoetoprotein. Although there is presumptive evidence to suggest that a factor released from granulocytes and other phagocytic cells may stimulate the liver to synthesize increased amounts of acute-phase globulins, the proof of this postulate can only be demonstrated when this mediator is purified.

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