Regulation of immune-aggregate-stimulated hepatic glycogenolysis and vasoconstriction by vicinal dithiols

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Evidence suggesting that vicinal dithiols regulate immune-aggregate-induced vasoconstriction and glycogenolysis in the perfused rat liver was obtained. Phenylarsine oxide (PhAsO) and other trivalent organic arsenicals inhibited in a dose-dependent manner hepatic glycogenolysis, vasoconstriction, Ca^{2+} mobilization and the stimulated O_2 consumption caused by immune-aggregate infusion. Polar trivalent and quinquivalent arsenicals were less effective than hydrophobic arsenicals. Prior infusion of Fc- but not Fab-fragments of IgG prevented partially immune-aggregate-stimulated hepatic metabolism, suggesting that immune aggregates elicit hepatic metabolic responses through Fc γ receptors. The inhibitory action of PhAsO on immune-aggregate-stimulated hepatic glycogenolysis was unique; inhibition of glycogenolysis was not observed when phenylephrine, isoprenaline or glucagon was used as a stimulant. Although PhAsO might be expected to sequester cellular thiols, no significant change in the oxidation–reduction state of the major cellular thiol, glutathione, was found during PhAsO infusion. In addition, PhAsO exerted its effects without producing changes in hepatic adenine nucleotides and cyclic AMP. Evidence suggesting the involvement of vicinal dithiols was obtained through thiol-competition experiments using mono- and dithiols. PhAsO inhibition of IgG-aggregate-stimulated hepatic vasoconstriction and glycogenolysis was reversed significantly by infusion of 2,3-dimercaptopropan-1-ol at 3-fold molar excess, whereas 2-mercaptoethanol at 40-fold molar excess was ineffective. The results of the present study provide evidence documenting the participation of vicinal dithiols during the coupling of hepatic immune-aggregate clearance by Kupffer cells with vasoconstriction of the hepatic vasculature (e.g. endothelial cells) and glycogenolysis (e.g. parenchymal cells).

INTRODUCTION

Immune complexes are cleared from the vasculature [1,2] predominantly by hepatic Kupffer cells of the reticuloendothelial system [3]. Several metabolic responses of the liver during exposure to immune complexes have been characterized recently [4]. Stimulated rates of glucose production and O_2 consumption were observed on infusion of heat-aggregated human IgG into perfused livers from fed rats. Since Kupffer cells do not contain sufficient glycogen stores to account for the glucose produced in this situation [5], and incubation of isolated parenchymal cells with heat-aggregated IgG results in neither stimulated glucose production nor phosphorylase *a* activation [6], it was assumed that a signal or mediator, directed at the metabolic responses of the parenchymal cells, originated in the Kupffer cells, which elicited the observed immune-aggregate-induced hepatic glycolytic response. Evidence suggesting that AGEPC [4] or prostaglandin E_2 [6] mediates the hepatic response to the immune aggregate has been presented.

Immune aggregates elicit physiological response through interactions with cell-surface receptors specific for the Fc domain of the immunoglobulin. Fc γ receptors isolated from peritoneal macrophages have been demonstrated to function as ligand-activated ion channels [7], and human B-lymphocyte receptors specific for murine IgG_{2b} possess an intrinsic ligand-activated phospholipase

A_2 activity [8]. Mast cells contain Fc receptors specific for IgE, which activate ion channels in a ligand-dependent manner [9]. Despite this growing body of evidence indicating bifunctional properties of immunoglobulin Fc receptors, few details of the biochemical mechanisms regulating receptor function are known. Nevertheless, the importance of cell-surface as well as intracellular thiols in the phagocytosis of immune complexes is documented [10,11]. However, the role of thiols in the stimulation of hepatic metabolism by immune complexes is less clear.

Trivalent arsenicals have been shown to inhibit processes dependent on intracellular vesicular transport in a variety of cell types. These processes include extracellular protein uptake by amphibian oocytes [12], agonist-induced endocytosis of cell-surface insulin, epidermal growth factor and β -adrenergic receptors [13–15], and insulin-stimulated hexose transport in cultured cells [16]. Quinquivalent arsenicals were inactive, and depletion of cellular ATP was not documented in these systems. PhAsO, the most potent of the trivalent arsenicals, elicits these effects by complexing vicinal dithiols in a manner initially described for the lipoic acid-dependent 2-oxo acid dehydrogenase [17]. The purpose of the present study was to characterize further IgG-aggregate-induced vasoconstriction and glycogenolysis in rat liver, and to determine whether vicinal thiols regulate these processes.

EXPERIMENTAL

Liver perfusions

Livers from male Sprague-Dawley rats (Harlan, Houston, TX, U.S.A.), 160–220 g body wt., fed with a standard laboratory diet *ad libitum*, were perfused *in situ* in a non-recirculating, constant-flow, haemoglobin-free perfusion system as described previously [18]. The perfusion medium, Krebs–Henseleit bicarbonate buffer, pH 7.4, saturated with O_2/CO_2 (19:1), was maintained at 37 °C. Perfusate $[CaCl_2]$ was 1.25 mM unless otherwise indicated. A Clark-type oxygen electrode, placed immediately after the liver in the perfusion circuit, was used to monitor continuously hepatic O_2 consumption. Portal-vein pressure, an index of intrahepatic pressure [19], was measured with a Statham P23ID pressure transducer, in combination with a Grass 7P1A/70AC polygraph, connected to the portal cannula line. Basal portal pressure in this series of experiments was 4.6 ± 0.7 mmHg. Effluent perfusate was collected for 30 s intervals for metabolite assays. Livers were perfused for 30 min before sample collection, to allow the removal of hormones and the stabilization of glucose output.

Ca^{2+} -mobilization studies

During Ca^{2+} -mobilization experiments (Fig. 4) the perfusate Ca^{2+} concentration was maintained at 0.5 mM. Ca^{2+} -efflux studies were performed by infusing livers with tracer doses of $^{45}CaCl_2$ for 45 min before the beginning of the wash-out period. Before the wash-out period, samples of effluent perfusate were used to evaluate the specific radioactivity of the perfusate Ca^{2+} . $^{45}Ca^{2+}$ was determined by liquid-scintillation counting of samples of the effluent perfusate. Calculations were performed essentially as described by Lapointe *et al.* [20]. Total calcium in the effluent perfusate was determined by atomic-absorption spectroscopy.

Metabolite assays

Glucose was measured enzymically [21]. Tissue metabolite concentrations during various metabolic conditions were obtained by freeze-clamping livers between aluminium paddles cooled in liquid N_2 before use. Tissue was powdered with a mortar and pestle cooled previously in a solid- CO_2 /acetone bath, and cooled in a bed of powdered solid CO_2 during grinding. Glutathione in extracts of powdered liver was measured enzymically [22]. Adenine nucleotides in liver extracts were measured by h.p.l.c. [23]. Cyclic AMP was measured with a commercial protein-binding assay kit (Diagnostic Products) after extraction of the liver powder [24].

Materials

Heat-aggregated human IgG was prepared as described previously [25,26] and was infused as a solution in Hepes-buffered saline containing 0.25% (w/v) bovine serum albumin. IgG fragments were prepared by digestion of IgG with papain, followed by purification on DEAE-Sephacel essentially as described by Spiegelberg & Weigle [27] and dissolved in albumin-containing Hepes-buffered saline. Fragments were analysed by SDS/polyacrylamide-gel electrophoresis to determine purity (> 95%). PhAsO, arsanilic acid and 2,3-dimercaptopropan-1-ol were purchased from Aldrich. 2-Mercaptoethanol was obtained from Bio-Rad. L-Phenylphrine hydrochloride and L-isoprenaline (isoproterenol)

bitartrate were purchased from Sigma. Glucagon was acquired from Eli Lilly. $^{45}CaCl_2$ was purchased from ICN. *p*-Aminophenylarsene oxide was generously given by Dr. Kenneth Stevenson, University of Calgary, Canada, and Dr. Leslie Werbel, Warner–Lambert, Ann Arbor, MI, U.S.A., and was converted into the water-soluble dichloroarsine before use.

Expression of results

Results presented in tabular form are expressed as means $\pm$ S.E.M. Statistical significance was determined by Student's *t* test. Perfusion experiments illustrated in the Figures are representative of at least three experiments with essentially identical results.

RESULTS

Effects of phenylarsine oxide on immune-aggregate-stimulated hepatic metabolism

Heat-aggregated IgG increases glucose production, O_2 consumption and hepatic portal pressure when infused into perfused livers from fed rats [4]. As illustrated in Fig. 1, PhAsO (10 μ M) prevents immune-aggregate-stimulated glucose production and the accompanying portal-pressure changes. The transient increase in O_2 consumption which normally occurs during immune-aggregate infusion was similarly prevented by prior infusion of PhAsO (results not shown). Infusion of PhAsO (10 μ M) alone resulted in a slight stimulation of O_2 consumption and glucose production, although basal portal pressure was not altered. Such small changes in O_2 consumption and glucose production were due to the small quantity of ethanol used to solubilize PhAsO (results not shown).

The relationship between PhAsO concentration and total glucose production stimulated by immune-aggregate infusion is shown in Fig. 2. Significant inhibition of immune-aggregate-stimulated glycogenolysis was observed with 0.2 μ M-PhAsO. A more polar trivalent arsenical, *p*-aminophenylarsene oxide, was a weaker inhibitor of immune-aggregate-stimulated glycogenolysis. Quinquevalent arsanilic acid did not affect

Fig. 1. Effect of PhAsO on immune-aggregate-stimulated portal pressure and glucose production in the perfused rat liver

Immune aggregate (11 μ g/ml) was infused into livers from fed rats in the absence (a, ●) or the presence (b, ○) of PhAsO (10 μ M).

Fig. 2. Dose-dependent effect of organic arsenicals on immune-aggregate-stimulated glucose production in the perfused rat liver

Points in the Figure represent the cumulative glucose release stimulated by immune aggregate through approx. 5 min from the start of infusion. Immune aggregate ($1 \mu\text{g} \cdot \text{ml}^{-1}$) was infused, as shown in Fig. 1, in the presence of ethanol (control) (●), PhAsO (○), *p*-aminophenylarsene oxide (■) or arsanilic acid (▲). Each point represents the mean of at least two determinations; bars represent S.E.M. for at least three determinations.

Table 1. Effect of immunoglobulin fragments on immune-aggregate-stimulated vasoconstriction and glycogenolysis in perfused rat liver

Livers were perfused with heat-aggregated IgG ($1 \mu\text{g}/\text{ml}$) for 2 min, essentially as shown in Fig. 1. Where indicated in the Table, immunoglobulin fragments were infused for a 12 min time period beginning 5 min before the start of immune-aggregate infusion. Results are means $\pm$ S.E.M. for three livers analysed as described in the Experimental section.

	Maximal increase in:	
	Glucose production ($\mu\text{mol}/\text{h}$ per g)	Portal pressure (mmHg)
Control	73 ± 8	1.8 ± 0.2
Fab ($20 \mu\text{g}/\text{ml}$)	67 ± 1	1.9 ± 0.1
Fc ($24 \mu\text{g}/\text{ml}$)	$30 \pm 5^*$	$0.4 \pm 0.3^*$

* $P < 0.01$ versus control.

immune-aggregate-stimulated glycogenolysis. The relationship between immune-aggregate-stimulated portal-pressure changes and PhAsO concentration was similar to that shown for total glucose production. Arsanilic acid did not affect significantly immune-aggregate-stimulated vasoconstriction (results not shown).

We examined next the effect of Fc and Fab fragments on metabolic responses to immune aggregate in order to determine whether these responses required hepatic receptors recognizing specific domains of the immunoglobulin. As shown in Table 1, only Fc fragments

Fig. 3. Effect of PhAsO on (a) phenylephrine-, (b) isoprenaline- and (c) glucagon-stimulated glucose production in the perfused rat liver

Livers were perfused with the agonists in the absence (●) or the presence (○) of PhAsO ($10 \mu\text{M}$).

attenuated to any significant extent vasoconstriction and glycogenolysis observed after immune-aggregate infusion. This observation suggests that hepatic Fc γ receptors mediate, at least in part, the metabolic response of the liver to immune aggregate.

The ability of trivalent arsenicals to inhibit immune-aggregate-stimulated glycogenolysis was not due to inhibition either of glycogenolytic enzymes or of various hepatic hormonal mechanisms. PhAsO infusion ($10 \mu\text{M}$) exerted little inhibitory effect toward glycogenolysis stimulated by sub-maximal doses of phenylephrine ($5 \mu\text{M}$), isoprenaline ($5 \mu\text{M}$) or glucagon (0.5 nM) (Fig. 3), although some delay in the initial onset of glucose production was evident. Interestingly, hepatic vasoconstriction upon infusion of phenylephrine was decreased significantly by PhAsO (results not shown).

The possibility that PhAsO mediates its effects through interactions with thiol groups on the infused immune aggregate, rather than with hepatic cellular sites, was considered. When PhAsO infusion preceded immune-

Table 2. Effect of PhAsO (10 μ M) and heat-aggregated IgG (1 μ g/ml) on GSSG and GSH concentrations in perfused rat liver

Values are means ($\pm$ S.E.M.) for four livers, freeze-clamped and assayed as described in the Experimental section.

Treatment	GSSG (nmol/g)	GSH (μ mol/g)
Ethanol (5 min)	38 (15)	4.95 (0.49)
PhAsO (5 min)	46 (18)	4.48 (0.56)
Ethanol (5 min), plus IgG aggregate (2 min)	42 (8)	3.76 (0.41)
PhAsO (5 min), plus IgG aggregate (2 min)	46 (10)	3.97 (0.34)

aggregate infusion by 5 min, a significant inhibition of immune-aggregate-stimulated glycogenolysis resulted (results not shown). Increasing the time period between PhAsO and immune-aggregate infusion resulted in little significant recovery of glycogenolysis (results not shown). In contrast with this finding, the inhibitory effect of PhAsO on hepatic [$1\text{-}^{14}\text{C}$]pyruvate decarboxylation (catalysed nominally by the lipoate-dependent pyruvate dehydrogenase complex) recovers (50%) within 5 min of arsenical removal (results not shown). To address this point more directly, immune aggregate was treated with or without PhAsO (0.1 mM) for 2 h at room temperature, followed by gel filtration through Sephadex G-25. The void-volume fraction, containing immune aggregate, was infused (1 μ g/ml) into perfused rat livers. Glycogenolysis stimulated by PhAsO-treated immune aggregate was 120% of the amount stimulated by untreated immune aggregate. Together, these observations suggest that PhAsO does not exert its effects by complexing thiols present in immune aggregates.

Hepatic thiol, adenine nucleotide and Ca^{2+} status

Because trivalent arsenicals complex thiols, the possibility that PhAsO mediated its effects through perturbation of hepatic glutathione contents was investigated. Measurement of tissue glutathione concentrations indicated little change during infusion of PhAsO (Table 2). In addition, hepatic nucleotide concentrations do not

change appreciably during infusion of PhAsO or immune aggregate; cyclic AMP concentrations were similarly unaffected (Table 3).

Lapointe *et al.* [20] demonstrated that both latex-coated polystyrene beads and AGEPC, when infused separately into livers previously equilibrated with $^{45}\text{CaCl}_2$ ($[^{40}\text{Ca}^{2+}] = 0.5$ or 0.05 mM), produced a significant efflux of $^{45}\text{Ca}^{2+}$ from the liver, without concomitant changes in perfusate $[^{40}\text{Ca}^{2+}]$. This finding contrasts with the known effects elicited by phenylephrine [28], e.g. a transient increase in perfusate Ca^{2+} , in addition to the mobilization of a pool of radiolabelled Ca^{2+} . Heat-aggregated IgG caused an efflux of $^{45}\text{Ca}^{2+}$ from the liver (Fig. 4) without any measurable increase in perfusate Ca^{2+} [6], and this $^{45}\text{Ca}^{2+}$ efflux was inhibited by over 80% by PhAsO (10 μ M). In five separate experiments identical with that illustrated in Fig. 4, PhAsO (10 μ M) did not diminish the phenylephrine-induced increase in perfusate $[^{40}\text{Ca}^{2+}]$, although some diminution (less than 20%) of $^{45}\text{Ca}^{2+}$ efflux was noted. This latter effect correlated with a modest inhibition by PhAsO (10 μ M) of phenylephrine (10 μ M)-induced glucose production. Computational analysis [20] of the steady-state release of $^{45}\text{Ca}^{2+}$ from livers perfused with PhAsO (10 μ M) or an ethanol control showed little significant difference in the fractional transfer coefficients for Ca^{2+} efflux during PhAsO infusion (results not shown). This finding suggests that PhAsO itself does not provoke gross alterations in hepatic Ca^{2+} homeostasis.

A role for hepatic vicinal dithiols in immune-aggregate-stimulated glycogenolysis

Although trivalent arsenicals can form complexes with both monothiol and dithiol [17], the cyclic dithioarsenical derivatives have greater association constants than monothioarsenicals, probably owing to stabilization by cyclization. To distinguish between a cellular monothiol (other than glutathione) or dithiol as the site of PhAsO action, a monothiol, 2-mercaptoethanol, and a dithiol, 2,3-dimercaptopropan-1-ol, were examined for their ability to compete with cellular thiols for PhAsO (Fig. 5). A 40-fold molar excess of 2-mercaptoethanol did not prevent PhAsO from exerting its effects. However, infusion of a small 3-fold molar excess of 2,3-dimercaptopropan-1-ol resulted in substantial recovery of immune-aggregate-stimulated hepatic vaso-

Table 3. Effect of PhAsO (10 μ M) and heat-aggregated IgG (1 μ g/ml) on adenine nucleotide and cyclic AMP contents in the perfused rat liver

Values are expressed as μ mol/g wet wt. and nmol/g wet wt. for adenine nucleotides and cyclic AMP respectively. Results are means ($\pm$ S.E.M.) for three to five livers, freeze-clamped and assayed as described in the Experimental section.

Treatment	ATP	ADP	AMP	Cyclic AMP
Ethanol (5 min)	2.72 (0.11)	0.47 (0.07)	0.32 (0.13)	0.19 (0.04)
PhAsO (5 min)	2.49 (0.19)	0.45 (0.07)	0.28 (0.09)	0.19 (0.02)
Ethanol (5 min), plus IgG aggregate (2 min)	2.37 (0.74)	0.44 (0.03)	0.38 (0.13)	0.20 (0.06)
PhAsO (5 min), plus IgG aggregate (2 min)	2.39 (0.46)	0.43 (0.05)	0.32 (0.12)	0.15 (0.01)

Fig. 4. Effect of PhAsO on immune-aggregate- and phenylephrine-stimulated glucose production and $^{45}\text{Ca}^{2+}$ efflux in livers equilibrated with $^{45}\text{CaCl}_2$

Livers were perfused for 45 min in the presence of $^{45}\text{CaCl}_2$; 5 min before the cessation of $^{45}\text{CaCl}_2$ infusion, either PhAsO ($10\ \mu\text{M}$) (●, ■) or an ethanol control (○, □) was infused. Immune aggregate ($1\ \mu\text{g}/\text{ml}$) and phenylephrine ($10\ \mu\text{M}$) were subsequently infused. $^{45}\text{Ca}^{2+}$ and glucose in the effluent perfusate were determined as described in the Experimental section.

constriction and glycogenolysis. Interestingly, when PhAsO and thiols were infused sequentially, 2,3-dimercaptopropan-1-ol was still effective in reversing PhAsO action (Table 4). 2-Mercaptoethanol or 2,3-dimercaptopropan-1-ol themselves produced no significant effect on immune-aggregate-stimulated glycogenolysis or vasoconstriction.

Fig. 5. Reversal of PhAsO inhibition of immune-aggregate-stimulated glycogenolysis by dithiol

Livers were perfused in the presence of PhAsO ($10\ \mu\text{M}$), either 2-mercaptoethanol ($0.4\ \text{mM}$) (○) or 2,3-dimercaptopropan-1-ol ($30\ \mu\text{M}$) (●), and immune aggregate rat liver

DISCUSSION

Several receptor-mediated responses have been shown to be dependent on the oxidation-reduction state of vicinal dithiols [13–15]. Although no information about the nature of these cellular thiols is available currently, it would appear that thiol-disulphide exchange may represent a general mechanism by which occupied receptors transmit intracellular signals. The aim of the present study was to identify whether vicinal dithiols are required for hepatic vasoconstriction and glycogenolysis after activation of the reticuloendothelial system with aggregated immunoglobulin.

The inhibition of immune-aggregate-stimulated glycogenolysis by tervalent arsenicals appears to be specific, as no significant alteration of the hepatic response to other glycogenolytic hormones (i.e. phenylephrine, isoprenaline or glucagon) was noted in the present study.

Table 4. Effect of sequential infusion of PhAsO and thiols on immune-aggregate-stimulated metabolism in the perfused rat liver

Where indicated in the Table, PhAsO or ethanol was infused from 35 to 45 min of perfusion and thiols were infused from 45 to 55 min of perfusion. All livers were infused with immune aggregate ($1\ \mu\text{g}/\text{ml}$) from 60 to 62 min of perfusion. Results are means ($\pm$ S.E.M.) for three livers analysed as described in the Experimental section.

Treatment	IgG-aggregate-stimulated glucose output ($\mu\text{mol}/\text{g}$)	Maximal increase in glucose output ($\mu\text{mol}/\text{h per g}$)	Maximal increase in portal pressure (mmHg)
Ethanol	1.79 (0.21)	39.0 (4.0)	3.1 (0.1)
PhAsO ($10\ \mu\text{M}$)	0.18* (0.12)	2.7* (1.3)	0.3* (0.1)
PhAsO ($10\ \mu\text{M}$), 2-mercaptoethanol ($400\ \mu\text{M}$)	0.17† (0.13)	5.0† (3.2)	0.3† (0.2)
PhAsO ($10\ \mu\text{M}$), 2,3-dimercaptopropan-1-ol ($30\ \mu\text{M}$)	0.97‡ (0.23)	24.7‡ (3.0)	1.0‡ (0.3)
Ethanol, 2-mercaptoethanol ($400\ \mu\text{M}$)	1.61§ (0.13)	39.7§ (3.9)	2.8§ (0.3)
Ethanol, 2,3-dimercaptopropan-1-ol ($30\ \mu\text{M}$)	1.60§ (0.35)	40.7§ (8.7)	3.1§ (0.9)

* $P < 0.005$ compared with ethanol.

† Not significant statistically ($P > 0.2$) compared with PhAsO.

‡ $P < 0.005$ compared with PhAsO.

§ Not significant statistically ($P > 0.2$) compared with ethanol.

This finding suggests that vicinal dithiols are not involved substantially in the mechanisms by which these hormones activate glycogenolysis. Interestingly, phenylephrine-stimulated vasoconstriction was inhibited completely by PhAsO, suggesting that α -adrenergic agonist-stimulated glycogenolysis is largely independent of hepatic haemodynamics. Sequestration of IgG-aggregate-responsive vicinal dithiols by PhAsO appears quite long-lived compared with the sequestration of other hepatic vicinal dithiols (e.g. lipoate-dependent pyruvate dehydrogenase).

Kupffer cells are likely sites for receptor-mediated uptake of IgG aggregate in the liver [3], although other studies have shown that hepatic endothelial cells may bind immune complexes [29,30]. It would seem likely that production and/or secretion of one or several autacoid mediators (arachidonic acid metabolites and AGEPC) may be required to link immune-aggregate uptake with increased vascular resistance and glycogenolysis. Macrophages from a variety of tissues produce and secrete autacoids in response to stimuli [31,32].

In contrast with hormones which act directly on parenchymal cells to elicit glycogenolysis (i.e. phenylephrine) [28], immune aggregates do not cause an increase in the effluent perfusate Ca^{2+} concentration [6]. However, immune-aggregate infusion does mobilize a pool of $^{45}\text{Ca}^{2+}$ from $^{45}\text{Ca}^{2+}$ -equilibrated liver (Fig. 4), which is similar to that mobilized by AGEPC [20]. The Ca^{2+} pool mobilized by immune aggregate is small relative to that mobilized by phenylephrine and perhaps reflects a sinusoidal-cell Ca^{2+} pool, as discussed by Lapointe *et al.* [20]. The ability of PhAsO to inhibit this response is notable, considering the ability of purified Fc γ receptors to transport Ca^{2+} in reconstituted membranes upon binding of ligand [7].

The purified human B-lymphocyte Fc γ receptor contains an intrinsic ligand-stimulated phospholipase A_2 activity [8]. It is appropriate to expect that the rat Kupffer-cell Fc γ receptor might contain a similar activity. Thus, binding of immune aggregate to a hepatic Fc γ receptor would result in the production of autacoids. Increased concentrations of AGEPC have been found in the effluent perfusate of livers perfused with immune aggregate [4]. In addition, various autacoids (AGEPC, prostaglandin E_2 and the thromboxane analogue U-46619) have been shown to increase portal vascular resistance and stimulate glycogenolysis [6,33,34]. The haemodynamic changes and the transient increases in the effluent lactate/pyruvate ratio (which indicates a more reduced cytosolic NADH/NAD $^{+}$ couple [35]) observed after the infusion of these various autacoids are consistent with transient hypoxia within sections of the liver microvasculature. Hypoxia stimulates hepatic glycogenolysis rapidly via activation of glycogen phosphorylase by AMP [36,37]. Thus PhAsO may elicit its effects either by preventing the production of autacoids and/or by preventing autacoid action within the liver. The precise mechanism and site of action of PhAsO in the liver remains to be established.

One would expect immune complexes to contribute little in regulating hepatic function during normal physiological states. Nonetheless, elevated immune-complex concentrations could affect liver function significantly during various disease states. Elevated serum immune-complex concentrations characterize disease states associated with hepatic insufficiency, such as

cholestasis [38] and acute viral hepatitis [39]. Interestingly, activation of Kupffer cells has been suggested as a primary cause for hepatic insufficiency observed in patients with multiple-system organ-failure syndrome [40]. Thus autacoids may contribute significantly to the development of hepatic insufficiency in various pathological states. Further chemical characterization of the molecular mechanisms which regulate autacoid production are of obvious importance to a fuller understanding of hepatic physiology and pathophysiology.

This study was supported by grants from the N.I.H. (AM-19473) and the Robert A. Welch Foundation (AQ-728). We thank Yvonne DeCento for technical assistance, Dr. Kris Hill for advice concerning the glutathione assays, and Dr. David Lapointe for comments regarding the Ca^{2+} -mobilization studies.

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Received 27 April 1987/20 July 1987; accepted 9 September 1987