

HEPATIC changes during inflammation were studied in rats bearing a carrageenan induced granuloma. In spite of a decrease in the metabolic capacity of microsomes to induce lipid peroxidation during inflammation, the endogenous lipid peroxidation remained unchanged and unrelated with the hepatic activities measured. The continuous increase in hepatic cAMP observed during acute and chronic phases could be related to adenylate cyclase stimulation by mediators, and could be an initial step in the hepatocyte adaptation leading to the increased level of hepatic caeruloplasmin, to the reduction of cytochrome P-450 level and to the modifications of  $\text{Ca}^{2+}$  sequestration by microsomes.

**Key words:**  $\text{Ca}^{2+}$  sequestration, Caeruloplasmin, cAMP, Cytochrome P-450, Inflammation, Lipid peroxidation, Liver, Microsomes

## Hepatic changes during a carrageenan induced granuloma in rats

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## Introduction

It is becoming evident that the liver has an important role in the development of inflammatory processes.<sup>1–3</sup> Physiological adaptive changes have been observed, such as a decrease in hepatic smooth reticuloendothelial system drug metabolism during adjuvant induced arthritis<sup>4–6</sup> and during carrageenan induced granulomatous inflammation in rats.<sup>7</sup> A reduction in cytochrome P-450 dependent activities has been observed after administration of IL-6.<sup>8</sup> The cytochrome P-450 system seems to have a central role in the control of hepatic activities during inflammation. It is also known that lipid peroxidation reduces the level of cytochrome P-450<sup>9,10</sup> and microsomal  $\text{Ca}^{2+}$  sequestration,<sup>9</sup> but the role of peroxidative degradation of liver phospholipids during inflammation remains poorly understood and controversial.<sup>7,11,12</sup> In previous papers, the authors reported that carrageenan induced granuloma increased the thiobarbituric (TBA) reactive substances in plasma and in the inflammatory exudate.<sup>13,14</sup> Mediators and reactants released locally can also modify the physiological activity of hepatocytes via the stimulation of adenylate cyclase.<sup>23</sup>

The purpose of this research was to study the relation between inflammation and the regulation of several hepatic microsomal activities. Thus, particular attention has been paid to the changes in endogenous lipid peroxidation and in cAMP in relation to the synthesis of acute phase proteins such

as caeruloplasmin, to  $\text{Ca}^{2+}$  sequestration, and to cytochrome P-450 levels.

## Materials and Methods

**Animals and materials:** Three groups of nine male Sprague–Dawley rats, each weighing approximately 225 g were used. The animals were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, 1978) in the Animal Care Centre of the Faculty of Biology. One group was used as control (Control) and the other two were killed at 1 (I-1 day) and 6 days (I-6 days) after the induction of a carrageenan granuloma, enabling discrimination between the acute and chronic phases of the inflammation. The granuloma was induced by a s.c. injection of 6 ml of air into the dorsum of the rats, followed 24 h later by 4 ml of carrageenan 2% (w/v) (Viscarin 402, Marine Colloids, Springfield, NJ, USA) in NaCl 0.9%.<sup>15</sup> The three groups were starved overnight before the experiment, but given water *ad libitum*.

Blood was obtained by cardiac puncture under ether anaesthesia with a heparinized syringe and plasma was separated after centrifugation at  $1\,800 \times g$  at 4°C for 10 min.

The liver was perfused with NaCl 0.9% through the subhepatic vein and weighed, and 0.1 and 2.0 g were immediately removed for cAMP

assay and for isolation of the microsomal fraction respectively.

NADP, ATP, isocitrate and isocitrate dehydrogenase were supplied by Sigma Chemical Co (St Louis, MO).  $^{45}\text{Ca}^{2+}$  was obtained as aqueous  $^{45}\text{CaCl}_2$  from the I.R.E. (Fleurus, Belgium). All other chemicals and reagents were of the highest purity available.

**Caeruloplasmin assay:** The caeruloplasmin level was evaluated in 0.1 ml of plasma by measurement of its *p*-phenylenediamine oxidase activity, according to the Sunderman and Nomoto technique.<sup>16</sup>

**Hepatic cAMP assay:** A 0.1 g sample of liver was homogenized in 10 ml of methanol/acetic acid (95:5). After centrifugation at  $2000 \times g$  for 10 min the protein concentration in 50  $\mu\text{l}$  of supernatant was measured by the method of Lowry *et al.*<sup>17</sup> using bovine serum albumin as a standard. The remaining supernatant was stored at  $-80^\circ\text{C}$  until cAMP assay was carried out by radioimmunoassay (RIA,  $^{125}\text{I}$ -cAMP, Immunotech, France) in a fraction containing 250  $\mu\text{g}$  of protein.

**Hepatic microsomal assays:** The hepatic microsomal fraction from 2 g of liver sample was obtained by the method of Lowrey *et al.*,<sup>18</sup> and the protein concentration was measured.<sup>16</sup> Cytochrome P-450 was evaluated by the capacity of its reduced form to react with CO.<sup>19</sup>  $\text{Ca}^{2+}$  sequestration was measured as described by Moore.<sup>20</sup> Endogenous lipid peroxidation was determined by the absorbance at 233 nm (conjugated dienes) of the extracted lipids dissolved in cyclohexane.<sup>21</sup> The metabolic capacity of microsomes to induce peroxidation was assayed after 30 min of incubation at  $37^\circ\text{C}$  in the presence of an NADPH generating system,<sup>18</sup>  $\text{CCl}_4$  or  $\text{FeSO}_4$ , at the concentrations given in Table 2, and the TBA reactive substances produced were measured by the method of Ghoshal and Recknagel.<sup>22</sup>

## Results

The concentration of caeruloplasmin in plasma (Fig. 1) increased 1 day after the induction of the granuloma (0.60 g/l), and even more after 6 days (1.13 g/l) compared to the control value (0.34 g/l). Figure 2 shows a significant increase in the relative weight of the liver 1 day after the induction of the inflammation. No difference was found between the values obtained at 1 day and at 6 days of inflammation. cAMP concentration in the liver (Fig. 3) increased gradually throughout the development of the inflammation, reaching 1.25  $\mu\text{g}/\text{mg}$  protein 6 days after the induction.

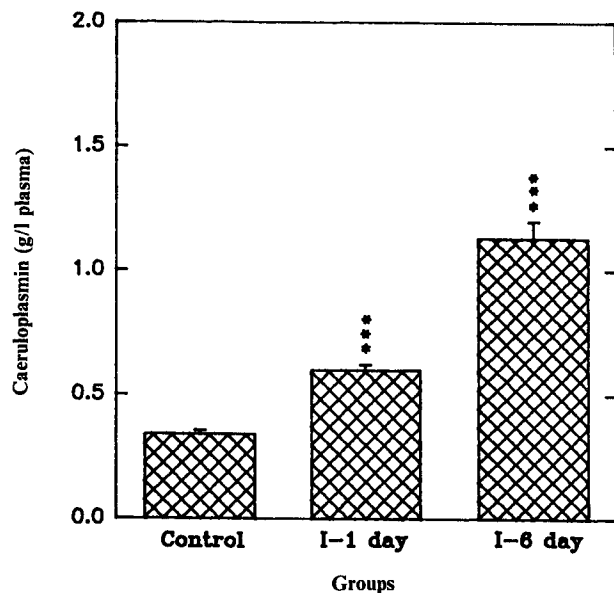


FIG. 1. Changes in the plasma concentration of caeruloplasmin induced by inflammation. Groups and assay were as described in Materials and Methods. Data represent mean  $\pm$  S.E.M. of nine rats. The results were analyzed using Student's *t* test. Inflamed groups (I) were compared with control group (Control): \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

At the hepatic microsomal membrane level, no significant difference between groups was found in the endogenous conjugated diene concentration (Table 1). The level of cytochrome P-450 (Table 1) decreased strongly after the induction of the inflammation (55% at I-1 day and 60% at I-6 day).  $\text{Ca}^{2+}$  sequestration by the microsomal fraction (Table 1) was substantially affected by the

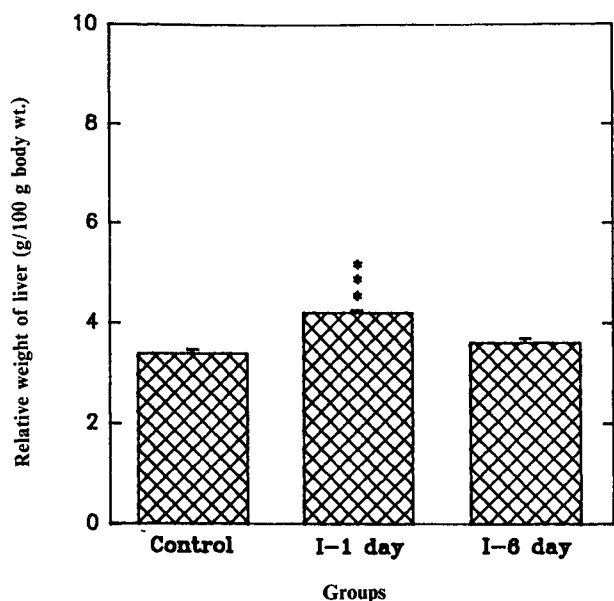


FIG. 2. Changes in the relative weight of the liver induced by inflammation. Groups were as described in Materials and Methods. Data represent mean  $\pm$  S.E.M. of nine rats. The results were analyzed using Student's *t* test. Inflamed groups (I) were compared with control group (Control): \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

**Table 1** Effect of inflammation on the level of conjugated dienes, cytochrome P-450 and Ca<sup>2+</sup> sequestration in the hepatic microsomal fraction

Groups	Conjugated dienes (absorbance at 233 nm of 1 mg lipids/ml cyclohexane)	Cytochrome P-450 (nmol/mg prot.)	Ca <sup>2+</sup> sequestration (nmol/mg prot. per 30 min)
Control	0.288 ± 0.0092	0.963 ± 0.0265	85 ± 4.8
I-1 day	0.311 ± 0.0158	0.433 ± 0.0222***	146 ± 4.1***
I-6 day	0.313 ± 0.0141	0.382 ± 0.0577***	58 ± 9.1***

Data represent mean ± S.E.M. of nine rats. The results were analyzed using Student's *t*-test. Inflamed groups (I) were compared with control group (Control). \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

inflammation, reaching the highest value 1 day after the induction (146 nmol Ca<sup>2+</sup>/mg protein) and showing a lower value 6 days after induction (58 nmol Ca<sup>2+</sup>/mg protein) than the control group (85 nmol Ca<sup>2+</sup>/mg protein).

The metabolic capacity of microsomes to induce lipid peroxidation decreased during inflammation (Table 2) when incubated in the presence of 1.5 μM FeSO<sub>4</sub> or 0.5 μM CCl<sub>4</sub>. The metabolic activity of microsomes in the presence of both inducers was lower in I-6 day than in I-1 day groups.

## Discussion

The increase in the relative weight of the liver during the acute phase of inflammation suggests intense activity of the Kupffer cells and it had been

observed that administration of zymosan intravenously<sup>23</sup> and rhamnose intraperitoneally<sup>24</sup> stimulated the reticuloendothelial system.

Some parameters show a change under the effect of inflammation while others, such as conjugated dienes, remain unchanged. In other situations it has been postulated that lipid peroxidation is responsible for a reduction in the activity of cytochrome P-450.<sup>9,25</sup> As to its role in inflammation, Robak<sup>11</sup> observed a reduction in the TBA reactive substances in the homogenate of the liver during inflammation induced by carrageenan (3 and 24 h after induction) and by Freund's adjuvant (1, 5, 10 and 21 days after induction). However, other authors reported an increase in the TBA reactive substances in the homogenate of the liver, at 21 days after adjuvant induced arthritis<sup>12</sup> and 7 days after carrageenan induced granuloma growing around subcutaneously implanted teflon chambers.<sup>7</sup> In our conditions the relation between microsomal lipid peroxidation and the decrease of cytochrome P-450 is difficult to assume since there is no difference in conjugated diene concentration between groups, although the authors have observed an increase of TBA reactive substances in plasma and in the inflammatory exudate.<sup>13,14</sup> So, there may

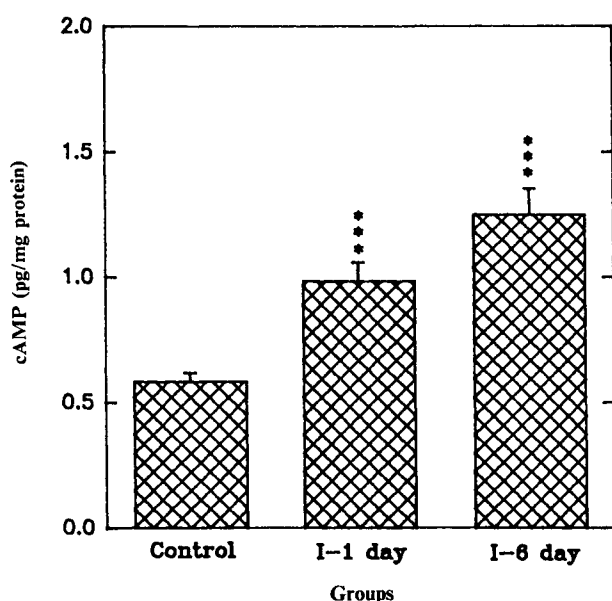


FIG. 3. Changes in the hepatic concentrations of cAMP induced by inflammation. Groups and assay were as described in Materials and Methods. Data represent mean ± S.E.M. of nine rats. The results were analyzed using Student's *t* test. Inflamed groups (I) were compared with control group (Control): \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

**Table 2.** Effect of FeSO<sub>4</sub> and CCl<sub>4</sub> on the level of TBA-reactive substances generated by hepatic microsomal fraction

Groups	TBA reactive substances (nmol malondialdehyde/mg protein)	
	FeSO <sub>4</sub> (1.5 μM)	CCl <sub>4</sub> (0.5 μM)
Control	5.56 ± 0.314	1.330 ± 0.0149
I-1 day	3.59 ± 0.309**	0.505 ± 0.0546***
I-6 day	2.89 ± 0.211***	0.097 ± 0.0493***

Data represent mean ± S.E.M. of nine rats. The results were analyzed using Student's *t* test. Inflamed groups (I) were compared with control group (Control). \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

be other mechanisms for the down-regulation of cytochrome P-450. Fukuda *et al.*<sup>24</sup> postulated haem deficiency which results from the induction of haem oxygenase or the increase in specific cytokines such as IL-6, but the phosphorylation of cytochrome P-450 by adrenalin and glucagon may also be involved.<sup>27</sup>

In the present work an increase in hepatic cAMP while cytochrome P-450 decreased. Inflammatory mediators and hepatocytes may thus be involved in the increase in cAMP. On the other hand, adrenalin and glucagon (factors present during acute phase reactions) added to hepatocytes in culture increased the phosphorylation of certain isoenzymes of cytochrome P-450.<sup>7,28,29</sup> It has thus been postulated that the increase in hepatic cAMP may be responsible for the phosphorylation of cytochrome P-450 and its degradation,<sup>29</sup> transforming the P-450 form to the inactive form P-420.<sup>30</sup> Such a mechanism could lead to changes in cytochrome P-450 concentration during inflammation and also to the decrease in its capacity to induce lipid peroxidation in the presence of FeSO<sub>4</sub> or CCl<sub>4</sub>, which is in accordance with studies carried out by Sakai *et al.*<sup>31</sup> showing that IL-1 treatment depresses superoxide production by microsomes. This study will be pursued in order to clarify the role of local and systemic mediators (IL-1, TNF, catecholamines, glucocorticoids) *in vitro*.

The increase in the Ca<sup>2+</sup> sequestration by microsomes during the acute phase of inflammation suggests an important role for this element as second intracellular messenger. It has been observed that the capacity of microsomes to sequester cytosolic Ca<sup>2+</sup> is modified by glucagon or cAMP analogues.<sup>32,33</sup>

Caeruloplasmin, as an acute phase protein, is a good indicator of inflammatory response.<sup>34</sup> The transcription of the caeruloplasmin gene in the liver is stimulated by several factors (catecholamines, IL-6, glucocorticoids) where cAMP is the intracellular messenger involved.<sup>3</sup> So, the increase in hepatic cAMP during inflammation may cause high concentrations of caeruloplasmin in plasma.

These results suggest that mediators released during carrageenan induced inflammation could increase cAMP in hepatocytes and then stimulate synthesis of caeruloplasmin, reduce the level of cytochrome P-450, and modify Ca<sup>2+</sup> sequestration by the microsomal fraction.

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