Biochemical Pharmacology, Vol. 35, No. 9, pp. 1491–1497, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

KUPFFER CELL FACTOR MEDIATED DEPRESSION OF HEPATIC PARENCHYMAL CELL CYTOCHROME P-450

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(*Received* 15 *June* 1985; *accepted* 10 *October* 1985)

Abstract—Following the administration of latex particles (0.46 μm), cytochrome P-450 dependent monooxygenase system was depressed in the livers of mice. These particles were taken up exclusively by Kupffer cells in the liver, and no particles were found in the hepatocytes which contain most of the monooxygenase capacity in that organ. Cytochrome P-450 was also depressed in isolated hepatocytes incubated with phagocytosing Kupffer cells or the cell free filtrate from an incubation mixture of Kupffer cells and latex particles. Kupffer cells and hepatocytes were then incubated in a double-chambered vessel in which the two cell types were separated by a semi-permeable membrane. When latex particles were added to the chamber containing Kupffer cells, a factor was released which crossed the semi-permeable membrane and depressed cytochrome P-450 and benzo[*a*]pyrene hydroxylase in hepatocytes contained in the other chamber. It is concluded that, during the process of phagocytosis (*in vivo* or *in vitro*) by Kupffer cells in the liver, the levels of cytochrome P-450 and related drug biotransformation were depressed in the adjacent parenchymal cells.

The hepatic cytochrome P-450 dependent monooxygenase system which is responsible for the metabolism of a large number of drugs, carcinogens, pollutants and other chemicals is often depressed by agents that alter host defence mechanisms. The depression of this enzyme activity occurs in both animals and man during active viral infections and causes a decline in the capacity of the liver to eliminate drugs [1–3]. A loss of cytochrome P-450 is observed when animals are treated with a number of non-infectious agents which affect the immune system including *Bordetella pertussis* vaccine, *Corynebacterium parvum*, BCG, tilorone and certain polymers [4]. In 1976, Renton and Mannering [5] proposed that the loss of drug biotransformation caused by some immunoactive agents is mediated via the production of interferon. This hypothesis was substantiated indirectly using a genetic model [6] and directly using highly purified human interferons obtained by recombinant DNA techniques [7, 8]. However, interferon cannot explain the depression of cytochrome P-450 caused by all immunoactive agents because not all of these agents are capable of inducing the formation of interferon.

Although most of the drug biotransformation capacity of the liver is contained in the hepatocytes [9], the status of the non-parenchymal cells of the reticuloendothelial system appears to play an important role in the maintenance of cytochrome P-450 levels within the hepatocyte. Whenever animals are treated with agents that are phagocytosed by the reticuloendothelial system, the levels of drug biotransformation in the liver appear to decrease simultaneously [10–12]. These studies did not provide a mechanism to explain why a loss of cytochrome P-450 accompanied the phagocytosis of particulate matter. Involvement of the reticuloendothelial system has also been suggested to explain the depression of cytochrome P-450 which is caused by BCG [13] or by *C. parvum* [14].

If the depression of drug biotransformation by immunoactive agents is mediated by the activation of the liver reticuloendothelial system, this hypothesis would require some form of communication between Kupffer cells which themselves contain little or no cytochrome P-450 and the parenchymal cells which contain the bulk of the cytochrome P-450 found in the liver [9]. In this paper, we report that phagocytosing Kupffer cells released a factor which was transferred to the hepatic parenchymal cells of the liver and lowered cytochrome P-450 and related drug biotransformation in this cell type.

MATERIALS AND METHODS

Materials. Latex beads with a mean particle of 0.46 μm diameter (standard deviation = 0.0059 μm) were obtained from the Sigma Chemical Co., St. Louis, MO. The doses of latex beads used *in vivo* and the concentrations used *in vitro* were those which produced the maximum effect on cytochrome P-450 and drug biotransformation.

Animals. Male Swiss strain mice of 25 g were obtained from Jackson Laboratories, Maine, and were acclimatized in our facility for at least 1 week before use. All animals were housed on clay chip bedding to avoid the possibility of enzyme induction and received standard Purina mouse chow and water *ad lib.*

Microsome preparation and drug biotransformation. Hepatic microsomes were prepared by differential ultracentrifugation by the method described by El Defrawy El Masry *et al.* [15]. The

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levels of cytochrome P-450, aminopyrine *N*-demethylase and benzo[*a*]pyrene hydroxylase in hepatic microsomes were determined by the methods of Omura and Sato [16], Cochin and Axelrod [17], and Nebert and Gelboin [18] respectively. In isolated cell preparations, cytochrome P-450 was determined by solubilizing whole cells in 4% Lubrol in 0.1 M (pH 7.4) phosphate buffer and then measuring the carbon monoxide difference spectrum as described by Omura and Sato [16]. Benzo[*a*]pyrene hydroxylase activity in isolated cells was determined by the method of Cantrell and Bresnick [9]. Protein determinations were carried out using the method of Lowry *et al.* [19] using bovine serum albumin (BSA) as a protein standard.

Electron microscopy. Portions of liver (approximately 2 mm^3) were fixed for 2 hr in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.29) at 4°. The sections were post fixed in 1% osmium tetroxide for 1 hr and then dehydrated in alcohols before embedding in TAAB resin. Thin sections (60 nm) were stained with uranyl acetate and lead citrate [20] and examined in a Phillips 300 electron microscope.

Interferon levels in blood and culture medium. Interferon was measured by the plaque reduction method of O'Shaughnessy *et al.* [21] using L-929 cells and vesicular stomatitis virus as the challenge virus. One unit of interferon was defined as the amount reducing plaque formation by 50%.

Cell preparations. Liver cells were prepared by perfusing the liver in a retrograde direction with 0.05% collagenase (Sigma) as described previously [22]. This procedure yielded a mixture of parenchymal and non-parenchymal cells which was suspended in 50 ml of the calcium-free *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer described by Seglen [23]. The cell types were then fractionated using a modification of the procedure described by Skilleter and Price [24] as outlined in Fig. 1. The non-parenchymal cell fraction contained primarily Kupffer cells which were identified by size and their ability to take up carbon particles [25]. The non-parenchymal cells usually contained about 30×10^6 cells/g liver with a viability of greater than 95% as determined by trypan blue exclusion. These cell preparations contained less than 4% parenchymal cells all of which were non-viable. The parenchymal cell fractions usually contained at least 100×10^6 cells/g liver and had a viability of greater than 80%. These cell preparations contained less than 1% Kupffer cells. The L-15 medium used in the final cell suspension was supplemented with 10% fetal calf serum, 2 mM glutamine, and antibiotics (100 units penicillin, 100 μg streptomycin and 0.25 μg fungizone per ml). Isolated cell preparations alone or in combination were incubated with latex particles in supplemented L-15 medium using the incubation conditions given in the respective table and figure legends.

Statistics. An unpaired Student's *t*-test was utilized to compare two variables, and a Student–Newman–Keuls test was used when more than two variables were compared [26].

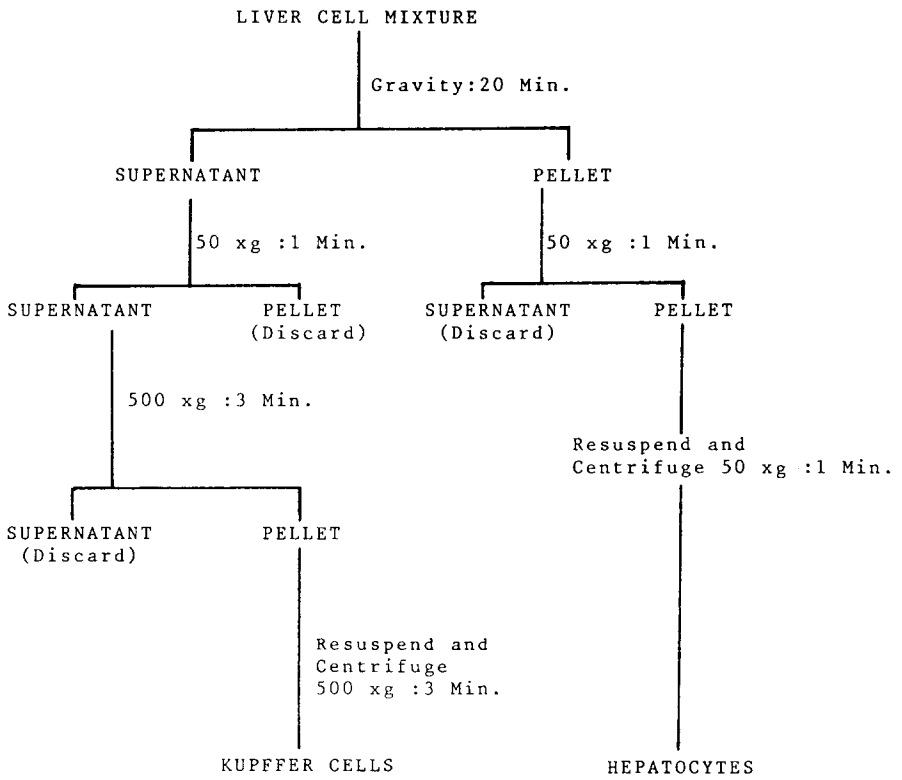

Fig. 1. Fractionation of Kupffer cells and hepatocytes from murine liver cell mixture.

Table 1. Cytochrome P-450 levels and aminopyrine *N*-demethylase activity in hepatic microsomes prepared from animals treated with latex particles

	Liver weight (g)	Microsomal protein (mg/g liver)	Cytochrome P-450 (nmoles/mg protein)	Aminopyrine *N*-demethylase (nmoles HCHO/ mg protein/hr)
Control	1.96 ± 0.04	13.0 ± 0.46	0.72 ± 0.04	315 ± 26
Latex	1.90 ± 0.30	11.28 ± 0.60	0.50 ± 0.04*	217 ± 16*

Latex particles (20 mg of 0.46 μm diameter) were injected i.v. in 0.2 ml saline 24 hr before the animals were killed. Values are means ± S.E., N = 4.
* Significantly different from control, $P < 0.05$.

RESULTS

Hepatic microsomal mixed-function oxidase following in vivo *phagocytosis.* The level of cytochrome P-450 and the activity of aminopyrine *N*-demethylase were decreased significantly in hepatic microsomes prepared from the livers of mice treated for 24 hr with latex beads (20 mg solids in 0.2 ml saline, given i.v.) as shown in Table 1. Body weight, liver weight, and hepatic microsomal protein levels were unaffected by the latex bead treatment. In both the control and latex bead treated groups, the plasma level of interferon was below the detectable limit of the assay used (10 PRD units/ml) at the time the animals were killed. In mice treated for 24 hr with the interferon inducer poly rI·rC (10 mg/kg), cytochrome P-450 in liver microsomes was depressed by a magnitude similar to that caused by the latex treatment, but in this case plasma interferon levels were greater than 2000 PRD units/ml.

An electron micrograph of a liver section taken from an animal treated with latex particles is illustrated in Fig. 2. Large numbers of latex particles have been taken up by a Kupffer cell, in contrast to the adjacent hepatocytes which contain no latex

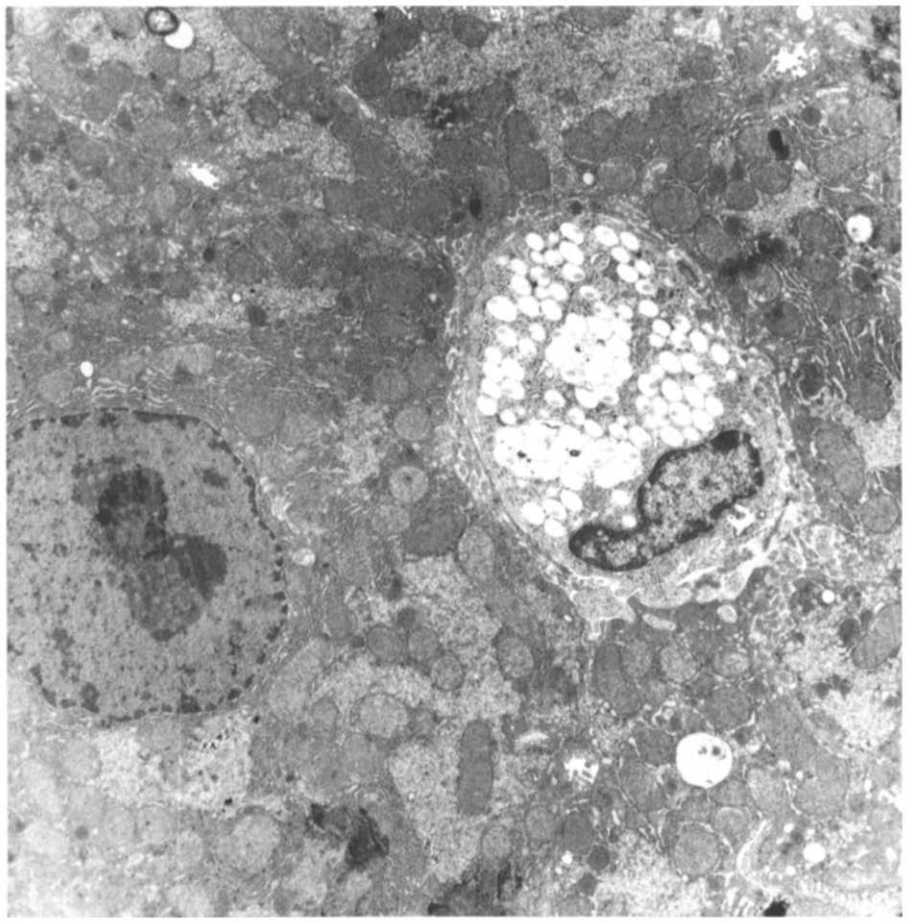

Fig. 2. Electron micrograph of a liver section taken from an animal treated with latex particles. Mice were treated i.v. with 0.46 μm latex particles 24 hr before being killed. Sections were prepared for microscopy as described in Materials and Methods. Magnification in the illustration is 8085×.

particles. Examination of liver sections from the other mice used in these experiments indicated that no latex particles were taken up by parenchymal cells, but in all of the animals examined the Kupffer cells were heavily loaded with latex particles.

Effect of latex beads on drug biotransformation in isolated cells. Preparations of isolated Kupffer cells were incubated with latex beads (1.0 mg/ml solids, final concentration) for 30 min. When this incubation mixture was added to a preparation of isolated hepatocytes and incubated for a further 30 min, the level of cytochrome P-450 in the cells was depressed by 34% compared to the control (Fig. 3). These incubation times represented when the maximum depression of cytochrome P-450 occurred. Trypan blue exclusion by these cells indicated that the hepatocytes remained viable throughout the period of incubation. The control in this experiment was treated in an identical manner except that latex particles were not added to the initial mixture of Kupffer cells. No changes in cytochrome P-450 were apparent in controls throughout the incubation period. When latex particles were incubated in medium for 30 min without Kupffer cells and then incubated further with hepatocytes, the level of cytochrome P-450 in the hepatocytes was unaffected.

Kupffer cells were then incubated with latex particles (1 mg/ml) for 1 hr before the incubation mixture was filtered through a 0.22 μm Millex filter to remove cells and latex particles. When this filtrate was added to preparations of hepatocytes and incubated for a further 60 min, the level of cytochrome P-450 in the hepatocytes was depressed by 53% compared to the level of cytochrome P-450 found in the control incubations (Fig. 4). A filtrate obtained by incubating latex particles without Kupffer cells had no effect on cytochrome P-450 in hepatocytes. In a similar experiment, the activity of benzo[*a*]-pyrene hydroxylase was depressed by 40% in hepatocytes incubated with the filtrate obtained from incubating latex particles with Kupffer cells (Fig. 4). A filtrate obtained by incubating latex particles without Kupffer cells had no effect on the activity of benzo[*a*]pyrene hydroxylase. The level of interferon in the filtrates obtained in these experiments was below the level of detection.

Further experiments were carried out using a double-chambered Marbrook vessel in which the two cell types were incubated separated only by a semi-permeable membrane with a molecular cut-off of 12,000 daltons. When Kupffer cells were incubated with latex beads in the inner chamber for 16 hr at 37°, the level of cytochrome P-450 in the hepatocytes in the outer chamber was depressed by 65% compared to control preparations that were incubated under similar conditions without the latex particles (Fig. 5). In preliminary experiments it was determined that the maximum effect occurred after an incubation time of 16 hr. The activity of benzo[*a*]-

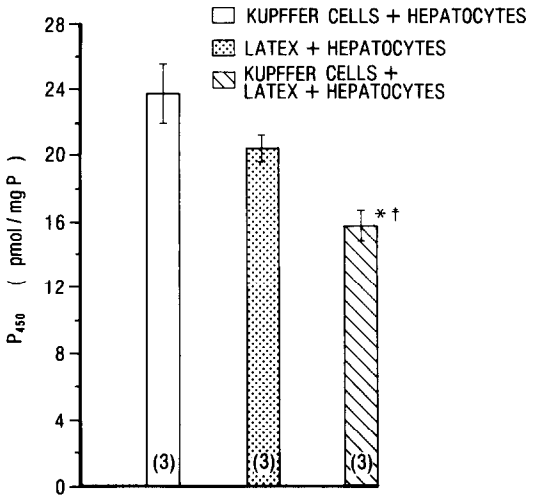

Fig. 3. Cytochrome P-450 levels in hepatocytes incubated with Kupffer cells and latex particles. Kupffer cells (1.0 ml of 7.5×10^6) were incubated with 1 mg/ml latex particles for 30 min. This mixture was then added to 1 ml of hepatocytes (14×10^6 cells) and incubated for a further 30 min. Control incubations were treated in an identical manner except that the initial incubation was carried out without the addition of latex particles. Latex particles were also incubated with medium in the absence of Kupffer cells and then added to hepatocytes. Each value is the mean ± S.E., N = 3; mg P indicates milligrams protein. Key: (*) significantly different from the control, $P < 0.05$; and (‡) significantly different from incubation without Kupffer cells, $P < 0.05$.

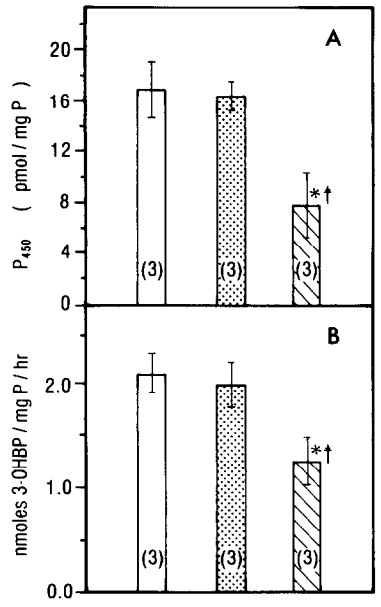

Fig. 4. Level of cytochrome P-450 (A) and benzo[*a*]pyrene hydroxylase (B) in hepatocytes incubated with filtrates obtained by incubating latex particles with Kupffer cells. Kupffer cells (7.5×10^6 cells/ml) were incubated with latex particles (1 mg/ml) for 60 min, and then the mixture was passed through a 0.22 μm Millex filter to remove cells and latex particles. The filtrate (0.5 ml) was added to 0.5 ml of hepatocytes (14.0×10^6 cells/ml) and incubated for 60 min. At the end of this time, cytochrome P-450 and benzo-[*a*]pyrene hydroxylase were determined as described in Materials and Methods. Incubation mixtures were (□) control which contained no latex particles; (▩) incubation without Kupffer cells; and (▨) incubation with Kupffer cells and latex particles. Each value is the mean ± S.E., N = 3. Key: (*) significantly different from control, $P < 0.05$; and (‡) significantly different from incubation mixture without Kupffer cells, $P < 0.05$.

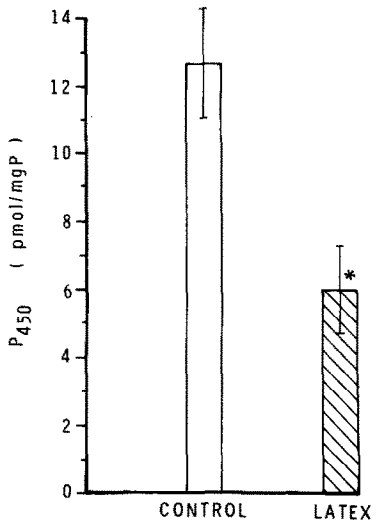

Fig. 5. Level of cytochrome P-450 in hepatocytes incubated with Kupffer cells and latex particles in a double-chambered Marbrook vessel. One milliliter of Kupffer cells (15×10^6 cells) and latex particles (1 mg/ml) were contained in the inner chamber of the vessel and were separated from 10 ml of hepatocytes (7×10^6 cells/ml) by a semi-permeable membrane which prevented the passage of molecules larger than 12,000 molecular weight. Incubations were carried out for 16 hr at 37°. Cytochrome P-450 levels in the hepatocytes were determined at the end of the incubation time. Marbrook vessels containing both cell types but without latex particles were treated in an identical manner and served as controls. Each value is the mean ± S.E., N = 3 different cell preparations. Key: (*) significantly different from control, $P < 0.05$.

pyrene hydroxylase was also depressed in hepatocytes incubated with latex particles and Kupffer cells in the Marbrook vessel (Table 2). In one experiment, the level of benzo[*a*]pyrene was depressed by 50% and in a second experiment the activity of the enzyme was abolished completely. In all of the experiments carried out in the Marbrook vessels, cytochrome P-450 levels and benzo[*a*]pyrene activity decreased in the control incubations to between 50 and 70% of the initial values during the 16-hr incubations. All of the data presented following incubations with latex particles is therefore compared to controls incubated for an identical time.

Table 2. Activity of aryl hydrocarbon hydroxylase in hepatocytes incubated with Kupffer cells and latex particles in a double-chambered Marbrook vessel

	Aryl hydrocarbon hydroxylase activity (nmoles 3-OH benzo[*a*]pyrene/mg protein/hr)	
	Control	Latex
Experiment 1	0.058 ± 0.017	0.029 ± 0.02*
Experiment 2	0.197 ± 0.006	0.000

Conditions for this experiment were identical to those described in the legend of Fig. 5. Benzo[*a*]pyrene hydroxylase activity was determined in the hepatocytes at the end of the incubation time. The two experiments were carried out using two separate preparations of cells. Values are means ± S.E., N = 3 (separate vessels).

* Significantly different from control, $P < 0.05$.

DISCUSSION

A large number of immunoactive agents have been reported to depress cytochrome P-450 and its related drug biotransformation [4], but the mechanism for this effect has been poorly understood. Renton and Mannering [5] reported that agents which can induce the formation of interferon can depress drug biotransformation in the liver. Subsequently, interferon itself was shown to depress cytochrome P-450 and related drug metabolism [7, 8, 27]. Many immunoactive agents, however, have little ability to induce the formation of interferon [2, 28, 29], but most of these agents do affect the reticuloendothelial system. Whenever this system is either stimulated or depressed, the liver loses some of its capacity to metabolize drugs [10]. During phagocytosis in the liver, most of the engulfed material is taken up by the Kupffer cells and few particles are taken up by parenchymal cells [30]. The drug biotransformation activity of the liver, however, is contained primarily in the parenchymal cells and only a small proportion of the overall activity in this organ is contained in the Kupffer cells [9]. Although it is possible that the agents which are phagocytosed in the liver have a direct action on cytochrome P-450 in the parenchymal cells, it is much more likely that the depression of cytochrome P-450 in the parenchymal cells results indirectly from the phagocytic action occurring in the Kupffer cells.

In the present studies, latex particles depressed the cytochrome P-450 drug-metabolizing system in the intact animal. No latex particles were observed in parenchymal cells in sections of the liver examined by electron microscopy. This contrasted with the Kupffer cells which were heavily laden with latex particles. The absence of latex particles in the hepatocytes support the hypothesis that the loss of drug biotransformation in the liver was not due to a direct action of these particles in the hepatocyte but more likely resulted indirectly from the phagocytosis of the particles by the Kupffer cells. The loss of cytochrome P-450 is unlikely due to the release of interferon into the circulation as none could be detected in the plasma of latex-treated animals. In contrast, the loss of cytochrome P-450 by poly rI·rC was accompanied by high plasma levels of interferon.

Further support for the hypothesis was provided in experiments utilizing isolated preparations of hepatocytes and Kupffer cells. Latex particles had no direct effect on cytochrome P-450 when incubated alone with hepatocytes, but cytochrome P-450 was depressed significantly when latex particles were preincubated with Kupffer cells and then this mixture further incubated with hepatocytes. This loss was not due to direct cell damage as the hepatocytes used in these experiments remained viable as measured by trypan blue exclusion throughout the periods of incubation. The effect was not dependent on contact between the two cell types as filtrates from incubations of Kupffer cells and latex particles also depressed cytochrome P-450 in hepatocytes. The

factor involved in the loss of cytochrome P-450 from hepatocytes *in vitro* was unlikely to be interferon as none could be detected in the filtrates from incubation mixtures containing Kupffer cells and latex particles. The experiment using the Marbrook vessels clearly indicates that a factor is released from Kupffer cells which crosses a semi-permeable membrane and depresses cytochrome P-450 in hepatocytes. The membrane used had a molecular weight cut off of about 12,000 daltons, which also suggests that the factor released is not interferon which has a molecular weight of 20,000 and has been shown previously not to cross this size dialysis membrane [21]. Although it is possible that the factor could induce the formation of interferon in the responding hepatocytes, this is unlikely as pure interferon requires a much longer time period to lower cytochrome P-450 when incubated with preparations of isolated hepatocytes.* These experiments indicate that, although some infections and immunoactive drugs lower cytochrome P-450 via the production of interferon [4], the level of this enzyme is also depressed by another factor which is released from Kupffer cells during the process of phagocytosis. Recently, in related experiments Williams and Szentivanyi [31] reported that the endotoxin-mediated loss of drug biotransformation occurred via a substance derived from peritoneal macrophages.

It has been suggested that macrophages are involved in the depressive action on drug biotransformation caused by *C. parvum* [14], *B. pertussis* [32], *M. butyricum* [33], and BCG [13]. In a recent paper, Farquhar *et al.* [34] suggested that the hepatic granulomas caused by *C. parvum* in the liver might release a factor which could damage the endoplasmic reticulum in proximate parenchymal cells. These authors, however, provided no experimental evidence to support such a mechanism. In addition, many other non-specific immunostimulants including Freunds adjuvant [35], *Escherichia coli* endotoxin [36], zymosan [37], colloidal carbon [38], dextran [39], latex beads [40], trypan blue [41], interferon [42] and pyran copolymers [43] all have major effects on the reticuloendothelial system and depress drug biotransformation in the liver. Recent experiments in our own laboratory have suggested that dextran sulfate depresses cytochrome P-450 in the liver by an indirect action on non-parenchymal cells [28]. It is likely that many immunoactive agents can depress drug biotransformation by the indirect release of a factor from the Kupffer cells which is similar to that described in the present paper for latex particles.

The results reported in this paper indicate that during the operation of host defence mechanisms the capacity of the liver to metabolize drugs is diminished by a factor which is released from Kupffer cells. The loss which occurs by this mechanism is in addition to the loss of cytochrome P-450 and related drug metabolism which occurs following the induction of interferon [4]. The decrease in drug biotransformation which occurs during infections is likely to occur via both mechanisms, but at the present time it is unknown if the effects of the two processes are related to each other in any way or if they are separate and act in an additive manner. The finding that Kupffer cell function plays an active role in determining the levels of cytochrome P-450 in proximate parenchymal cells provides yet another factor that can alter the ability of the liver to eliminate drugs. This mechanism may play an important role in the alteration of drug biotransformation which occurs during active infections in man.

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