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# Stimulation of Monooxygenation and Conjugation after Liver Transplantation in the Rat: Involvement of Kupffer Cells

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## SUMMARY

The success rate of liver transplantation has improved markedly during the last few years and, although this patient population receives multiple drug therapies, the effect of liver transplantation on drug metabolism has been studied very little. Therefore, the purpose of this study was to assess the metabolism of model drug substrates after liver transplantation in the rat. Rat livers were stored for 4 hr in cold Euro-Collins solution, transplanted orthotopically, and then perfused 2 hr later with oxygenated Krebs-Henseleit buffer, using a nonrecirculating system. Rates of monooxygenation of the model compound *p*-nitroanisole, conjugation of *p*-nitrophenol, and uptake of oxygen were measured. All parameters studied were elevated significantly, by nearly 2-fold, by transplantation. Specifically, monooxygenation was increased from 2.9 ± 0.2 to 5.1 ± 0.4 μmol/g/hr, conjugation was elevated from 3.3 ± 0.6 to 7.7 ± 0.1 μmol/g/hr, and $O_2$ uptake was stimulated from basal values of 114 to 197 μmol/g/hr. Transplantation did not, however, alter rates of monooxygenation and conjugation in isolated microsomes supplemented with excess cofactor. When donor rats were pretreated with the Kupffer cell toxicant gadolinium chloride (10 mg/kg, intravenously) 30 hr before liver storage, the elevation after transplantation in all parameters studied was prevented. Depletion of carbohydrate reserves by fasting of donor rats did not prevent stimulation of monooxygenation and conjugation. On the other hand, urea synthesis from ammonium chloride, a process dependent on mitochondrial NADPH, was increased and monooxygenation was diminished after transplantation, suggesting the involvement of mitochondria in this phenomenon. Indeed, mitochondria isolated 2 hr postoperatively exhibited significantly elevated respiratory control ratios and higher state 3 rates of respiration. Taken together, these data support the hypothesis that Kupffer cells, activated by transplantation, release mediators that stimulate mitochondria in parenchymal cells and enhance drug metabolism by increasing cofactor supply (e.g., NADPH for monooxygenation and UDP-glucuronic acid for glucuronidation).

Liver transplantation is now a recommended therapeutic option for a number of liver diseases (1). Although liver transplant patients receive multiple drugs, very little information is available on the effect of liver transplantation on drug metabolism. Because there is a narrow window between therapeutic efficacy and toxicity of immunosuppressive drugs, a thorough understanding of drug metabolism after liver transplantation is essential to optimize drug therapy in this important patient population (2).

Many factors, such as a decrease of hepatic energy status by cold storage or hormone release after surgical stress, could influence drug metabolism after liver transplantation (3–5). It is also well known that transplantation activates Kupffer cells (6), the hepatic macrophages. Kupffer cells are nearly devoid of cytochrome P-450 (7) but have been reported to modify hepatocyte cytochrome P-450 activity assessed *in vitro* (8). However, studies carried out to date have not considered the complex regulation of drug metabolism in intact cells (9). Therefore, the purpose of this study was to evaluate monooxygenation and conjugation of model drug substrates in the perfused liver after liver transplantation in the rat.

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## Materials and Methods

**Experimental animals.** Female Sprague-Dawley rats (200–250 g), with free access to food and water, were used in this study. For some experiments, rats were fasted 24–30 hr before experiments. When indicated, rats were treated with gadolinium chloride (10 mg/kg, intravenously) 30 to 36 hr before experiments, to destroy Kupffer cells (10–11).

**Drug metabolism in perfused liver.** Details of the perfusion technique have been described elsewhere (12). Briefly, livers were perfused at 37° with Krebs-Henseleit bicarbonate buffer, pH 7.4, saturated with an oxygen/carbon dioxide mixture (95:5), in a nonrecirculating system. The fluid was pumped through the liver via a cannula placed in the portal vein, was collected with a cannula in the vena cava,

**ABBREVIATION:** UDPGA, UDP-glucuronic acid.

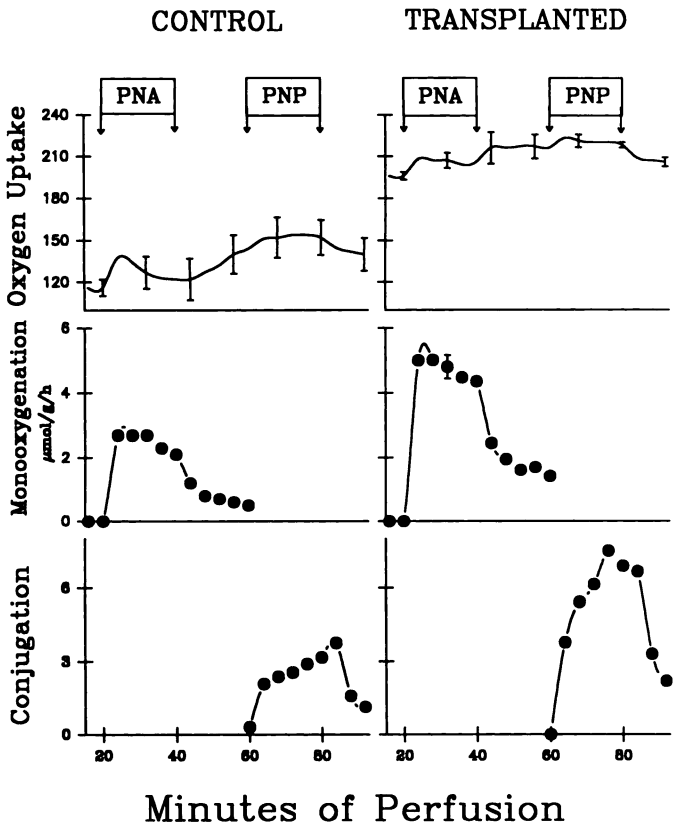


**Fig. 1.** Oxygen uptake and drug metabolism in perfused livers from normal and transplanted rats. Livers were transplanted and subsequently perfused 2 hr later with Krebs-Henseleit buffer, as described in Materials and Methods. After 20 min, *p*-nitroanisole (*PNA*) (200 μM) was infused for 20 min, followed by infusion of *p*-nitrophenol (*PNP*) (70 μM) between 60 and 80 min, as indicated by the *horizontal bars* and *arrows*. Samples of effluent perfusate were collected for determination of free and conjugated *p*-nitrophenol. Rates of monooxygenation of *p*-nitroanisole, conjugation of *p*-nitrophenol, and uptake of oxygen were calculated from influent minus effluent concentration differences, the flow rate and liver wet weight. *Left*, data from normal control liver; *right*, data from liver perfused 2 hr after transplantation. Results, with representative error terms, are from typical experiments that were repeated four times in each group.

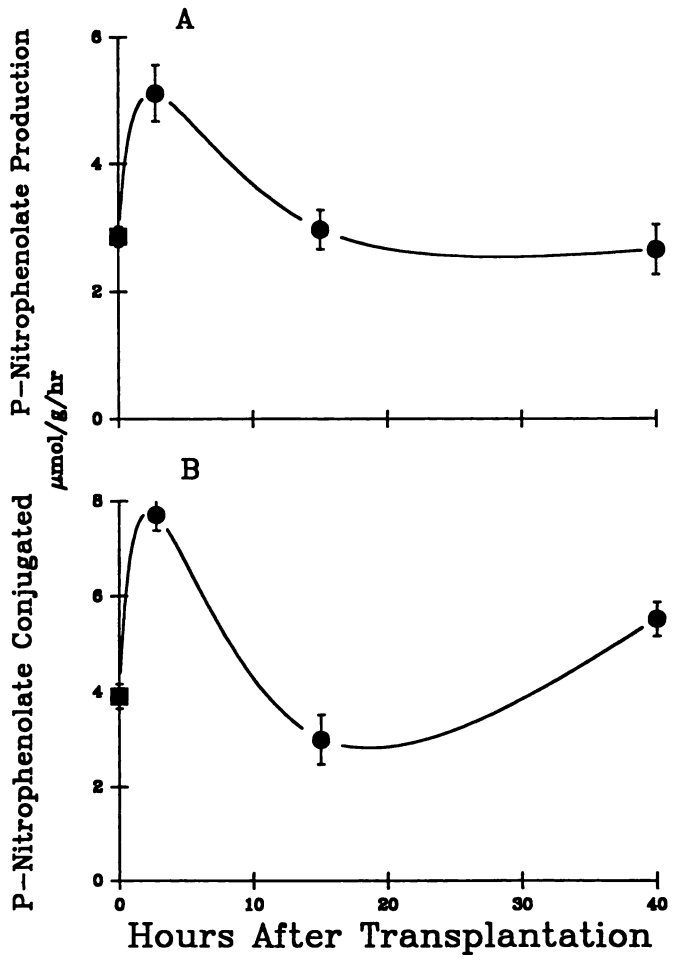


**Fig. 2.** Time course of changes in monooxygenation and conjugation in perfused liver after transplantation. Livers from fed donor rats were transplanted and perfused 2, 16, and 40 hr postoperatively, as described in Materials and Methods, using experimental designs depicted in Fig. 1. A, Maximal rates of monooxygenation of *p*-nitroanisole; B, conjugation of *p*-nitrophenol. Values are mean ± standard error ($n$ = 4/group).

TABLE 1

**Effect of liver transplantation and gadolinium chloride treatment on hepatic drug metabolism in perfused livers from fed rats**

Livers from fed donor rats were transplanted as described in Materials and Methods. Sham-operated animals were opened surgically but livers were not removed. Cold storage was for 24 h, in Euro-Collins solution, before perfusion. Some rats were treated with gadolinium chloride (10 mg/kg, intravenously, 30 hr before cold storage of the liver). Livers were perfused 2 hr after transplantation, as described in Materials and Methods and depicted in Fig. 1. Data represent maximal rates of *p*-nitrophenol production and conjugation. Oxygen uptake data are from 20 min of perfusion just before addition of drug. Results are mean ± standard error ($n$ = 4 or 5 livers/group).

| Treatment | Oxygen uptake | Monoxygenation | Conjugation |
|---|---|---|---|
| | μmol/g/hr | μmol/g/hr | μmol/g/hr |
| Control | 114 ± 6 | 2.9 ± 0.2 | 3.3 ± 0.6 |
| Sham-operated | 105 ± 10 | 2.6 ± 0.3 | 2.9 ± 0.2 |
| Cold-stored | 81 ± 11 | 2.6 ± 0.1 | 2.5 ± 0.6 |
| Transplant | 197 ± 3[a] | 5.1 ± 0.4[a] | 7.7 ± 0.1[a] |
| Transplant + $GdCl_3$ | 138 ± 11[b] | 3.4 ± 0.2[b] | 5.6 ± 0.4[b] |
| Control + $GdCl_3$ | 119 ± 14 | 3.2 ± 0.5 | 5.0 ± 0.6 |

[a] $p < 0.05$ for the comparison between transplant and control.
[b] $p < 0.05$ for the comparison between transplant and transplant plus $GdCl_3$ treatment.

and flowed past a Clark-type oxygen electrode before being discarded. The bile duct was cut, allowing bile to mix freely with the effluent perfusate. To assess monooxygenation, *p*-nitroanisole (0.2 mM) dissolved in Krebs-Henseleit buffer was infused, and total *p*-nitrophenolate was measured spectrophotometrically in samples of effluent perfusate collected at 4-min intervals after enzymatic hydrolysis of conjugates (13). Conjugation *per se* was measured by infusion of *p*-nitrophenol (0.07 mM) and assessment of *p*-nitrophenol liberated in the effluent by β-glucuronidase (14). Release of lactate, pyruvate, and glucose from endogenous glycogen was measured enzymatically in the effluent perfusate, as described elsewhere (15). Rates were calculated from influent minus effluent concentration differences, the flow rate, and the liver wet weight.

**Orthotopic liver transplantation.** Livers were transplanted orthotopically according to procedures described by Kamada and Calne (16) and Zimmerman *et al.* (17). Briefly, explanted livers were rinsed with 10 ml of Ringer's solution at 4°, followed by 15 ml of Euro-Collins solution at 4°. Cuffs were placed on the portal vein and inferior hepatic vena cava, and livers were stored for 4 hr in Euro-Collins solution at 1°. Implantation was performed by connecting the suprahepatic vena cava with a running suture, inserting cuffs into the appropriate vessels, and anastomosing the bile duct with an intraluminal splint. Just before reperfusion with blood, grafts were rinsed with 5 ml of Ringer's solution at 21°. The ischemic interval due to clamping of the portal vein during transplantation did not exceed 20 min.

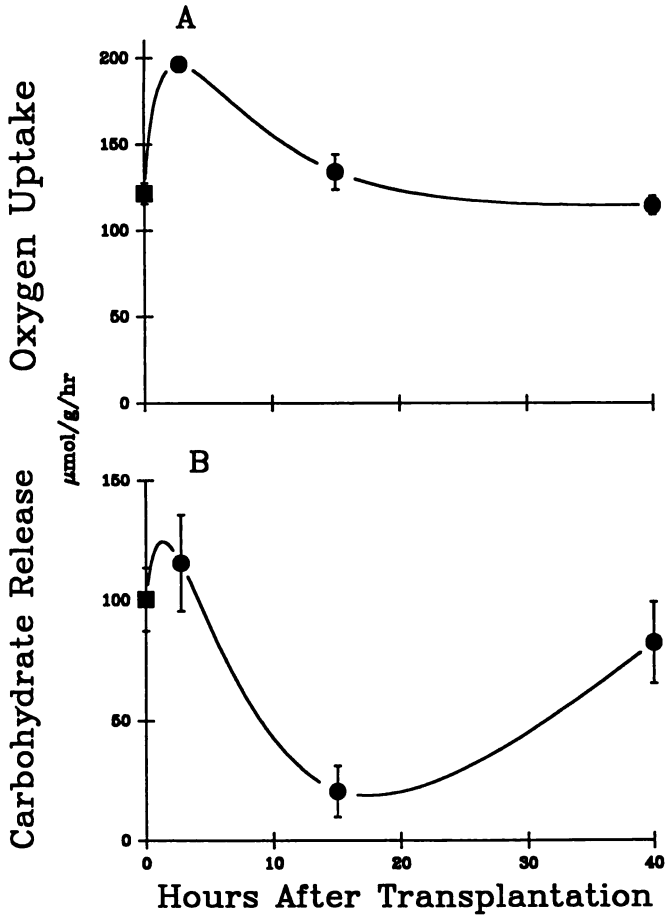


**Fig. 3.** Time course of changes in $O_2$ uptake and carbohydrate release in perfused livers after transplantation. Conditions as in Figs. 1 and 2. A, Maximal rates of $O_2$ uptake; B, carbohydrate release (glucose plus lactate plus pyruvate). Values are mean ± standard error ($n$ = 4/group).

TABLE 2

**Effect of liver transplantation and gadolinium chloride treatment on microsomal drug metabolism**

Hepatic microsomes were prepared and incubated with *p*-nitroanisole (0.5 mM) or *p*-nitrophenol (0.2 mM) and rates of monooxygenation and conjugation were determined as described in Materials and Methods. Results are mean ± standard error ($n$ = 5–7/group).

| Treatment | *p*-Nitrophenol production | *p*-Nitrophenol glucuronidation |
|---|---|---|
| | *nmol/min/mg of protein* | *nmol/min/mg of protein* |
| Control | 3.0 ± 0.3 | 2.4 ± 0.2 |
| Transplant | 3.3 ± 0.4 | 2.6 ± 0.3 |
| Transplant + $GdCl_3$ | 4.1 ± 0.6 | 2.7 ± 0.4 |

**Microsomal monooxygenation and glucuronidation.** Hepatic microsomes were prepared by standard techniques of differential centrifugation (18). *p*-Nitroanisole *O*-demethylase activity was determined in microsomal pellets resuspended in 0.15 M KCl. Assays were performed at 37° in 25-ml Erlenmeyer flasks containing 5 mM $MgCl_2$, 0.5 mM *p*-nitroanisole, microsomes (11–13 mg of protein/ml), and an NADPH-generating system consisting of 0.4 mM $NADP^+$, 30 mM isocitrate, and 0.2 units of isocitrate dehydrogenase, in a final volume of 2.0 ml of 0.18 M potassium phosphate buffer, pH 7.4. Incubations were initiated by the addition of an NADPH-generating system (19) and were terminated after 10 min by the addition of 0.5 ml of 0.6 M perchloric acid. After centrifugation to remove denatured protein, 1.0 ml of supernatant was mixed with 0.1 ml of 12 N NaOH. This mixture was recentrifuged, and the supernatant was used for the spectrophotometric determination of *p*-nitrophenol ($\epsilon_{436}$ = 7.11 $mM^{-1}$ $cm^{-1}$). Mi-

TABLE 3

**Effect of liver transplantation and gadolinium chloride treatment on hepatic drug metabolism in perfused livers from fasted rats**

All condtions were as in Table 1, except that rats were fasted for 24 hr before liver perfusion or organ cold storage. Data repesent maximal rates of *p*-nitrophenol production and conjugation. Oxygen uptake data are from 20 min of perfusion just before addition of drug. Results are mean ± standard error ($n$ = 4 livers/group).

| Treatment | Oxygen uptake | Monoxygenation | Conjugation |
|---|---|---|---|
| | *μmol/g/hr* | *μmol/g/hr* | *μmol/g/hr* |
| Control | 122 ± 2 | 3.2 ± 0.3 | 1.5 ± 0.1 |
| Transplant | 159 ± 9[a] | 4.2 ± 0.3[a] | 3.9 ± 0.5[a] |
| Transplant + $GdCl_3$ | 130 ± 13 | 3.4 ± 0.2[b] | 2.5 ± 0.2[b] |

[a] $p < 0.05$ for comparison between transplant and control.
[b] $p < 0.05$ for comparison between transplant and transplant plus $GdCl_3$ treatment.

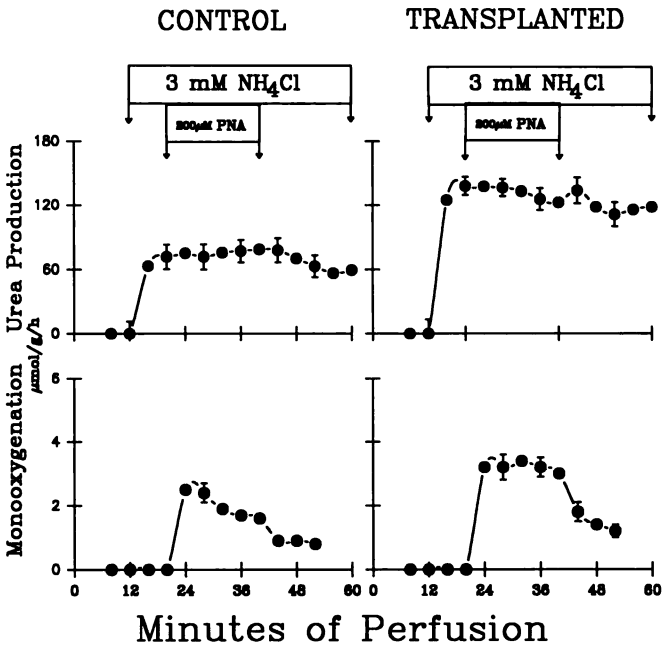


**Fig. 4.** Effect of ammonium chloride on *p*-nitroanisole metabolism in perfused livers from normal and transplanted rats. Ammonium chloride (3 mM) was infused for 48 min and *p*-nitroanisole (*PNA*) was infused for 20 min, as indicated by the *horizontal bars* and *arrows*. Other conditions were as in Fig. 1. Values are mean ± standard error ($n$ = 4/group).

TABLE 4

**Effect of ammonium chloride on hepatic drug metabolism in perfused livers from fed rats**

Livers were perfused for 12 min, to establish a steady rate of oxygen uptake (e.g., see Fig. 4). Ammonium chloride (3 mM) was then infused to stimulate urea synthesis. After perfusion with $NH_4Cl$ for 8 min, *p*-nitroanisole (200 μM) was infused for 20 min (Fig. 4). Samples of effluent perfusate were collected every 4 min, and *p*-nitrophenol and urea production were measured. Data represent maximal rates of *p*-nitrophenol production and urea production. Oxygen uptake data are from 20 min of perfusion just before addition of drug. Results are mean ± standard error ($n$ = 4/group).

| Treatment | Oxygen uptake | Monoxygenation | Conjugation |
|---|---|---|---|
| | *μmol/s/hr* | *μmol/g/hr* | *μmol/s/hr* |
| Control | 129 ± 14 | 2.7 ± 0.5 | 82 ± 12 |
| Transplant | 194 ± 12[a] | 3.5 ± 0.5 | 145 ± 13[a] |
| Transplant + $GdCl_3$ | 165 ± 23 | 2.7 ± 0.3 | 93 ± 13 |

[a] $p < 0.05$ for the comparison between transplant and control groups.

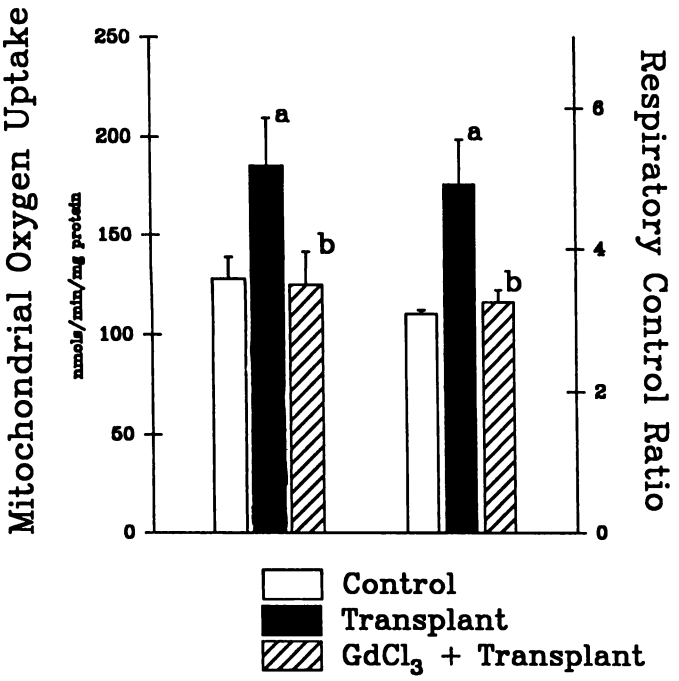


**Fig. 5.** Effect of transplantation on mitochondrial respiration. Liver mitochondria from control and transplanted rats (2 hr postoperatively) were isolated and incubated as described in Materials and Methods. *Left*, state 3 rates of oxygen uptake were calculated from changes in oxygen concentration per minute per milligram of protein; *right*, respiratory control ratios (state 3/state 4). Values are mean ± standard error. *a*, $p < 0.05$ for comparison between control and transplant groups; *b*, $p < 0.05$ for comparison between transplant and $GdCl_3$-treated groups ($n$ = 7/group).

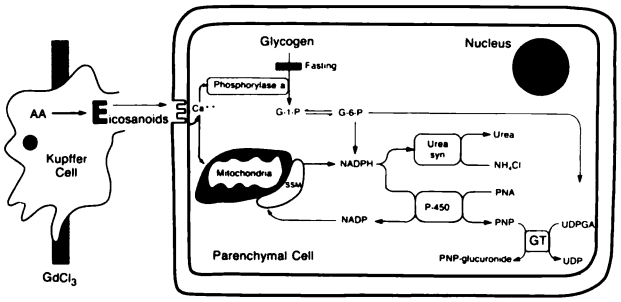


**Fig. 6.** Scheme depicting role of Kupffer cells in stimulation of drug metabolism by liver transplantation. *GT*, glucuronosyltransferase; *AA*, arachidonic acid; *SSM*, substrate-shuttle mechanism; *PNP*, *p*-nitrophenol; *PNA*, *p*-nitroanisole; *G-1-P*, glucose-1-phosphate; *G-6-P*, glucose-6-phosphate.

crosomal *p*-nitrophenol glucuronosyltransferase activity was assayed using UDPGA (0.2 mM) as cofactor and *p*-nitrophenol (0.2 mM) as substrate, as described in detail elsewhere (20). Protein was determined by the biuret reaction (21).

**Isolation of mitochondria.** Rat liver mitochondria from untransplanted control or transplanted livers were isolated by standard procedures of differential centrifugation (22). Isolated mitochondria were incubated at room temperature in buffer (pH 7.2) containing 100 mM KCl, 50 mM sucrose, 20 mM Tris·HCl, 5 mM Tris-phosphate, and 10 μM rotenone. Oxygen uptake was measured in a closed vessel (1.0 ml) with a Clark-type oxygen electrode, after addition of succinate (1.0 μmol) and ADP (0.5 μmol) (23).

**Statistical analyses.** Statistical analyses were performed by using analysis of variance (24). Results were presented as mean ± standard error and the criterion for significance was $p < 0.05$.

## Results

**Effect of liver transplantation and gadolinium chloride treatment on hepatic drug metabolism in perfused livers from fed rats.** Rates of oxygen uptake, *p*-nitroanisole, *O*-demethylation, and conjugation of *p*-nitrophenol in perfused livers from normal and transplanted rats are depicted in typical experiments in Fig. 1 and summarized in Table 1. Two hours after liver transplantation, all parameters studied were elevated significantly, by nearly 2-fold. However, no significant differences between perfused livers from cold-stored and control rats were detected. Also, no significant differences between perfused livers from sham-operated and control rats were detected. Because transplantation activates Kupffer cells (6), donor rats were pretreated with the Kupffer cell toxicant gadolinium chloride 30 hr before liver storage. This treatment largely prevented the increase in oxygen uptake, monooxygenation, and conjugation observed after liver transplantation. However, gadolinium chloride treatment had no effect on the parameters studied in livers from control rats (Table 1).

**Time course of changes in monooxygenation, conjugation, oxygen uptake, and carbohydrate release in perfused liver after transplantation.** Livers from fed donor rats were transplanted and perfused 2, 16, and 40 hr postoperatively, using the experimental design depicted in Fig. 1. Monooxygenation and conjugation were increased significantly 2 hr after transplantation before returning to normal values in about 16 hr (Fig. 2). Oxygen uptake was also increased dramatically 2 hr after transplantation but returned to near-normal values in 16 hr. Carbohydrate release, however, was decreased significantly 16 hr after surgery (Fig. 3).

**Effect of liver transplantation and gadolinium chloride treatment on microsomal drug metabolism.** To test the hypothesis that transplantation stimulated drug metabolism by increasing cytochrome P-450 and glucuronosyltransferase activities, *O*-demethylation of *p*-nitroanisole and glucuronidation of *p*-nitrophenol were studied in isolated hepatic microsomes incubated with an NADPH- or UDPGA-generating system, respectively. In contrast to data obtained with the perfused liver, neither monooxygenation of *p*-nitroanisole nor conjugation of *p*-nitrophenol was altered by transplantation (Table 2). Thus, the increase in drug metabolism observed in the intact organ after transplantation and its prevention by $GdCl_3$ treatment cannot be explained at the level of the endoplasmic reticulum.

**Effect of liver transplantation and gadolinium chloride treatment on hepatic drug metabolism in perfused liver from fasted rats.** Fasting decreases substrate for the generation of NADPH via the pentose cycle (20). To investigate whether transplantation stimulated drug metabolism by affecting the pentose cycle, livers from fasted rats were studied. The increase in all parameters due to transplantation and the blockage by gadolinium chloride treatment were similar in livers from fasted and fed rats (compare Tables 1 and 3). Therefore, it was concluded that NADPH from the pentose cycle was not necessary for the observed increase in drug metabolism due to transplantation.

**Effect of ammonium chloride on *p*-nitroanisole metabolism in perfused livers from normal and transplanted rats.** To test the hypothesis that transplantation stimulated drug metabolism by increasing mitochondrial NADPH supply, a competing pathway for NADPH supply was activated. Urea

synthesis from ammonium chloride has an absolute requirement for mitochondrial NADPH (25). In these experiments, transplantation increased urea production from $NH_4Cl$ significantly (Fig. 4). Concomitantly, the increase in monooxygenation was minimized, suggesting the involvement of mitochondrial NADPH in the activation due to transplantation. Furthermore, gadolinium chloride treatment prevented this increase in urea synthesis, suggesting that mediators from Kupffer cells increase mitochondrial NADPH supply (Table 4).

**Effect of transplantation on mitochondrial respiration.** To test this hypothesis, the effect of transplantation on isolated mitochondria was examined, because oxygen uptake in the liver is predominantly due to mitochondrial respiration (23). Both state 3 rates of oxygen uptake and the respiratory control ratio were increased significantly after liver transplantation (Fig. 5). Further, increases in both of these parameters were prevented by gadolinium chloride treatment.

## Discussion

### Changes in Drug Metabolism after Liver Transplantation

**Stimulation of monooxygenation and conjugation.** The goal of this study was to document any changes in the metabolism of drugs after liver transplantation. Although transplantation was performed under survival conditions, drug metabolism was nearly doubled after only 2 hr. This led to the following question: how are monooxygenation and conjugation activated by transplantation? Activities of cytochrome P-450 and glucuronosyltransferase were not elevated in hepatic microsomes (Table 2); therefore, effects at the level of the endoplasmic reticulum can be ruled out. On the other hand, it is well known that monooxygenation of *p*-nitroanisole and conjugation of *p*-nitrophenol require the cofactors NADPH and UDPGA, respectively (9). In experiments with microsomes, cofactors were added in excess and thus could not be rate limiting; however, in the whole organ, cofactor supply limits monooxygenation (9). Thus, we tested the hypothesis that cofactor supply was increased after transplantation.

**Cofactor-mediated stimulation of monooxygenation and conjugation.** Cofactor for monooxygenation arises from the pentose cycle or mitochondrial oxidations (9). By donor rat fasting, NADPH supply from the pentose cycle was decreased in the graft, due to substrate limitation (9). Under these conditions, stimulation of drug metabolism was still observed after transplantation (Table 3). Therefore, we conclude that the pentose cycle is not a major source of cofactor supply for drug metabolism after liver transplantation.

Thurman and Kauffman (9) have pointed out that transport of reducing equivalents across intracellular membranes influence rates of mixed-function oxidation and conjugation in whole-cell preparations (e.g., NADPH supply is an important rate-controlling factor for intact cells). In addition, Belinsky *et al.* (26) have demonstrated that mitochondrial oxidations supply most of the reducing equivalents for the mixed-function oxidation of *p*-nitroanisole in the fed state. Monooxygenation and glucuronidation changed in parallel with oxygen uptake after surgery (Figs. 2 and 3). It is possible, therefore, that the increase in oxygen uptake explains the stimulation of monooxygenation, because NADPH is produced by mitochondria. Moreover, Sies *et al.* (25) have reported that synthesis of urea from $CO_2$ and $NH_4^+$ utilizes mitochondrial NADPH. ATP of mitochondrial origin is also required for UDPGA synthesis (9). In support of the hypothesis that mitochondria are involved in stimulation of monooxygenation by transplantation, it was observed that urea synthesis from ammonium chloride, a mitochondrial reaction that requires NADPH, was increased after transplantation. Further, the stimulation of monooxygenation due to transplantation was blunted (Table 4). Weigl and Sies (27) demonstrated that rates of urea formation are about 5-fold higher than rates of monooxygenation. Further, when mitochondria were isolated 2 hr after transplantation, state 3 rates of $O_2$ uptake and respiratory control ratios were increased significantly (Fig. 5). Taken together, these data strongly support the hypothesis that mitochondrial cofactor supply for monooxygenation and conjugation is increased after transplantation. Thus a "hypermetabolic state" was observed as soon as 2 hr after transplantation. Yuki and Thurman (28) have described a similar stimulation of oxygen uptake, which involves hormones, after acute ingestion of alcohol in rats. However, in the transplant model, hormonal release induced by surgical stress alone failed to induce a hypermetabolic state (Table 1). The question then becomes the following: how does this hypermetabolism occur after transplantation?

### Involvement of Kupffer Cells in the Stimulation of Drug Metabolism by Liver Transplantation

Kupffer cells are hepatic resident macrophages, and several studies have reported that they are activated after cold storage and reperfusion (6, 29, 30). Kupffer cells are also the major source of eicosanoids and cytokines in the liver (31, 32), mediators that are increased after transplantation (33, 34). This led to the idea that Kupffer cells were involved in the stimulation of oxygen uptake after transplantation (see Fig. 6). The stimulation of oxygen uptake could increase cofactor supply and, therefore, explain the stimulation of drug metabolism observed in this study. Therefore, we tested the hypothesis that Kupffer cells stimulated monooxygenation and conjugation after transplantation by influencing cofactor supply. For this purpose, we used gadolinium chloride to destroy Kupffer cells (35). Gadolinium chloride treatment did not influence oxygen uptake in perfused livers from normal rats (Table 1); however, in transplanted livers, it blocked the stimulation of oxygen uptake in both the fed and fasted states (Tables 1 and 3). Stimulation of urea production and mitochondrial respiration was also blocked (Table 4; Fig. 5). Therefore, we conclude that transplantation activates Kupffer cells, leading to the release of mediators that produce a hypermetabolic state in parenchymal cells. This, in turn, stimulates monooxygenation and conjugation dramatically (see Fig. 6). Further work should focus on the mechanism of this important phenomenon.

### Clinical Applications

It has been reported that oxygen uptake was increased in humans after liver transplantation (36), suggesting that a hypermetabolic state also occurs immediately after graft reperfusion in humans. It is also known that the early postoperative period is critical because of the risk of primary graft nonfunction, which occurs in about 15% of the cases and necessitates retransplantation (37). The acute stimulation of oxygen uptake observed in this study could play a role in the mechanism of graft failure, making the oxygen gradient along the hepatic sinusoid steeper. This could produce hypoxic injury in downstream pericentral regions of the liver lobule. Indeed, pericen-

tral injury is observed commonly after liver transplantation (38).

Many drugs are used postoperatively, including the all-important immunosuppressive therapy. However, excessive immunosuppression leads to life-threatening infections and toxic effects, especially kidney injury, whereas insufficient treatment leads to graft rejection. By modulation of Kupffer cells or their products, it may be possible to manage blood levels of immunosuppressive drugs with much more accuracy than is now possible in the clinic.

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