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In situ formation of the acetaminophen metabolite covalently bound in kidney and lung Supportive evidence provided by total hepatectomy

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Acetaminophen may produce extrahepatic, as well as hepatic, lesions in rodents [1–3], and in humans [4]. After administration of acetaminophen, a metabolite covalently bound to macromolecules is found mainly in the liver but also in extrahepatic organs [1–3]. It is not yet clear, however, whether the metabolite bound in extrahepatic tissues is directly formed *in situ* [1, 2] or is formed in the liver and exported elsewhere [3]. Although the liver is probably the main site of formation of the reactive metabolite in the body, the metabolite may be too unstable to leave the liver and reach extrahepatic organs. McMurtry, Snodgrass and Mitchell [1] reported that pretreatment of male Fischer rats with 3-methylcholanthrene increased the *in vitro* covalent binding of the reactive metabolite of acetaminophen to hepatic microsomes but not to renal microsomes. This pretreatment increased the *in vivo* covalent binding to hepatic proteins but not to renal proteins [1]. It was suggested that the reactive metabolite covalently bound to proteins in the kidney did not come from the liver but was instead directly formed in the kidney [1]. In this communication, we report the effect of total hepatectomy on the *in vivo* covalent binding of the reactive metabolite of acetaminophen in kidney and lung. Results obtained by this different approach confirm the view that bound metabolites are mainly formed *in situ*.

Unlabeled acetaminophen was purchased from Sigma Chemical Co. (St Louis, MO). [^{3}H]Acetaminophen (generally labeled, sp.act. 5.6 Ci/mmole) was purchased from New England Nuclear (Boston, MA). Its radiochemical purity, checked by thin-layer chromatography, was found to be higher than 99%. Male Sprague–Dawley rats were purchased from Charles River, Saint-Aubin-lès-Elbeuf, France. Rats were fed a standard diet (Autoclavé 113, UAR, Villemoisson-sur-Orge, France) given *ad lib.* Animals were operated upon under ethyl ether anaesthesia. Total hepatectomy was performed in 3 stages as previously reported [5]; a catheter was inserted in the inferior vena cava and an infusion of glucose was started; another catheter was placed in a femoral artery. Control rats were subjected to a laparotomy and catheters were placed in the inferior vena cava and in a femoral artery. Animals weighed 300–360 g at the time of hepatectomy or laparotomy. Hepatectomized or laparotomized rats were held in restraining cages that were placed in heating chambers where the ambient temperature was automatically adjusted to maintain a rectal temperature of 37.5°. Hepatectomized rats remained in good hemodynamic and general condition during the whole period of the metabolic study.

The administration of acetaminophen was started 10 min after the completion of surgery. Acetaminophen was dis-

Table 1. Plasma concentrations of unchanged acetaminophen, and metabolite irreversibly bound to proteins in various tissues*

	Plasma concentration of unchanged acetaminophen			Metabolite irreversibly bound to proteins at 90 min		
	30 min	60 min	90 min	Liver	Kidney	Lung
		(μg/ml)			(nmoles/g tissue)	
Laparotomized rats	439 ± 39	440 ± 29	453 ± 40	19 ± 4	15 ± 4	7 ± 3
Hepatectomized rats	440 ± 28	441 ± 38	448 ± 31		11 ± 3	6 ± 3

* In laparotomized rats, a loading dose of 400 mg/kg [^{3}H]acetaminophen (sp.act., 0.2 mCi/mmole) was followed by the infusion, during the next 90 min of 160 mg/kg/hr of [^{3}H]acetaminophen. In hepatectomized rats, a loading dose of 350 mg/kg was followed by the infusion of 40 mg/kg/hr. Blood was drawn 30, 60, and 90 min after the onset of the infusions and rats were killed after obtention of the last blood sample. Plasma concentration of unchanged acetaminophen was measured by the method of Glynn and Kendal [6]. The amount of metabolite irreversibly bound to proteins in various tissues was measured as previously reported [8, 9].

Results are means ± S.D. for 5 rats.

solved in 0.08 M NaCl, 0.5 M NaOH at a concentration of 100 mg/ml; the pH of the solution was 10.6. This solution was infused through the catheter placed in the inferior vena cava: we first used a high infusion rate to deliver a loading dose of acetaminophen in 8 min; this loading dose was followed by a slower infusion rate for the next 90 min. Blood was drawn from the femoral artery 30, 60 and 90 min after the onset of the slow infusion rate of acetaminophen. Plasma concentrations of acetaminophen were measured by the method of Glynn and Kendal [6]. This method accurately measures unchanged acetaminophen [7]. In preliminary experiments, we determined (starting from an arbitrary loading dose of 400 mg/kg in control rats) the infusion rates which maintained a constant and similar plateau in the plasma concentrations of unchanged acetaminophen in control (laparotomized) and hepatectomized rats. The selected loading doses and infusion rates are given in the legend for Table 1. The same experiment was now repeated with [^{3}H]acetaminophen (sp. act., 0.2 mCi/mmole) and rats were killed after the last blood sample (90 min). Tissue fragments were homogenized in 3 vols. of 0.154 M NaCl and the amount of metabolite irreversibly bound to tissue proteins was measured as previously described [8, 9].

The results are set out in Table 1. The selected loading doses and infusion rates ensured a constant and similar plateau in the plasma concentrations of unchanged acetaminophen in control (laparotomized) and in hepatectomized rats. The amount of metabolite irreversibly bound to proteins in kidney and lung was not significantly lower in the hepatectomized rats than in the control rats (Student's *t*-test).

It is apparent from these observations that most of the metabolite bound in extrahepatic tissues cannot come from the liver but is probably directly formed *in situ*. Total hepatectomy (with maintenance of similar concentrations of the parent compound in control and in liverless animals) may prove a useful approach to determine the hepatic or *in situ* origin of metabolites covalently bound in extrahepatic tissues.

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