tion of Sarcoma 180 DNA was also observed after mixing IQ-1 and purified DNA *in vitro*. The use of EDTA to minimize enzymatic degradation of DNA during the cell lysis on top of sucrose gradients, as well as in the alkaline sucrose environment employed for DNA denaturation, suggests that IQ-1 induces the fragmentation of Sarcoma 180 DNA *in vivo*, rather than on the sucrose gradient. The precise mechanism(s) responsible for fragmentation is unknown; however, it is conceivable that it is due to prolonged inhibition of the enzyme, ribonucleoside diphosphate reductase. No attempt was made to estimate the molecular weight of the IQ-1-induced DNA fragments for, although some reports have been published which indicate that a direct relationship exists between sedimentation distance and the molecular weight of single strands [17–19], the relationship appears to be more complicated [18, 20]. It has been demonstrated that the sedimentation velocity of DNA in an alkaline sucrose gradient is dependent upon the cell concentration [18, 21]. This relationship was also found to be of importance in this system, and the addition of $>1 \times 10^6$ Sarcoma 180 cells to the gradient led to aggregation of high molecular weight DNA.

The experiments employing gel filtration techniques demonstrate that binding between DNA and IQ-1 takes place, at least *in vitro*. The association of IQ-1 with a new fraction of apparently lower molecular weight DNA suggests that IQ-1 is primarily bound to modified DNA, possibly implying that the binding of IQ-1 to DNA increases its susceptibility to shearing as it passes through the Sephadex column. The IQ-1 retained by the Sephadex column is presumably either free or bound to DNA degradation products, such as nucleotides.

Regardless of the precise mechanism by which IQ-1 induces the breakage of Sarcoma 180 DNA, the finding of this second site of action appears to be of major significance to the cytotoxic mechanism of action of α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazones, since it creates a lesion in the genome which is reinforced by blockade of ribonucleoside diphosphate reductase.

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Effects of activators *in vitro* on rabbit lung and liver microsomal UDP-glucuronyltransferase activity

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UDP-glucuronate glucuronyltransferase (acceptor unspecific) (EC 2.4.1.17) activity toward several exogenous acceptor substrates has been reported in lung microsomes from various animal species [1–5]. Aitio [6] detected levels of microsomal UDP-glucuronyltransferase activity in rat and guinea pig lung equal to more than one-third the liver specific activity. Furthermore, the pulmonary enzyme exhibited a 2 to 3-fold activation by digitonin *in vitro*, suggesting a latent state. Activation of UDP-glucuronyltransferase *in vitro* by a number of materials has been previously described [7–14]. Recent work in our laboratory failed to demonstrate measurable "native" UDP-glucuronyltransferase activity in rabbit lung microsomes toward *p*-nitrophenol or phenolphthalein as acceptor substrates [15]. The present study was designed to determine if the enzyme was truly absent from rabbit lung or if it was present in

a latent form which could be revealed by various activators added *in vitro*.

Microsome preparation. Male New Zealand white rabbits (2–3 kg) that were allowed free access to laboratory chow and tap water were used in all experiments. The animals were killed by cervical fracture and the lungs and livers quickly excised and rinsed in chilled KCl–Tris buffer (150 mM KCl–50 mM Tris), pH 7·4. Microsomes were then isolated as previously described [15].

Enzyme assays. UDP-glucuronyltransferase activity was measured aerobically at 37° using *p*-nitrophenol [16] and *o*-aminophenol [17, 18] as acceptor substrates. Assays were conducted under zero order kinetics regarding acceptor and donor substrates, and activities measured were linear with protein concentration and time. Incubation mixtures were as follows:

(1) *p*-Nitrophenol as substrate: microsomal protein, 1·5 mg; UDP-glucuronic acid (NH_4 salt) (UDPGA), 3·3 mM; *p*-nitrophenol, 0·23 mM; Tris-HCl buffer, pH 7·4, 66 mM final concentration; all in a final volume of 1·5 ml. Since the assay for UDP-glucuronyltransferase utilizing *p*-nitrophenol as substrate measures substrate loss, it was important to verify that the product formed was indeed the glucuronide. When incubations were performed as usual, then stopped by placing them into boiling water, substrate lost as a result of conjugation could be quantitatively recovered (99–100 per cent) by adjusting the pH to 5·0, adding β-glucuronidase and reincubating at 37°. No products were detected nor was substrate lost in the absence of UDPGA, or in the presence of uridine-5′-diphospho-*N*-acetylglucosamine (UDPNAG), revealing the absolute requirement of UDP-glucuronyltransferase for UDPGA as donor substrate.

(2) *o*-Aminophenol as substrate: microsomal protein, 6·0 mg; UDP-glucuronic acid, 5 mM; *o*-aminophenol, 1·0 mM; ascorbic acid, 0·38 mM; Tris-HCl buffer, pH 7·4, 83 mM; in a final volume of 3·0 ml. Reactions were initiated by adding substrate, and incubations were conducted aerobically for either 15 or 30 min using a Dubnoff metabolic shaker. Appropriate substrate, zero time and boiled enzyme blanks were included, and activity at 0–4° was found to be negligible. Under the conditions of assay, there was no significant metabolism of *p*-nitrophenol, *o*-aminophenol or the glucuronide conjugates via alternate biotransformation pathways.

Activators. The following materials were added to incubation mixtures in a wide range of concentrations to obtain optimum activating concentrations: the detergents Triton X-100 (nonionic type), cetyl trimethyl ammonium bromide (cationic type) (CETAB), and mersalyl acid (anionic type and —SH group reagent); UDPNAG, $MgCl_2$ and *N*-nitroso diethylamine (diethyl nitrosamine; DENA). Several of these activators at optimal concentrations were combined in incubation mixtures to investigate the possibility of additive or synergistic activation of UDP-glucuronyl-transferase [19]. Other additives used for specific purposes were: tetra-potassium pyrophosphate, β-glucuronidase and D-glucaro-1,4-lactone. All chemicals were obtained from Sigma with the exception of *o*-aminophenol which was purchased from Eastman Chemical Co. and purified by sublimation.

Microsomal protein was measured by the method of Lowry *et al.* [20], using bovine serum albumin as a standard.

Animals treated with phenobarbital received 50 mg/kg of the sodium salt in normal saline intraperitoneally once a day for 4 days, and they were sacrificed 24 hr after the last injection. Control rabbits received normal saline.

Mersalyl acid, Triton X-100 and cetyl trimethyl ammonium bromide produced biphasic effects on hepatic UDP-glucuronyltransferase activity—up to 4-fold activation at low concentrations and inhibition at higher concentrations. In addition, they exhibited a quantitative similarity in optimum concentrations for both acceptor substrates. However, pulmonary enzyme could not be activated to measurable levels. Activation by Mg^{2+} was very slight, with essentially equal hepatic UDP-glucuronyltransferase activity at concentrations of 1–25 mM. Addition of UDPNAG resulted in an increase in the rate of glucuronidation, with concentrations in excess of 5 mM failing to further activate the liver enzyme. When Mg^{2+} was combined with UDPNAG, a synergistic effect was achieved. Table 1 summarizes the activation of UDP-glucuronyltransferase which exhibited a 4 to 5-fold increase with liver microsomes, yet showed no activation of pulmonary enzyme.

Several other combinations of activators also were employed in a further attempt to reveal glucuronide synthesis by lung microsomes. No combination of chemicals activated hepatic UDP-glucuronyltransferase beyond the maximum activation achieved using the individual activators; thus, there was no synergism observed using DENA combined with UDPNAG, CETAB, Triton X-100, or mersalyl acid, as has been reported for the rat [19]. Under no circumstances was activity demonstrable in lung microsomes.

In order to examine the possibility of endogenous inhibitors or alternate biotransformation pathways in the lung, several other experiments were conducted. Addition of lung microsomes (6 mg protein) to incubation mixtures containing liver microsomes (3 mg protein) resulted in no diminution of the hepatic UDP-glucuronyltransferase activity, suggesting that no freely diffusible endogenous inhibitor was present in lung microsomes. Failure to detect

Table 1. UDP-glucuronyltransferase activity*

Additions to incubation mixtures including Mg^{2+}	*p*-Nitrophenol as substrate		*o*-Aminophenol as substrate	
	Liver	Lung	Liver	Lung
None ("native")	2·16 ± 0·67 (14)†	ND‡(6)	1·60 ± 0·36 (13)	ND (10)
UDP-*N*-acetylglucosamine	10·49 ± 2·01 (6)	ND (6)	7·56 ± 0·36 (4)	ND (3)
Cetyl trimethyl ammonium bromide	11·18 ± 1·58 (3)	ND (2)	14·25 ± 1·11 (3)	ND (2)
Mersalyl acid	9·52 ± 0·16 (3)	ND (2)	13·97 ± 2·24 (5)	ND (2)
Triton X-100	8·15 ± 1·83 (3)	ND (2)	9·66 ± 1·38 (3)	ND (2)
Diethyl nitrosamine	2·43 ± 0·29 (2)	ND (2)	1·20 ± 0·10 (3)	ND (1)

* Activation of UDP-glucuronyltransferase activity by additions *in vitro* of materials to incubation mixtures at optimum activation concentrations. Data represent statistically significant difference from native enzyme activity ($P < 0·05$) with the exception of diethyl nitrosamine. Enzyme activities are expressed as nmoles glucuronides formed mg^{-1} min^{-1} at 37°.

† The number of animals used is shown in parentheses.

‡ ND = non-detectable activity.

UDP-glucuronyltransferase activity in the lung could conceivably be accounted for by high levels of pulmonary β-glucuronidase (EC 3.2.1.31). The activity of this enzyme using both substrates was undetectable in both liver and lung microsomes when assayed at pH 7·4. When the assay was conducted at pH 5·5, liver microsomes hydrolyzed 4–7 per cent of the glucuronides in 60 min, while lung microsomes yielded only 2–3 per cent hydrolysis. D-Glucaro-1,4-lactone has been shown to inhibit β-glucuronidase [21]. Inclusion of up to 10 mM D-glucaro-1,4-lactone in incubation mixtures failed to increase apparent UDP-glucuronyltransferase activity from "native" and activated liver microsomes, or allow activity to be detected in lung microsomes. These experiments suggest that interference by β-glucuronidase is minimal under our conditions of assay.

The possibility was investigated that lung microsomal preparations might be unusually rich in UDPGA-pyrophosphatase (EC 3.6.1.6.), which could hydrolyze UDP-glucuronic acid and thus inhibit glucuronide formation. Inclusion of 0·4 mM tetra-potassium pyrophosphate, a substrate which can serve as a competitive inhibitor of UDPGA-pyrophosphatase [22, 23], did not permit the detection of pulmonary UDP-glucuronyltransferase activity. Similarly, UDPGA in concentrations as high as 25 mM did reveal UDP-glucuronyltransferase activity from rabbit lung.

In contrast to the ability of rabbit hepatic microsomal UDP-glucuronyltransferase to be significantly activated *in vitro* by various agents, the results presented here reveal no detectable enzyme activity in pulmonary microsomes. This suggests the enzyme activity toward *o*-aminophenol and *p*-nitrophenol may be absent in the rabbit lung or completely latent under conditions of assay *in vitro*. However, one must be cautious not to generalize and conclude that UDP-glucuronyltransferase is totally absent in rabbit lung microsomes. Indeed the enzyme appears to be distinct in its specificity toward acceptor substrates; DeBernardi *et al.* [4] have shown that microsomes from rabbit lungs are capable of forming oxazepam glucuronide. The possibility exists that UDP-glucuronyltransferase activity from rabbit lung might be detectable using acceptor substrates such as 4-methylumbelliferone or bilirubin. Yet the data presented here suggest that the enzyme is lacking in the rabbit lung, and trans-species comparisons with the rat and guinea pig pulmonary UDP-glucuronyltransferase present a picture of an organ-specific species difference.

Activation of UDP-glucuronyltransferase by detergents has been proposed to involve a perturbation of the microsomal membrane phospholipid environment that reveals previously unaccessible active sites [11, 24], or releases constraining forces by phospholipids on the active conformation of the enzyme [8]. It is unlikely that the phospholipid environment of the enzyme in pulmonary microsomes of the rabbit differs so drastically from that found in hepatic microsomes that it is refractory to the agents used in this study [25]. Likewise UDPNAG, which acts as an allosteric effector of hepatic UDP-glucuronyltransferase [26], was ineffective as an activator on lung microsomal enzyme.

Native pulmonary UDP-glucuronyltransferase activity has been shown to be unresponsive to pretreatment with phenobarbital [14, 27]. However, when activated enzyme was studied, phenobarbital pretreatment markedly increased the UDP-glucuronyltransferase activity measured in rats and mice [24, 28]. In the present investigation, no native or activatable UDP-glucuronyltransferase activity toward *p*-nitrophenol or *o*-aminophenol was found in lung microsomes from rabbits that had been treated with phenobarbital.

The effects of endogenous β-glucuronidase and UDPGA-pyrophosphatase on UDP-glucuronyltransferase activity were found to be negligible and could not account for the lack of enzyme activity found in rabbit lung microsomes. In addition, detergent-type activators have been shown to enhance the activity of microsomal UDP-glucuronyltransferase without appreciably changing the activities of either pyrophosphatases or β-glucuronidase [29]. Evidence presented in this paper suggests that rabbit pulmonary microsomes are devoid of UDP-glucuronyltransferase activity toward *o*-aminophenol and *p*-nitrophenol, and reflects the multiplicity of this enzyme found in the lung of other mammalian species.

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