treatment with SKF-525A would be expected to increase the $LD_{50}$ values. In fact, the opposite effects were observed with both phenobarbital and SKF-525A.

Considering that VLB and VCR are responsible for their own observed toxic effects, the results obtained in the present work are consistent with the findings that 50–65% of a dose of tritiated VCR in rats is excreted unchanged (urine and bile)[6], but only 2–5% of a dose of tritiated VLB is excreted unchanged under similar conditions[5]. That is, agents which affect the metabolism of a drug have a greater effect on the toxicity of those drugs which are more extensively metabolized.

Whether similar relationships hold for the antitumor effects of these drugs is not known.

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## Elevation of Serum Xanthine Oxidase Following Halothane Anesthesia in the Rat

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*Summary.* Halothane anesthesia was found to be hepatotoxic in the rat, as demonstrated by a significant elevation of serum xanthine oxidase (SXO) level. SXO appeared to be a more sensitive marker of liver damage than serum glutamic oxalacetic transaminase. SXO was found to be elevated also following exposure to relative hypoxia.

In man, halothane anesthesia has been occasionally associated with hepatic damage, as evidenced clinically, histologically or biochemically[1–3]. Various animal species are believed to be less prone than man to halothane-induced liver damage, as for instance the dog[4–7], the monkey[4,7,8], the mouse[9,10] and the rat[9,11,12] in which administration of halothane by inhalation failed to produce elevation in the serum level of hepatocellular enzymes or histologically detectable abnormality. Only in the guinea-pig halothane has been found to cause early focal diffuse hepatitis, which, however, was not associated with a detectable release of the hepatocellular enzymes, glutamic oxalacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT) or lactic dehydrogenase (LDH)[13].

It has recently been shown by several investigators[14,15], and confirmed in our laboratory[16], that in the human as well as in several animal species, including the rat[17], serum xanthine oxidase activity (SXO) is a highly sensitive indicator of hepatocellular damage. It was the aim of the present study to reevaluate the possible hepatotoxicity of halothane in the rat by utilizing SXO as a marker.

*Materials and methods.* 70 Wistar rats of either sex between 100 and 120 g, were randomly divided into 7 groups of 10 animals each, as shown in the Table; one group of 10 rats served as non-anesthetized control. The rats, 2 at a time, were placed in a 6-liter glass chamber. The anesthetic agents, nitrous oxide ($N_2O$) oxygen and halothane, were delivered from an Fluotec Mark III vaporizer at a flow rate of 7 l/min. The oxygen concentration in the delivered gas mixture was regulated by an Oxygen Analyzer IL 406. The non-anesthetized control rats were kept in cages in the room.

Rats selected for reexposure were removed from the anesthetic chamber after the first exposure and kept in cages for 1 week following which the same anesthetic procedure was repeated. Blood samples obtained immediately following anesthesia by cardiac puncture, were allowed to clot, centrifuged, and the separated sera were assayed for SXO and for SGOT.

SXO was assayed radiochemically, as described by OLIVER and SPERLING[18] by measuring the enzymatic conversion of $^{14}C$-labelled hypoxanthine to uric acid. The activity of the enzyme is expressed in units/l, a unit being the amount of enzyme catalyzing the oxidation of 1 nmole of hypoxanthine to uric acid in 1 min at 37 °C. SGOT was measured according to FURUNO and SHEEMA[19].

*Results and comment.* The mean SXO and SGOT values in the non-anesthetized rats, $5062 \pm 572$ and $96.4 \pm 14.05$ units/l, respectively (see Table), were markedly higher than the serum levels of these enzymes found in healthy human subjects in our laboratory, $1.3 \pm 2.1$ and $21 \pm 8.4$ units/l, respectively[16]. This finding is in accordance with reports of other investigators[20–22]. The strikingly high

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Effect of halothane and hypoxia on SXO and SGOT levels

| Anesthetic procedure (nitrous oxide-oxygen mixture) | | Time of exposure (min) | SXO | | | SGOT | | |
|---|---|---|---|---|---|---|---|---|
| | | | units/l | significance $P_1$ | $P_2$ | units/l | significance $P_1$ | $P_2$ |
| Non-anesthetized | | | 5062 ± 572 (4232 — 6006) | | | 96.4 ± 14.05 (73 — 113) | | |
| Anesthetized | | | | | | | | |
| at 30% $O_2$ | Control | 60 | 4886 ± 698 (4156 — 6488) | | | 110.2 ± 13.2 (92 — 133) | | |
| | Halothane 1% | 60 | 9800 ± 6508 (5862 — 26650) | $< 0.05$ | | 125.6 ± 35.1 (93 — 204) | $> 0.2$ | |
| | Halothane 1% (2 exposures) | 60 | 9648 ± 6096 (4060 — 22776) | $< 0.05$ | | 138 ± 52.1 (82 — 261) | $> 0.1$ | |
| at 18% $O_2$ | Control | 30 | 9738 ± 4276 (4392 — 15890) | | $< 0.01$ | 143.7 ± 50.2 (93 — 238) | | $< 0.05$ |
| | Halothane 1% | 30 | 13624 ± 4472 (4816 — 19730) | $> 0.05$ | $< 0.001$ | 187.1 ± 49.5 (109 — 255) | $> 0.05$ | $< 0.001$ |
| | Halothane 1% (2 exposures) | 30 | 13938 ± 7088 (4756 — 29932) | $> 0.1$ | $< 0.001$ | 157 ± 46.8 (101 — 253) | $> 0.5$ | $< 0.01$ |

Values represent mean ± 1 SD (for each group of the rats) and the range is given in parenthesis. $P_1$, statistical significance (Student's $t$-test) in comparison to corresponding anesthetized control group. $P_2$, statistical significance in comparison to the control group anesthetized at 30% $O_2$.

level of SXO in the normal rat reflects the high concentration of xanthine oxidase in its liver, being the highest among all mammals studied[23]. Anesthesia with $N_2O$-70%, $O_2$-30% gas mixture did not cause elevation in SXO but did cause a small, though significant ($p < 0.05$), increase in SGOT activity. This finding may possibly be attributed to the slight hemolysis observed in some of the sera studied.

In the present study it was biochemically demonstrated that halothane anesthesia causes acute hepatocellular damage in the rat. The parameters used to reflect hepatocellular damage were the serum levels of the intracellular enzymes xanthine oxidase and glutamic oxalacetic transaminase. Determination of SGOT, despite the low tissue specificity of this enzyme, is used in man widely for assessment of myocardial infarction and hepatitis. On the other hand, xanthine oxidase is confined mainly to the liver[20, 24] and its release to the serum has been found to be highly sensitive to acute hepatocellular damage[14–16, 25]. The high sensitivity of SXO was verified again in the present study. In comparison to SGOT, SXO exhibited a markedly higher increase following the hepatotoxic procedures employed. Addition of halothane 1% to the anesthetizing gas mixture at 30% oxygen resulted in a significant 2-fold increase in SXO, as compared to the corresponding anesthetized control group, but in only a slight, non-significant, increase in SGOT ($P_1$ in the Table). Exposure of the rats to a relative hypoxic condition (18% $O_2$) resulted in a significant increase in both SXO and SGOT, as compared to the control group anesthetized at 30% $O_2$ ($P_2$ in the Table). Combination of relative hypoxia and halothane resulted, when compared with the control group anesthetized at 30% $O_2$, in the highest increase of SXO and SGOT ($P_2$ in the Table). However, this increase was not significantly higher than the levels found for these enzymes in the corresponding control group at 18% $O_2$ ($P_1$ in the Table).

Repeat experiments were designed to detect a possible sensitizing effect of halothane on the liver cells. However, reexposure to halothane anesthesia after 1 week, both at 30% and 18% oxygen, did not cause an additional increase in SXO and SGOT. These results are compatible with those obtained by other investigators in several animal species[6, 8, 9].

It is noteworthy, that, in contradistinction to our findings in the rat, halothane anesthesia has yet not been found to be hepatotoxic in other animal species, as gauged by serum levels of the commonly assayed hepatocellular enzymes[7, 8, 13]. Possibly, the failure to demonstrate hepatotoxicity of halothane in these animal species thus far may reflect the lesser sensitivity of SGOT, SGPT and LDH than of SXO, as markers.

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