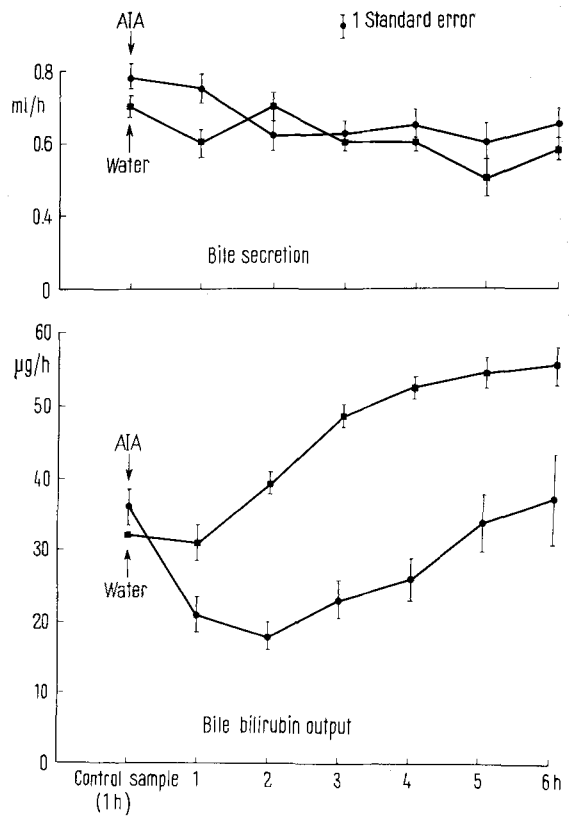
Biliary Pigment Changes During Sedormid and Allylisopropylacetamide (AIA) Administration

In a previous study it was reported that the administration of allylisopropylacetamide (AIA) to rats produces liver injury and lipid storage[1]. In the course of those experiments an increased biliary secretion with normal bile bilirubin output was observed. While these problems were under study the inhibitions of UDPG-glucoronyl transferase activity by sedormid was reported[2]. In this paper we present the results obtained on biliary pigment excretions when AIA and sedormid were given to rats.

Methods. Wistar strain albino rats of both sexes weighing between 160 and 180 g were used; they were kept on a complete rat diet and were not fasted before any of the experiments. Sedormid was given in doses of 40 mg/100 g body weight each day for 4 days, by gastric intubation under light ether anesthesia. The substance was suspended in mineral oil. 15 rats were given AIA in aqueous solution, in the same dose and time, intraperitoneally. 1 ml of mineral oil or 2 ml of water/100 g body weight were given to control rats in the same manner. Since no significant difference between the 2 treatments was observed, the results were pooled and referred to as only one control group. When the treatment was finished, each animal was submitted to a middle laparatomy and common bile duct intubated under pentobarbital anesthesia (4 mg/100 g body weight). The bile was collected for 3 h in darkness and the volume measured. Bile bilirubin concentration was determined by a modification of the JENDRASSIK and CLEGHORN method[3]. Bile and fecal urobilin concentrations were determined by the LOZZIO and ROYER method[4]. The fecal urobilin includes the amount found in the colon as well as in the feces excreted during 24 h. In another 10 rats the common bile duct was cannulated and a 1 h bile specimen was obtained. At that time, a single injection of AIA (40 mg/100 g body weight) was given i.p. Bile secretion and bile pigment output were determined hourly for 6 additional hours. In addition, 10 rats were given water and studied in the same way. Blocks of tissue from liver were submitted to microscopic light observation.

Results. The administration of sedormid or AIA for 4 days produced an increase of biliary secretion with normal bilirubin output. However, the bilirubin concentration was about 44% less than control rats. The bile urobilin output and concentration were constantly reduced by 50 and 70% of the normal values. Total fecal urobilin excretion was found to be in the normal range (Table).

The hourly study of bile secretion and bilirubin in control rats shows that, despite the decrease in bile volume, the bilirubin output increased progressively after bile duct intubation. The pigment output was twofold higher 6 h later. A single injection of AIA produced a striking decrease in the bile bilirubin output. The effect of the drug started immediately after administration and produced the maximum impairment of bilirubin excretion in the second hour. From this time, the bilirubin output began to increase, making a parallel rise to that of the control rats, but the bilirubin output was still lower after 6 h. Bile secretion diminished in this group similarly to the control group (Figure).



Bile secretion and bilirubin output after a single injection of AIA (40 mg/100 g body weight) as compared with the control No. 1. Each point is the mean of 10 rats.

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Rate of biliary pigment excretion in sedormid and AIA treated rats, for 4 days

Group	Number of rats	Bile secretion	Bile bilirubin		Bile urobilin		Total fecal urobilin
		ml/3 h	µg/ml	µg/3 h	µg/ml	µg/3 h	µg
Control	30	1.5 ± 0.3	75 ± 10	122 ± 30	10 ± 6	14.5 ± 7	115 40
Sedormid	30	3.0 ± 0.3[a]	43 ± 13[a]	129 ± 55	2.5 ± 2[a]	7 ± 3[a]	135 53
AIA	15	2.4 ± 0.8[a]	41 ± 10[a]	93 ± 22	1.8 ± 1.6[a]	4.5 ± 3[a]	97 16

[a] $P = < 0.01 > 0.001$.

The histologic observations revealed that liver structure was preserved. Hypertrophy of histiocytes and liver cells, vacuolization with scarce lipid storage, phagocytosis of hyaline body by Kuppfer's cells, and an increase in the number of binucleated cells, were the most frequent findings in the sedormid treated rats. The AIA treated rats showed the same histologic picture described in another work[1].

Discussion. The formation of the glucoronide is an obligatory step in the excretion of bilirubin in the bile of rats, since rats of the Gunn strain, which cannot form bilirubin glucoronide from UDPG[5], are unable to excrete free bilirubin, although they can excrete bilirubin glucoronide at the normal rate[6,7]. Since the inhibitory effect of one of these drugs (sedormid) on the UDPG-glucoronyl transferase activity has been demonstrated[1], these observations present further evidence that the drugs employed produce an impairment on the enzyme system involved in the conjugation of bilirubin with glucoronic acid. It should be noted that bile urobilin output and concentration were decreased at the same time as that of bilirubin. It is not known how urobilin is transported and excreted by the liver, but these findings might indicate that the liver uptake and excretion of urobilin is an active process.

Resumen. La administración de sedormid o AIA durante cuatro días, o una sola inyección de AIA reducen las excreciones de bilirubina y urobilinas en ratas. El mecanismo probable de la falla de la excreción de estos pigmentos puede ser atribuída a una inhibición de la actividad de la UDPG-glucoronil transferasa.

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Influence of Serotonin on the Radiation Sensitivity of Lactate Dehydrogenase[1]

Recently we have shown[2] that glycerol protects lactate dehydrogenase (LDH) against radiation damage when added to the enzyme solution prior to irradiation. In these experiments all of the results obtained, including an optimum concentration for the protective effect (approx. 5 λ/10 ml), are similar to results obtained using glycerol to protect catalase. In order to explain this protective effect we proposed a chelating mechanism by which glycerol forms a complex with the metal ions present in the LDH molecule. This metallo-enzyme has been found to contain zinc bound coordinately[3].

If the protective action of glycerol can be attributed to this mechanism, then it should also be possible to produce a modification in the radiation response of this enzyme with other compounds which exhibit a close interaction with bound metal ions. In a previous investigation[4] we could show by means of electron spin resonance and optical absorption studies that serotonin creatinine sulfate (5-HT) forms a complex with metal ions. Such a complex with Fe^{3+}, the active site of catalase, was thought to be responsible for the radioprotective effect observed with this enzyme. In our present investigation, the action of 5-HT on LDH was studied.

12 λ of a rabbit muscle LDH stock solution (49 mg/ml) (Mann Research Laboratories, New York, New York) were diluted to 100 ml with 0.035 M phosphate buffer, pH 7.4, making a final LDH concentration of $4.3 \cdot 10^{-8} M$. 5-HT (Nutritional Biochemical Corporation, Cleveland, Ohio) was dissolved in the LDH solution prior to irradiation. The 5-HT concentrations used are mentioned in Figure 1. The reduced form of diphosphopyridine nucleotide, NADH (Mann Research Laboratories, New York, New York), was used as a substrate in a concentration of $3.45 \cdot 10^{-4} M$.

1 ml samples of LDH solution were irradiated in open Lucite containers with different exposure doses ($0–9 \cdot 10^5$ R). The irradiations were done with a beryllium-window X-ray tube (100 kV, 12 mA, HVL 0.064 mm Al;

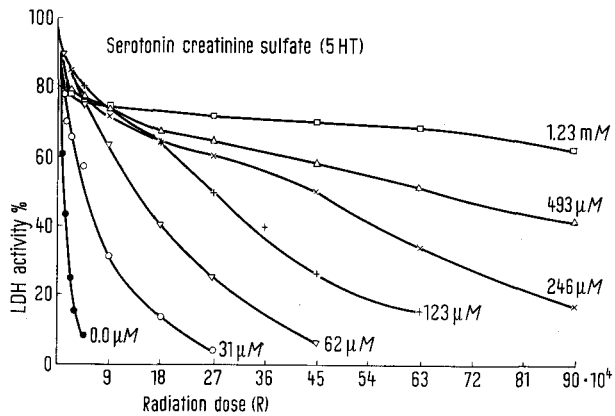

Fig. 1. The influence of different concentrations of serotonin creatinine sulfate (5-HT) on the radiation sensitivity of lactate dehydrogenase.

[1] This investigation was supported in part by the Public Health Service Research Grant No. RH 00474, Division of Radiological Health.
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