acid from D-glucuronolactone results in a higher D-glucaric acid production, leading to a higher D-glucaric acid excretion. A similar explanation might fit for the sex difference in the L-ascorbic acid response upon stimulation. Recently sex difference in the activity of liver glucuronolactone reductase and of liver L-gulonolactone oxidase has been shown by Stubbs and McKernan.[11]

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Effect of sodium diethylbarbiturate on xanthine dehydrogenase

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IN A PREVIOUS paper it was shown that xanthine oxidase and dehydrogenase activities (XO, XD) decrease sharply in the blood serum and brain of rats 3 hr after the beginning of narcosis produced by sodium diethylbarbiturate (Medinal).[1] Since XO is associated with lipids through lipoprotein bonds[2,3] and that there is a correlation between enzymatic activity and unsaturated fatty acids[4] it seemed of interest to know if the breakage of the lipid-enzyme association *in vitro* and *in vivo* could account for some of the inhibition produced by barbiturates. In the present note results obtained with the purified enzyme submitted to different treatments are reported. Carbon tetrachloride exerts *in vivo* a dissociation of the lipid enzyme complex increasing its activity. Therefore, the experiments with CCl_4 were also included to show the effect on the barbiturate *in vivo*.

Medinal is inhibitory *in vitro* when incubated with serum or brain homogenates for 30–60 min at 37° but only slightly if not incubated. However, when liver preparations were submitted to treatments which liberate the lipid material before Medinal addition such as heating at 56°/30 min, treatment with *Tween 80* (1%)/30 min, extraction with butanol, addition of sodium desoxycholate (1·5 mg/ml

enzyme solution), there is an increase in the inhibitory effect of Medinal. Fig. 1 summarizes these results. XD was prepared from rat liver homogenates (0·2 M Na_2HPO_4, 1:5 w/v). To each 100 ml of the homogenate 47 ml of saturated ammonium sulphate solution were added. The protein salted out was separated and to each 100 ml of the supernatant was added 47 ml of saturated ammonium sulphate solution. The precipitated protein was dissolved in phosphate buffer pH 7·4 and dialysed

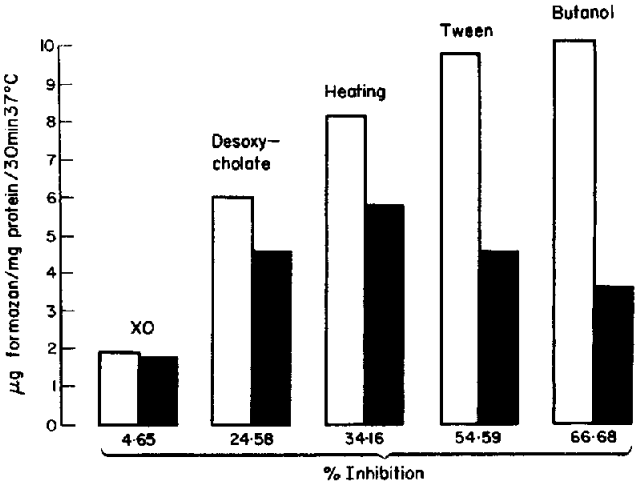

FIG. 1. *In vitro* inhibition of purified liver XD by Medinal. Enzyme activity was determined in Thunberg tubes using 0·5 ml of enzyme solution (13·16 mg protein/ml), 0·1 ml of a 0·05 M hypoxanthine solution as substrate and 0·3 ml of a 0·1% triphenyltetrazolium chloride solution as hydrogen acceptor. Phosphate buffer pH 7·4 to give a final volume of 3·4 ml.

White bars: without Medinal.

Black bars: with Medinal (final concentration 0·3 mg/ml)

against twice distilled water at 5°. After centrifugation a solution free of turbidity with a reddish colour was obtained with $E_{280}/_{450} = 15·5$ to 14·7. XD activity was determined anaerobically as described elsewhere.[7] The experiments with CCl^4 were performed with Wistar white rats injected intraperitoneally with 0·1 ml/100 g body wt. Medinal (30 mg/100 g body wt.) was injected s.c. 22 hr after the administration of CCl_4.

In the experiments with purified liver XD the enhanced inhbition by Medinal is probably due to the dissociation of the enzyme-lipid complex, the free enzyme becoming therefore more vulnerable to the effect of the barbiturate. CCl_4 which activates the XD and liberate the enzyme from its lipid linkage has shown also to increase the inhibition by barbiturate as shown in Table 1. The *in vivo* and *in vitro*

TABLE 1. INHIBITION OF THE BLOOD SERUM XD ACTIVITY BY MEDINAL IN NORMAL AND POISONED RATS WITH CCl_4

	Xanthine dehydrogenase activity†	
Dose of medinal*	Normal	Poisoned
0	20·00	39·00
1	13·36	11·48
2	9·42	3·94

* Intervals of doses: 24 hr (30 mg/100 g body wt.)

† μg formazan/ml serum/30 min.

experiments indicate that the dissociation of the enzyme lipid complex increases the activity of the enzyme permitting a more effective inhibition by the barbiturate.

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Induction of lambda phage by hydroxyurea

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U.V. IRRADIATION and inhibitors of DNA synthesis are able to induce phage production in lysogenic strains of bacteria. In our laboratory we are studying the induction of lysogenic *Escherichia coli* λ-28 strain by various substances under different culture conditions. According to our previous results[1] prophage detachment in a certain proportion of the population took place immediately after the addition of mitomycin C (MC), even at 2°. This step is followed by a relatively slow increase in the number of infective centers.

There are many reports that hydroxyurea (HU) is an antitumour substance[2] and that it has a specific effect on DNA synthesis[3,4] and phage synthesis.[5] In this report the effect of this substance on *E. coli* λ-28 are presented. A 16-hr-old broth culture was inoculated in a 1:10 proportion to the same medium and after incubating for 2 hr at 37° the cells were washed twice with water and suspended at a concentration of 10^6 cells/ml in minimal medium of Davis and Mingioli[6] plus 0·25% casein hydrolysate* (complete medium). In the case of shift down state, we transferred the inoculum grown in broth to the above mentioned minimal medium supplemented with 1 ml of 0·01 % casein hydrolysate/1l., that is, a state of transitory starving of the cells due to the depressed state of biosynthetic enzymes was performed. Incubation of cultures was generally made at 37°. The number of infective centers was determined by the plaque count on the indicator strain C_{600}. For measuring the number of complete phages the cells were treated with chloroform at 2° for 5 min. Incubation of the plates was carried out at 28° in the case of the count of infective centers and at 37° when determining the number of complete phages.

In complete medium 0·005 M HU induced phage production and higher concentrations (above 0·05 M) inhibited complete phage production although the count of infective centers was increased.

* Bacto casein hydrolysate (Difco).