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Inhibition of Ethanol Metabolism by Oral Antidiabetics.* (27122)

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Ethanol is metabolized in the body by the following processes:

$$\text{Ethanol} \xrightarrow{1} \text{Acetaldehyde} \xrightarrow{2} \text{Acetate} \rightarrow CO_2 + H_2O.$$

Reaction 1 is catalysed by the DPN-dependent enzyme alcohol dehydrogenase (ADH), reaction 2 by the likewise DPN-dependent acetaldehyde dehydrogenase (AcDH).

In vitro studies(17) have shown that tolbutamide inhibits a number of DPN-dependent enzymes, among these ADH. These findings have been confirmed(5) and it was also reported that AcDH is inhibited by tolbutamide. The inhibition seems to be explained by a substrate competition between tolbutamide and DPN(5). This may account for the uncomfortable flushes experienced by some tolbutamide or chlorpropamide treated patients upon exposure to alcoholic beverages(2,3,4,7,9,10,16). During the reaction, which resembles the ethanol-antabuse reaction, high concentrations of acetaldehyde have been found in the blood and the expiratory air(6,8).

As tolbutamide inhibits both ADH and AcDH *in vitro*, it would be expected that the metabolism of ethanol would be diminished after tolbutamide *in vivo*, but in two reports (6,8) this effect could not be demonstrated. However, we considered it pertinent to reinvestigate this question under conditions which permitted a more exact determination of the metabolism of ethanol in the hours preceding and following intravenous injection of tolbutamide. Furthermore, the effect of a tolbutamide metabolite and of carbutamide was tested with the same technic.

Methods. The experiments were performed on intact, nembutal anaesthetized cats in the postabsorptive state. Cannulas were placed in a femoral artery and a saphenous vein for blood sampling and injections. A priming dose of ethanol was injected intravenously. This resulted in a plasma concentration between 50 and 150 mg/l. To maintain this concentration a continuous infusion of ethanol was given at a rate calculated from the average elimination rate of ethanol for cats. The first blood sample was taken one hour after the priming dose when diffusion equilibrium of ethanol had been established. Arterial blood samples were then taken every 30 minutes for 4 hours. Two hours after the sampling was started, the compound to be investigated was injected slowly intravenously in an amount of 100 mg/kg. Ethanol concentration in the plasma was determined enzymatically by the method of Lundquist and Wolthers(12) (coeff. of var. 0.7%); plasma glucose by the Somogyi-Nelson technic(14).

From the weight of the animal, the volume of distribution of ethanol and rise or decline of ethanol concentration, the disappearance rate was determined before and after sulphonylurea injection.

In 8 experiments tolbutamide was given. In one experiment its primary elimination product N - (4-hydroxymethyl-benzolsulphonyl)-N'-n-butylurea (HBBU) and in 3 other experiments carbutamide was given. In 3 control experiments saline was injected instead of sulphonylurea.

Results. The results appear in Table I.

* Tolbutamide (Rastinon) and HBBU were given by the Copenhagen Representative for Farbwerke Hoechst, Lautrup-Larsen A/S. Carbutamide (Nadisan) was provided by C. F. Boehringer & Soehne through A. H. Allesen Holm A/S. The technical assistance of Miss Ulla Bengtsson and Mrs. Inga Almdal is gratefully acknowledged.

TABLE I. Changes in Ethanol Disappearance Rate in Cats Given Sulphonylureas.

Exp.	Wt, kg	Sex	Ethanol disappearance rate, mg/min., before and after inj. Before	After	Change, %	Blood sugar fall, % of initial value
Tolbutamide:						
524	2.42		3.96	2.80	−29	not measured
543	3.49	♂	6.11	3.74	−39	64
544	4.39	♂	6.54	4.39	−33	7
549	3.12	♀	4.52	2.58	−43	21
556	3.22	♂	4.42	3.45	−22	13
565	3.14	♀	4.45	3.70	−17	3
566	3.58	♀	5.18	3.18	−39	6
570	3.24	♀	3.15	1.57	−50	43
					−34 ± 11‡	
Carbutamide:						
561	3.58	♀	5.34	5.34	0	56
574	3.54	♂	5.18	3.94	−24	61
577	3.08	♀	4.68	4.68	0	16
HBBU*:						
582	3.80	♂	6.70	3.73	−44	9
Control†:						
546	2.84	♀	3.96	3.96	0	not measured
547	3.65	♂	4.69	4.69	0	—
562	3.26	♂	7.67	7.67	0	—

* HBBU = N-(4-hydroxymethyl-benzolsulphonyl)-N'-n-butylurea.
† Control animals were inj. with saline.
‡ Mean ± S.D.

In all 8 experiments where tolbutamide was injected, the ethanol disappearance rate was markedly reduced, average reduction being 34%. There seemed to be no relation between the effect of sulphonylureas on blood sugar and the metabolism of ethanol, as marked inhibition of ethanol metabolism occurred when the effect on blood sugar was insignificant (Exp. No. 544, 565, 566). Furthermore the metabolite HBBU inhibited the metabolism of ethanol without having any hypoglycemic effect.

Of the 3 cats treated with carbutamide only 1 showed any reduction in disappearance rate of ethanol.

Discussion. There have been three publications dealing with the influence of sulphonylureas on ethanol metabolism. Czyzyk & Mohnike(8) found no alteration in disappearance rate in 5 human diabetics after 7-9 days of treatment with tolbutamide. However, ethanol was given by mouth in a single dose, which makes the disappearance rate less comparable than when the ethanol is injected. Furthermore, variations in the metabolism from one day to another(15) may disguise a possible effect of the tolbutamide. Using the same procedure the effect of carbutamide treatment on alcohol metabolism in rabbits was investigated. Only in one case—after a very high dosage of carbutamide—a slight effect on the alcohol metabolism was found. In similar experiments on healthy humans, Büttner(6) also failed to demonstrate any effect of tolbutamide on alcohol disappearance. Finally, Forney and Hulpieu(11) have examined the disappearance rate in 6 dogs after one week of treatment with carbutamide. They found no alterations.

We found that carbutamide affected the metabolism of ethanol in only one of 3 experiments. On the other hand tolbutamide caused a marked inhibition in all experiments. This discrepancy between our material and the above mentioned findings may be due to differences in species, to a smaller DPN content in the livers of our animals under the experimental conditions used or to differences in sulphonylurea doses. In other experiments one of us found(13) that tolbutamide, 100 mg/kg intravenously, produces plasma concentrations of 30-35 mg %. These concentrations are about twice the concentrations found in humans in the first hours after in-

gestion of 1.5 g tolbutamide(1). The difference between our findings and the results mentioned above might, however, also be due to differences in the methods used for determination of the disappearance rate.

As even a moderate inhibition of ethanol metabolism in man would be of considerable forensic interest, we feel that further investigations of this tolbutamide effect should be carried out.

Summary. With a sensitive technic it is demonstrated that tolbutamide (100 mg/kg) injected intravenously inhibits the metabolism of ethanol in intact cats, average inhibition being 34%.

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Reaction of the Subcutaneous Tissue of Rats to Injected Air.* (27123)

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The formation of localized, circumscribed pockets by injecting air or specific gases into the subcutaneous tissue of animals and man has proved to be a useful means for physiological studies of gas tissue tensions and rate of absorption of various gases from the subcutaneous tissue(1,8). These air pockets are essentially tonometers where a definite gas volume is continuously perfused by blood and the rate of exchange between gas in the pocket and the tissue-blood environment depends upon the structural and physiological characteristics of this environment. While the structural reactions of the subcutaneous tissues to air injections have been described in the guinea pig(2) and rat(3), little attention has been given to the blood supply of the air pockets which is of prime importance in such studies. It was felt therefore that air pockets in rats provide an exceptional case for study since the depot could be maintained in the same area by air refills(4) for weeks and months, thus establishing a very stable adjustment to the air as a foreign body. In fact, such gas pockets eventually form a very discrete structure with a highly developed vasculature and an organized lining. A well established pocket can actually be dissected out and removed as a complete sac, which in

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