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Effect of Ethanol on Plasma Glucose and Insulin in the Fasted Dog.* (28916)

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Recent studies have demonstrated that the hypoglycemic potential of ethanol may be unmasked in normal human subjects by administering ethanol after 2 to 3 days of prior fasting(1-4). In the search for a more suitable experimental preparation for the study of "alcohol† hypoglycemia," we have investigated the effects of ethanol upon blood sugar in the dog. Although most previous studies have failed to disclose an acute hypoglycemic action of ethanol in this species (5-8), we felt that our efforts might be facilitated by the institution of preliminary dietary deprivation. The present studies have indicated that ethanol can acutely lower blood sugar in the fasted dog and that such "alcohol hypoglycemia" in dogs, as in humans(1,2) is not attended by elevations of plasma insulin.

Materials and methods. Food was withheld for 1.5 to 8.0 days from 8 male and 1 female mongrel dogs. Initial weights ranged from 13 to 32 kg. During the period of fasting, the animals were housed in individual cages, weighed daily, and permitted unlimited access to water. For the individual experiments, the animals were anesthetized with intravenous sodium pentobarbital (30 mg/kg) except in one instance, where chloralose was employed. Supplemental amounts of pentobarbital were administered as necessary during the studies.

Following induction of anesthesia, the left femoral artery was cannulated, and a catheter was introduced into the right femoral vein for infusion of 0.85% saline, via Bowman pump, at the rate of 1.9-2.0 ml/min. The control infusion of saline was continued for a minimum period of 120 minutes to stabilize blood sugar. For the subsequent 120 minutes, 15% ethanol (absolute ethanol in isotonic saline, vol/vol) was infused. In most experiments, sodium tolbutamide (0.5 g) was introduced through the infusion tubing 60 or 120 minutes after cessation of alcohol administration, and intravenous glucose (0.5 g/kg) was given one hour later. The tolbutamide and glucose were designed to contrast the effects of known insulinogenic agents with those of ethanol.

Control specimens of arterial blood were obtained 30 and 5 minutes prior to the start of alcohol infusions, and experimental samples were secured at timed intervals thereafter

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† The terms "ethanol" and "alcohol" are used interchangeably in subsequent portions of text.

TABLE I. Effects of Intravenous Ethanol on Plasma Glucose and Insulin in the Fasted Dog.*

Fast:	36 hr		5 days		5 days		6 days		8 days	
Wt (kg) Initial:			31.8		15.0		22.9		22.7	
Final:	19.1		29.9		12.9		20.9		18.6	
Time (min)	Glucose†	Insulin†	Glucose	Insulin	Glucose	Insulin	Glucose	Insulin	Glucose	Insulin
− 30	106	25	91	18	95	16	125	18	122	9
− 5	101	20	86	15	94	15	123	18	117	18
				Alcohol administration: 118 g/l isotonic saline (2 ml/min)						
+ 15	134 (67)‡	42	87 (94)	10	82 (47)	9	108 (45)	8	—	—
+ 30	138	47	89 (124)	10	65 (78)	10	106 (68)	7	121 (58)	13
+ 60	153 (111)	73	81 (187)	12	40 (111)	5	107 (108)	5	122 (90)	18
+ 90	—	—	66 (280)	8	26 (131)	14	—	—	127 (125)	14
+120	141 (237)	143	50 (286)	12	Expired at +110		90 (161)	4	126 (153)	18
				Discontinue alcohol administration						
+ 15	122 (201)	42	47 (202)	11			82 (147)	1	123	23
+ 60	118 (191)	45	57 (173)	13			69 (136)	6	132 (135)	11
+120			91 (155)	26					146 (116)	12
				I. V. Tolbutamide: 0.5 g						
+ 15	105 (153)	128	105 (147)	49			69 (114)	24		
+ 60	104 (141)	146	109 (132)	59			60 (111)	20		
				I. V. Glucose: 0.5 g/kg						
+ 15	302 (135)	340	334 (129)	174			251	31		
+ 60	175 (117)	305	— —	—			168	14		

* Control infusions of saline were started immediately after induction of anesthesia and continued for a minimum of 120 min prior to substitution of 15% ethanol (v/v) in the infusate. Serial observations for the subsequent 5 to 6 hr were secured at timed intervals as indicated above. Tolbutamide was administered 60 or 120 min following cessation of alcohol administration.

† "Glucose" and "insulin" refers to concentrations in plasma in mg % and μU/ml respectively.

‡ Values for plasma alcohol in mg % are denoted by figures in parentheses.

(Table I). Blood samples were introduced into chilled tubes containing heparin. Plasma was separated by centrifugation at 4°C and stored at −18°C for subsequent analyses.

All measurements were obtained in duplicate or triplicate. Plasma glucose was estimated by the Nelson modification of the Somogyi method(9). Plasma alcohol was measured by the micro-diffusion procedure of Newman and Newman(10). In 3 experiments, plasma alcohol was also assayed enzymatically with alcohol dehydrogenase. The paired comparisons yielded excellent agreement and did not differ by more than ± 10%. Plasma concentrations of immunologically-reactive insulin were assessed by the immunoassay procedure of Yalow and Berson(11) as described alsewhere(2). In the absence of canine insulin, duplicate 13-point standard curves were prepared with human insulin. Thus, the derived values represent "human insulin equivalents" since the immunological interactions between guinea-pig antiserum to porcine insulin and to insulin from different species need not be identical. Assays were conducted with 1:5 to 1:20 dilutions of plasma and, in each instance, the final values were appropriately corrected for damage to the I^{131}-insulin which occurred during the 4 days of incubation for the immunoassay procedure.

For preparation of the ethanol infusions, freshly opened bottles of absolute ethanol (Fisher Scientific Corp., New York) were employed.

Results. Three types of blood sugar responses were observed in 9 experiments with ethanol infusion: In 6 animals (*i.e.,* 1 of 2 dogs fasted for 3 days; 1 dog fasted for 4 days; 2 dogs fasted for 5 days; 1 dog fasted for 6 days; and 1 of 2 dogs fasted for 8 days), plasma glucose fell to 70% or less of control values during or immediately following administration of ethanol. Two of these animals expired during the alcohol infusion, possibly due to hypoglycemia, since antemortem blood sugar values had already declined to precariously low values. Acute challenge with alcohol did not alter plasma glucose in 2 dogs fasted respectively for 3 and 8 days. In a single animal which had been fasted for only 36 hours, plasma glucose rose coincident with infusion of ethanol. Representative examples from each category are listed in Table I. The variegated blood sugar responses were not attended by systematic differences in plasma alcohol nor in the rate at which alcohol disappeared from the circulation after the infusion had been terminated. Peak plasma ethanol concentrations ranged from 143 to 286 mg % and did not exceed the range of mild intoxication (Table I).

Plasma insulin paralleled the alterations in blood sugar (Table I). Thus, in the animal in whom ethanol effected hyperglycemia, plasma insulin rose concomitantly. Contrariwise, where alcohol reduced plasma glucose, there was usually a modest reduction in the circulating immunologically-reactive insulin. Despite the prior fast, and the presence of alcohol in the blood, the responsiveness to known insulinogenic agents was preserved. Intravenous tolbutamide effected a measureable increment in plasma insulin (even though the changes in plasma glucose were minimal), and a further rise in plasma insulin was produced by intravenous administration of glucose (Table I).

Discussion. In the present studies, acute challenge with 0.5 to 1.0 g per kg ethanol effected hypoglycemia in 6 of 8 dogs which had been fasted for 3 to 8 days and hyperglycemia in one dog from whom food had been withheld for only 36 hours. Ethanol-induced hyperglycemia in the dog is not at variance with the experience of others(6). On the other hand, previous attempts(5-8) to demonstrate an acute blood-sugar lowering action have been unsuccessful unless pretreatment with oxythiamine(8) had been instituted. However, in all of these earlier efforts, dogs were deprived of food for 48 hours or less (5,6,8) or for as long as 14 days(7). Thus, the ethanol was administered after minimal or extreme starvation. Intermediate situations have not been heretofore examined. Only Clark, Wilson and Hulpieu observed any *acute* hypoglycemia following ethanol, and this was in dogs which had received no food for 5 days but had been given 4 g per kg ethanol on each of the preceding 4 days (7). They concluded that "repeated intoxi-

cation plus inadequate food intake appear to be essential" for induction of alcohol hypoglycemia in the dog. On the basis of the present findings, "repeated intoxication" may not be required. Rather, in dog as in man, there may be a finite phase of starvation which constitutes an appropriate metabolic setting for alcohol hypoglycemia.

The metabolic interrelationships following withdrawal of food may be subdivided into 3 sequential stages: 1) the immediate postprandial period during which the liver and periphery are packed with substrates derived directly from the preceding alimentation, and mobilization of peripheral fat has not yet been augmented; 2) early starvation during which peripheral needs are largely fulfilled by mobilized and locally-derived products of fat metabolism so that carbohydrate is spared and normoglycemia can be sustained with minimal hepatic gluconeogenesis; and 3) late starvation where stores of fat have become depleted and hepatic gluconeogenesis must be augmented to provide adequate substrate for the periphery(12).

Elsewhere we have shown that the variegated effects of alcohol upon blood sugar may be mediated principally within the liver since all of them can be reproduced with liver slices *in vitro*(13). We proposed that the interactions may be formulated in terms of: a) amount of alcohol available; b) intrahepatic complement of enzymes for oxidizing alcohol; c) generation of "reducing equivalents" during this enzymatic oxidation; and d) resultant increases in the DPNH/DPN ratio within the liver (as conditioned by total stores of pyridine nucleotides and preexisting interrelationships of DPNH/DPN). We suggested that the subsequent direction of change in hepatic glucose production could depend upon the alternative pathways for hydrogen disposition that are employed to reoxidize the "extra" DPNH and to reestablish oxidation-reduction equilibrium(13).

In the present studies, the actions of ethanol upon plasma glucose was not uniform. Since the delivery of alcohol was kept constant, and since starvation causes only modest changes in alcohol metabolism in the dog(14), it is doubtful whether the variable results can be attributed to differences in the absolute availability of alcohol or in the generation of DPNH. Rather, they may have resulted from differences in the remaining components of the interactions outlined above. Immediately after feeding, or during heightened gluconeogenesis, an active turnover of abundant intrahepatic 3-carbon fragments could furnish the hydrogen acceptors for optimal extramitochondrial disposition of electrons. Under such circumstances, hepatic glucose production might be unaffected or actually increased by ethanol. On the other hand, during limited gluconeogenesis and pyruvate turnover, the necessity for a proportionately greater reoxidation of DPNH within the mitochondria, and preemption of already marginal pyruvate by dismutation to lactate could compromise the renewal of phospho-enol-pyruvate and arrest gluconeogenesis. Conceivably, if in the dog, as in the starved rat(15), intrahepatic DPNH/DPN ratios are already elevated, and the total DPNH + DPN stores are reduced at the time of peak fat mobilization, smaller amounts of H^+ could effect even greater insult to prevailing oxidation-reduction equilibrium. Thus, one might conjecture that the vulnerability to alcohol hypoglycemia would be maximal when the oxidation of alcohol contributes additively to an already heightened reducing environment within the liver, and to an already attenuated potential for the endogenous formation of 3-carbon hydrogen acceptors.

The formulation does not define the precise biochemical steps which may restrict gluconeogenesis and/or pyruvate turnover at that phase of normal starvation during which extracerebral metabolic demands are normally fulfilled by fat. Moreover, it need not exclude the possibility that products of incomplete alcohol oxidation (*i.e.* acetaldehyde) or of the peroxidation of unsaturated fatty acids could accumulate at that time, and inhibit gluconeogenesis directly. However, by ascribing the *direction* of blood sugar response to ethanol during normal starvation to the prevailing gluconeogenic setting(13), the formulation is compatible with the *in vivo* observations. It could account for our inability to

elicit hypoglycemia in all of the animals which had been fasted for 3 to 8 days and for the failure of others to induce hypoglycemia by the acute administration of 4 g per kg ethanol to dogs which had been fasted for 14 days(7).‡

Despite all the persisting enigmas about the effects of alcohol upon blood sugar, the role of insulin can now be defined with more certitude. First, our immunoassays in the dog, and earlier measurements in man(1,2) indicate that immunologically-reactive insulin is not the *primary* mediator when alcohol lowers blood sugar. Secondly, our findings that tolbutamide or exogenous glucose elevate plasma insulin even in the presence of alcohol, and that plasma insulin also rises during ethanol-induced hyperglycemia would suggest that alcohol need not compromise the responsiveness of pancreatic islet cells. The latter may have relevance for the disparate preservation of body fat that is seen in certain otherwise poorly-nourished alcoholics. In such patients, a redistribution of endogenous (or exogenous) carbohydrate towards selective repletion of adipose stores might ensue whenever ethanol boosts blood sugar through an activation of hepatic glycogenolysis or a facilitation of gluconeogenesis.

Summary. 1. Plasma alcohol levels of 143 to 286 mg % were achieved by intravenous infusion of ethanol (0.5 to 1.0 g per kg) into 9 dogs which had been deprived of food for varying periods. Three patterns of blood sugar response were observed: Blood sugar rose in a single animal which had been fasted for 36 hours; hypoglycemia occurred in 6 of 8 animals which had been starved for 3 to 8 days; and blood sugar was unaltered in the remaining 2 animals. 2. Concurrent changes in circulating immunologically-reactive insulin paralleled the alterations in blood sugar. Alcohol-induced hypoglycemia was neither preceded nor attended by a rise in plasma insulin. 3. The capacity to respond to known agents which stimulate pancreatic insulin release was preserved in starved dogs treated with ethanol. Immunoassay disclosed measureable elevations of plasma insulin following intravenous administration of tolbutamide (0.5 g) or glucose (0.5 g per kg) to such animals. 4. An hypothesis is advanced to explain the varied effects of ethanol upon blood sugar on the basis of the intrahepatic oxidation of alcohol and the prevailing level of gluconeogenesis.

‡ Since the completion of the present studies, Lochner and Madison(16) have reported that ethanol *invariably* lowers blood sugar in dogs with chronic end-to-side portacaval shunts which have been fasted for 3 days. Their more uniform experience might reflect a more percarious balance of endogenous glucose production in the "shunted" animal, a suggestion which is reinforced by the recent demonstration that such dogs exhibit an altered response to exogenous insulin(17).

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