# Potentiation of the Plasma Insulin Response to Glucose by Prior Administration of Alcohol

## An Apparent Islet-Priming Effect

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SUMMARY

**Ethyl alcohol which has been reported to be without effect on insulin secretion apparently modifies beta-cell function nevertheless, as indicated by the plasma insulin responses to glucose loading after prior administration of alcohol. Glucose was injected intravenously in nine young adults on three separate occasions at intervals of at least two weeks. During the twelve hours preceding each test the subjects received ethanol either by mouth or by vein or, as a control, no ethanol. Plasma insulin and glucose concentrations were not noticeably affected by the ethanol alone but alcohol pretreatment was followed in all instances by heightened plasma insulin responses to the glycemic pulse stimulus and by accelerated rates of plasma glucose disappearance. The mean plasma insulin response was increased 50 per cent by the alcohol, irrespective of the route of administration. Unlike recognized insulin secretogogues, therefore, ethanol appears to augment insulin secretion only on demand. The route of administration appeared not to be a factor in determining the magnitude of the alcohol effect. Other alcohol effects included blunting of the plasma pyruvate and exaggeration of the plasma lactate elevations after glucose. DIABETES *18*:517-22, August, 1969.**

Ethyl alcohol, a metabolic fuel which can be utilized without benefit of insulin,[1] apparently does not itself stimulate insulin secretion.[2,3] Yet in the course of an investigation into the effects of alcohol on glucose tolerance we encountered evidence suggesting that ingestion of ethanol induced an exaggerated plasma insulin response to a subsequent glucose load.

The study reported here grew out of that unexpected observation. The data seem clearly to confirm a synergistic effect of ethanol and glucose on insulin secretion. This phenomenon differs from examples of synergism between substances which independently stimulate insulin secretion, in that of the two agents only glucose is itself an insulin secretogogue while ethanol is inert when acting alone. We propose the term "priming" to describe this change of function in the direction of a heightened responsiveness to a secretory stimulus.

EXPERIMENTAL SUBJECTS AND METHODS

The experimental subjects were eight women and one man, aged between twenty and twenty-eight years, without any evidence of ill health. None of the subjects exceeded "desirable" body weight (Metropolitan Life Insurance Co. Statistical Bulletin, No. 40, 1959) by more than 10 per cent, nor were any of them obese by inspection. In only two was there a history of a close relative with diabetes (a mother and father respectively). None of them was taking any medications. A series of three intravenous glucose tolerance tests comprised the experimental study, with each of the tests being preceded by a different preparatory overnight regimen as described below. No individual subject was tested more frequently than at two-week intervals and tests were not performed during menstruation or if menstruation was imminent. The experimental subject entered the hospital the evening before the test and went to bed after a standardized 980-calorie steak and vegetable dinner at 6 p.m. At 8 p.m. the study proper began with one of the following three preparatory regimens, given in random order. (1) *Control (GTT):* At 8 p.m. the subject drank 160 ml. of noncaloric ginger ale, and this was repeated every two hours for six doses, the last dose being administered at 6 a.m. (2) *Alcohol glucose tolerance test (alcohol GTT):* Performed exactly as above except that 12 per cent ethyl alcohol in

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ginger ale was substituted for the unmodified ginger ale. (3) *Intravenous-alcohol glucose tolerance test (intravenous-alcohol GTT)*: Nothing was taken orally. Instead 960 ml. of 12 per cent ethanol in 0.9 per cent NaCl solution was administered as a continuous intravenous infusion between 8 p.m. and 6 a.m. One of the subjects was unavailable for this test.

The rate of administration of the ethanol was chosen with the objective of providing alcohol at about the anticipated maximum rate of its oxidation,[4] so that little accumulation in the plasma was expected. As will be seen in the results, this expectation was essentially fulfilled. Most of the subjects tolerated the alcohol without undue discomfort although a few experienced some nausea after ingestion. None showed any evidence of inebriation.

At 7:30 a.m. two indwelling cannulas (one for the glucose injection, the other for blood sampling) were inserted into forearm veins and shortly thereafter two blood samples were drawn to determine baseline plasma concentrations of glucose, insulin, pyruvate, lactate, free fatty acids (FFA) and alcohol. At 8 a.m. 70 ml. of 50 per cent dextrose was injected over two-and-one-half minutes. Blood samples were collected at five minutes after the start of the injection and then at ten minute intervals for two hours. Plasma glucose was measured in the AutoAnalyzer by a glucose oxidase method, insulin by a double antibody technic,[5] pyruvate, lactate and alcohol by enzymatic methods (Boehringer kits), and FFA by a colorometric method.[6]

The insulin secretory response to the glucose load was expressed as sums of increments of plasma insulin, i.e., the summed net increases (above baseline values) in plasma insulin concentrations in the first four post-glucose blood samples. Changes in plasma lactate and pyruvate were also expressed as sums of increments. Glucose tolerance was determined by the disappearance rate, designated K, obtained by visual fit of a straight line to the semilogarithmic plot of the absolute plasma glucose values against time. Since the disappearance rate in a given individual is unaltered by varying the glucose dose from 25 gm. to 35 gm.,[7] we have adopted standard criteria, and K values above unity are considered normal.[8] The theoretical peak increment of glucose ($\triangle G$) was derived by extrapolating the straight line plot back to zero time and subtracting the baseline value. The total urinary output in the hour after the glucose injection was collected and the glucose, pyruvate[9] and lactate (Boehringer kit) concentrations were determined. Statistical evaluation of differences between means was performed by the $t$ test for paired experiments.

## RESULTS

Plasma ethanol concentrations ($\bar{x} \pm$ S.E.M.), measured approximately one-and-three-quarter hours after the last dose of alcohol, were 26.8 ± 6.1 mg./100 ml. after oral alcohol, and 16.2 ± 2.0 mg./100 ml. after intravenous alcohol (table 1). These low levels explain the absence of objective evidence of intoxication, for plasma levels of the order of 200 mg. per cent are generally required for "mild to moderate intoxication."[4]

Urinary excretion of glucose, lactate and pyruvate was negligible in all tests and will not be reported in detail.

*Effect of alcohol alone on various plasma constituents:*

The effects of the overnight administration of alcohol were assessed in plasma samples collected before glucose administration, and results were compared with corresponding samples taken during the control experiments (table 1). Ethanol by either route failed to exert a discernible effect on baseline glucose or insulin concentrations. A 50 per cent increase in plasma lactate was seen after oral alcohol; pyruvate and FFA were not appreciably affected. The latter three plasma constituents were not measured after intravenous alcohol.

**TABLE 1**

Effects of ethanol alone on plasma constituents (mean ± S.E.M.)

| | | Route of ethanol administration | |
|---|---|---|---|
| | Control n = 9 | Oral n = 9 | Intravenous n = 8 |
| Ethanol mg./100 ml. | — | 26.8±6.1 | 16.2±2.0 |
| Glucose mg./100 ml. | 84.0±2.7 | 78.6±2.0 | 81.8±1.9 |
| Insulin μU./ml. | 19.9±4.8 | 20.0±4.4 | 19.9±6.5 |
| Lactate mg./100 ml. | 8.5±0.7 | 13.8±1.3 | — |
| Pyruvate mg./100 ml. | 0.14±0.02 | 0.15±0.01 | — |
| FFA mEq./L. | 773±100 | 828±122 | — |

*Effect of oral alcohol on plasma glucose responses to glucose loading:*

The metabolic consequences of overnight ethanol administration on carbohydrate metabolism, so inconspicuous in the fasting state, were clearly revealed by glucose loading. The most obvious effect of the alcohol



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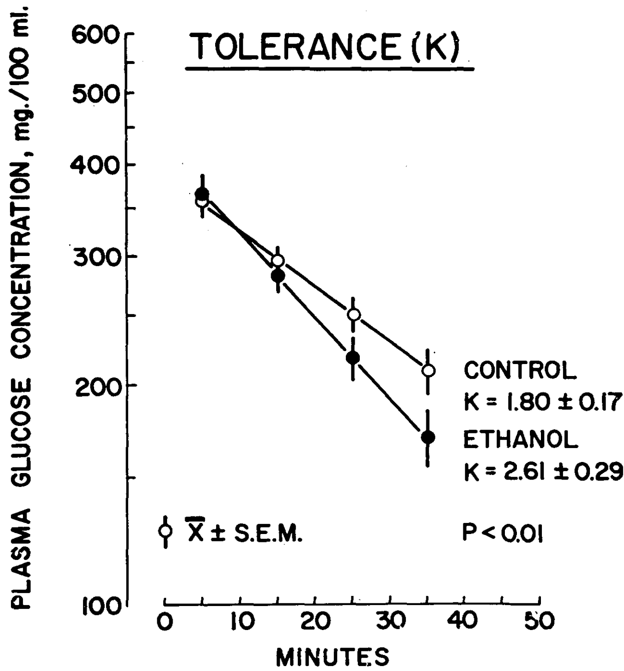


FIG. 1. Effect of oral ethanol on intravenous glucose tolerance.

pretreatment was a brisker rate of assimilation of the intravenous glucose load (figure 1). The mean time for the plasma glucose to fall to half the peak concentration was twenty-seven minutes in the alcohol GTT as compared to thirty-eight minutes in the control GTT. To express this significant ($p<.01$) acceleration of the glucose disposal rate in more familiar terms, the mean assimilation constant, K, was increased from 1.80 ± 0.21 to 2.61 ± 0.29 by alcohol pretreatment.

Note that the theoretical mean peak glucose increment (*see* Methods) was essentially unchanged by the oral alcohol pretreatment (table 2). Thus the enhanced insulin secretory responses described below were not attributable to a more intense glycemic stimulus.

*Effect of oral alcohol on plasma insulin responses to glucose:*

In every case the plasma insulin response to glucose was increased when alcohol was included in the pre-

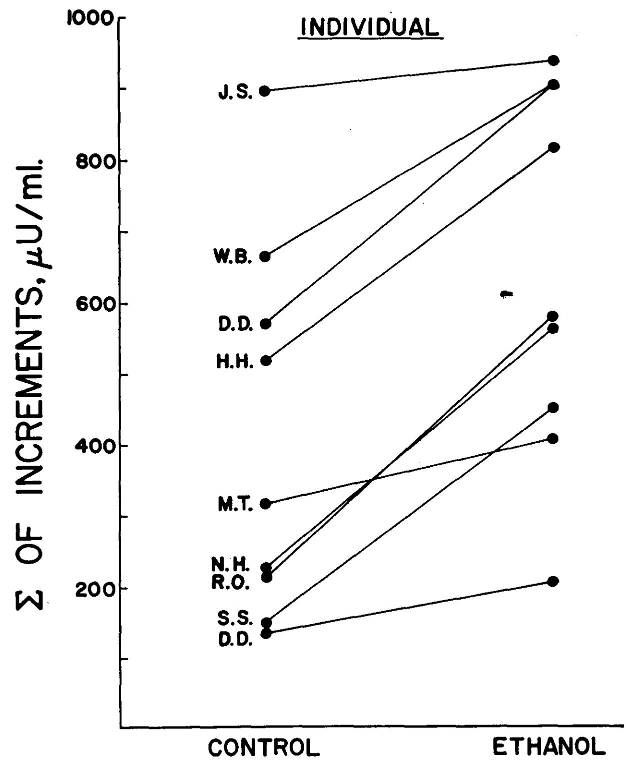


FIG. 2. Effect of oral ethanol on plasma insulin responses to an intravenous glucose load.

TABLE 2

Plasma glucose and insulin responses to intravenous glucose in alcohol pretreated subjects (mean ± S.E.M.). The derivation of the various parameters in the table is given under Methods.

| Parameter | None (control) | Preglucose alcohol administration Oral | Intravenous |
|---|---|---|---|
| Glucose assimilation constant (K) | 1.80±0.17 | 2.61±0.29* | 2.72±0.12* |
| Theoretical peak increment of glucose (△G) (mg./100 ml.) | 317±16 | 331±18 | 322±15 |
| Insulin increment at five minutes after start of glucose injection (△I) (μU./ml.) | 166±36 | 252±39* | 245±47 |
| $\frac{\Delta I}{\Delta G} \times 100$ | 524 | 761 | 761 |
| Insulin sum of increments (μU./ml.) | 416±87 | 656±85* | 602±95* |

*Significantly different from control ($p < .01$)



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paratory regimen. As shown in figure 2, the increase was substantial in most cases although in a few it was marginal. The mean difference attributable to the oral alcohol pretreatment was 57 per cent (table 2). The heightened response was discernible quite promptly. As shown in table 2, the effect of alcohol on insulin secretion was evident by five minutes after administration of the glucose load. The accompanying increments in plasma glucose concentrations were approximately the same with and without alcohol pretreatment. Hence, the immediate insulin responses were disproportionate to the glycemic stimulus, as shown by the marked change in the ratio of the increment in insulin at five minutes to that of the peak increments of glucose. No difference was seen in the pattern of the plasma insulin responses, i.e., the time of appearance of the peak and the return to baseline, between the control and the alcohol series.

*Effect of intravenous alcohol on plasma insulin responses to glucose:*

Parenteral administration of the alcohol was included in this study so as not to overlook the possibility that the alcohol effect on insulin secretion might be mediated by a gastrointestinal factor. As noted earlier, the plasma alcohol concentrations at the time of the glucose injection were higher after oral alcohol. This finding is probably attributable to the fact that in the alcohol GTT one sixth of the dose was given at 6 a.m. whereas in the intravenous-alcohol GTT the infusion was stopped at 6 a.m. Perhaps because of the lower plasma concentration, intravenous alcohol may have been associated with a slightly smaller potentiation of the glucose-induced insulin secretion. Nevertheless, the patterns of plasma glucose and insulin responses were very similar following either route of administration of the ethanol. Table 2 presents data relating to these responses in the three test procedures.

*Changes in plasma concentrations of lactate and pyruvate after glucose:*

Further evidence of an effect of alcohol on carbohydrate metabolism was provided by changes in the plasma lactate response to glucose loading (figure 3). Plasma lactate rose almost twice as high during the alcohol glucose tolerance test when compared to the control studies ($p < 0.01$). There appeared to be a reciprocal effect on plasma pyruvate responses but here the differences were not statistically significant.

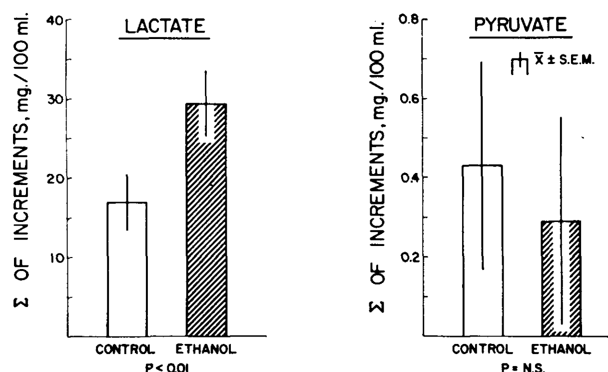


FIG. 3. Effect of ethanol on plasma lactate and pyruvate responses during intravenous GTT.

## DISCUSSION

The plasma insulin and glucose concentrations in our subjects were unaffected by overnight administration of alcohol, an observation in accord with previous reports[2,3] that ethanol does not itself stimulate insulin secretion. Nevertheless the data showed that pretreatment with ethanol augments the plasma insulin response to a pulse increase in the blood glucose concentration.

Several examples of two or more agents exerting a synergistic effect on insulin secretion have been reported. Tolbutamide-leucine,[10] and glucose-tolbutamide-glucagon,[11] for instance, are well known partners in such synergism. What serves to distinguish the alcohol-glucose potentiation from these and other examples of synergism is that each of the synergistic agents is independently capable of stimulating insulin secretion. In contrast, ethanol is inert in this respect, hence the term priming rather than synergism.

Other instances of potentiation of an insulin secretogogue by a substance which is itself not an insulin secretogogue have been reported. Galactose apparently does not provoke insulin secretion,[12,13] yet individuals with galactosemia exhibit an exaggerated plasma insulin response to glucose following a loading dose of galactose.[13] As another example, treatment with the anabolic steroid methandienone increased tolbutamide-induced hyperinsulinemia, even though the steroid itself had no effect on insulin levels.[14]

Implicit in the term priming is the assumption that the alcohol acts directly on the B-cell. However, with respect to mechanism, we obtained only the negative evidence that the route of administration is not critical. Therefore a gastrointestinal factor, humoral or neural, presumably is not involved. Our data provide no further clue to the mechanism. The action of alcohol may not even be exerted directly on the islets. A change in the

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insulin extraction ratio[15] of the liver could conceivably account for the observed effect on the plasma insulin responses to glucose. However, except for the probability that the amount of insulin reaching the liver itself controls the percentage of insulin retained,[15,16] factors which might acutely affect the "setting" of the process responsible for hepatic removal of insulin have not been shown to exist. Although the liver is virtually the sole site of alcohol oxidation, a hepatic action does not seem a likely source of the enhanced plasma insulin response.

The liver obviously is not the sole site of many other biological effects of alcohol. The presumed effect on the islets could be direct or indirect. Alcohol can affect nerve cell function as well as cause vasodilatation.[4] It could conceivably influence islets indirectly by acting through their rich innervation or by modifying their blood supply, perhaps thereby causing more islets to be perfused. It would certainly be of interest in this connection to know whether the augmented insulin secretory response results from more islets responding at one time, or alternatively whether exposure to alcohol heightens the responsivity of individual islets, causing them to secrete more insulin on demand. However, as already noted, we have no useful information bearing on the question of mechanism. Furthermore we cannot even assume the specificity of the alcohol effect. Whether priming depends simply on the caloric value of the alcohol or on some more particular property remains to be determined.

Accompanying, and presumably resulting from the augmented insulin output, was a striking increase in glucose disposal rates. This observation clearly indicates that the heightened insulin output was not a compensatory response to impaired glucose tolerance, as in the hyperinsulinemic response to glucose after cortisone pretreatment.[17] The improvement in glucose tolerance after alcohol inevitably raises the question of whether diabetics, specifically mild diabetics, would be susceptible to the priming phenomenon. Will further study permit substitution of "glucagon" by "alcohol" in the following conclusion reached by others?:[18] "Thus, in mild diabetics, an available reserve of insulin appears to exist which can be released by glucose plus glucagon but not by glucose alone." The possibility exists of therapeutic applications, should diabetics prove to be responsive to priming agents.

Indeed it is tempting to relate the present findings to those reported in the literature of half a century ago. Benedict and Torok,[19] Neubauer[20] and Allen[21] reported beneficial effects of ethanol in diabetics using doses comparable, and in some cases identical, to those used in the present study. Before suggesting that islet priming may have contributed to their observations, however, some reservations are in order. For one, we as yet have no evidence to indicate that diabetic islets can be primed to deliver more insulin. More important, the beneficial effects of alcohol on diabetics may have been more apparent than real. Alcohol lowers blood sugar by inhibition of gluconeogenesis[2,22,23] and can substitute lactic- for ketoacidosis.[1] These changes could of course superficially mimic an insulin effect. Nevertheless the possibility of exploiting the priming phenomenon, with the objective of improving the impaired B-cell reserve of the diabetic,[24] is surely worth looking into.

Finally, it may be noted that the increase in plasma lactate brought about by ethanol probably indicates that more of the glucose taken up by the liver was shunted into lactate. This effect is an expected result of the increase in the ratio of NADH to NAD[1,22,23] that results when alcohol is oxidized in the liver. The resulting disturbance in the intrahepatic redox potential accounts for the observed effects on the ratio of the redox pair lactate-pyruvate in the plasma.[22,23]

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## *Capillary Hemorrhage in Scorbutic Guinea Pigs*

One of the most characteristic signs of ascorbic acid deficiency is hemorrhage, apparently due to capillary fragility. The lesions are frequently found around the joint and are accompanied by swelling and tenderness. Hemorrhages have also been found in the intestinal mucosa and, less frequently, in the myocardium in studies in scorbutic guinea pigs. The depletion of collagen that occurs has been known for many years and was one of the earliest findings; there are also changes in supporting tissue of the endothelium and in the so-called cement substance.

I. Gore, M. Fujinami, and T. Shirahama (*see Nutrition Reviews 24*:179, 1966) previously suggested that reduction in collagen may lead to an increase in the width of intercellular spaces and that blood cells could move through these gaps. In a more recent study, Gore, M. Wada, and M. L. Goodman (*Arch. Path. 85*:493, 1968) have expanded their earlier work, using electron microscopy to examine changes in the areas where capillary hemorrhage was most frequently found in scorbutic guinea pigs.

Thirty-six male guinea pigs were fed a diet deficient in ascorbic acid for a period of one to five weeks. Half of the guinea pigs were young, averaging about 500 gm. in weight; the remainder were adults, 700 to 1,000 gm. Twenty-seven animals were studied using optical microscopy and nine using the electron microscope. Most of the twenty-seven animals studied using optical procedures suffered from infection and were either sacrificed or had died spontaneously. The nine animals studied using electron microscopy were sacrificed, three after two weeks, five animals after three weeks, one after five weeks on the deficient diet. Seven controls were maintained for five weeks. Four of them were studied using the optical procedures and three using the electron microscope.

To identify vascular leakage, animals who were studied using the electron microscope received an intravenous injection of thorium dioxide approximately five minutes prior to being sacrificed. Samples of skin, heart muscle, kidney, and the small intestine were taken for study.

Animals on the deficient diet had a loss of appetite

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