Differential Plasma Insulin Response to Glucose and Glucagon Stimulation Following Ethanol Priming

Ralph Friedenberg, M.D., Robert Metz, M.D., Mary Mako, M.S. and Barbara Surmaczynska, M.D., Chicago

SUMMARY

The early phase of the insulin secretory response to glucose may not be derived from the total pool of stored insulin, but possibly from a specific and relatively small fraction in a state or locus necessary for its immediate release. In an attempt to deplete this hypothetical fraction, and thereby to support the concept of its existence and obtain an estimate of its size under different circumstances, normal and mildly diabetic subjects were given a series of three large glucose loads followed by glucagon, with and without alcohol "priming". Results indicate that repetitive glucose or glucagon challenges over a two-hour period failed to exhaust the supply of immediately releasable insulin in either group of subjects as judged by the increments in plasma insulin during the first few minutes after each challenge. Alcohol pretreatment resulted in a larger early insulin secretory response to the first of three glucose loads, but a decreasing response to the subsequent two, possibly indicating that the initially augmented secretory response had resulted in depletion of a finite supply of immediately available insulin. An unexpected finding was the inhibitory effect of alcohol on glucagon-induced insulin release, in direct contrast to its effect when glucose is the insulinogogue, suggesting that glucose and glucagon exert their effects through different mechanisms or on different fractions of the insulin pool. DIABETES *20*:397-403, June, 1971.

Administration of glucose to normal subjects produces a prompt increase in their plasma insulin concentration. The magnitude of this initial increase has been claimed by several workers[1,2] to be the critical factor in determining an individual's capacity to assimilate a glucose load. Recent evidence raises the possibility that a blunted insulin secretory response may be a characteristic defect of diabetes mellitus, and one which possibly antedates detectable impairment of glucose tolerance.[3-5] Thus, overall adequacy of β-cell function may depend not so much on the total quantity of insulin secreted in response to a challenge but rather on the proportion of the total that is secreted in the first minutes. An inadequate early response would presumably indicate either paucity of insulin available for rapid release or sluggishness of the secretory process. Since the quantity of biologically active insulin extractable from the pancreas of persons with mild, insulin-independent diabetes may be in the normal range,[6] the former alternative might seem unlikely, particularly since the extent of the increase in plasma insulin concentration and the rate of loss of insulin from plasma[7] would indicate that only a very small fraction of the total insulin stores is released during the first five or ten minutes after glucose administration. If, however, only a small fraction of the insulin stores is in a state or site permitting prompt release,[8] effective secretory reserve might be very limited, despite an adequate total insulin content.

The present study was designed to test the possibility that several large stimuli, administered in relatively rapid sequence, might deplete the stores of insulin immediately available on demand, if not in normal subjects then perhaps in diabetic subjects, whose supply of rapidly releasable insulin might be limited.

A prime objective of the experimental design was to elicit a maximal β-cell response to stimulation, but without resorting to a combination of insulin secretogogues which might evoke secretion from more than one pool.[9] We sought to achieve this by the administration of ethanol, a β-cell "primer".[10] Administration of ethanol has no effect by itself on plasma insulin concentra-

From the Department of Medicine, Northwestern University Medical Center, Chicago, Illinois.

Requests for reprints should be addressed to Doctor Robert Metz, Virginia Mason Medical Center, 1118 Ninth Avenue, Seattle, Washington 98101.

tion, but results in modification of the function of the normal β-cell so that it hyperresponds when challenged with a glucose load.[10] Since the alcohol-conditioned enhancement of the plasma insulin response is detectable within five minutes of the glucose challenge, the "priming" effect presumably depends either on enlargement of the pool of insulin available for rapid discharge from the β-cell or on facilitation of the release mechanism. In either case, it was expected that alcohol might prove potentially useful as a tool for investigating the early phase of the insulin secretory response to glucose and possibly to other β-cell stimuli.

SUBJECTS AND METHODS

Nine women and three men, aged between twenty-one and thirty years (mean twenty-four) comprised the normal group. The diabetic group was composed of five women and one man, aged between twenty-five and fifty-two (mean thirty-nine). One of the diabetic subjects had been treated with tolbutamide for the previous four months following the discovery of glycosuria and ketonuria. Another had not been treated but had exhibited impaired glucose tolerance over a number of years. The other four subjects were recruited on the basis of a strong family history of diabetes but were not previously known to be diabetic. They were so classified in the course of this investigation, however, on the basis of the criteria discussed below. None of the subjects in either group appeared obese and all weighed within 10 per cent of their "desirable weight" (Metropolitan Life Insurance Company Statistical Bulletin Number 40, 1959). None of the subjects took any medication during the week prior to the start of the study period, and all were consuming normal mixed diets without carbohydrate restriction.

Each subject was tested twice, with an interval of at least two weeks between the two procedures. The two tests were identical except for the preparatory overnight period, the details of which have been described previously.[10] In the twelve hours before the test proper, the subject consumed either 1 L. of 12.5 per cent (by volume) ethyl alcohol in a noncaloric vehicle ("diet" ginger-ale) or the vehicle alone. The respective tests, which were performed in random sequence, will be referred to as the "alcohol test" or the "no-alcohol test." The experimental design is outlined in the following scheme:

Time	Procedure
—15 hours	Admitted to Clinical Research Center.
—14 hours	Standardized 960-calorie meal.
—12 to —2 hours	Every two hours subject is wakened and given either 160 ml. of 12.5 per cent alcohol in sugar-free ginger-ale (alcohol test) or the ginger-ale without the alcohol (no-alcohol test). A total of six doses is administered, so that in the alcohol test the subject consumes the equivalent of about 1 L. of wine over the ten-hour period.
—15 to —5 min.	Venous blood samples obtained.
0 (approx. 8 a.m.)	First glucose dose of 60 ml. of 50 per cent dextrose in buffered water is administered intravenously over 2½ minutes. Venous blood samples are drawn into heparinized syringes via an indwelling plastic cannula at 5, 10, 20, 30, 40 and 50 minutes after the load.
60 minutes	Second glucose dose given and blood samples obtained as above.
120 minutes	Third glucose dose given and blood samples obtained at 5, 10, and 20 minutes.
150 minutes	Glucagon (0.25 mg. in 10 ml. of saline) given intravenously over two minutes, and blood samples obtained at 5, 10 and 20 minutes.

A supplementary study (to clarify a point arising out of the primary investigation) was performed as follows: Five healthy young women (including two from the group described above and three new recruits) aged between twenty and twenty-six were studied. Instead of three glucose loads followed by glucagon, these subjects received three doses of glucagon (0.25 mg. intravenously at half-hour intervals) followed by glucose, both with and without alcohol pretreatment.

All blood samples were centrifuged in the cold immediately after withdrawal and aliquots of plasma were frozen pending analysis. Insulin was assayed immunochemically[11] using a pork insulin standard and guinea pig antiserum to pork insulin. Glucose was measured on a Technicon AutoAnalyzer by a glucose oxidase method.

The plasma insulin responses were computed as the sums of increments in the 5, 10, and 20-minute samples. "Increment" refers to the increase in concentration above the baseline, "baseline" being the concentration in the sample preceding the administration of each of the respective glucose loads. The plasma glucose concentrations were plotted on semilogarithmic paper and a straight line was fitted to the points representing

TABLE 1

Plasma concentrations of glucose, insulin and alcohol prior to each insulinogenic stimulus (mean ± S.E.)

		Glucose (mg./100 ml.)		Insulin (μU./ml.)		Alcohol
		No Alcohol	Alcohol	No Alcohol	Alcohol	(mg./100 ml.)
Before glucose load 1	Nondiabetic	92.9 ± 1.69	85.5 ± 1.47	7.7 ± 0.59	7.2 ± 1.00	30.1 ± 4.65
	Diabetic	105.0 ± 5.10	96.0 ± 3.70	8.0 ± 3.20	8.2 ± 2.95	22.3 ± 4.50
Before glucose load 2	Nondiabetic	157.0 ± 6.35	82.8 ± 7.70	26.0 ± 4.50	13.6 ± 4.30	21.3 ± 3.50
	Diabetic	204.0 ± 2.80	155.3 ± 2.80	22.0 ± 3.00	26.0 ± 5.10	15.6 ± 4.70
Before glucose load 3	Nondiabetic	124.5 ± 10.40	74.1 ± 8.90	12.0 ± 5.30	3.2 ± 2.20	18.6 ± 3.64
	Diabetic	180.6 ± 17.90	127.8 ± 24.90	32.0 ± 11.40	11.0 ± 5.50	5.2 ± 2.50
Before glucagon	Nondiabetic	230.0 ± 13.43	199.0 ± 21.14	65.0 ± 9.67	61.3 ± 10.30	
	Diabetic	*253.0 ± 14.60*	*198.0 ± 25.50*	*50.0 ± 28.20*	*26.6 ± 7.11*	

the 10, 20, 30, 40, and 50-minute values (after the third load only the first three points were available). The fractional disappearance rate (designated K) was derived from this straight line ($K = 0.639/T\text{-}½$). In the two known diabetics and four other subjects, the value for K after the first glucose load in the no-alcohol test was less than 1.1 which is by definition the lower limit of normal in our laboratory, in close agreement with the practice of other workers.[12] These subjects were accordingly classified as diabetic.

Urine specimens were collected before and after the three-dose test and examined for glucose. In no subject did the urinary loss reach 1 gm.; these results will not be reported in detail.

Statistical significance was calculated using Student's *t-test. Four of the subjects (three controls and one diabetic)* included in this report were studied under an earlier protocol which called for only two glucose loads, but which was otherwise identical.

RESULTS

Baseline (pre-glucose) concentrations of plasma constituents

Table 1 presents the mean concentration of glucose, insulin and alcohol, before glucose administration. Plasma glucose was lowered by overnight alcohol in both normals (86 mg./100 ml. after alcohol compared to 93 mg./100 ml. without alcohol) and diabetics (96 mg./100 ml. compared to 105 mg./100 ml.). These differences attributable to alcohol, although small, were

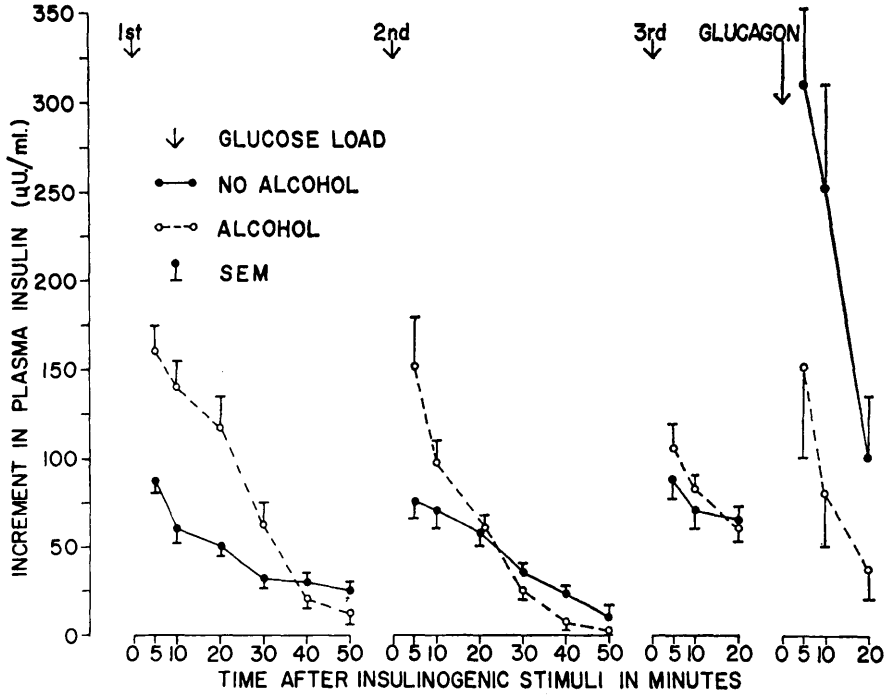

FIG. 1.

Mean plasma insulin responses to multiple insulinogenic stimuli with and without alcohol pretreatment in nondiabetic subjects. The data are expressed as increments of plasma insulin concentrations above baseline values (see "Methods"). The successive insulinogenic stimuli are indicated by the arrows.

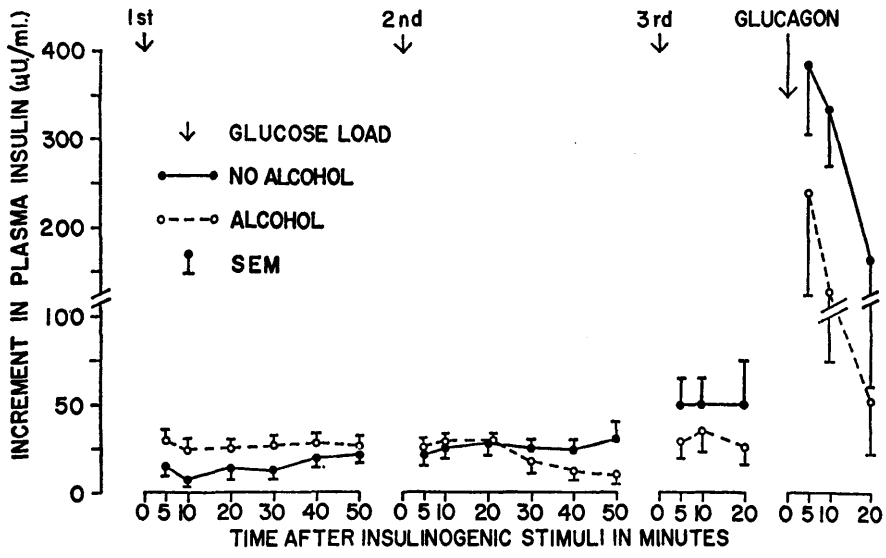

FIG. 2.

Mean plasma insulin responses to multiple insulinogenic stimuli with and without alcohol pretreatment in diabetic subjects. The data are expressed as increments of plasma insulin concentration above baseline values. Successive insulinogenic stimuli are indicated by arrows.

statistically significant ($p < .05$). Baseline plasma insulin concentrations were not discernibly affected by alcohol. The mean plasma alcohol concentrations after alcohol were well below those generally associated with inebriation.[13]

Plasma insulin responses to glucose loading with and without alcohol

The mean plasma insulin responses in the nondiabetic subjects are shown in figure 1. The heights of the three peaks in the no-alcohol series were remarkably similar; the respective increments at five minutes were 87, 74 and 86 μU./ml. In the alcohol series, the first and second glucose loads resulted in significantly ($p < 0.01$) exaggerated peak insulin concentrations (160 and 153 μU./ml., respectively). By the third glucose load, however, the effect of alcohol pretreatment was no longer apparent: The peak increment of 105 μU./ml. was not significantly different ($p > .05$) from the corresponding peak in the no-alcohol test.

The effect of alcohol on the plasma insulin responses to glucagon appeared to be quite different from its effect on glucose-induced insulin secretion. The peak insulin concentration produced by glucagon in the alcohol series (161 μU./ml.) was only half that in the no-alcohol series (311 μU./ml.), a highly significant ($p < .001$) difference.

The plasma insulin responses in the diabetics differed markedly from those just described (figure 2). The first glucose challenge in the no-alcohol test produced a very much smaller increment in insulin concentration than in the nondiabetics. In contrast to the similarity in the sequential response patterns in the nondiabetic group, the diabetics exhibited progressively larger responses to the second and third loads. Instead of the sharp peak increments seen in the nondiabetic, the diabetic curves exhibited no early peaks. The effects of alcohol pretreatment were in general similar to those in the nondiabetics: The response to the first glucose load was enhanced ($p < .05$) and the response to glucagon was depressed. However, in the alcohol test, the insulin output after the second load was no greater than in the no-alcohol control and the response to the third glucose load was smaller, but not significantly so ($p > .05$), than in the control.

Figures 3 and 4 present the insulin responses expressed as sums of increments, in the nondiabetic and diabetic groups, respectively. The constancy of the insulin responses to glucose in the normal subjects is clearly seen, as is the initially large but progressively smaller responses to the same stimuli after alcohol pretreatment. In the diabetics, insulin secretion was very low to begin with in the no-alcohol series, but then improved with subsequent glucose loading. In both diabetics and nondiabetics, the inhibitory effect of alcohol on the responses to glucagon appeared clear-cut. However, the difference does not attain statistical significance ($p > .05$) in the small number of diabetics studied.

Since glucose and glucagon exert synergistic effects on insulin secretion,[14] evaluation of the smaller glucagon-induced insulin output after alcohol requires examination of the plasma glucose concentrations at the time of glucagon administration. The mean plasma glucose concentrations in the normal subjects just before the glucagon injection were 230 ± 13.4 and 199 ± 21.1 mg./

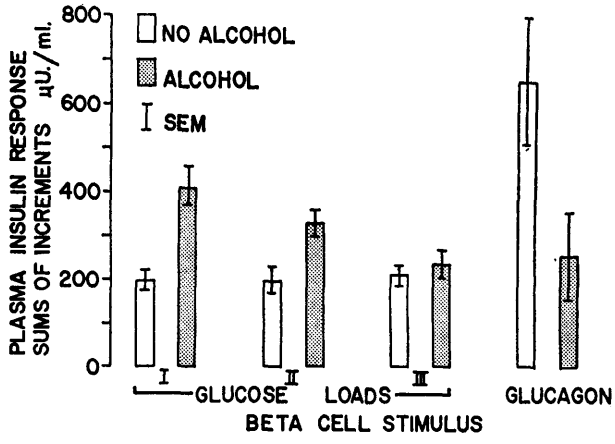

FIG. 3. Insulin secretory responses in the first twenty minutes following each of a succession of β-cell stimuli in nondiabetics with and without alcohol pretreatment. The secretory responses are computed as sums of increments (above respective baselines) at 5, 10 and 20 minutes.

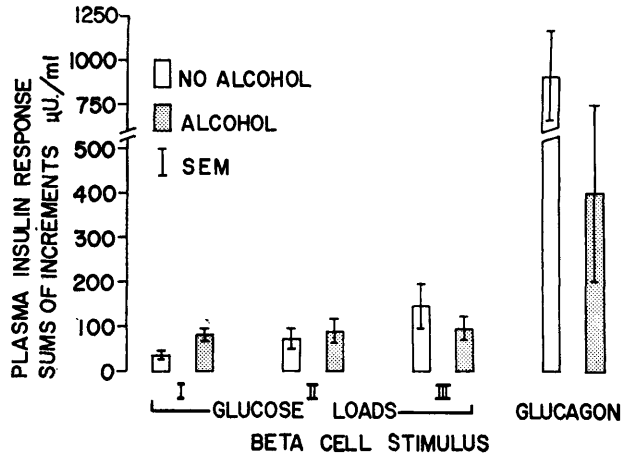

FIG. 4. Insulin secretory responses in the first twenty minutes following each of a succession of β-cell stimuli in diabetics with and without alcohol pretreatment. The secretory responses are computed as sums of increments (above respective baselines) at 5, 10 and 20 minutes.

100 ml. in the no-alcohol and alcohol tests, respectively. This difference is not statistically significant. Furthermore, in four of the twelve subjects, the pre-glucagon blood sugar was higher in the alcohol series, yet these too showed the depressed insulin response to glucagon after alcohol. In the diabetic group, however, the respective mean glucose concentrations just before glucagon (253 ± 14.6 mg./100 ml. in the no-alcohol test and 198 ± 25.5 mg./100 ml. in the alcohol test) were significantly different ($p < .05$).

Effect of alcohol on insulin responses to glucagon not preceded by glucose

The smaller insulin response to glucagon after alcohol pretreatment raised the alternative possibilities either of a direct suppressive effect on this process by alcohol or of a depletion of insulin reserves by the alcohol-induced exaggerated secretory responses to the preceding glucose loads. In order to choose between these two possibilities, another experiment was performed in which glucagon was given to a small group of nondiabetic subjects without prior glucose loading.

The mean glucagon-induced plasma insulin responses, expressed as sums of increments (figure 5) appear to be attenuated by previous alcohol ingestion. The difference was not statistically significant ($p > .05$) after the first glucagon dose, but was significant ($p < .05$) with respect to the second and third doses. In the no-alcohol series the responses to the second and third glucagon injections were larger than to the first ($p < .05$), and the difference between the alcohol and no-alcohol series was exaggerated. The plasma insulin response to the subsequent glucose challenge was again higher in the alcohol test ($p < .05$). Thus, glucagon administration did not abolish the priming effect of alcohol on the glucose-induced insulin response.

Glucose tolerance

In both the diabetic and nondiabetic groups, glucose tolerance improved from the first to the second and improved further from the second to the third glucose loads (Staub-Traugott Effect). In the alcohol series, the glucose disposal rates after each of the first two loads were faster than in the corresponding no-alcohol tests (figures 6 and 7).

In the normal subjects after alcohol pretreatment, glucose tolerance was not as good after the third as after the second loads, paralleling the concomitant decline in insulin secretion after the third glucose load.

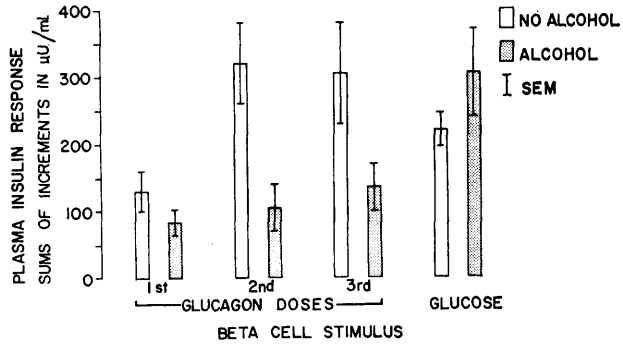

FIG. 5. Insulin secretory response in the first twenty minutes following a succession of β-cell stimuli administered in reverse sequence (glucagon before glucose) in normal subjects. Data expressed as in figure 3.

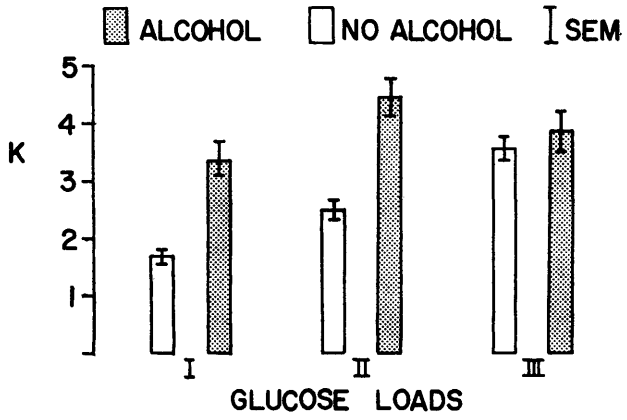

FIG. 6. Mean fractional disappearance rates of glucose (K) after successive glucose stimuli in no-alcohol series in normal subjects.

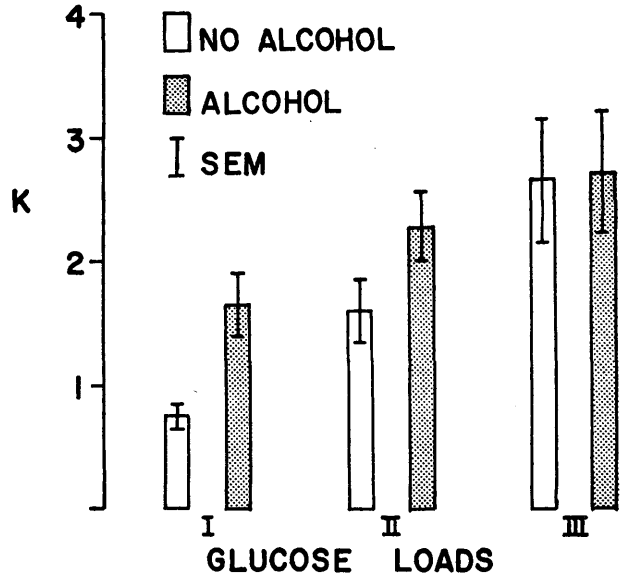

FIG. 7. Mean fractional disappearance rates of glucose (K) after successive glucose stimuli in no-alcohol and alcohol series in diabetic subjects.

DISCUSSION

The remarkable uniformity of the plasma insulin response in normal subjects to repetitive glucose loading has been reported previously.[15] Their undiminished increment in plasma insulin after the third of three large glucose loads indicates that the preceding two loads had not depleted their secretory reserve capacity. When the same successive demands were preceded by alcohol, however, constancy of insulin secretion was not maintained. The mean secretory response to the first load was much larger than in the corresponding period in the no-alcohol test (thus confirming a previous report of the "priming" action of alcohol[10]) but showed a progressive decline with successive stimuli. By the third load, the alcohol-induced enhancement of the early phase of insulin output was no longer apparent. Possibly the alcohol-primed hyperresponse to the first two loads had depleted the pool of immediately releasable insulin, although apparently not to levels below those existing in the absence of alcohol. Thus, even with the alcohol preparation, the goal of β-cell exhaustion proved to be elusive. The results appear to suggest that the mechanism of alcohol "priming" may involve a modest enlargement of the supply of insulin available for quick release, and that only this supernormal component is readily exhaustible. In the diabetics, alcohol pretreatment did not result in a progressively smaller plasma insulin response with successive stimuli. Perhaps it might be said that the successive responses after alcohol pretreatment were diminished in relation to the progressively increasing responses seen in the absence of alcohol.

The magnitude of the alcohol effect was much smaller in the diabetics. Comparison of the mean increments in plasma insulin at five minutes after the first glucose load reveals a difference of only 15 μU./ml. attributable to alcohol. This small difference does represent a doubling of the early response, however. That this may well have been functionally significant is suggested by the improvement in glucose tolerance.

The effect of glucagon on insulin output in the no-alcohol test confirms the observation of others that glucagon is a potent insulin secretogogue in diabetics as well as normals.[9] When the depressant effect of alcohol on glucagon-stimulated insulin output was noted, two possibilities were considered. Firstly, the diminished plasma insulin response to glucagon may have reflected β-cell exhaustion from the alcohol-induced hypersecretion to the preceding glucose loads. In support of this possibility was the observation discussed above, that following alcohol ingestion, the plasma insulin response to the third glucose load was smaller than to the first. The other possibility was that alcohol itself depressed β-cell responsiveness to glucagon. The experiments in which glucagon was given without preceding glucose favor the latter possibility. The effect of alcohol on β-cell function would thus appear to be a complex one, since it primes "positively" for one stimulus and "negatively" for another. To look at the situation another way, the disparate effects of alcohol on the β-cytotrophic effects of glucose and glucagon would appear to indicate that glucose and glucagon either activate different mechanisms for rapid release or tap different, rapidly releas-

able, insulin pools. Other studies in both animals[16,17] and man[9] have led to similar conclusions.

The β-cell priming effect of alcohol was first observed in nondiabetic subjects.[10] The data reported above suggest that the glucose-stimulated acutely releasable insulin in some diabetics can be augmented by pretreatment with alcohol. In our small group of subjects with impaired glucose tolerance, alcohol pretreatment resulted in improved glucose tolerance. This observation raises the prospect that the use of agents which induce β-cell priming might possibly constitute an approach to therapy in diabetes mellitus. It is tempting to speculate that the successful use of alcohol in the treatment of diabetes (reported by several authors[18,19] over fifty years ago) may have represented an early application to clinical medicine of the now-recognized action of alcohol on β-cell responsiveness.

ACKNOWLEDGMENT

This work was supported in part by USPHS Training Grant AM 5071; it was performed in part in the Northwestern University Clinical Research Center (USPHS Grant FR 48).

REFERENCES

[1] Samols, E., and Marks, V.: Interpretation of the intravenous glucose test. Lancet *1*:462-63, 1965.

[2] Garcia, M. J., Soeldner, J. S., Gleason, R. E., and Williams, R. F.: Relationship of blood glucose and serum immunoreactive insulin during repeated intravenous glucose tolerance tests in normals. J. Clin. Invest. *45*:1010-11, 1966.

[3] Colwell, J. A., and Lein, A.: Diminished insulin response to hyperglycemia in prediabetics and diabetics. Diabetes *16*: 560-65, 1967.

[4] Luft, R.: Some considerations of the pathogenesis of diabetes mellitus. New Eng. J. Med. *279*:1086-92, 1968.

[5] Seltzer, H. S., Allen, E. W., Herron, A. L., and Brennan, M. T.: Insulin secretion in response to glycemic stimulus: Relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J. Clin. Invest. *46*:323-35, 1967.

[6] Wrenshall, G. A., Bogoch, A., and Ritchie, R. C.: Extractable insulin of pancreas. Diabetes *1*:87-107, 1952.

[7] Stern, M., Farquhar, J., Silvers, A., and Reaven, G.: Insulin delivery rate into plasma in normal and diabetic subjects. J. Clin. Invest. *47*:1947-57, 1968.

[8] Lerner, R., and Porte, B.: A two pool mechanism for insulin secretion in man. Clin. Res. *17*:388, 1969.

[9] Simpson, R. G., Benedetti, A., Grodsky, G. M., Karam, J. H., and Forsham, P. H.: Stimulation of insulin release by glucagon in noninsulin-dependent diabetics. Metabolism *15*: 1046-49, 1966.

[10] Metz, R., Berger, S., and Mako, M.: Potentiation of the plasma insulin response to glucose by prior administration of alcohol. Diabetes *18*:517-22, 1969.

[11] Herbert, V., Lay, K. S., Gottlieb, C. W., and Bleicher, S. J.: Coated charcoal immunoassay of insulin. J. Clin. Endocr. *25*:1375-84, 1965.

[12] Lundbaek, K.: Intravenous glucose tolerance as a tool in definition and diagnosis of diabetes mellitus. Brit. Med. J. *1*:1507-13, 1962.

[13] Ritchie, J. M.: *In* The Pharmacological Basis of Therapeutics. L. S. Goodman and A. Gilman, (Eds.). New York, MacMillan, 1965, pp. 149, 150.

[14] Samols, E., Marri, G., and Marks, V.: Interrelationship of glucagon, insulin and glucose. Diabetes *15*:855-66, 1966.

[15] Metz, R., and Friedenberg, R.: The effects of repetitive glucose loads on plasma concentrations of glucose, insulin and free fatty acids: Parodoxical plasma insulin responses in subjects with mild glucose intolerance. J. Clin. Endocr. *30*:602-08, 1970.

[16] Brech, W. J., Kaess, H., Schlierf, G., and Rohs, M.: Der Einfluss von Diazoxid auf die Glucagon-induzierte Insulinsekretion. Klin. Wschr. *46*:449-50, 1968.

[17] Lebovitz, H. E., and Pooler, K.: Puromycin potentiation of corticotropin-induced insulin release. Endocrinology *80*:656-67, 1967.

[18] Benedict, H., and Torok, B.: Der Alcohol in der Ernahrung der Zuckerkranken: Experimentelle Untersuchungen. Z. Klin. Med. *60*:329-48, 1906.

[19] Neubauer, O.: Uber die Wirkung des Alcohols auf die Ausscheidung der Azotonkorper. Munchen Med. Wschr. *53*: 790-93, 1906.