

Restoration of the Acute Insulin Response by Sodium Salicylate

A Glucose Dose-related Phenomenon

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SUMMARY

Adult-onset diabetics have markedly diminished or absent acute insulin responses to glucose that can be partially restored by sodium salicylate infusion. To determine whether this restoration of the acute insulin response is glucose dose dependent and whether complete restoration can be achieved, adult-onset diabetics with a mean fasting plasma glucose value of 216 ± 20 mg. per deciliter ($\bar{x} \pm$ S.E.) were stimulated with various doses of intravenous glucose. Restoration occurred in a glucose dose-dependent manner. Complete restoration could not be achieved with the maximal tolerable glucose dose (80 gm.). Second phase insulin secretion also improved in a glucose dose-dependent manner. These findings are compatible with the hypothesis that defective insulin secretion in adult-onset, hyperglycemic diabetics is not due to absolute deficiency of insulin but may be a result of defective recognition of glucose signals by pancreatic B-cells—a defect that can be partially reversed by sodium salicylate. *DIABETES* 27:750-56, July, 1978.

It is well known that adult-onset diabetics with fasting hyperglycemia have markedly diminished or absent acute insulin responses to intravenous glucose.¹⁻⁶ However, the pathogenesis of this defect is unclear. In order to elucidate the underlying mechanism, we and others have examined several endogenous substances that can inhibit acute insulin responses in nondiabetics, such as catecholamines⁷⁻⁹ and somatostatin.^{10,11} We have more recently reported that prostaglandin E₂ (PGE₂) inhibits acute insulin responses to glucose in normal man.¹² This effect appeared to be glucose specific since the acute insulin response to arginine was not affected by PGE₂.

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Infusion of sodium salicylate, an inhibitor of endogenous prostaglandin synthesis,¹³ augmented acute insulin responses in normal subjects and partially restored absent acute insulin responses to glucose in adult-onset diabetics. Thus, we suggested that endogenous PGE may play a role in defective insulin secretion in diabetes mellitus.

In order to determine the maximal extent to which sodium salicylate can restore absent acute insulin responses to glucose, the studies described herein were performed. They were designed to answer (1) whether restoration of acute insulin responses in diabetics by sodium salicylate is glucose dose dependent, and (2) whether complete normalization of acute insulin responses can be achieved.

MATERIALS AND METHODS

All subjects (table 1) were between the ages of 41 and 75 years with body weight between 100 and 166 per cent of ideal ($\bar{x} \pm$ S.E. = 122 ± 5 per cent). They had fasting plasma glucose levels of between 122 and 320 (216 ± 20) mg. per deciliter and basal insulin levels of between 5 and 47 (16 ± 3) μ U. per milliliter. None were receiving insulin; those being treated with oral agents were withdrawn from these drugs at least five days before the studies. Special care was taken to insure against the recent use of drugs that contain aspirin.

All studies were performed at 8 a.m. after a 12-hour fast and while at bed rest. Scalp vein needles were placed in each antecubital vein (one for infusion and one for blood sampling) and kept patent with a slow infusion of saline. Intravenous glucose tolerance tests were performed by injecting various doses of glu-

TABLE 1
Clinical profiles and changes in circulating insulin and glucose after various glucose doses

| Patient | Age (yr.) | %IBW* | Fasting | | 5-gm. Dose | | | | 20-gm. Dose | | | |
|-----------|-----------|-------|-------------------------|-------------------|-----------------------|--------------|-----------------|--------------|----------------------|--------------|-----------------|--------------|
| | | | insulin (μ U./ml.) | glucose (mg./dl.) | Control Δ AIR* | Peak glucose | SS Δ AIR | Peak glucose | Control Δ AIR | Peak glucose | SS Δ AIR | Peak glucose |
| 1 | 48 | 107 | 8 | 146 | -1 | 252 | 0 | 232 | 0 | 326 | 16 | 284 |
| 2 | 57 | 133 | 25 | 195 | 0 | 265 | 1 | 246 | -1 | 358 | 13 | 327 |
| 3 | 73 | 105 | 5 | 265 | 0 | 306 | 1 | 298 | 1 | 435 | -1 | 447 |
| 4 | 55 | 143 | 8 | 303 | 1 | 284 | 2 | 294 | 2 | 446 | 0 | 454 |
| 5 | 62 | 100 | 11 | 206 | 3 | 204 | 5 | 198 | -2 | 354 | 18 | 398 |
| 6 | 47 | 118 | 5 | 363 | -2 | 381 | 2 | 338 | | | | |
| 7 | 65 | 130 | 23 | 129 | | | | | -5 | 300 | 23 | 270 |
| 8 | 62 | 141 | 8 | 208 | | | | | 1 | 322 | 6 | 288 |
| 9 | 47 | 129 | 31 | 232 | | | | | 4 | 400 | 25 | 352 |
| 10 | 73 | 109 | 7 | 143 | | | | | 1 | 325 | 4 | 228 |
| 11 | 53 | 111 | 16 | 122 | | | | | 5 | 262 | 24 | 252 |
| 12 | 41 | 127 | 20 | 126 | | | | | 3 | 292 | 51 | 278 |
| 13 | 42 | 166 | 47 | 276 | | | | | -11 | 418 | 28 | 445 |
| 14 | 75 | 110 | 18 | 208 | | | | | | | | |
| 15 | 58 | 102 | 15 | 320 | | | | | | | | |
| \bar{x} | 57 | 122 | 16 | 216 | 0 | 282 | 2 | 268 | 0 | 353 | 17 | 335 |
| S.E. | 3 | 5 | 3 | 20 | 1 | 27 | 1 | 23 | 1 | 18 | 4 | 25 |
| | | | | $\Delta=28$ | | $\Delta=35$ | | $\Delta=157$ | | $\Delta=161$ | | ± 10 |
| | | | | | | ± 7 | | ± 3 | | ± 6 | | |

| Patient | 40-gm. Dose | | | | 80-gm. Dose | | | |
|-----------|----------------------|--------------|-----------------|--------------|----------------------|--------------|-----------------|--------------|
| | Control Δ AIR | Peak glucose | SS Δ AIR | Peak glucose | Control Δ AIR | Peak glucose | SS Δ AIR | Peak glucose |
| 1 | -2 | 489 | 23 | 505 | -1 | 772 | 12 | 768 |
| 2 | | | | | | | | |
| 3 | 0 | 560 | 7 | 535 | 0 | 816 | 10 | 828 |
| 4 | | | | | | | | |
| 5 | | | | | | | | |
| 6 | | | | | | | | |
| 7 | 3 | 339 | 9 | 330 | 3 | 680 | 8 | 640 |
| 8 | 4 | 506 | 16 | 488 | 9 | 660 | 39 | 764 |
| 9 | 12 | 536 | 56 | 538 | 37 | 716 | 31 | 708 |
| 10 | | | | | | | | |
| 11 | | | | | | | | |
| 12 | | | | | | | | |
| 13 | | | | | | | | |
| 14 | 19 | 555 | 40 | 428 | | | | |
| 15 | | | | | -1 | 812 | 9 | 844 |
| \bar{x} | 6 | 496 | 25 | 471 | 8 | 743 | 18 | 758 |
| S.E. | 4 | 41 | 9 | 36 | 7 | 30 | 6 | 34 |
| | | $\Delta=281$ | | $\Delta=250$ | | $\Delta=516$ | | $\Delta=524$ |
| | | ± 20 | | ± 17 | | ± 23 | | ± 19 |

*%IBW=per cent ideal body weight, SS=sodium salicylate, Δ AIR=change in acute insulin response.

cose (5, 20, 40, and 80 gm. as a 50 per cent dextrose solution) at a rate greater than 1 gm. per second on different days. Sodium salicylate (SS) was infused intravenously at a rate of 40 mg. per minute, which resulted in serum salicylate levels of 19 ± 1 mg. per deciliter by 60 minutes. Blood samples were drawn at -15, -10, -5, and 0 minutes before the glucose pulse and at 2, 3, 4, 5, 10, 15, 20, 25, 30, 45, and 60 minutes after the pulse.

Basal plasma insulin levels were calculated as the mean of the four samples drawn before the first glu-

cose pulse. The acute insulin response (AIR) was defined as the mean of the 3-, 4-, and 5-minute values after the glucose pulse minus the basal insulin level, and it was expressed as per cent of the basal level (\bar{x} 3-5' Δ IRI, per cent basal). Second phase insulin secretion was defined as the insulin area above basal from 10 to 60 minutes after the glucose pulse and was expressed as per cent basal. Calculations for the responses to the second glucose pulse used the insulin level just before this pulse instead of initial basal insulin value. Glucose disappearance (Kg) was calculated

as the slope of the natural logarithm of glucose concentration between 10 and 30 minutes after the glucose pulse.

Blood samples were collected in EDTA (1 mg. per milliliter) tubes and kept in ice until centrifuged at 4° C. The plasma was kept frozen until future analysis for glucose by an AutoAnalyzer-ferricyanide method¹⁴ and for insulin by a modification of the method of Morgan and Lazarow.¹⁵ Serum level of salicylate was determined by a commercial salicylate test set.¹⁶ Statistical analysis was performed by student's *t*-test, paired *t*-test, and Wilcoxon rank sum test. Results are reported as mean and standard error of the mean.

RESULTS

The Effect of Sodium Salicylate Infusion on Insulin Secretion in Adult-onset Diabetics

The infusion of sodium salicylate for 60 minutes caused a statistically significant increase of about twofold in basal insulin levels in each glucose dose group (figures 1-4). The magnitude of the restorative effect of sodium salicylate on the acute insulin response and second phase insulin secretion was glucose dose dependent (table 1, figure 5). Sodium salicylate (SS) failed to improve the acute insulin response (AIR) to the 5-gm. glucose pulse (before SS: AIR = 6 ± 17 per cent; during SS: AIR = 23 ± 8 per cent; $n = 6$, $p = \text{N.S.}$; figure 1). However, 10 of 12 diabetics with no significant AIR during the control period developed a partial restoration of the AIR to the 20-gm. glucose pulse during SS infusion (before SS: AIR = 5

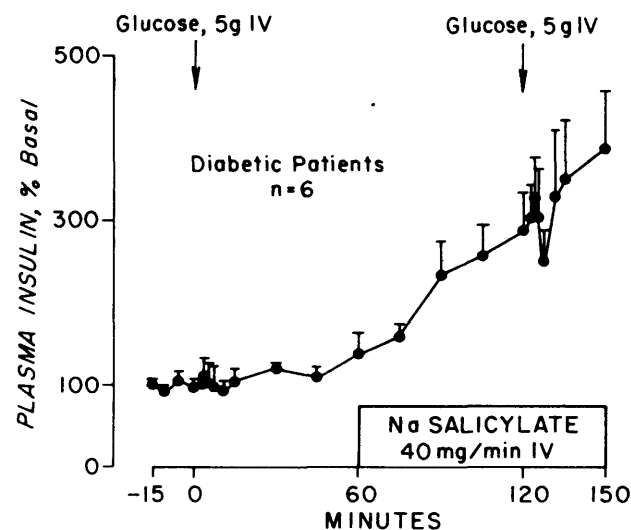


FIG. 1. Circulating insulin levels in response to 5-gm. glucose pulses before and during infusions of sodium salicylate in diabetics.

± 6 per cent; during SS: AIR = 97 ± 23 per cent; $n = 12$, $p < 0.005$; figure 2). The glucose dose of 40 gm. without sodium salicylate failed to elicit significant acute insulin responses (AIR = 27 ± 21 per cent; $n = 6$, $p = \text{N.S.}$; figure 3); however, during SS infusion all six diabetics tested had acute insulin responses (AIR = 154 ± 43 per cent; $n = 6$, $p < 0.02$). The 80-gm. glucose dose also failed to elicit significant responses (AIR = 32 ± 20 per cent; $n = 6$, $p = \text{N.S.}$; figure 4); during SS infusion there was a significant AIR (AIR = 145 ± 46 per cent; $n = 6$, $p < 0.02$). Although there was an inverse correlation ($r = -0.69$, $p < 0.05$) between the AIR during SS with fasting plasma glucose level, the mean fasting plasma glucose levels for the four glucose doses are not significantly different statistically (5 gm., 254 ± 27 ; 20 gm., 196 ± 19 ; 40 gm., 217 ± 19 ; and 80 gm., 227 ± 31 ; all $p > 0.05$).

The effect of SS on second phase insulin secretion was similar. The 5-gm. glucose pulse before or during SS infusion elicited no significant second phase insulin secretion (figure 1). The 20-gm. glucose pulse before SS infusion elicited significant second phase insulin secretion, but this was enhanced during the infusion (before SS, second phase = $1,704 \pm 446$ per cent; during SS, second phase = $6,851 \pm 994$ per cent; per cent basal; $p < 0.001$, $n = 12$; figure 2). In contrast, during the control study with normal saline infusion in diabetics (figure 2), no significant difference in second phase insulin secretion after the two pulses was observed (first pulse = $1,137 \pm 562$ per cent; second pulse = $1,950 \pm 915$ per cent; $n = 6$, $p = \text{N.S.}$). Second phase insulin secretion after the 40-gm. glucose pulse was also augmented during SS infusion (before SS, second phase = $2,649 \pm 887$ per cent; during SS = $9,213 \pm 2,489$ per cent; $n = 6$, $p < 0.025$; figure 3). After the 80-gm. glucose dose, second phase was again augmented (before SS, second phase = $3,734 \pm 1,689$ per cent; during SS, second phase = $10,072 \pm 2,449$ per cent; $n = 6$, $p < 0.05$).

The Effect of Sodium Salicylate on Circulating Glucose Levels

After 60 minutes of SS infusion, the mean plasma glucose level in all four glucose dose groups invariably and comparably decreased. For example, in the group receiving the 20-gm. glucose pulse, plasma glucose levels just before and after 60 minutes of SS were 219 ± 17 mg. per deciliter and 208 ± 19 mg. per deciliter, respectively ($p < 0.001$). However, similar changes also occurred during one hour of saline infusion (before saline = 222 ± 18 ; after one hour = 209

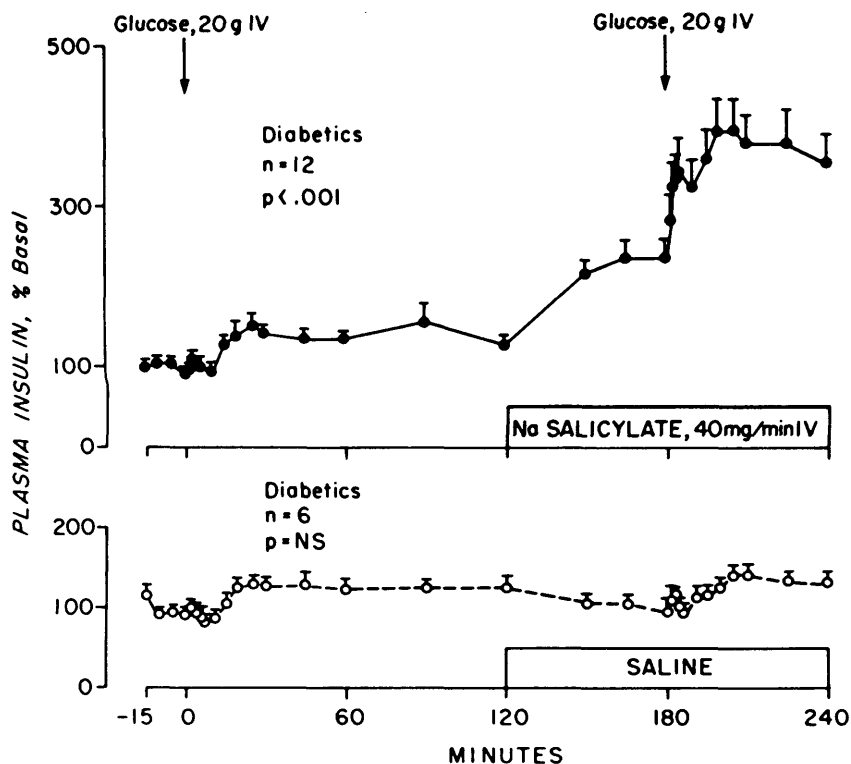


FIGURE 2

Comparison of the insulin responses to 20-gm. glucose pulses before and during sodium salicylate or saline infusions in diabetics.

± 18 ; $n = 6$, $p < 0.01$). There was no significant difference between the magnitude of glucose decreases during sodium salicylate and during saline infusion. *The Effect of Sodium Salicylate on Glucose Disappearance Rates (KG)*

There was a slight but insignificant improvement in KG after the 5-gm. glucose dose during the SS infusion (before SS = 0.21 ± 0.05 ; during SS = 0.37 ± 0.05 ; per cent per minute, $n = 6$, $p = N.S.$). However, after the 20-gm. glucose pulse there was a

significant increase in mean KG (before SS, $KG = 0.56 \pm 0.06$; during SS, $KG = 1.02 \pm 0.17$; per cent per minute, $n = 12$, $p < 0.005$). In contrast, there was no change in KG in the control group given saline rather than sodium salicylate (before saline = 0.65 ± 0.08 ; during saline = 0.72 ± 0.09 ; per cent per minute, $n = 6$, $p = N.S.$). After 40-gm. and 80-gm. glucose pulses, the mean KG increased slightly but insignificantly during SS infusion (40 gm. glucose: control $KG = 0.57 \pm 0.08$; KG during SS = $0.81 \pm$

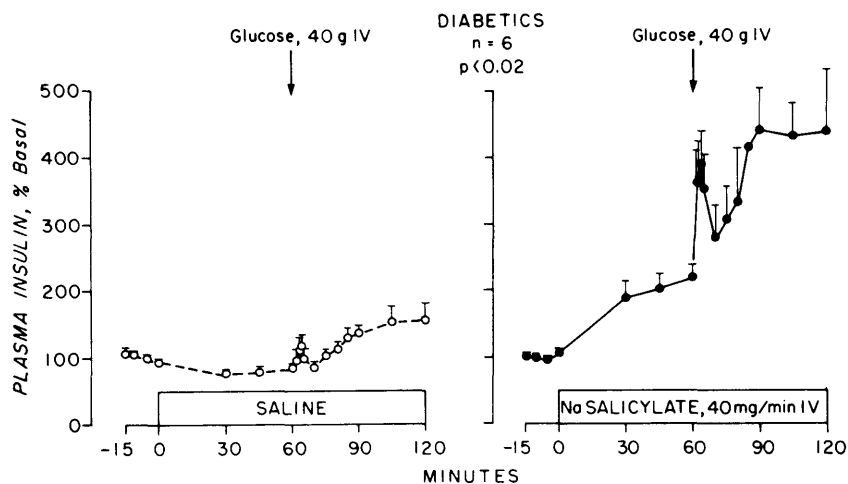
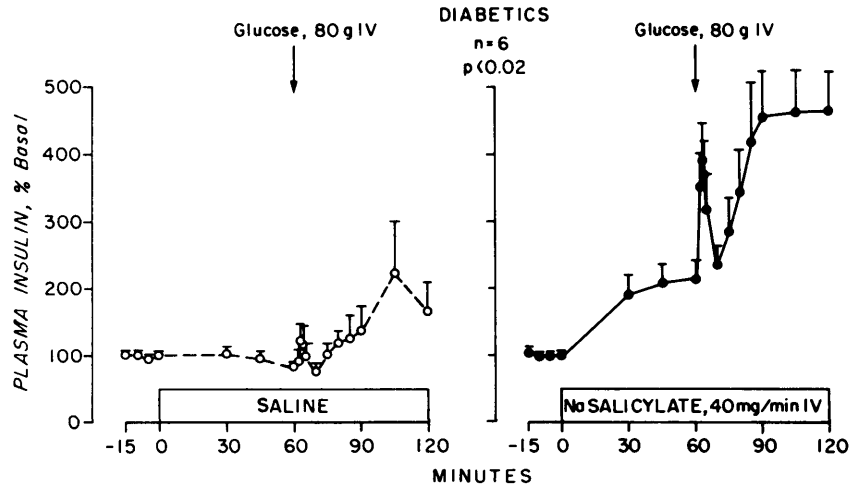


FIGURE 3

Comparison of the insulin responses to 40-gm. glucose pulses during saline or sodium salicylate infusions in diabetics.

FIGURE 4

Comparison of the insulin responses to 80-gm. glucose pulses during saline or sodium salicylate infusions in diabetics.



0.26, $n = 6$, $p = N.S.$; 80 gm. glucose; control $K_G = 0.71 \pm 0.11$; K_G during SS = 0.98 ± 0.17 , $n = 6$, $p = N.S.$)

DISCUSSION

The data from this study uniquely demonstrate that restoration of the acute insulin response and augmentation of second phase insulin secretion by sodium salicylate in hyperglycemic adult-onset diabetics occur in a glucose dose-dependent fashion. A related goal of the study was to assess maximal restoration; thus, the glucose dose-response studies were designed to determine whether complete restoration of the AIR to glucose could be achieved. The glucose dose-response curve seemed to plateau between the 40-gm. and

80-gm. dose, suggesting that a maximal glucose stimulus had been achieved. However, we were unable to test larger glucose doses because 80 gm. consistently caused a transient dizzy sensation, severe flushing, transient (one minute) mild hypotension, and palpitations. This complicates the interpretation of our data with the 80-gm. dose, since stress hormones may have been stimulated, which could have dampened the AIR. Another possibility is that salicylate alone was unable to completely restore defective acute responses because of partial depletion in these diabetics of releasable insulin in pancreatic B-cells. Still another possibility is that brief exposure of the islet to one hour of sodium salicylate is inadequate to produce its maximal restorative effect on the AIR. In

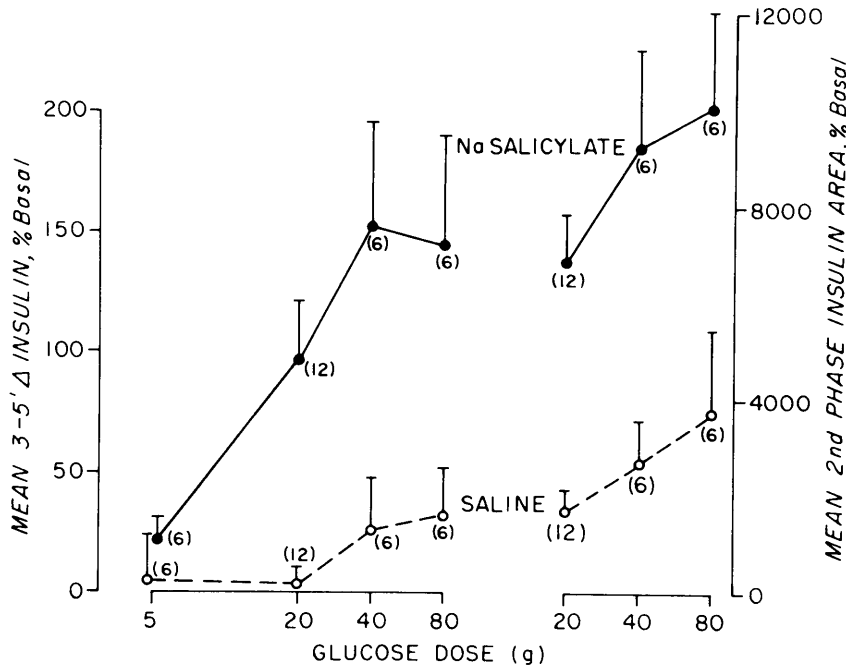


FIGURE 5

Acute insulin responses and second phase insulin responses to various doses of intravenous-glucose pulses during saline or sodium salicylate infusions in diabetics.

spite of the failure to normalize the AIR in diabetes, it is clear from this study that the pathogenesis of defective insulin secretion in diabetes is not due to absolute deficiency of insulin in the B-cells. Rather, it may be a result of defective recognition of glucose signals by the B-cells—a defect that can be partially reversed by sodium salicylate infusion.

Salicylate has been used sporadically in the treatment of diabetes mellitus since 1876.¹⁷ It has been shown to improve glycosuria¹⁸⁻²¹ and to have a hypoglycemic effect.^{22,23} The mechanisms of this hypoglycemic action have been attributed to (1) increased peripheral utilization of glucose due to increased uncoupling of oxidative phosphorylation;²⁴ (2) decreased synthesis of glucose secondary to the decreased activities of the enzymes of gluconeogenesis;²⁵ and (3) augmentation of insulin secretion.²⁶ Since the doses required to uncouple oxidative phosphorylation and to inhibit enzymes of gluconeogenesis are far larger than those used in our investigation, it is difficult to accept the first two alternatives to explain our findings. Since glucose disposal is a function of the AIR, the improvement of Kg after the 20-gm. glucose pulse during salicylate infusion in our study and the significant reduction in the half-time disappearance of intravenous glucose in both the normal subjects and normoglycemic diabetics in the study of Field et al.²⁶ favor enhanced insulin secretion as the explanation. This action of salicylate on glucose disposal should be taken into consideration when it is used to treat diabetics already receiving other hypoglycemic agents or insulin, since its additional effect may lead to unanticipated hypoglycemia.

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