Insulin Secretion Studied in the Perfused Rat Pancreas

II. Effect of Glucose, Glucagon, 3′5′ Adenosine Monophosphate, Theophylline, Imidazole and Phenoxybenzamine; Their Interaction with Diazoxide

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SUMMARY

This communication reports studies dealing with the action of glucagon, theophylline, cyclic adenosine monophosphate (3′5′-AMP) and phenoxybenzamine on insulin secretion, and their possible interaction with glucose and diazoxide.

Diazoxide inhibited both phases of insulin secretion produced by glucose. This blocking action was suppressed when the alpha-blocking phenoxybenzamine was present.

Glucagon produced a uniform pattern of insulin secretion when perfused alone. A one-minute pulse of 3′5′-AMP produced a sharp peak of insulin secretion. Neither glucagon nor 3′5′-AMP effects were inhibited by diazoxide.

Theophylline did not increase the basal insulin-secretion. However, when perfused together with glucose, theophylline stimulated additional insulin secretion, significantly higher than that produced by glucose alone.

When combinations of glucagon plus glucose, 3′5′-AMP plus glucose and theophylline plus glucose, were perfused, diazoxide blocked the secretion of insulin stimulated by glucose, but not that produced by glucagon, 3′5′-AMP or theophylline. This suggests that glucagon, 3′5′-AMP and theophylline stimulate insulin secretion by acting on mechanisms different from the ones stimulated by glucose. DIABETES *20*: 457-66, July, 1971.

The hyperglycemic action of diazoxide (3-methyl-7-chloro-1, 2-benzothiadiazine-1, 1-dioxide) both in man and experimental animals[1-3] has led to its use as a therapeutic agent,[4,5] and especially as a tool in studies related to insulin secretion and diabetes mellitus.[6,7]

The mechanism of diazoxide hyperglycemia has been correlated with direct inhibitory action on insulin secretion. An extrapancreatic mechanism involving the adrenal glands has also been reported.[8-10]

The mode of action of diazoxide on the pancreas has not been settled yet. Although it has been shown that this drug inhibits insulin secretion both in vivo[6,11] and in vitro,[7,12] the mode of action is not clear. Previous work from our laboratory[13] showed that diazoxide inhibits insulin release, but not synthesis, in the isolated perfused rat pancreas, when glucose is used as stimulus.

A considerable amount of evidence has been accumulated in recent years concerning inhibition or stimulation of insulin secretion by hormones and/or substances involved in the regulation of the adenyl cyclase system.[14-17] Most studies trying to correlate the mode of action of diazoxide with the adenyl cyclase system are contradictory.[18-20]

This paper is a continuation of Part 1, and reports a series of experiments performed in the perfused isolated pancreas investigating (a) the possible interaction of diazoxide with glucagon and theophylline, 3′5′-AMP, imidazole, and the alpha-blocker phenoxybenzamine, (b) the blocking action of diazoxide as a tool in the study

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of patterns of insulin secretion produced by the above-mentioned substances and their effects on the mechanism of glucose action, and (c) the pancreatic levels of 3′5′-AMP produced by glucose and by theophylline and the response to diazoxide and imidazole.

MATERIAL AND METHODS

Animals (rats) used in all pancreas perfusions and the treatment of the perfusate (buffer) were identical to those described in Part I of our communication.

Diazoxide was dissolved at pH 10.5, and in all cases was added to the perfusate at zero minute of the perfusion, in the same way as in previously described experiments (Part I). The introduction of this solution into the perfusion system results in a 100 to 200-fold dilution, which is easily buffered by the perfusate to its pH of 7.4. A concentration of 15 mg. per cent of diazoxide added to control samples in the insulin assay showed no difference in insulin values over a range of 10 to 250 μU./ml.

All other substances studied except 3′5′-AMP were added to the perfusate at three minutes, and perfused at a constant rate during the sixty minutes of perfusion. They reached the pancreas between 1.30 to 2.15 minutes after they were introduced into the perfusate.

Glucagon,* in concentrations of 5 μg./ml., showed, as determined by our immunoassay, an insulin contamination within a range of 6 to 10 μU./ml. This amount was deducted from insulin values in all experiments using glucagon. Phenoxybenzamine† produced a slight turbidity. Control samples in the radioimmunoassay showed that this did not interfere with the insulin assay.

3′5′-AMP (free acid or dibutyryl sodium salt) was infused as a one-minute pulse directly into the aortic cannula. In all cases, the flow rate was 10 ml./min.; consequently the 3′5′-AMP pulse (1.7 mg./ml.) resulted in a total dose of 5 mM. Tissue content of 3′5′-AMP was measured in pieces of pancreas (80 to 180 mg.), taken at —2,6,12,15,30, and 60 minutes of the perfusion. The samples were weighed, boiled for two minutes in 2 ml. distilled water, frozen, and stored for 3′5′-AMP determinations. 3′5′-AMP measurements were based on the method described by Krishna et al.,[22] modified for continuous Dowex column chromatography, without carrier ATP.

Insulin was assayed by the radioimmunoassay of Hales and Randle[21] using pork insulin as standard. The amount was expressed as μU./min.

The results were evaluated with standard procedures of statistical analysis, differences being judged significant only if p-values were lower than $p < 0.05$.

RESULTS

Figure 1 shows the effect of glucose and diazoxide on insulin secretion by the perfused pancreas. All other figures are compared with this basic pattern.

Crystalline glucagon at concentrations of 5 μg./ml. and 10 μg./ml. produced a quick and significant increase of insulin secretion (figure 2). This increased secretion rate remained almost constant during the sixty minutes of perfusion. A 10 μg./ml. concentration of glucagon produced significantly higher levels of insulin than the 5 μg./ml. concentration. Diazoxide at 10 mg. per cent or 25 mg. per cent concentration had no inhibitory effect on the rate of insulin secretion produced by the stimulus of 5 μg./ml. glucagon.

The effect of 5 μg./ml. glucagon perfused with 300 mg. per 100 ml. glucose is shown in figure 3. When perfused together, they produced the same diphasic pattern of insulin secretion typical for glucose stimulation, but significantly higher. The same figure shows that

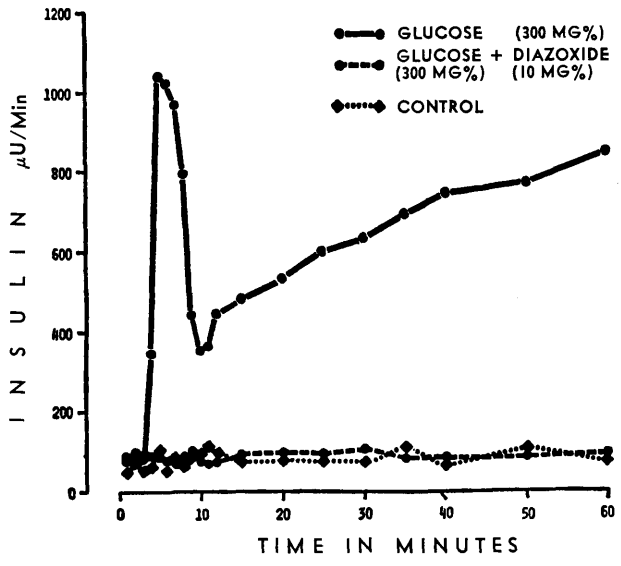

FIG. 1. Effect of buffer alone (control, n = 6), glucose (300 mg. per cent) (n = 6) and glucose plus diazoxide (10 mg. per cent) (n = 6) on the levels of insulin secreted by the pancreas. Statistical analysis shows $p < 0.001$ from five to eight minutes (first phase) and from twelve to sixty minutes (second phase), when insulin levels produced by the glucose stimulus are compared with control group.

*Crystalline (lot 258-234B-167-1), kindly provided by Dr. M. A. Root, Lilly Research Laboratories.

†Dibenzyline RN, kindly supplied by Smith, Kline and French Research Laboratories.

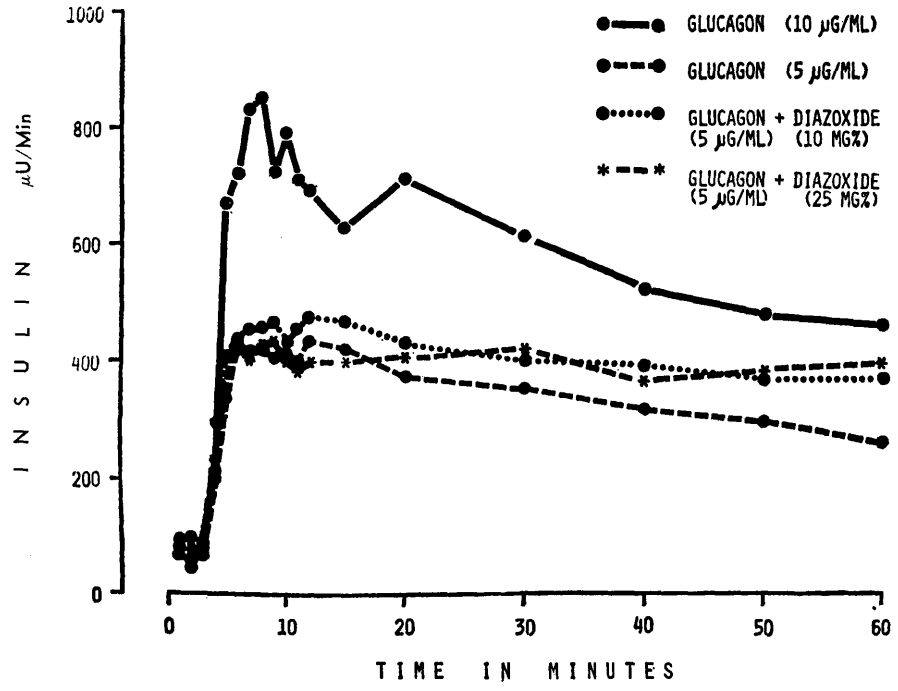

FIG. 2.

Effect of glucagon (10 μg./ml.) on insulin secretion (n = 4), and 5 μg./ml. (n = 6) when perfused alone, and of 5 μg./ml. glucagon with the addition of 10 mg. per cent (n = 6) and 25 mg. per cent (n = 5) of diazoxide. Statistical analysis: (a) glucagon (5 μg. and/or 10 μg./ml.)—$p < 0.001$ from five to sixty minutes when compared with control; (b) glucagon at 10 μg./ml. produces higher values than at 5 μg./ml. concentration—$p < 0.005$ at seven and ten minutes, and $p < 0.001$ for the rest of the points from five to sixty minutes.

10 mg. per cent diazoxide reduced the amount of insulin produced by glucose (300 mg. per 100 ml.) plus glucagon (5 μg./ml.) to a lower, constant level. This amount and pattern is not significantly different from that produced by 5 μg./ml. glucagon alone.

Theophylline, in concentrations of 0.44 mM, 5 mM or 12 mM, failed to stimulate insulin secretion (figure 4). However, when theophylline (0.44 mM) was perfused together with glucose (300 mg./100 ml.), it produced a clear increase in the secretion rate, significantly higher than the increase produced by glucose alone. The same situation resulted when theophylline was used at a 5 mM concentration, but there was no statistically significant difference between the amounts of insulin secreted in both experimental conditions.

Figure 5 demonstrates that the addition of diazoxide (10 mg. per cent) diminished significantly the rate of insulin secretion produced by glucose (300 mg./100 ml.) plus theophylline (0.44 or 5 mM concentration). However, the amount of insulin that was still secreted was significantly higher than the control levels. This elevated rate of insulin secretion was suppressed to control levels by imidazole (5 mM).

Table 1 shows changes of 3'5'-AMP levels in pan-

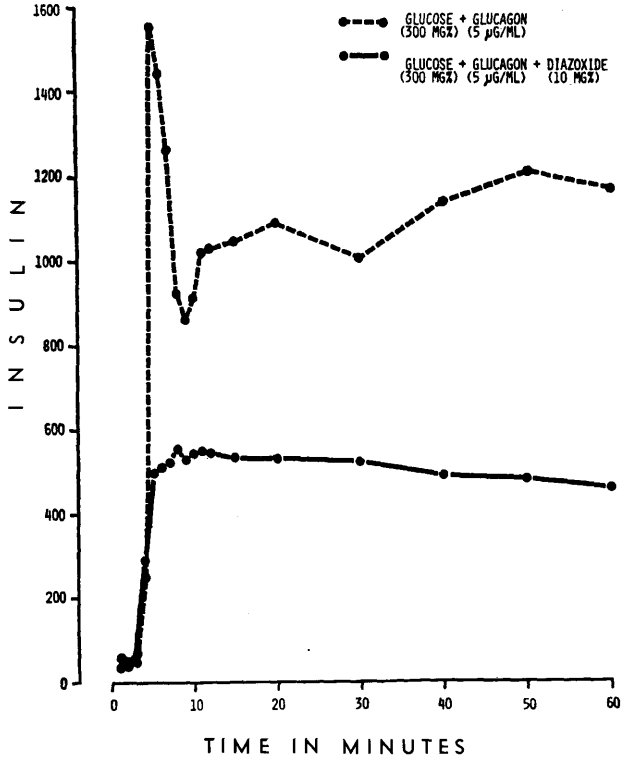

FIG. 3. Insulin secretion produced by glucose (300 mg. per cent) plus glucagon (5 μg./ml.) (n = 6) and the effect of diazoxide (10 mg. per cent) (n = 6). Statistical analysis: glucagon (5 μg./ml.) plus glucose (300 mg. per cent) produces higher amounts of secreted insulin when compared with the one produced by glucose alone—$p < 0.001$ at five and from nine to sixty minutes, and $p < 0.005$ at six and seven minutes.

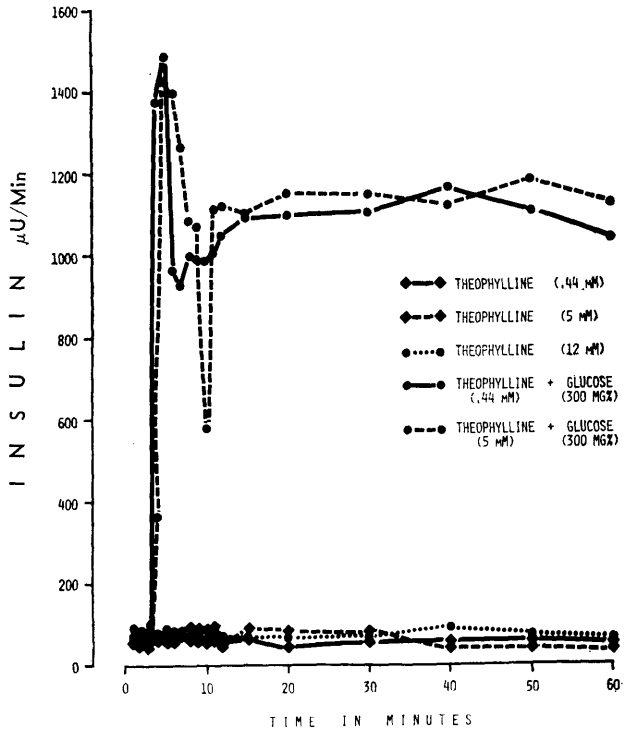

FIG. 4. Lack of effect of theophylline on insulin secretion at 0.44, 5 and 12 mM concentrations (n = 3 in each group). Effect of glucose plus 0.44 or 5 mM theophylline (n = 6 in each group). Statistical analysis: (a) glucose (300 mg. per cent) plus theophylline (0.44 mM) —$p < 0.001$ at four and five minutes and from nine to fifty minutes, $p < 0.005$ at minute 8, and 0.05 at sixty minutes when compared with glucose alone; (b) glucose (300 mg.per cent) plus theophylline (5 mM)— $p < 0.001$ at four and from ten to fifty minutes, $p < 0.005$ at five and sixty minutes, $p < 0.01$ at seven minutes, and $p < 0.05$ at eight and nine minutes, when compared with glucose alone.

creatic tissue in different treatment groups. The percentages are average increments obtained during the perfusion, compared to values before perfusion (considered 100 per cent). There were three to five perfusions in each treatment group; n-values refer to number of samples in each treatment group. The basal levels of 3′5′-AMP before perfusion ranged from 13.44 ± 0.89 to 21.65 ± 1.40 gamma/100 mg.

As established by one-way analysis of variance, increases in groups 4-7 where theophylline was used, were significantly different from the control group.

The effect of a one-minute pulse of 3′5′-AMP (free acid) on the secretion of insulin is illustrated in figure 6. There was a quick and significant increase of the insulin output of about four minutes duration ($p < 0.001$ from three to six minutes), followed by a return to basal levels. The same figure shows that the constant perfusion of diazoxide at 10 mg. per cent or 25 mg. per cent concentrations from zero to sixty minutes had no effect on the insulin secretion stimulated by 3′5′-AMP at any of the doses used. Sodium-dibutyryl 3′5′-AMP was reported to enter the cell better than the free acid form. Experiments summarized in table 2 indicate that there was no difference in the levels of insulin secretion induced by pulses of sodium-dibutyryl 3′5′-AMP. It also shows that diazoxide had no effect on the insulin secretion induced by sodium-dibutyryl 3′5′-AMP.

The effect of a one-minute pulse of 3′5′-AMP (free acid form) on insulin levels produced by glucose stimulation is illustrated in figure 7. The glucose (300 mg./ 100 ml.) perfusion was started so that both glucose and 3′5′-AMP reached the pancreas practically at the same time. 3′5′-AMP significantly increased the first

FIG. 5.
Effect of diazoxide (10 mg. per cent) on the insulin levels produced by glucose (300 mg. per cent) plus 0.44 or 5 mM theophylline concentrations (n = 6 in each group). Blocking effect of imidazole (5 mM) when perfused together with glucose, diazoxide, and theophylline (5 mM) (n = 6). Statistical analysis: (a) glucose plus theophylline (0.44 mM) plus diazoxide—$p < 0.001$ from 5 to 60 min., and (b) glucose plus diazoxide and theophylline (5 mM)—$p < 0.001$ from 7 to 60 min., when both groups are compared with the corresponding group in the absence of diazoxide; (c) glucose plus diazoxide plus theophylline (0.44 mM) compared with control group—$p < 0.001$ from 7 to 60 min., and $p < 0.01$ at 5 and 6 min.; (d) glucose plus diazoxide plus theophylline (5 mM) compared with control group—$p < 0.001$ from 9 to 60 min., and $p < 0.005$ from 5 to 8 min.

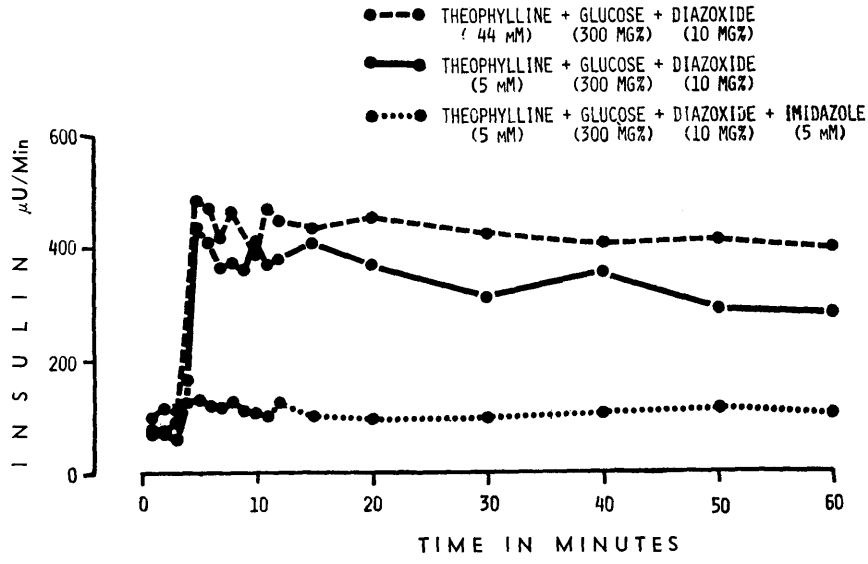

TABLE 1

Endogenous 3′5′-AMP levels in pancreatic tissue. All values: x ± SEM.

Group Number	Composition of Perfusate	n	3′5′-AMP Average Percentage Changes Compared with Zero Time†
1.	control	21	105.6 ± 3.3
2.	glucose (300 mg. per cent)	34	116.7 ± 3.9
3.	glucose (300 mg. per cent) diazoxide (10 mg. per cent)	26	115.6 ± 7.4
4.	glucose (300 mg. per cent) theophylline (0.44 mM)	34	141.3 ± 14.6*
5.	glucose (300 mg. per cent) theophylline (5 mM)	35	154.0 ± 8.2*
6.	glucose (300 mg. per cent) theophylline (0.44 mM) diazoxide (10 mg. per cent)	35	143.1 ± 10.3*
7.	glucose (300 mg. per cent) diazoxide (10 mg. per cent) theophylline (5 mM)	27	144.3 ± 12.0*
8.	glucose (300 mg. per cent) theophylline (5 mM) diazoxide (10 mg. per cent) imidazole (5 mM)	28	100.9 ± 6.7

* Indicates values significantly different from control.
† The average percentage changes represent the mean of 3′5′-AMP levels measured at the same time intervals as indicated for insulin.

phase of insulin secretion produced by glucose ($p < 0.001$ at three and four minutes), but had no stimulatory effect on the second phase. Diazoxide blocked both phases of insulin secretion produced by glucose, but the increase caused by 3′5′-AMP (first phase) was not inhibited ($p < 0.001$, from four to seven minutes).

Figure 8 shows that when 3′5′-AMP was added between ten and eleven minutes of perfusion (at the time when the insulin levels between both phases were at their minimum), it produced a new peak of insulin secretion followed by the second phase. Diazoxide reduced this maximum to a level identical with the one produced by 3′5′-AMP alone.

The effect of the alpha-blocker phenoxybenzamine on the rate of insulin secretion is illustrated in figure 9. Phenoxybenzamine, when perfused at 2 mM concentration, produced a slight but significant increase in the rate of insulin secreted. When 2 mM phenoxybenzamine was perfused together with 10 mg. per cent diazoxide, most of the increases in insulin levels were abolished. When glucose (300 mg./100 ml.) plus diazoxide (10 mg. per cent) were perfused together with phenoxybenzamine (2 mM), a diphasic pattern of insulin secretion appeared.

DISCUSSION

The diphasic insulin secretion by the isolated perfused pancreas, in response to glucose stimulation, and its possible connection and physiological implication with a two-compartment model of the mechanism of insulin secretion has been discussed.[13,23,24] Diazoxide blocks both phases of insulin secretion; this has also been reported and discussed earlier.[13] Glucagon is known to stimulate insulin secretion both in vivo[25,26] and in vitro.[15,27]

Some authors have reported that the presence of glucose is necessary for glucagon stimulation of insulin release; others have shown that glucagon can produce insulin secretion in vitro in the absence of glucose.[28-30] Our results show that when perfused at a constant rate, glucagon produces a distinct increase in the levels of secreted insulin in the absence of glucose stimulation.

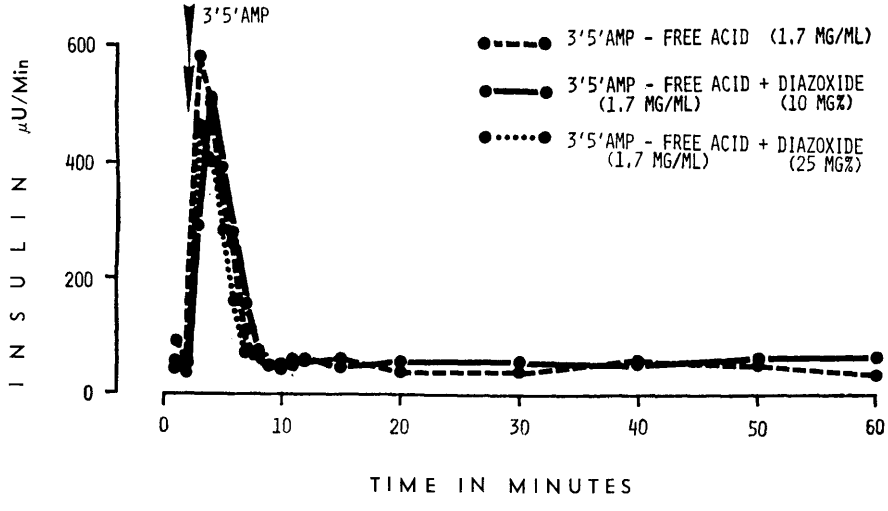

FIG. 6.

Insulin secretion produced by a one-minute pulse of 3′5′-AMP, administered between minutes 2 and 3 (n = 5). No inhibition can be observed when 10 mg. per cent (n = 5) or 25 mg. per cent (n = 4) of diazoxide was added to the perfusion.

TABLE 2

Effect of diazoxide on the insulin secretion produced by Na-dibutyryl-3′5′-AMP.

Time in Minutes		1	2	3	4	5	6	7	8	9	10	11	12
Group 1* (n = 3)	Mean	57	36	416	318	228	145	55	40	45	55	47	57
	± SEM	10	11	29	58	42	32	10	10	9	8	6	16
Group 2† (n = 3)	Mean	35	40	314	296	225	96	45	45	45	46	54	43
	± SEM	6	12	53	49	26	14	14	16	13	9	10	9

* One-minute pulse of 1.7 mg./ml. of Na-dibutyryl-3′5′-AMP (flow 10 ml./min.) given between the second and third minutes of perfusion.
† Na-dibutyryl-3′5′-AMP administered in the same way as in Group 1; diazoxide (10 mg. per cent) perfused from zero to twelve minutes.

The pattern of insulin release stimulated by glucagon is a uniform one, and no peaks or phases can be observed during the time of perfusion. Curry et al.[31] have reported recently that puromycin has no effect on insulin secretion produced by glucagon, and postulated that glucagon stimulates release of preformed rather than de novo synthesized insulin.

Diazoxide, which showed a marked inhibitory effect on the insulin secretion stimulated by glucose, has no effect on the rate of insulin secretion stimulated by

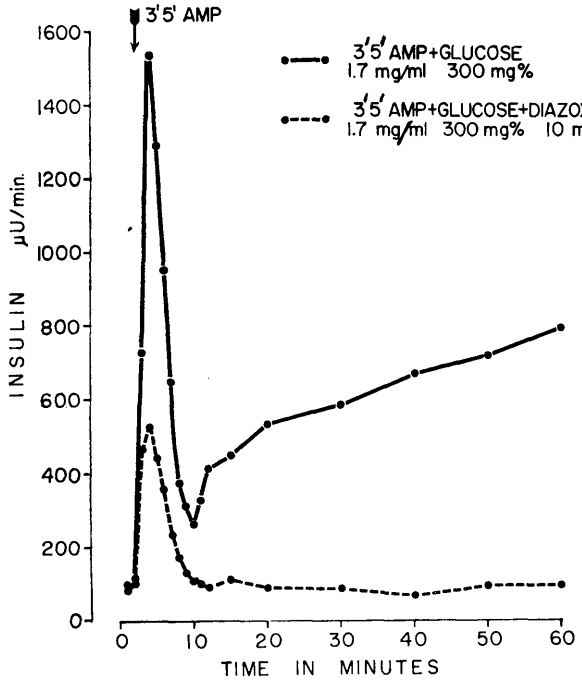

FIG. 7. Rate of insulin secretion produced by a pulse stimulus of 3′5′-AMP (between two and three minutes) plus a constant stimulus of glucose (n = 4) and the effect that 10 mg. per cent diazoxide has on it (n = 4). Statistical analysis: 3′5′-AMP plus glucose plus diazoxide compared with control group—$p < 0.001$ at 4,5,7 and 8 minutes, $p < 0.005$ at six minutes.

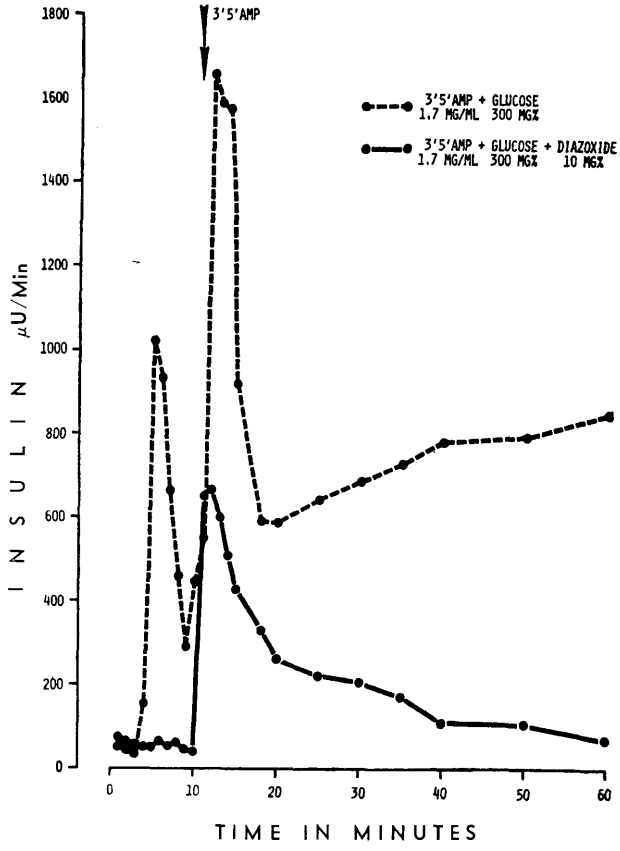

FIG. 8. Insulin levels produced by a constant perfusion of glucose plus a pulse dose of 3′5′-AMP, administered between ten and eleven minutes of perfusion (n = 4). The partial inhibitory effect of 10 mg. per cent diazoxide (n = 6). Statistical analysis: differences between glucose plus 3′5′-AMP, compared with glucose plus 3′5′-AMP plus diazoxide—$p < 0.001$ from twelve to fifteen minutes.

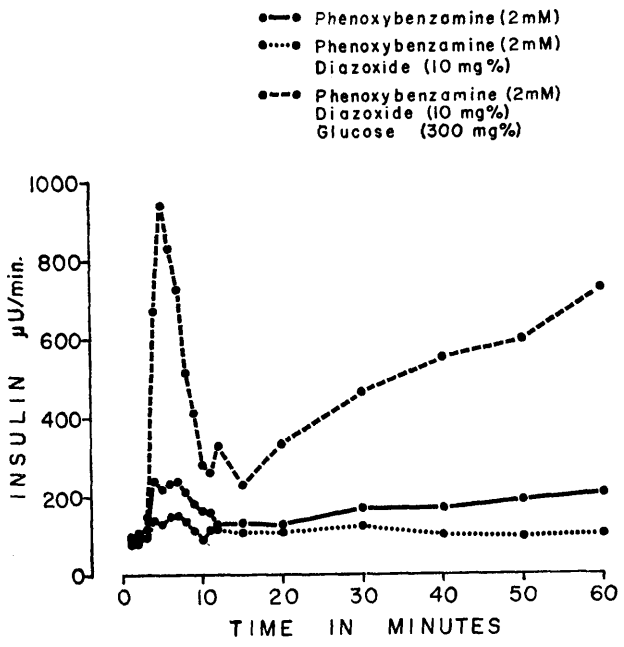

FIG. 9. Levels of insulin produced by the action of 2 mM phenoxybenzamine alone ($n = 4$), phenoxybenzamine (2 mM) plus 10 mg. per cent diazoxide ($n = 3$) and by glucose (300 mg. per cent) plus phenoxybenzamine (2 mM) and 10 mg. per cent diazoxide ($n = 4$). Statistical analysis: (a) phenoxybenzamine (2 mM) compared with control group—$p < 0.001$ at four and from six to eight minutes, $p < 0.005$ at 5, 40, 50 and 60 minutes, and $p < 0.01$ at thirty minutes; (b) phenoxybenzamine (2 mM) plus diazoxide (10 mg. per cent) compared with control group—$p < 0.01$ at six and seven minutes; (c) phenoxybenzamine (2 mM) plus diazoxide (10 mg. per cent) plus glucose (300 mg. per cent) compared with control group—$p < 0.05$ at 4, 8, and 12 minutes, $p < 0.01$ at 11, 20 and 40 minutes, $p < 0.005$ at nine and ten minutes, and $p < 0.001$ at 5, 6, 7, 15, 30, 50 and 60 minutes; (d) phenoxybenzamine (2 mM) plus glucose (300 mg. per cent) plus diazoxide (10 mg. per cent) compared with glucose (300 mg. per cent) alone—$p < 0.05$ at fifteen and fifty minutes, and $p < 0.01$ at seven minutes.

glucagon. Diazoxide was used in concentrations of 10 mg. per cent and 25 mg. per cent sufficient to block the stimulus of glucose.

A synergism of glucagon and glucose effects on insulin secretion has been reported by others.[15,31,32] Our results show that glucose and glucagon perfused together produce a pattern which is exactly additive.

When glucose, glucagon, and diazoxide are perfused together, the pattern and amount of insulin secreted is the same as the response to glucagon stimulation. We conclude that in a medium containing glucose plus glucagon as stimulus, diazoxide inhibits the mechanism by which glucose stimulates insulin secretion, but has no effect on the mechanism by which glucagon is acting.

Apart from, or in connection with, its effect on insulin secretion, glucagon has been shown to stimulate the enzyme adenyl cyclase, increasing in turn the levels of cyclic 3′5′ adenosine monophosphate (3′5′-AMP) in islets of Langerhans of the rat pancreas.[16] Theophylline also enhances insulin levels both in vivo[33] and in vitro,[15,39] and increases the levels of 3′5′-AMP in pancreatic islets.[16] The main action of theophylline seems to be the inactivation of P-diesterase, which catalyzes the breakdown of 3′5′-AMP.

In our experimental arrangement, theophylline alone did not stimulate insulin secretion at 0.44, 5 or 12 mM concentration, but 0.44 or 5 mM theophylline in the presence of glucose produces a marked increase of insulin secretion. The insulin levels are significantly higher in both phases, than those obtained by the stimulus of glucose alone (compare figures 1 and 4). We conclude that this difference is due to a stimulatory effect of theophylline on insulin secretion. The lack of difference of 0.44 and 5.0 mM theophylline can be explained by the fact that the main action of theophylline is a blocking effect and not a stimulatory one. If the 0.44 mM concentration of theophylline is sufficient to block P-diesterase activation, any further increase will have no additional effect.

The results also suggest that diazoxide blocks glucose-induced insulin release, but not the facilitating effect of glucose, which is necessary to permit an effect of theophylline on insulin release.

Where glucose and theophylline are perfused together with diazoxide, the insulin output is diminished. This decrease is of the same order and pattern which diazoxide exhibits on glucose-stimulated insulin release. This indicates that while diazoxide inhibits the amount and pattern of insulin produced by glucose, it has no effect on the stimulatory action of theophylline at any of the concentrations used. That the remaining levels of insulin are due to the theophylline stimulatory effect is supported by the fact that imidazole (stimulating the P-diesterase[34] accelerated breakdown of 3′5′-AMP) reduces the insulin secretion rate to basal levels.

Both glucagon and theophylline under the conditions described, produced an increase in the rate of insulin secreted by the pancreas. This effect is not inhibited by diazoxide. Both glucagon and theophylline have been reported to increase the levels of endogenous 3′5′-AMP in pancreatic islets.[16] It should be emphasized that both substances may have many other actions which are not related to the adenyl cyclase system.[35,36] A good amount of evidence has been accumulated for the role of 3′5′-AMP in regulating secretion of insulin.[15-17]

Our results dealing with the effect of exogenous 3′5′-

AMP on insulin secretion and its interaction with glucose and/or diazoxide are illustrated in table 1 and figures 6-8. Both sodium dibutyryl salt and the free acid form produce a clear increase in levels of insulin, where administered as a one-minute pulse resulting in a total dose of 5 mM of 3′5′-AMP. As is well known, 3′5′-AMP does not penetrate tissues readily;[37] therefore, higher than physiologic concentrations are necessary. In our experiments the same insulin response is obtained using the free acid or dibutyryl form of 3′5′-AMP. Even though the total dose was 5 mM of 3′5′-AMP, our determinations of 3′5′-AMP in pieces of pancreas show the pancreatic levels to be in the range of 16 to 32 μg./100 mg. of tissue. Diazoxide does not show any influence on the peak of insulin produced by the pulse stimulation of 3′5′-AMP.

The pulse stimulus of 3′5′-AMP is additive with glucose. Since 3′5′-AMP is administered in a one-minute pulse, the above-mentioned effect can be observed on the first peak of insulin stimulated by glucose. The fact that a synergistic effect of 3′5′-AMP can be produced at ten minutes (minimal secretion period) of the perfusion, indicates that this period is not refractory to 3′5′-AMP stimulus. The peak value observed at this particular time is higher, resembling an additive 3′5′-AMP plus glucose effect. This suggests also that glucose could produce a new peak at this moment if 3′5′-AMP is present. Whether this is dependent on the presence of 3′5′-AMP or could be produced by glucose alone, was not clarified by our studies.

Diazoxide reduces the amount of insulin produced by 3′5′-AMP in the presence of glucose, by a degree corresponding to the glucose stimulation. The residual peak shows no statistical difference from the one produced by exogenous 3′5′-AMP alone. One can therefore summarize that diazoxide inhibits the pattern and amount of insulin stimulated by glucose, but has no effect on the stimulus of glucagon, theophylline or exogenous 3′5′-AMP.

The role of alpha and beta-adrenergic receptors in insulin secretion has been clearly shown.[16] The beta antagonist, isoproterenol, showed an increase in insulin secretion when added to the isolated perfused pancreas.[17] Propranolol, a beta-blocker, produced a fall in the levels of insulin when given alone. Phentolamine, an alpha-blocker, inhibits the epinephrine blocking action on insulin; when given alone, phentolamine had no effect on the insulin levels in man, but increased the rate of insulin secretion in anesthetized dogs.[38] Turtle et al.[39] reported that stimulation of beta-receptors increases, and stimulation of alpha-receptors decreases the levels of endogenous 3′5′-AMP in pancreatic islets.

The evidence of diazoxide effect on alpha-receptors has been controversial. The fact that the alpha-blocker phenoxybenzamine prevented diazoxide suppression of insulin release in the rat in vivo[17] led to the suggestion that diazoxide action on insulin secretion could be related to alpha-receptors. On the other hand, Malaisse et al.[20] were unable to find any effect of the same alpha-blocker on diazoxide action. Our results show that phenoxybenzamine produces a small, but significant increase in the rate of insulin secretion.

Diazoxide counteracts this effect, returning insulin levels to basal values. When phenoxybenzamine is perfused together with glucose and diazoxide, phenoxybenzamine prevents the blocking effect of diazoxide on the glucose stimulus, and the typical biphasic pattern of insulin secretion appears. Whether this phenoxybenzamine effect is due only to its action on the alpha-receptors or elsewhere, is not clarified by this study. However, if we assume that the phenoxybenzamine effect takes place at the alpha-receptors, we have to infer that one of the possible modes of action of diazoxide could be the stimulation of alpha-adrenergic receptors. This stimulation would implicate a decrease of 3′5′-AMP levels in the beta cell.

In vivo evidence for the interaction of diazoxide with alpha and beta-adrenergic receptors as presented by Blackard[40] and Porte,[41] is supported by the findings of the present report.

In conclusion, our results show that diazoxide inhibits the stimulatory action of glucose on insulin secretion. Phenoxybenzamine counteracts this inhibitory effect. This suggests that one of the possible mechanisms of action of diazoxide could be connected with the alpha-receptors.

On the other hand, diazoxide has no effect on the secretion of insulin produced by glucagon, theophylline or exogenous 3′5′-AMP. When any of these substances is perfused in the presence of glucose, diazoxide blocks only the amount and pattern of insulin secretion stimulated by glucose, but has no effect on the insulin secretion produced by glucagon, theophylline and exogenous 3′5′-AMP. We conclude that glucose on one side and glucagon, theophylline and exogenous 3′5′-AMP on the other, stimulate insulin secretion by mechanisms that are totally or partially different for each group.

Since the processes that led to insulin secretion are not known, all interpretations have to be taken merely as a possibility. The mechanism of insulin secretion involves a series of steps, transport or movement of the granules inside the beta cell, solubility of the granules, interaction between the granules and membranes, etc.

Our results support the hypothesis that more than one type of mechanism is involved in the regulation of these processes and that probably more than one type of signal is involved in the mechanism or mechanisms connected with insulin secretion.

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REFERENCES

[1] Wolff, F., Parmley, W., White, K., and Okun, R.: Drug-induced diabetes. Diabetogenic activity of long-term administration of benzothiadiazines. J.A.M.A. *185*:568, 1963.

[2] Rubin, A. D., Roth, F., Taylor, R., and Rosenkilde, H.: Pharmacology of diazoxide, an antihypertensive, nondiuretic benzothiadiazine. J. Pharmacol. Exp. Ther. *136*:344, 1962.

[3] Wolff, F., Langdon, R., Ruebner, B., Hollander, C., and Skoglund, R.: A new form of experimental diabetes. Diabetes *12*:335, 1963.

[4] Drash, A., and Wolff, F.: Drug therapy in leucine sensitive hypoglycemia. Metabolism *13*:487, 1964.

[5] Graber, A., Porte, D., and Williams, R.: Clinical use of diazoxide and the mechanism for its hypoglycemic effects. Diabetes *15*:143, 1966.

[6] Seltzer, H., and Crout, J.: Inhibition of insulin secretion by diazoxide and epinephrine. Clin. Res. *14*:101, 1966.

[7] Loubatières, A., Alric, R., Mariani, M., deMalbose, H., and Ribes, J.: Le diazoxide exerce expérimentalement une action inhibitrice sur la sécrétion basale d'insuline. C. R. Soc. Biol. *160*:168, 1966.

[8] Nabwangu, Y., Staquet, M., Viktora, J., Yabo, R., and Wolff, F.: Hormonal regulation of hyperglycemia due to diazoxide. Clin. Res. *13*:246, 1965.

[9] Tabachnick, I., Gulbenkian, A., and Seidman, F.: Further studies on the metabolic effect of diazoxide. J. Pharmacol. Exp. Ther. *150*:455, 1965.

[10] Staquet, M., Yabo, R., Viktora, J., and Wolff, F.: An adrenergic mechanism for hyperglycemia induced by diazoxide. Metabolism *14*:1000, 1965.

[11] Fajans, S., Floyd, J. C., Jr., Knopf, R., Rull, J., Guntsche, E., and Conn, J.: Benzothiadiazine suppression of insulin release from normal and abnormal islet tissue in man. J. Clin. Invest. *45*:481, 1966.

[12] Wong, K., Symchowicz, S., Staub, M., and Tabachnick, I.: The in vitro effect of catecholamines, diazoxide and theophylline in insulin release. Life Sci. *6*:2285, 1967.

[13] Basabe, J., Lopez, N., Viktora, J., and Wolff, F.: Insulin secretion studied in the perfused rat pancreas. I. Effect of tolbutamide, leucine and arginine; their interaction with diazoxide, and relation to glucose. Diabetes *20*:449-56, July, 1971.

[14] Samols, E., Marri, J., and Marks, V.: Interrelationship of glucagon, insulin and glucose: The insulinogenic effect of glucagon. Diabetes *15*:855, 1966.

[15] Malaisse, W., Malaisse-Lagae, F., and Mayhew, D.: A possible role for the adenylcyclase system in insulin secretion. J. Clin. Invest. *46*:1724, 1967.

[16] Turtle, J., and Kipnis, D.: An adrenergic mechanism for the control of cyclic 3′5′ adenosine monophosphate synthesis in tissues. Biochem. Biophys. Res. Commun. *28*:797, 1967.

[17] Sussman, K., Vaughan, J., and Stjernholm, M.: Factors controlling insulin secretion in the perfused isolated rat pancreas. Proceedings of the Sixth Congress, International Diabetes Federation, Excerpta Medica, 1969, p. 123.

[18] Tabachnick, I., and Gulbenkian, A.: Mechanism of diazoxide hyperglycemia in animals. Ann. N.Y. Acad. Sci. *150*: 204, 1968.

[19] Senft, G.: Biochemical aspects of hyperglycemic action of diazoxide. Ann. N.Y. Acad. Sci. *150*:242, 1968.

[20] Malaisse, W., and Malaisse-Lagae, F.: Effect of thiazides upon insulin secretion in vitro. Arch. Int. Pharmacodyn. *171*: 235, 1968.

[21] Hales, C., and Randle, P.: Immunoassay of insulin with insulin antibody precipitate. Biochem. J. *88*:137, 1963.

[22] Krishna, G., Weiss, B., and Brodie, B.: A simple sensitive method for the assay of adenylcyclase. J. Pharmacol. Exp. Ther. *163*:379, 1968.

[23] Curry, C., Bennett, L., and Grodsky, G.: Dynamics of insulin secretion by the perfused rat pancreas. Endocrinology *83*:572, 1968.

[24] Porte, D., Jr., and Pupo, A.: Insulin responses to glucose: Evidence for a two pool system in man. J. Clin. Invest. *48*: 2309, 1969.

[25] Samols, E., Marri, G., and Marks, V.: Promotion of insulin secretion by glucagon. Lancet *II*:415, 1965.

[26] Crockford, P., Porte, D., Jr., Wood, F., Jr., and Williams, R.: Effect of glucagon on serum insulin, plasma glucose and free fatty acids in man. Metabolism *15*:114, 1966.

[27] Turner, D., and McIntyre, N.: Stimulation by glucagon of insulin release from rabbit pancreas in vitro. Lancet *I*:351, 1966.

[28] Devrim, S., and Recant, L.: Effect of glucagon on insulin release in vitro. Lancet *II*:1227, 1966.

[29] Grodsky, G., Bennett, L., Smith, D., and Schmid, F.: Effect of pulse administration of glucose or glucagon on insulin secretion in vitro. Metabolism *16*:222, 1967.

[30] Sussman, K., and Vaughan, G.: Insulin release after ACTH, glucagon, and adenosine 3′5′-monophosphate (cyclic AMP) in the perfused isolated rat pancreas. Diabetes *16*:449, 1967.

[31] Curry, D., Bennett, L., and Grodsky, G.: Glucagon potentiation of insulin secretion by the perfused rat pancreas. Endocrine Society, Program of the 51st meeting, p. 36 (Abstract).

[32] Ryan, W., Nibbe, A., and Schwartz, T.: Beta-cytotrophic effect of glucose, glucagon and tolbutamide in man. Lancet *I*: 1255, 1967.

[33] Turtle, J., and Kipnis, D.: Stimulation of insulin secretion by theophylline. Nature (London) *213*:727, 1967.

[34] Butcher, R., and Sutherland, E.: Adenosine 3′5′ monophosphate in biological materials. I. Purification and properties of cyclic 3′5′ nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3′5′ monophosphate in human urine. J. Biol. Chem. *237*:1244, 1962.

[35] Halkerston, I., Feinstein, M., and Hechter, O.: An anomalous effect of theophylline on ACTH and adenosine 3′5′-

monophosphate stimulation. Proc. Soc. Exp. Biol. Med. *122*: 896, 1966.

[36] Montague, W., and Taylor, K.: Islet-cell metabolism during insulin release; effect of glucose, citrate, octanoate, tolbutamide, glucagon and theophylline. Biochem. J. *115*:257, 1969.

[37] Sutherland, E., Oye, J., and Butcher, R.: The action of epinephrine and the role of the adenylcyclase system in hormone action. Recent Progr. Hormone Res. *21*:263, 1964.

[38] Frohman, L., Ezdinli, C., and Javid, R.: Effect of vagotomy and vagal stimulation on insulin secretion. Diabetes *16*: 443, 1967.

[39] Turtle, J., Littleton, K., and Kipnis, D.: Stimulation of insulin secretion by theophylline. Nature (London) *213*:727, 1967.

[40] Blackard, W. G., Aprill, C. N., III: Mechanism of action of diazoxide. J. Lab. Clin. Med. 69:960, 1967.

[41] Porte, D., Jr.: Inhibition of insulin release by diazoxide and its relation to catecholamine effects in man. Ann. N.Y. Acad. Sci. *150*:281, 1968.

The Metabolism of Mannoheptulose-C-14 by the Rat

(*Continued from page* 456)

which permitted collection of CO_2. In one experiment three rats were used, each was injected with 1 μC. of mannoheptulose-C-14; one rat also received 100 mg. of unlabeled mannoheptulose, and another animal was nephrectomized. CO_2 was collected for a two-hour period. During this period 0.5 per cent or less of the administered radioactivity appeared in expired CO_2. In the two rats with intact kidneys 64 and 80 per cent of administered radioactivity appeared in the urine. The total recovery of administered radioactivity in CO_2, urine, and the carcass ranged from 91 to 94 per cent.

In a second experiment each of two rats was injected with 15 μC. of mannoheptulose-C-14, and CO_2 was collected for a six-hour period. During this time 3.3 and 3.4 per cent of the administered radioactivity appeared in expired CO_2, and 87.2 and 82.0 per cent in the urine respectively. Total recovery of administered radioactivity in expired CO_2, urine, and the carcass was 98 and 100 per cent.

Urine samples and homogenates of liver, other tissues, and the carcass were subjected to chromatographic analysis to identify the radioactive materials. Two different chromatographic systems were employed. In all cases only a single component was observed which corresponded to authentic mannoheptulose. Also, the authors indicate that only one radioactive component was observed and again it corresponded in chromatographic mobility to mannoheptulose.

The results of this study show that mannoheptulose is metabolized to only a very limited extent, and that 80 to 90 per cent of the sugar is excreted in the urine unchanged within six hours. Also, as the authors state, in view of the fact that mannoheptulose is not extensively metabolized and that no metabolite of this sugar could be detected in tissues, mannoheptulose itself seems to be responsible for the reported diabetogenic effects. It also appears from these findings that mannoheptulose is metabolically inert except for its effect on the pancreas. In this organ mannoheptulose appears to block not only the release of pre-formed insulin but also synthesis of the hormone (see Nutrition Reviews 27:206, 1969). This specificity of action of mannoheptulose, if indeed it has no other direct metabolic effects, should be extremely valuable in studying various aspects of insulin metabolism.

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