Effect of Diazoxide on Glucose U-C-14 Utilization in Mice

G. R. Jansen, Ph.D., C. F. Hutchison, B.S., and M. E. Zanetti, M.S., Rahway, New Jersey

SUMMARY

(1) The effects of diazoxide on the conversion of oral doses of glucose-U-C-14 to fatty acids in adipose tissue, and to fatty acids and glycogen in liver have been studied in intact mice.

(2) Diazoxide in a subcutaneous dose of 10 mg. per kilogram inhibited conversion of the glucose-U-C-14 to fatty acids of epididymal fat approximately 50 per cent. The inhibition in conversion of isotopic glucose to fatty acids in liver was slightly less than that found for adipose tissue. These inhibitory effects as well as hyperglycemia occurred within twenty minutes after drug administration and ten minutes after the glucose-U-C-14 was given.

(3) In spite of the sensitivity of the system, insulin overcame the inhibitory effects of 200 mg. per kilogram diazoxide on all these parameters. This large dose of diazoxide did not antagonize the action of exogenous insulin.

(4) Although diazoxide inhibited conversion of glucose-U-C-14 to liver fatty acids, net incorporation into liver glycogen was stimulated one-hundredfold. A net increase in the weight of total liver glycogen also was found. The significance of the striking elevation in glycogen synthesis in terms of proposed mechanisms of action for diazoxide will be discussed.

(5) The inhibition in conversion of the glucose-U-C-14 to fatty acid and the hyperglycemia were accompanied by an increase rather than a decrease in the specific activity of the plasma glucose. This, combined with the fact that no antagonism to the action of exogenous insulin was observed, is consistent with the hypothesis that diazoxide-mediated hyperglycemia, at least at low doses, results primarily from an inhibition in insulin secretion. At higher doses adrenergic mechanism could also become involved in potentiating the hyperglycemia. DIABETES *16*:777-83, November, 1967.

Diazoxide is a benzothiadiazine drug with hypotensive activity which, rather than being a diuretic, has antidiuretic properties.[1] It soon was found to cause hyperglycemia when given in combination with benzothiadiazine diuretics.[2,3] Although most workers allow for the possibility that more than one mechanism could be operating, emphasis has been placed mainly on either decreased insulin release[4-7] or an adrenergic-mediated glycogenolysis[8-13] as being responsible for the hyperglycemia diazoxide causes when given alone as well as in combination with diuretics. Some workers have emphasized both mechanisms,[14] or have attempted to integrate them into unitarian hypotheses such as (1) the inhibition in insulin secretion caused by epinephrine[15,16] or (2) an effect of diazoxide on 3'5'-cyclic AMP metabolism in pancreas and other tissues.[17]

In previous papers we have described experiments in which conversion of glucose-U-C-14 to various lipid fractions in the mouse has been studied in vivo.[18,19] By using this system it should be possible to distinguish between the alternate mechanisms that have been proposed to explain diazoxide's action. Accordingly the effects of diazoxide on conversion of orally given glucose-U-C-14 to liver glycogen and fatty acids and to adipose tissue fatty acids have been investigated using intact mice. Concurrent changes in the level and specific activity of plasma glucose also were measured.

METHODS

The technics used in dosing, bleeding, preparing and analyzing tissues, and determining radioactivity have been described previously.[18] The mice were all males and were from the Merck Sharp & Dohme colony (ICR strain). They were maintained on a purified diet containing 1 per cent corn oil, 70 per cent glucose and 20 per cent casein along with the necessary vitamins and minerals (Diet 2[18]). Details as to the number of animals in each group, their body weights, and additional details of the experimental protocols used for the individual experiments are given in the legends to each of the tables. The mice were housed in individual screen-bottomed cages in an air conditioned room maintained at approximately 24° C. Food and water were supplied ad libitum. The glucose-U-C-14* was diluted

From the Merck Institute for Therapeutic Research, Rahway, New Jersey.

*The glucose-U-C-14 was obtained from New England Nuclear Corp., Boston, and had a specific activity of 10-15 mc./m-mole.

with unlabeled glucose (125 mg./mouse) and given as a 50 per cent solution (w:v) by stomach tube. Diazoxide* was dissolved in saline by the dropwise addition of dilute NaOH (final *p*H 11.1-11.8) and the saline used for control injections also was adjusted to this *p*H. Crystalline zinc insulin containing 24 U. per milligram was obtained from Eli Lilly and Co., Indianapolis, Indiana. Since all experiments were acute and there were no significant differences in fat pad or liver weights the incorporation of radioactivity has been expressed per fat pad or per gram of liver. Statistical analyses were performed using an analysis of variance and the "F" test† as described by Snedecor.[20]

RESULTS

The effect of subcutaneous doses of 50-200 mg. per kilogram of diazoxide on plasma glucose levels and incorporation of glucose-U-C-14 into fatty acids of epididymal fat pads was determined following 125 mg. glucose-U-C-14 (5 μc.) given orally. As shown in table 1 plasma glucose levels were elevated approximately threefold at sixty minutes with relatively small differences among the three doses. By 120 minutes, however, a dose-response relationship was apparent with 200 mg. per kilogram diazoxide increasing plasma glucose fivefold over controls. The lowest dose tested, 50 mg. per kilogram, inhibited conversion of the glucose-U-C-14 into adipose tissue fatty acid 97 per cent. The rise in plasma glucose appeared to be associated more with an inhibition in glucose utilization than increased glycogenolysis since the specific activity of the circulating glucose in the diazoxide treated animals was increased rather than depressed. These data suggest that under these conditions diazoxide treatment resulted in a decreased output of unlabeled glucose from the liver. In view of the pronounced inhibitory effect on fatty acid synthesis of 50 mg. per kilogram diazoxide, an experiment was carried out to determine the lowest dose of diazoxide that would inhibit the conversion of glucose-U-C-14 to fatty acid. As shown in table 2, 10 mg. per kilogram diazoxide inhibited both total glucose-U-C-14 uptake and conversion into fatty acid approximately 50 per cent as measured in the epididymal fat pads.

These results indicated that diazoxide had a potent inhibitory effect on peripheral glucose utilization. The effect of diazoxide on C-14-O_2 evolution was not measured, but one might expect that this also would be depressed. In view of the previous reports it seemed likely that either the peripheral action of insulin or the secretion of insulin from the pancreas was involved. Therefore an experiment was carried out to determine the effectiveness of exogenous insulin in overcoming the inhibitory action of a large dose of diazoxide. In this experiment the incorporation of glucose-U-C-14 into both liver and adipose tissue fatty acids was measured and the results along with plasma glucose changes are presented in table 3. The effect of 200 mg. per kilogram diazoxide on plasma glucose levels and incorporation of the glucose-U-C-14 into the fatty acid fraction of epididymal fat was consistent with the results obtained earlier (table 1). The inhibitory effect on incorporation into fatty acid was larger than the effect on total uptake of label by epididymal fat pads. Insulin reversed these changes in the fat pad to a large extent as early as ten minutes after glucose-U-C-14 administration (twenty minutes after drug and insulin treatment), and completely by 120 minutes. Similarly insulin reversed the effect of diazoxide on plasma glucose measurably at ten minutes and completely at 120 minutes following glucose-U-C-14 administration. The effectiveness of insulin in overcoming diazoxide induced hyperglycemia has been shown previously.[9] As shown in table 3, diazoxide inhibited conversion of glucose-U-C-14 to fatty acids in the liver. For time periods of ten minutes, thirty minutes, and 120 minutes, inhibitions of 55 per cent, 95 per cent and 76 per cent respectively were obtained. Insulin also reversed the inhibitory effect of diazoxide on incorporation of label into liver fatty acid almost completely. The increase in liver esterified fat that one would expect with the reported lipolytic activity of diazoxide[10] did not occur until two hours after dosage.

The foregoing experiments demonstrated that diazoxide can inhibit the synthesis of fatty acids in the liver as well as in adipose tissue and that insulin can reverse this inhibitory activity. The increase in the specific activity of plasma glucose in the diazoxide treated animals suggested that, rather than increased glycogenolysis, a lowered utilization of glucose in the face of continued glucose absorption is responsible for the hyperglycemia. It appeared to be important to determine if any important changes in liver glycogen were taking place under these experimental conditions. Therefore an experiment was set up in which changes in liver glycogen and fatty acids, adipose tissue fatty acids and plasma glucose all were determined for time periods of ten to 120 minutes following oral administration of 125

*3-Methyl-7-chloro-1,2,4-benzothiadiazine-1,1-dioxide.

†Duncan, D. B.: Multiple range and multiple F tests. Biometrics *11*:1-42, 1955.

TABLE 1

Effect of diazoxide on plasma glucose levels and incorporation of a glucose-U-C-14 meal into epididymal fatty acid*

Treatment	Plasma glucose		Epididymal fat pad fatty acid	
	Mg./100 ml.	Cpm./mg.	Cpm/pad	Per cent inhibition
		60 min.		
Saline	125 ± 3	26,300 ± 6,400	11,960 ±1,760	—
50 mg/kg. diazoxide	316 ± 20	42,500 ± 1,800	300 ± 120	97†
100 mg./kg. diazoxide	303 ± 10	35,000 ± 2,400	307 ± 84	97†
200 mg./kg. diazoxide	376 ± 23	22,200 ± 1,600	166 ± 40	99†
		120 min.		
Saline	125 ± 12	8,120 ± 2,400	10,400 ± 100	—
50 mg./kg. diazoxide	260 ± 16	25,100 ± 2,900	320 ± 64	97†
100 mg./kg. diazoxide	395 ± 53	32,300 ± 4,100	182 ± 31	98†
200 mg./kg. diazoxide	696 ± 38	18,600 ± 2,200	54 ± 13	99†

*Male mice, weighing 23 to 26 gm. (8/group), were each given alkaline saline or diazoxide subcutaneously followed in fifteen minutes by 125 mg. glucose-U-C-14 (5 μc.) given orally. The mice were bled and then killed sixty minutes or 120 minutes after isotope administration. Food was removed from the cages at the time the diazoxide was given. The results are presented as means ± S.E.M.

For each parameter measured those means that differ by more than three or four times the standard error are considered to be significantly different at $p < 0.05$ and $p < 0.01$ respectively. See Duncan, D. B.: Multiple range and multiple F tests. Biometrics *11*:1-42, 1955.

†Significant at $p < 0.01$.

TABLE 2

Effect of low doses of diazoxide on incorporation of a glucose-U-C-14 meal into epididymal fatty acid*

Dose (mg./kg.)	Total incorporation Cpm/pad	Per cent inhibition	Fatty acid incorporation Cpm/pad	Per cent inhibition
0	591±56	—	450±48	—
5	608±99	—	454±85	—
10	313±46	48†	203±43	55†
20	185±33	69†	79±30	82†

*Male mice, weighing 26 to 28 gm. (8/group), were each given alkaline saline or diazoxide subcutaneously followed in five minutes by 125 mg. glucose-U-C-14 (0.125 μc.) given orally. The mice were killed sixty minutes later. Food was removed from cages at the time the diazoxide was given. The results are presented as means ± S.E.M.

For each parameter measured those means that differ by more than three or four times the standard error are considered to be significantly different at $p < 0.05$ and $p < 0.01$ respectively. See Duncan, D. B.: Multiple range and multiple F tests. Biometrics *11*:1-42, 1955.

†Significant at $p < 0.01$.

mg. glucose-U-C-14. The results are presented in tables 4 and 5. The hyperglycemia was apparent as early as ten minutes after isotope administration and the plasma glucose specific activity was only slightly lower in the treated mice at ten minutes and thirty minutes. However, by sixty minutes the specific activity was higher in the treated groups and by 120 minutes there was almost a sixfold elevation in the specific activity of the circulating glucose in the diazoxide treated mice. The inhibition in incorporation of glucose-U-C-14 into epididymal fat was virtually complete at ten minutes after 50 mg. per kilogram diazoxide and remained so for the entire period measured. Diazoxide decreased the proportion of fat pad radioactivity present as fat and increased the proportion present as glyceride-glycerol. These changes in distribution of fat pad radioactivity are similar to those found after starvation or feeding fat.[18,19]

As shown in table 5, diazoxide strongly inhibited conversion of the glucose-U-C-14 to liver fatty acid at all doses and times studied, but quantitatively the inhibition was not quite as large as that occurring in epididymal fat. As found previously (table 3) the increase in total esterified fatty acid in the liver did not become apparent until two hours following isotope administration. In contrast, incorporation of the glucose-U-C-14 into liver glycogen was greatly increased by diazoxide administration. With a 50 mg. per kilogram dose incorporation of the label into liver glycogen was increased twofold, tenfold and 100-fold at time intervals of thirty minutes, sixty minutes, and 120 minutes, respectively. At 120 minutes after the glucose-U-C-14 was given the weight of liver glycogen was elevated 40 per cent by 50 mg. per kilogram diazoxide.

DISCUSSION

A considerable amount of experimental work on diazoxide has led to two general theories as to the mechanism of action of this compound in causing hyperglycemia. As previously mentioned these are (1)

TABLE 3

Effect of diazoxide and insulin on incorporation of a glucose-U-C-14 meal into liver fatty acid and the fatty acid of epididymal fat pads*

Treatment	Plasma glucose mg./100 ml.	Liver fatty acid: Gm. fatty acid/ 100 gm. liver	Liver fatty acid: Cpm/gm. liver	Epididymal fat pad: Total cpm/pad	Epididymal fat pad: Fatty acid cpm/pad	Epididymal fat pad: Per cent of Pad C-14 as fatty acid
			10 min.			
Saline	228±20	5.88±0.49	1,300± 260	1,050±200	710±150	61
Saline + 0.25 U. insulin	178± 9	5.45±0.49	1,600± 260	1,060±120	660± 90	62
200 mg./kg. diazoxide	428±28	6.09±0.63	580± 130	280± 60	86± 35	25
200 mg./kg. diazoxide + 0.25 U. insulin	325± 8	5.32±0.43	580± 150	570± 50	330± 40	57
			30 min.			
Saline	195± 8	5.68±0.40	8,730±2,390	3,840±390	2,240±250	58
Saline + 0.25 U. insulin	59± 2	6.35±0.61	9,870±1,430	5,460±510	3,670±360	67
200 mg./kg. diazoxide	463±80	6.10±0.63	420± 110	800±130	28± 9	4
200 mg./kg. diazoxide + 0.25 U. insulin	71± 4	5.72±0.39	4,660±1,310	2,340±240	1,310±160	56
			120 min.			
Saline	123± 2	5.19±0.27	11,320±2,460	4,290±680	3,220±520	75
Saline + 0.25 U. insulin	49± 3	4.74±0.38	13,680±1,660	5,000±610	3,760±510	75
200 mg./kg. diazoxide	539±23	7.02±0.67	2,600± 680	940± 90	97± 30	10
200 mg./kg. diazoxide + 0.25 U. insulin	39± 1	5.11±0.43	9,320±1,380	4,880±490	3,000±300	61

*Male mice, weighing 25 to 29 gm. each (8/group), were given subcutaneously diazoxide, insulin or diazoxide and insulin followed by oral administration of 125 mg. glucose-U-C-14 (1.25 μc.). The mice were bled and killed ten minutes, thirty minutes, or 120 minutes following isotope administration. The saline, insulin, diazoxde, or insulin + diazoxide was given subcutaneously ten minutes or fifteen minutes before the isotopic glucose when the sacrifice times were ten minutes, or thirty to 120 minutes, respectively. Food was removed from the cages at the time the diazoxide or insulin was given. The results are presented as means ± S.E.M.

For each parameter measured those means that differ by more than three or four times the standard error are considered to be significantly different at $p < 0.05$ and $p < 0.01$ respectively. See Duncan, D. B.: Multiple range and multiple F tests. Biometrics *11*:1-42, 1955.

an inhibition in insulin secretion and (2) adrenergic-mediated glycogenolysis. A review on the mechanism of action of diazoxide has recently been published[21] and the following discussion will be restricted to an interpretation of our data in the light of these previously reported observations.

In our system as little as 10 mg. per kilogram diazoxide inhibited conversion of glucose-U-C-14 to adipose tissue fatty acid approximately 50 per cent. The peaks in hyperglycemic responses for diazoxide doses of 20, 50, 100 and 200 mg. per kilogram were reached in ten minutes, thirty minutes, 120 minutes, and 120 minutes, respectively, following glucose-U-C-14 administration. The plasma glucose specific activity changes following diazoxide administration strongly suggest that the most important reason for the hyperglycemia that occurred under the conditions we employed was an inhibition in glucose utilization. This block in utilization along with continued absorption of glucose from the intestinal tract resulted in the observed hyperglycemia. In the saline injected controls the specific activity of the plasma glucose at times of ten minutes to thirty minutes following administration approximated the specific activity of the glucose-U-C-14 solution given. Following the return of the plasma glucose level to the preprandial level its specific activity dropped rapidly from 30,000 cpm to 6,700 cpm (table 4). Diazoxide blocked this normal turnover and thus the level and specific activity of the plasma glucose remained elevated. The fact that a diazoxide dose twenty times as large as necessary to inhibit glucose utilization did not antagonize the hypoglycemic activity of exogenous insulin supports the hypothesis that diazoxide inhibits the release of insulin from the pancreas rather than insulin action on sensitive tissues. A slight lowering of plasma glucose specific activity was observed at the earliest time periods studied. However, in the procedure that was used the drug was given ten to fifteen minutes before the labeled glucose. Therefore part of the increase in plasma glucose levels took place before the glucose-U-C-14 had been given and could also have resulted from an inhibition in glucose utilization rather than glyco-

TABLE 4

Effect of diazoxide on plasma glucose specific activity and distribution of radioactivity in epididymal fat pads following a glucose-U-C-14 meal*

Treatment	Plasma glucose		Epididymal fat pads				
	Mg./100 ml.	Cpm/mg.	Total cpm/pad	Per cent of pad C-14 as fat	Per cent of fat C-14 as fatty acid	Fatty acid C-14 Cpm/pad	Per cent inhibition
			10 min.				
Saline	298±20	43,400± 600	3,760± 600	63±4	77±1	1,920± 340	—
20 mg./kg. diazoxide	432±29	38,000± 600	1,420± 260	35±5	38±5	262± 104	86†
50 mg./kg. diazoxide	437±29	35,200±1,600	1,040± 240	12±1	27±4	28± 4	99†
			30 min.				
Saline	148± 6	43,400±3,200	13,120±1,960	86±2	83±1	4,720± 750	—
20 mg./kg. diazoxide	368±46	38,400±1,600	3,400± 600	59±7	60±6	1,340± 350	86†
50 mg./kg. diazoxide	530±53	37,400±2,800	1,420± 220	26±3	33±4	122± 26	99†
			60 min.				
Saline	123± 2	30,000±2,800	12,420±2,860	91±2	76±3	8,980±2,240	—
20 mg./kg. diazoxide	280±17	38,400±3,000	5,900± 960	86±2	57±6	2,880± 600	68†
50 mg./kg. diazoxide	395±22	49,600±3,800	1,640± 140	45±5	29±5	242± 80	97†
			120 min.				
Saline	120± 7	6,720± 600	14,060±2,720	94±2	76±2	10,400±2,220	—
20 mg./kg. diazoxide	147± 6	34,200±3,400	20,060±4,100	93±2	68±5	14,200±3,660	—
50 mg./kg. diazoxide	293±39	36,400±2,600	1,920± 340	59±5	19±4	302± 144	97†

*Male mice weighing 22 to 26 gm. each (8/group) were dosed with alkaline saline or diazoxide subcutaneously fifteen minutes before 125 mg. glucose-U-C-14 (5 μc.) given orally. The mice were bled and killed ten minutes, thirty minutes, sixty minutes, or 120 minutes after isotope administration with ten-minute and thirty-minute groups run one morning and sixty-minute and 120-minute groups the next morning. Food was removed from the cages when the diazoxide was given. The results are presented as means ± S.E.M.

For each parameter measured those means that differ by more than three or four times the standard error are considered to be significantly different at $p < 0.05$ and $p < 0.01$ respectively. See Duncan, D. B.: Multiple range and multiple F tests, Biometrics *11*:1-42, 1955.

†Significant at $p < 0.01$.

genolysis. The diazoxide was given early in these experiments to insure that adequate blood levels would be reached before the labeled glucose was given. It is apparent that diazoxide has a rapid onset of activity and it would be desirable in future experiments to measure its effect on plasma glucose specific activity when given at the same time as the labeled glucose.

The large increase in incorporation of glucose-U-C-14 into liver glycogen was unexpected in view of the fact that previous workers have reported that glycogenolysis results from diazoxide administration.[9] These earlier experiments were not, however, performed under conditions of glucose loading. Increased incorporation into glycogen appeared to be associated with increased net synthesis and decreased turnover rather than increased turnover. The plasma glucose radioactivity rather than being lowered, as one might expect from dilution with unlabeled glucose coming from glycogen breakdown, was considerably increased. Also diazoxide increased the weight of liver glycogen. Assuming a specific activity for plasma glucose of 40,000 cpm per milligram, the data in table 5 suggest that an additional 12 mg. of glycogen-U-C-14 should be found, per gram liver, in mice given 50 mg. per kilogram diazoxide as compared with those saline injected, i.e., (472,000—5,000)/40,000 = 12. Since the average weight of the liver in the mice was 1.9 gm. these data suggest that, out of the 125 mg. glucose-UC-14 given, approximately 23 mg. or 18 per cent of the dose was converted to liver glycogen. We have previously reported a similar, although less marked, increase in conversion of orally given glucose-U-C-14 to liver glycogen in starved mice.[22] The difference in weight of liver glycogen between diazoxide and saline injected mice was 23 mg. per gram liver as measured gravimetrically supporting the thesis that a net increase in the amount of liver glycogen occurred under the conditions employed.

Glucose metabolism in the liver after diazoxide administration would appear to be inhibited between glucose-6-phosphate and fatty acid, but not between glucose-6-phosphate and glycogen. The inhibition in liver fatty acid synthesis could be reversed by insulin as could the inhibition in fatty acid synthesis in adipose tissue. An important question is whether the in-

TABLE 5

Effect of diazoxide on incorporation of a glucose-U-C-14 meal into liver glycogen and fatty acids*

Treatment	Liver fatty acid		Liver glycogen	
	Gm. fatty acid/ 100 gm. liver	Cpm/gm. liver	Gm. glycogen/ 100 gm. liver	Cpm/gm. liver
		10 min.		
Saline	4.94±0.48	3,990±1,020	5.90±0.47	3,450± 600
20 mg./kg. diazoxide	4.68±0.58	1,680± 460	5.87±0.50	2,770± 960
50 mg./kg. diazoxide	5.20±0.50	1,590± 580	5.85±0.73	2,470± 340
		30 min.		
Saline	5.16±0.45	44,800±6,270	7.08±0.52	25,700± 5,300
20 mg./kg. diazoxide	4.86±0.35	6,040±1,200	6.11±0.56	91,020±17,300
50 mg./kg. diazoxide	5.51±0.56	4,740±1,080	6.68±0.43	55,800±11,100
		60 min.		
Saline	4.05±0.42	33,000±3,360	7.29±0.73	19,900± 8,060
20 mg./kg. diazoxide	4.28±0.35	16,600±4,080	6.51±0.49	157,000±20,600
50 mg./kg. diazoxide	4.42±0.42	6,860±1,600	7.08±0.53	225,000±33,000
		120 min.		
Saline	4.00±0.33	26,700±3,870	5.60±0.70	5,060± 1,330
20 mg./kg. diazoxide	4.94±0.32	9,270±1,650	7.03±0.43	285,400±51,000
50 mg./kg. diazoxide	5.61±0.65	8,700±2,040	7.91±0.36	472,000±57,000

*Male mice weighing 24 to 29 gm. each (8/group) were dosed with alkaline saline or diazoxide subcutaneously fifteen minutes before 125 mg. glucose-U-C-14 (5μc.) given orally. The mice were killed ten minutes, thirty minutes, sixty minutes, or 120 minutes after isotope administration, and the the livers removed and quickly frozen in a deep freeze. The ten-minute and thirty-minute groups were run one morning and the sixty-minutes and 120-minute groups the next morning. Food was removed from the cages when the diazoxide was given. The results are presented as means ± S.E.M.

For each parameter measured those means that differ by more than three or four times the standard error are considered to be significantly different at $p < 0.05$ and $p < 0.01$ respectively. See Duncan, D. B.: Multiple range and multiple F tests. Biometrics *11*:1-42, 1955.

hibition in liver fatty acid synthesis is a direct effect or whether it is secondary to an increased transport of free fatty acids from adipose tissue to liver via the plasma. Other workers have shown that administration of anti-insulin serum to rats results in a rapid reduction in fatty acid synthesis in the liver from acetyl CoA, but not from malonyl CoA, and they suggest that mobilization of fatty acids from adipose tissue to liver is the cause via an inhibition of acetyl CoA carboxylase.[23] In our experiments the inhibition in conversion of glucose-U-C-14 to liver fatty acid occurred within twenty minutes while the increase in the level of total esterified fatty acids in the liver required two hours. Our data are not sufficient to answer the question as to whether the inhibition in liver fatty acid synthesis was caused by a direct effect of diazoxide on liver. Measurement of fatty acyl CoA, and free fatty acid levels in the liver at early times following diazoxide administration as well as in vitro experiments would help to resolve this question.

It is apparent that whatever mechanism is responsible for the inhibition in fatty acid synthesis does not generally inhibit glucose uptake or phosphorylation by the liver. The liver has been reported to be freely permeable to glucose[24] but a role for insulin in increasing glucose utilization by liver is still being debated.[25] Our data suggest that either insulin is not required for glucose uptake and glycogen synthesis by liver or that liver glycogen synthesis can take place with less insulin than is required for glucose uptake by adipose tissue. The considerable increase in the rate of incorporation of glucose-U-C-14 into liver glycogen following diazoxide administration may be interpreted as follows: The considerable elevation in plasma glucose level raised the cellular content of glucose in the liver cell. No barrier to phosphorylation existed and glycogen synthesis was apparently increased secondary to a large increase in the rate of glucose-6-phosphate formation with the other major pathway of glucose metabolism blocked. It has been reported that in the rat glucokinase and not hexokinase is the most important phosphorylating enzyme in regulating glucose uptake by liver[26] and this enzyme is not inhibited by glucose-6-phosphate.[27] Lauris and Cahill[28] have reported that mouse liver contains a high level of glucokinase. In view of the high plasma glucose levels following diazoxide it appears reasonable

that the high Km glucokinase was in fact the important phosphorylating enzyme under these conditions and that no reduction in the activity of this enzyme occurred after diazoxide administration. Any effects of insulin deficiency on glucokinase synthesis would not have had time to become manifest in the short time of these experiments. Alternatively if there was a reduction of glucokinase activity following diazoxide, it may be that a higher cellular concentration of glucose overcame this lower activity or another phosphorylating mechanism was used. It is difficult to compare an acute effect of diazoxide with alloxan diabetes where many secondary adaptations including effects on enzyme synthesis can occur prior to the experiment. Nevertheless it is interesting that Friedman, Goodman and Weinhouse[29] have reported that liver glycogen is maintained at a higher level during starvation in alloxan diabetic rats than in normals. The authors speculated that the high blood glucose level in the alloxan diabetic rat was a contributing factor to the high level of liver glycogen. The difference that we have observed between the effect of diazoxide on fatty acid synthesis in liver and adipose tissue on the one hand and liver glycogen synthesis on the other remains an intriguing observation, however, because one would not expect to see an increased rate of glycogen synthesis at a time of depressed insulin secretion. It is obvious that much more work on this phenomenon is required before an understanding is reached.

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