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Portal venous injection of insulin in the diabetic rat: time of induction of changes in hepatic lipogenesis, cholesterogenesis, and glycogenesis*

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SUMMARY

The time of onset of changes in liver metabolism of the 14-hour-insulin-deprived, alloxan-diabetic rat following continuous portal vein infusion of insulin was studied. The earliest effect of insulin—on glycogen content of the liver and on the capacity of the liver slice to synthesize cholesterol from acetate—occurred between 10 and 30 minutes after the start of the infusion. The liver's capacity for incorporating acetate carbon into cholesterol rose dramatically between 30 and 60 minutes after the start of the hormone infusion, and returned to normal in 90 minutes. The significance of this biphasic response in hepatic cholesterogenesis to portal vein insulin infusion is discussed. Fatty acid synthesis was slowest to respond to insulin. An effect on hepatic lipogenesis was first detected between 60 and 90 minutes after injection, and the liver's capacity for incorporation of acetate-1-C^{14} into fatty acids continued to increase with duration of insulin administration up to 180 minutes.

The importance of duration of insulin action in evoking a response in hepatic lipogenesis of the diabetic rat was pointed out by Chernick and Chaikoff in 1950 (1). They demonstrated that pretreatment of diabetic rats with insulin for 1 day resulted in an increased utilization of glucose for fatty acid and CO_2 formation; insulin treatment for as short a period as 3 hours failed to induce this increased glucose utilization. The time element in insulin action on liver was later studied in greater detail by Hastings and his associates. The earliest effect observed by these workers occurred in 6 hours in chronic diabetic rats (2), and in 4 hours in 14-hour-insulin-deprived diabetic rats (3). In all the studies cited above, insulin was administered by a subcutaneous or by a nonportal, intravenous route. Since insulin produced by the pancreas is secreted directly into the portal circulation, we raised the question whether the reported values for the time element might, in some part, be an artifact of the peripheral route of insulin administration. For this reason we have investigated the time of onset of hepatic insulin action in diabetic rats in which the hormone was injected directly into the portal vein. When this route of administration is used, not only does the hormone enter the liver first, but also all of it reaches the liver.

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EXPERIMENTAL

Male Long-Evans rats weighing from 225 to 275 g. were made diabetic by intravenous injections of alloxan-monohydrate (Eastman). Every day, each diabetic rat used in this study ingested about 30 g. of a stock diet (Diablo Labration), drank from 75 to 200 ml. of water, and excreted from 75 to 200 ml. of urine containing about 2 to 4 g. of glucose. Their blood sugars in the fed state ranged from 325 to 700 mg. per 100 ml. (Table 1).

At least 3 weeks were allowed to elapse after the alloxan injection before the diabetic rats were treated with insulin as described by Spiro and Hastings (4). On the first day each rat was injected subcutaneously with 10 units of protamine-zinc insulin per 100 g. body weight; on the second day, with 5 units per 100 g. body weight; on the third day, with 2 units per 100 g. body weight; and for the next 2 to 3 weeks, with 1 unit per 100 g. body weight. During the 2 days preceding the experiment, each rat was injected, in addition, with 1 unit of unmodified crystalline insulin (glucagon-free) 3 times daily.

The test diabetic rats had been deprived of insulin for exactly 14 hours at the time the cannulae were inserted into their portal veins. These test rats were first given, via the cannulae, a priming dose of 25 units of unmodified crystalline insulin (glucagon-free), followed immediately by infusion of a solution containing, per ml., 320 mg. of glucose and 5 units of unmodified insulin. Infusion was at the rate of 2.4 ml. per hour and lasted from 10 minutes to 3 hours.

The control rats were infused by portal vein with glucose only, from 10 minutes to 3 hours. The time of injection was adjusted so that, when the infusion was completed, the control rats had been deprived of insulin for exactly 14 hours. Thus the hepatic metabolism of each test rat was compared with that of a control which had been deprived of insulin for exactly 14 hours and which, in addition, had received the glucose infusion intraportally for the same period as had its corresponding test rat.

Approximately one-half hour before the start of the portal vein infusion, the rats were anesthetized with Nembutal® (10- and 30-minute-infused rats) or with ether (1- to 3-hour-infused rats). The duodenal portion of the small intestine was exteriorized, and small-bore polyethylene tubing (with an internal diameter of 0.023 inch reduced to proper diameter by stretching) was inserted deep into the inferior hepatico-duodenal vein so that the infusion fluid was delivered directly into the portal vein. The animals that were to be infused for 1 to 3 hours were placed in restraining cages. Each experiment consisted of at least 6 rats—from 3 to 5 test and from 2 to 4 control diabetic rats.

At the end of the infusion period the rats were rapidly killed by a blow on the head. Immediately thereafter blood was taken for determination of glucose, and a sample of liver was taken for determination of glycogen. The preparation of liver slices, the procedures used for their incubation with acetate-1-C^{14} and glucose evenly labeled with C^{14}, and the methods for determining C^{14}-fatty acids and C^{14}-cholesterol have been described elsewhere (5 to 8). Each incubation flask contained 2 μmoles of labeled acetate or 400 mg. per 100 ml. as labeled glucose. The amount of radioactivity in each flask was approximately 2×10^5 cpm.

Glycogen was isolated by the method of Sjögren *et al.* (9), and determined colorimetrically by the procedure of Mendel and Hoogland (10). In those cases in which the incorporation of the C^{14} of C^{14}-glucose into glycogen by liver slices was determined, the isolated glycogen was dialyzed to remove salts before it was plated on aluminum planchets for C^{14} counting.

RESULTS

Glycogenesis. The effects of infusion of glucose alone and of glucose plus insulin on blood glucose are recorded in Table 1. The effects of these treatments on liver glycogen are shown in this table and in Figure 1.

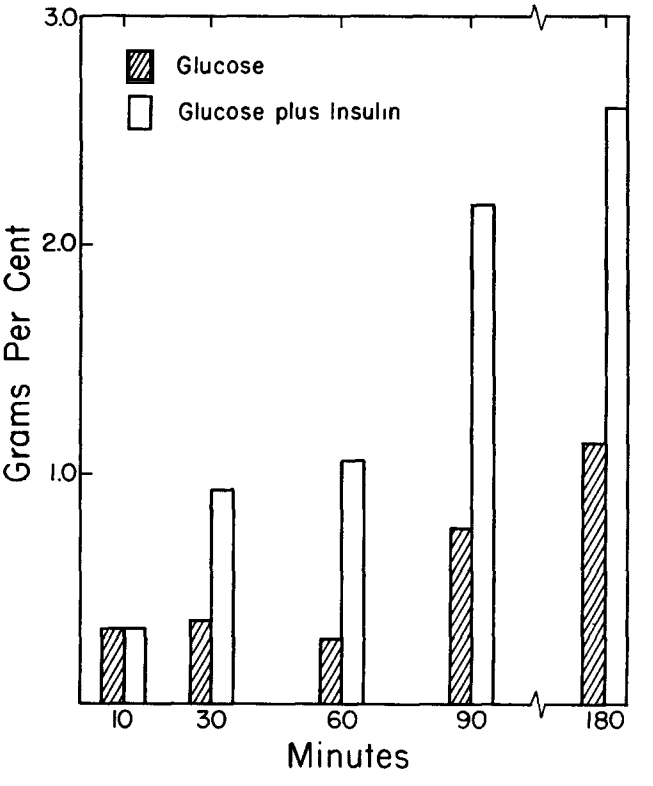

FIG. 1. Time course of the effects of continuous portal vein infusion of insulin on liver glycogen of intact diabetic rats. The control and test values for the 30-, 60-, 90-, and 180-minute experiments were significantly different at the 5% level as determined by the rank sum test (15).

The infusion of glucose alone (control diabetic rats) had no effect on the glycogen content of the liver for the first 60 minutes, but at later intervals of 90 and 180 minutes, the glycogen content of the livers of these control rats rose significantly.

No effect of insulin infusion for 10 minutes was detected, but by the time 30 minutes had elapsed, the administration of the hormone had increased the glycogen content of the liver.

The conversion of C^{14}- glucose to glycogen by slices prepared from the livers of insulin-treated and control rats was also studied. No effect of a 10-minute insulin infusion was noted. The infusion of the hormone for 30 minutes did, however, result in a fourfold increase in the liver's capacity for glycogenesis.

Lipogenesis. The continuous infusion of insulin for as long as 60 minutes failed to influence the liver's

TABLE 1. EFFECT OF INTRAPORTAL VEIN INFUSION OF INSULIN ON HEPATIC LIPOGENESIS, CHOLESTEROGENESIS, AND GLYCOGENESIS

Experiment	Number of Rats	Blood Sugar Before Infusion Treatment *		Infusion Treatment	Duration of Infusion	Liver Glycogen		cpm. of C^{14}-acetate Incorporated into				cpm. of Glucose-C^{14}† Recovered as				Blood Sugars at End of Infusion	
								Fatty Acid		Cholesterol		Fatty Acid		Glycogen			
		Range	Avg.			Range	Avg.	Range	Avg.	Range	Avg.	Range	Avg.	Range	Avg.	Range	Avg.
		mg./100 ml.	*mg./100 ml.*		*min.*	*per cent*	*per cent*										
1	5	386–511	446	Insulin + glucose	10	0.22–0.52	0.32	0–29	6	339–600	459			250–550	405	346–457	391
	4	380–467	431	Glucose	10	0.22–0.43	0.33	0–10	3	435–561	498			350–525	438	491–620	560
2	5	360–470	412	Insulin + glucose	30			0–17	3	958–1496	1122					190–222	206
	4	380–430	404	Glucose	30			0–21	9	525–991	729					510–690	588
3	5	383–450	414	Insulin + glucose	30	0.65–1.22	0.94							728–4527	1807	172–311	252
	4	376–428	398	Glucose	30	0.22–0.60	0.37							335–453	409	516–669	568
4	5	325–377	348	Insulin + glucose	60	0.45–1.51	1.06	0–147	56	1440–4378	2410	0–0	0			280–412	335
	4	336–531	431	Glucose	60	0.24–0.32	0.29	0–96	33	384–676	541	0–0	0			680–809	746
5	4	388–556	413	Insulin + glucose	90	1.35–3.20	2.19	190–525	316	308–1165	616	0–0	0			215–291	252
	2	462–570	516	Glucose	90	0.59–0.99	0.79	0–80	40	680–895	788	0–0	0			655–806	730
6	3	522–700	587	Insulin + glucose	180	2.24–2.86	2.61	1339–7215	3769	159–970	557	54–583	207			92–102	99
	3	442–630	505	Glucose	180	0.29–1.94	1.14	497–955	697	646–3460	1849	6–60	38			592–814	689

* These measurements were made during the 3-week (or longer) interval between the injection of the alloxan and the start of the insulin treatments as described by Spiro and Hastings (4).

† Evenly labeled.

capacity to synthesize fatty acids from acetate; an effect was, however, observed at 90 minutes (Table 1 and Fig. 2). An even greater delay was encountered in our attempt to obtain an action of insulin on fatty acid synthesis from C^{14}-glucose; a slight effect was detected in the 3-hour experiment, but not at 90 minutes.

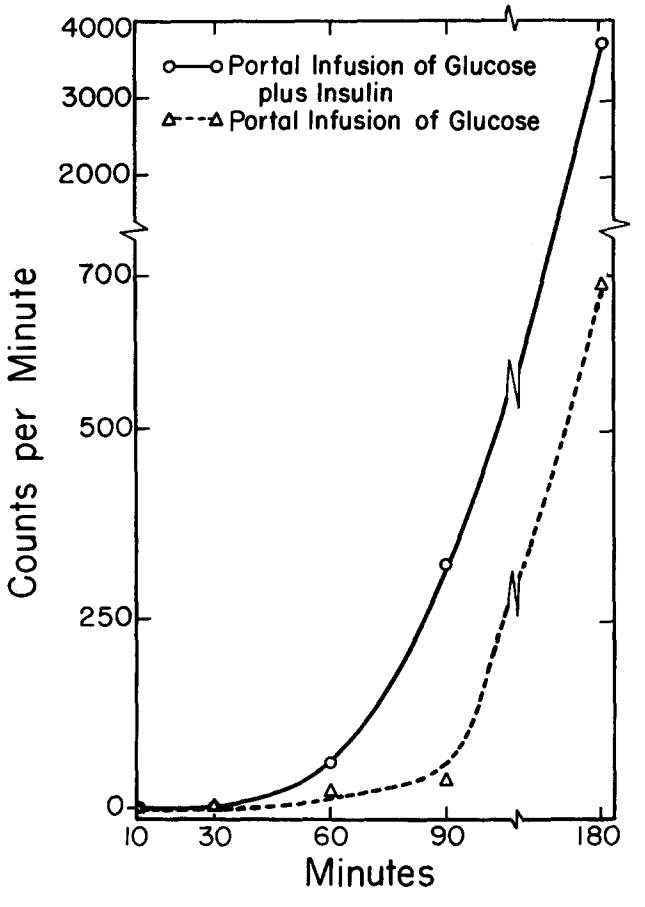

FIG. 2. Time course of the effects of continuous portal vein infusion of insulin on the capacity of liver slices of diabetic rats to convert the C^{14} of acetate-1-C^{14} to fatty acids. The control and test values differed significantly only in the 90-minute (at the 6% level) and in the 180-minute (at the 5% level) experiments, as judged by the rank sum test (15).

A significant increase in lipogenesis from acetate was also observed at 3 hours in the control rats. But it should be noted that these 3-hour-glucose-infused rats started to received glucose when they had been deprived of insulin for only 11 hours.

Cholesterogenesis. The earliest effect of insulin on the capacity of the diabetic rat liver to synthesize cholesterol from acetate occurred at 30 minutes (Table 1 and Fig. 3). A biphasic response was noted. The augmented cholesterogenesis observed at 60 minutes was followed by a sharp decline in cholesterogenesis at 90 and 180 minutes. The prolonged infusion of glucose alone increased cholesterogenesis from acetate in control diabetic rats.

DISCUSSION

In order to study the time element in the induction of changes in the metabolism of the liver by portal infusion of insulin, we used the 14-hour-insulin-deprived, alloxan-diabetic rat. No effects on lipogenesis, cholesterogenesis, and glycogenesis were observed in 10-minute experiments. Effects of the hormone on glycogen formation were noted as early as 30 minutes after the start of the insulin infusion; this was shown not only in the levels of glycogen in the intact animal but also in the capacity of liver slices to incorporate C^{14}-glucose into glycogen *in vitro*. An unexpected result was the rapidity with which insulin affected cholesterol metabolism. Significant changes in the liver's capacity to synthesize this sterol occurred not later than 30 minutes. The greatest delay in the hormone's action was noted in the response of fatty acid synthesis from acetate, which occurred between 60 and 90 minutes after the start of the injection of insulin by way of the portal vein.

We reported earlier that the previous nutritional state of the rat determines the level of cholesterogenesis in the liver. Thus it was shown that fasting drastically reduces hepatic cholesterogenesis (11), and that liver slices prepared from normal rats fed a synthetic

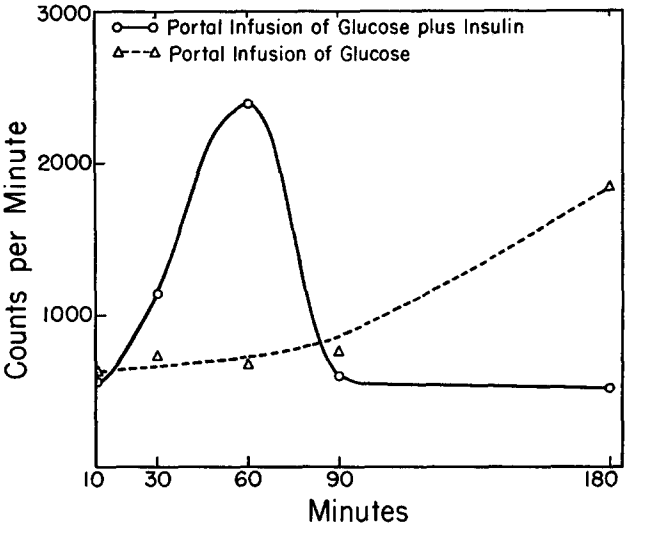

FIG. 3. Time course of the effects of continuous portal vein infusion of insulin on the capacity of diabetic rat liver slices to incorporate the C^{14} of acetate-1-C^{14} into cholesterol. Differences were significant at the 2% level in the 30-minute experiment (rank sum test) and at the 1% level in the 60-minute experiment. The values in the 180-minute experiment differed significantly at the 10% level.

diet containing 60 per cent glucose have a much greater capacity for incorporating acetate carbon into cholesterol than do slices from rats fed a 25 per cent glucose diet (12). A similar phenomenon was observed here—hepatic cholesterogenesis in the 14-hour-insulin-deprived, alloxan-diabetic rat is also sensitive to the availability of large amounts of carbohydrate.

An interesting aspect of the action of insulin on hepatic cholesterogenesis is the biphasic response shown in Figure 3. We have already seen that an augmented cholesterogenesis is associated with an increased glucose metabolism in the liver, and this accounts for the first effect of insulin (see 30 and 60 minutes in Fig. 3). The subsequent fall occurs at a time when the lipogenic effect of insulin has begun to take hold, and would appear to result from diversion of acetate toward fatty acid synthesis. A similar inverse relation between hepatic cholesterogenesis and lipogenesis was observed in experiments with normal rats fed high fat diets. In this case the depression in lipogenesis that followed fat feeding was accompanied by a rise in the extent of cholesterogenesis (13, 14).

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