The Effect of Insulin on the Formation of Glycogen by the Mouse Diaphragm in vitro

LARNER and VILLAR-PALASI[1] have shown that insulin stimulates muscle UDPG-α-glucan transglucosylase and this effect is independent of the presence of glucose in the medium[2]. The experiments reported here were undertaken to estimate the importance of this effect of insulin in the mouse muscle in vitro.

Methods. Mouse hemidiaphragms were obtained from fasted male mice of between 15 and 20 g. Before incubation, the tissues were soaked in Krebs' bicarbonate buffer at 4 °C. Incubation media were prepared by adding glucose (or (^{14}C) glucose) and insulin to Krebs' buffer which contained 2 mg of gelatin/ml. Incubations were carried out for 1 h at 37 °C after gassing the incubation flasks with 95% 0.2–5% CO_2. The glycogen was extracted from the tissues after incubation by 30% KOH, precipitated by alcohol and isolated by centrifugation. The glycogen was determined for each tissue by the anthrone method[3] and the radiocarbon in the glycogen measured by liquid scintillation counting[4]. The results of the glycogen and radiocarbon measurements are presented as μg of glycogen (or labelled glycogen)/mg wet tissue. The factor for converting the radiocarbon content of glycogen to μg of glycogen was determined by measuring the glucose content of mouse muscle glycogen, by acid hydrolysis, and the specific activity of the (^{14}C) glucose in the incubation medium. It was assumed that the molecules of glucose entering the diaphragms are incorporated into glycogen without their carbon atoms undergoing exchange reactions. The data were then subjected to an analysis of variance[5] to estimate the statistical significance of the results.

Results. Table I shows that an increase in either the concentration of glucose or of insulin stimulates the formation of glycogen. At all levels of glucose tested, including zero, 125 μU of insulin/ml further increases the formation of glycogen. The results presented in Table II(A) demonstrate that, in the presence of a fixed amount of (U^{14}C) glucose, an increase in the concentration of insulin causes an increase in the total labelled and unlabelled glycogen formed by the tissues. In Table II(B) an increase in the concentration of insulin is shown to increase the total glycogen formed by the diaphragms in the absence of glucose in the incubation medium. Table III summarizes the results of experiments in which dia-

Table I. The influence of the concentrations of insulin and of glucose on the formation of glycogen by the mouse diaphragm in vitro.

Glucose (m*M*)	Total glycogen A. Without insulin	B. With 125 μU insulin/ml
0.0	0.74	2.26
5.4	1.02	2.72
11.4	1.61	2.83
17.1	2.20	3.44
D = 0.40		

The glycogen content in unincubated tissues is 0.54 ± 0.04 μg/mg tissue (mean ± s.d. for 40 tissues). Each value is the mean (as μg/mg wet tissue) of the glycogen content of six tissues. D is the required minimum difference between the means for them to be statistically different at $P = 0.01$.

Table II. The influence of insulin on the composition of glycogen formed by the mouse hemidiaphragm in vitro

Insulin (μU/ml)	Glycogen composition A. With 12.6 m*M* (U^{14}C) glucose			B. Without glucose
	Total	Labelled	Unlabelled	Total
0	3.98	2.60	1.32	0.46
5	5.80	4.30	1.42	0.70
25	6.50	4.50	2.00	3.10
50	10.60	5.65	4.95	4.80
100	13.90	7.60	7.90	8.15
D	3.02	0.76	2.60	3.02

Each value is the mean (as μg of glycogen/mg tissue) of the glycogen content of eight tissues which were incubated in two groups of four. The difference between the groups is not significant at $P = 0.05$, whereas the difference between the response to different conditions of incubation is significant at $P = 0.001$.

Table III. The effect of incubation with and without insulin on the composition of glycogen formed by the mouse diaphragm during a preliminary incubation with (^{14}C) glucose

Incubation	Composition of the medium	Total	Glycogen composition Labelled	Unlabelled
No. 1	A. 5.8 m*M* (^{14}C) glucose	3.80	0.89	2.20
No. 2	B. 5.8 m*M* (^{14}C) glucose	4.65	0.64	4.00
	C. 5.8 m*M* (^{14}C) glucose + 100 μU insulin/ml	8.80	1.36	7.44
	D. 5.6 m*M* glucose	2.48	0.46	2.01
	E. 5.6 m*M* glucose + 100 μU insulin/ml	7.10	0.45	6.65
	F. buffer alone	0.83	0.46	0.37
	G. buffer + 100 μU insulin/ml	5.75	0.12	5.60
Unincubated diaphragms		0.44	0.00	0.44
D (for $P = 0.001$)		1.58	0.26	0.14

The values represent the combined results of two experiments. For each experiment 24 tissues were incubated in medium A for 1 h and batches of four tissues incubated in the media B–G one more h. Four tissues were frozen at the end of the first incubation. There was no significant difference between the results of the two experiments at $P = 0.05$, whereas the response of the tissues to the different media was significant at $P = 0.001$. The values are the means of the glycogen contents (in μg/mg tissue) of eight tissues.

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phragms were incubated in buffer containing (1^{14}C) glucose and then incubated in buffer containing different combinations of glucose (either labelled or unlabelled) and insulin. These experiments demonstrate that insulin does not alter the rate of break-down of labelled glycogen when tissues are transferred to buffer which does not contain labelled glucose. The presence of insulin alone in the second medium of incubation stimulates the formation of unlabelled glycogen; a similar amount of unlabelled glycogen is formed in the presence of insulin and labelled glucose.

Discussion. These results show that insulin stimulates the incorporation of exogenous glucose into the glycogen of the mouse hemidiaphragm in vitro. This effect can be stimulated by increasing the concentration of glucose in the incubation medium. Earlier work in this laboratory[6] has shown that insulin increases not only the amount of glucose entering the mouse diaphragm, but also the percentage of this glucose which is incorporated into glycogen. An increase in the concentration of glucose produces an increase in the glucose entering the tissues with a proportional increase in the incorporation of glucose into glycogen. The stimulating effect of insulin on the incorporation of exogenous glucose into glycogen is then not solely occasioned by the increased penetration of glucose into the tissues, and could be mediated by the known effect of insulin on UDPG-α-glucan transglucosylase[1]. Our experiments show that, at high concentrations of insulin (100 μU/ml), less than half the glycogen increase is due to the incorporation of exogenous glucose into glycogen. Insulin, in the absence of glucose, induces an increase in the glycogen of the mouse diaphragm and this increase in glycogen is of the same order of magnitude as the increase in unlabelled glycogen when incubations are carried out with labelled glucose. These results could be interpreted as meaning that insulin permits the diaphragm to utilize a pool of glucose-1-phosphate for glycogen synthesis and that this pool is not in equilibrium with glucose entering the tissues. We have shown that, after 1 h incubation in (1 ^{14}C) glucose, a second incubation with insulin alone results in the formation of unlabelled glycogen. This argues in favour of a route of glycogen synthesis which is independent of glucose entering the tissues, as it is to be expected (although not proven) that the pool of glucose-1-phosphate would be in equilibrium with exogenous glucose after 1 h incubation.

We conclude that the fasting mouse diaphragm contains a glycogen precursor which does not equilibrate with glucose entering the tissues and which is transformed into glycogen by the action of insulin. Insulin also stimulates the incorporation of exogenous glucose into glycogen, by a mechanism which may correspond to that involving the known action of insulin on UDPG-α-glucan transglucosylase.

Zusammenfassung. Insulinzugabe in ein Milieu mit (U^{14}C) Glukose stimuliert gleichfalls die Bildung des radio- wie des nicht-radioaktiven Glykogens in vitro. Insulin vermehrt ebenso die Bildung von Glykogen durch das Mäusezwerchfell bei Abwesenheit von Glukose im Inkubationsmilieu. Es wird eine doppelte Glykogenbildung angenommen: 1. Inkorporation von exogener Glukose in Glykogen; 2. Inkorporation eines endogenen Vorläufers im Glykogen, der mit Glukose nicht identisch ist.

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Initial Myocardial Succino-Oxidase Activity Changes Induced by Intravenous Infusion of *Escherichia coli* Endotoxin in Rabbit[1]

Previously, we have observed certain myocardial enzymatic changes and cardiac functional changes after acute histaminic shock[2], acute hemorrhagic shock[3], and acute endotoxin or hypoxic shock[4] in dogs. These myocardial changes, however, might be brought about by the primary vascular changes induced by chemical substances (e.g. histamine, 5-hydroxytryptamine, catecholamines, bradykinin etc.), which could be released during hypotension, endotoxin or other forms of shock[5]. It was also observed by others[6] that endotoxin did not directly affect the mammalian heart, but rather reacted primarily to the peripheral vessels, whereby the venous return to the heart would be reduced and, secondarily, cardiac functions would be affected. In order to clarify this problem, endotoxin was injected into the ear vein of rabbits, and its initial effect on the myocardial succino-oxidase system was studied. Also, no blood pooling in the hepatosplanchinic system has been observed in the rabbit by endotoxin[7], even though endotoxin can induce other vascular reactions similar to those of anaphylax[8]. Thus, histamine was also infused into the rabbit and its early effect on the heart was studied.

Method and materials. A total of 100 albino rabbits of both sexes, weighing 2 to 4 kg each, was anesthetized

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