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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with sodium pentobarbital (20 mg/kg). Endotoxin (0111:B4 Lipopolysaccharide of *Escherichia coli*, obtained from Difco Lab, Detroit, Michigan) (1.0 mg/kg) or histamine (1.0 mg/kg) was infused into the ear vein of a rabbit, which was sacrificed after 15 min. Another group of rabbits was pretreated with reserpine (3 mg/kg/day) for three days, and the third group received the compound 48/80 twice daily for two days and once in the morning of the experiment (on the first day, 0.2 mg/kg; on the second, 0.5 mg/kg of 48/80; and on the experimental day, 1.0 mg/kg), and cortisone acetate (200 mg/day for three days). Endotoxin (1.0 mg/kg) was also injected in these drug-pretreated groups. A pair of heart muscles obtained from two identically treated rabbits were homogenized and mitochondria isolated[8]. The mitochondria were disrupted in the cold by a Sonifier (Branson Sonic Power Co., Danbury, Conn.) and a cooling jacket for 3 to 5 min. The succinate dehydrogenase (SDH) and cytochrome oxidase (COase) activities were measured at 26° with a Zeiss spectrophotometer at wave-lengths of 400 nm and 550 nm, respectively[9].

Results and discussion. The SDH activity was significantly decreased with the infusion of endotoxin or histamine in rabbits (Table I); however, the COase activity was not changed by endotoxin or histamine within the given period of time (Table III). Probably, the SDH is a very sensitive enzyme responding during the early period of shock. The SDH activity was also decreased by depletion of catecholamines and 5-hydroxytryptamine (by reserpine), and by endogenous histamine (by 48/80) (control 0.7904 ± 0.06197 compared to that of reserpine–0.3818 ± 0.01794; $p < 0.001$; control – 0.7904 ± 0.06197 compared to that of 48/80 – 0.5819 ± 0.7505; $p < 0.02$). Therefore, infusion of endotoxin did not cause additional changes in the SDH activity (Table II) in these drug-pretreated groups. The COase activity was not altered by drug-pretreatment; however, the endotoxin infusion in these groups brought about significant depression of COase activity (Table IV). Apparently, the myocardial

Table I. Cardiac succinate dehydrogenase activity of rabbits (activity per mg protein of Mitochondria per min)

Exper. No.	Control	Endotoxin	Histamine
1	0.722	0.440	0.569
2	0.718	0.420	0.594
3	0.944	0.529	0.638
4	0.666	0.484	0.586
5	0.902	0.468	0.578
Mean ± SEM	0.7904 ± 0.06197	0.4682 ± 0.02101	0.5930 ± 0.01341
t[a] (Control and exper.)		$t = 4.92359$	$t = 3.11337$
p[b] (Control and exper.)		$p < 0.01$	$p < 0.02$

[a] $t = \frac{\text{Difference in Means}}{\sqrt{(\text{SEM}_1)^2 - (\text{SEM}_2)^2}}$; $\text{SEM} = \frac{\text{Standard Deviation}}{\sqrt{n-1}}$.

[b] p = Obtained from Student's t Distribution (R. A. Fisher and F. Yates, *Statistical Tables for Biological, Agricultural, and Medical Research* (Oliver & Boyd Ltd., Edinburgh 1949), Table III.

Table II. Cardiac succinate dehydrogenase activity of pretreated rabbits (activity per mg protein of mitochondria per min)

Exper. No.	Reserpine (control)	Reserpine + toxin	48/80 (control)	48/80 + toxin
1	0.426	0.335	0.797	0.570
2	0.335	0.414	0.844	0.638
3	0.345	0.418	0.867	0.461
4	0.401	0.395	0.856	0.549
5	0.398	0.478	0.450	0.615
6	–	0.485	0.433	0.527
7	–	0.414	0.428	0.630
8	–	0.466	0.357	0.645
9	–	0.491	0.392	0.662
10	–	0.505	0.395	0.556
Mean ± SEM	0.3818 ± 0.01794	0.4401 ± 0.01787	0.5819 ± 0.07505	0.5853 ± 0.02119
t (control and exper.)		$t = 2.30235$		$t = 0.4359$
p (control and exper.)		$p < 0.05$		$p < 0.7$

Table III. Cardiac cytochrome oxidase activity of rabbits (activity per mg protein of mitochondria per min)

Exper. No.	Control	Endotoxin	Histamine
1	8.06	6.93	9.17
2	8.16	8.00	12.42
3	8.10	7.00	11.50
4	7.22	6.13	–
5	8.06	6.57	–
Mean ± SEM	7.920 ± 0.2794	6.926 ± 0.3463	11.030 ± 1.41507
t (control and exper.)		$t = 2.2387$	$t = 1.4852$
p (control and exper.)		$p < 0.1$	$p < 0.1$

Table IV. Cardiac cytochrome oxidase activity of pretreated rabbits (activity per mg protein of mitochondria per min)

Exper. No.	Control (reserpine)	Reserpine + toxin	48/80 (control)	48/80 + toxin
1	5.57	5.12	8.75	10.00
2	6.32	4.88	10.00	9.80
3	7.93	4.81	9.13	5.73
4	6.83	4.42	9.47	6.00
5	5.79	5.58	9.79	4.70
6	–	–	8.21	8.10
7	–	–	10.11	5.85
8	–	–	9.63	6.40
9	–	–	10.74	5.70
10	–	–	9.29	6.60
Mean ± SEM	6.488 ± 0.4713	4.962 ± 0.2137	9.512 ± 0.2401	6.888 ± 0.6020
t (control and exper.)		$t = 2.9488$		$t = 4.04938$
p (control and exper.)		$p < 0.02$		$p < 0.001$

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enzymatic changes are enhanced by the drug-pretreatment. Also, the cardiac changes induced by endotoxin and histamine in rabbits were identical, suggesting that endotoxin shock might be mediated by histamine in vivo[8].

It was known that no significant blood pooling in the hepato-splanchinic system of rabbit after endotoxin infusion[7], and the observed myocardial SDH activity changes might have been brought about by primary action of endotoxin on the heart at the given period of time (15 min). Previously, streptolysin-O was the only known bacterial toxin to induce primary cardiac depression[10]. Even though no depression of function has been found after endotoxin infusion in the isolated rabbit heart[11], the present study is consistent with our recent findings made after coronary infusion of endotoxin in the canine heart, in situ. It was seen that cardiac mechanical and biochemical functional changes that occurred[12], strongly suggested that the endotoxin might react primarily on the heart as well as on the peripheral vessels. We also found that exogenous histamine infusion into the coronary artery resulted in myocardial functional changes similar to that of endotoxin shock[13,14].

Zusammenfassung. *Escherichia coli*-Endotoxin (1 mg per kg) wurde in die Ohrvene von Kaninchen injiziert: Die Succinat-dehydrogenase- und Cytochromoxydase-Aktivität in den Mitochondrien des Herzmuskels wurde nach 15 min verändert. Es ist wahrscheinlich, dass das Endotoxin sowohl auf das Herz, als auch auf die peripheren Gefässe direkt wirkt.

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Effect of Acute and Exhaustive Exercise Upon the Fine Structure of Heart Mitochondria

Mitochondria are among the most labile of myocardial structures, and the first to react to different stimuli. Most of the studies concerning the modifications of mitochondrial fine structure in different stages of cardiac functional overload have been made after long-lasting stimuli. Acute and exhaustive exercise represents perhaps one of the best ways to obtain cardiac functional overload in a short time. In the present report the modifications of mitochondrial fine structure after acute and exhaustive exercise are described.

The observations were performed on the hearts of 12 healthy adult dogs which were submitted to acute and exhaustive exercise forcing them to swim in a tank filled with warm water (24 °C) until they became exhausted and sank into the water. This occurred after variable periods of time, ranging between 40 and 90 min, according to the strength of the animals. Immediately after that the dogs were anaesthetized and a small piece of the tip of the heart was removed and fixed in cold 6.25% glutaraldehyde in cacodylate buffer[1]. The material was post-fixed in osmium tetroxide and embedded in Araldite[2].

The electron micrographs of the heart sections revealed the existence of mitochondria of unusual size. In some cases the mitochondrial mass represented more than one half of the whole myocardial area (Figure 1). The increase in mitochondrial size could be seen under three different aspects. In some cases there appeared to be a fusion of neighbouring mitochondria, appearing as long and slender

Fig. 1. Increase of the mitochondrial mass. In this micrograph most of the myocardium is represented by mitochondria with some swelling of the matrix. × 12,000.

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