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EFFECTS OF ENDOTOXIN ON GLUCOSE UPTAKE BY THE ISOLATED FORELIMB--ETC(U)  
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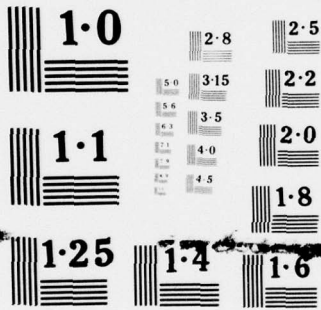
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<sup>9</sup> FINAL REPORT

<sup>6</sup> Effects of Endotoxin on Glucose Uptake by the Isolated Forelimb of the Dog.

by

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### ABSTRACT

Recent research has demonstrated an increase in glucose utilization by skeletal muscle occurs in hemorrhagic shock. It is conceivable that the hypoglycemia of gram negative septic shock is, in part, due to an increased glucose utilization by peripheral tissues. The hypothesis tested in this study was there is an increase in glucose uptake by the isolated innervated and/or denervated forelimb of the dog subjected to endotoxin shock. Results indicate that endotoxin does not affect a net increase of glucose uptake by the isolated forelimb. No increase in uptake occurred when blood glucose concentration was normal. However, when endotoxin hypotension induced a significant hyperglycemia or when arterial glucose concentration was elevated by glucose administration, an apparent increase in forelimb glucose uptake occurred. It is concluded that endotoxin does not increase the uptake of glucose by skin and muscle except that it causes a hyperglycemia secondary to an increased sympathoadrenal discharge in the shock state. If the dog becomes sufficiently hyperglycemic, an apparent increase in glucose uptake occurs probably because of accumulation of glucose in the interstitial space of skin and muscle.

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## INTRODUCTION

It is established that hypoglycemia is a hallmark of severe sepsis in many species (1, 2, 3). It has been shown that dogs become hypoglycemic following the administration of an LD<sub>70</sub> of E. coli endotoxin (4). Further, Peyton et al. showed that endotoxin treated eviscerated dogs become progressively hypoglycemic in contrast to controls administered saline alone (5). The fact that these endotoxin treated animals become hypoglycemic lends support to the view that an increase in peripheral utilization of glucose may be a causative factor in the hypoglycemia of endotoxin shock. Additionally, it has been indicated that neither the heart nor the lungs (6) or the central nervous system (7) are sites of increased glucose utilization. Thus, since skeletal muscle and skin make up such a large proportion of the body weight in the eviscerated animal, these seem to be the most promising sites for increased glucose utilization leading to hypoglycemia in endotoxin shock.

The aim of this research was to determine if endotoxin caused an increase in glucose utilization in the innervated and/or denervated isolated forelimb of the dog with and without glucose administration.

## METHODS

Mongrel dogs of either sex weighing 13 to 27 kg, fasted 24 hours with water ad lib were anesthetized with sodium pentobarbital (30 mg/kg). The left femoral artery and vein were catheterized for monitoring blood pressure and administration of infusions respectively. The brachial artery and vein and the cephalic vein were isolated and the limb amputated. The cephalic and brachial veins were cannulated for collecting blood for chemical analysis and timed determination of limb blood flow. The forelimb was positioned on a tray with unobstructed venous return and suspended from a counter-balanced strain gauge device for monitoring limb weight. Venous blood from the brachial and cephalic vein cannulae was collected in a reservoir through a nylon screen and returned to the animal via a Sarnes pump to the external jugular vein. Systemic arterial pressure and changes in limb weight were continuously monitored. Before the extracorporeal circulation was begun, the animal was heparinized (500 units/kg) followed by hourly maintenance doses of sodium heparin (250 units/kg). Rectal temperature was monitored and core temperature was maintained with heating pads.

Five groups of animals were used in this study:

- Group I Denervated with purified E. coli endotoxin (Difco) 3 mg/kg. (8 animals)
- Group II Denervated with saline alone (8 animals)
- Group III Innervated with endotoxin (8 animals)
- Group IV Innervated with saline alone (8 animals)
- Group V Innervated with endotoxin and 50% glucose infusion (6 animals)

All animals received a sustaining infusion of 9% NaCl at 2 ml/min. In addition, Group V received a 50% glucose infusion sufficient to maintain the plasma glucose concentration above 170 mg%. In Groups I-IV blood samples were collected every 30 minutes for 270 minutes. In the animals with innervated limbs after 270 minutes, the limb was denervated and an additional blood collection was made 30 minutes later. In Group V blood samples were collected every 15 minutes for 75 minutes.

Femoral arterial, brachial and cephalic venous plasma glucose determinations were made with a Beckman glucose analyzer and blood gases were determined with an IL Blood Gas/pH analyzer. Plasma insulin levels were determined by radioimmunoassay provided in kit form (8).

The unpaired t-test was used to determine significance between parameters of the control and endotoxin treated groups for each period of time. A linear regression and a simple linear correlation were used when analyzing the change in one parameter as a function of change in another (9). The Newman-Keul's test (10) of significance was applied to the means of the parameters reported from the additional group of six experiments whenever a one-way analysis of variance showed significance.  $p < 0.05$  was used for the level of significance.

## RESULTS

### Denervated Forelimbs.

Figure 1 shows the arterial glucose concentration, arterial glucose load and limb uptake of glucose against time for control and endotoxin-treated animals. In the control group the arterial blood glucose concentration ( $[Gl_c]_a$ ) decreased with time to a level 22% below baseline. In the endotoxin group the  $[Gl_c]_a$  was increased to 171 mg% at 30 minutes after the start of the endotoxin infusion. Thereafter  $[Gl_c]_a$  decreased and was not significantly different from control values. The glucose delivery in mg/min (AGI) is markedly depressed throughout the endotoxin infusion reflecting the decreased limb blood flow. The rate of glucose uptake ( $UGl_c$ ) was significantly increased at 30 min corresponding with the peak in  $[Gl_c]_a$ . Beyond 30 minutes  $UGl_c$  was not significantly different from the controls.

Table 1 presents the changes in blood pressure (MAP) forelimb blood flow (FBF) and forelimb vascular resistance (FVR). Endotoxin caused a significant decrease in MAP and FBF. There was no weight change in the control group. The endotoxin group showed a slight but insignificant decrease. Innervated Forelimbs.

Figure 2 depicts the glucose parameters in the innervated forelimb experiments. In contrast to the denervated forelimb experiments a significant hyperglycemia could not be demonstrated 30 minutes following administration of endotoxin. Further, no significant difference between the control and endotoxin groups could be demonstrated for either  $[Gl_c]_a$  or  $UGl_c$ . A significant decrease in the AGI occurred following endotoxin administration.

The MAP and FBF through the innervated isolated limb decreased with endotoxin infusion as shown in Table 2. With denervation at 240 minutes, FVR decreased and FBF increased in both groups. The change in forelimb weight for both control and endotoxin treated groups was insignificant.

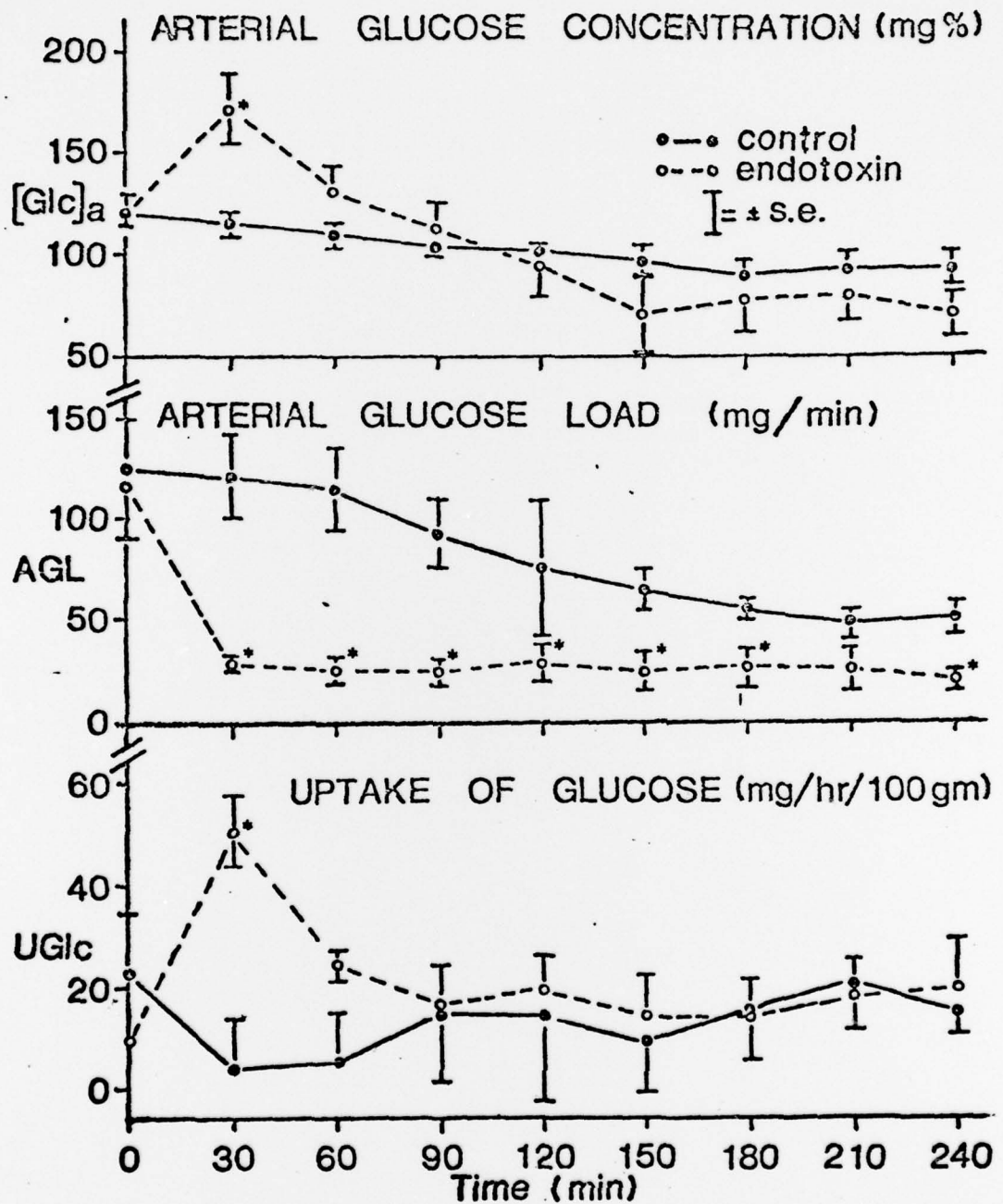


FIGURE 1. Arterial glucose concentration ([Glc]a), arterial glucose load (AGL), and uptake of glucose (UGlc) results from the denervated isolated forelimb preparation. Values are means (N = 8)  $\pm$ SE. \*Significantly different from control, (p < 0.05).

TABLE 1 Changes in mean systemic arterial blood pressure (MAP), total blood flow through the denervated isolated forelimb (FBF), and the forelimb vascular resistance (FVR) after *E. coli* endotoxin infusion.

Parameter	Time (min)								
	0	30	60	90	120	150†	180	210	240
Control (N = 8)									
MAP	134 ±6	131 ±6	136 ±7	137 ±8	138 ±7	140 ±7	140 ±7	139 ±8	134 ±9
FBF	17.9 ±3.3	18.0 ±2.8	17.9 ±2.9	15.1 ±2.6	13.0 ±2.0	12.0 ±2.0	10.6 ±1.1	9.0 ±1.1	8.9 ±1.1
FVR	1.6 ±0.3	1.5 ±0.3	1.6 ±0.3	1.9 ±0.3	2.2 ±0.3	2.4 ±0.4	2.5 ±0.3	3.0 ±0.4	3.1 ±0.5
Endotoxin (N = 8)									
MAP	134 ±2	66* ±11	59* ±3	54* ±9	63* ±12	66* ±14	73* ±16	77* ±16	78* ±16
FBF	14.3 ±1.0	2.6* ±0.4	3.3* ±0.8	3.6* ±0.9	5.0* ±1.4	5.0* ±1.2	4.5* ±1.4	4.6* ±1.5	4.0* ±1.1
FVR	1.6 ±0.2	4.4* ±0.8	4.1* ±1.0	3.7 ±0.9	3.0 ±0.7	3.1 ±0.8	4.6 ±1.4	4.8 ±1.5	4.5 ±1.1

Values are expressed as the mean ±SE. MAP, mean systemic arterial pressure (mm Hg). FBF, total forelimb blood flow (ml/min/100 gm). FVR, forelimb vascular resistance (mm Hg/ml/min).

\*Significant at the p<0.05 level.

†Two animals died after 150 minutes in the endotoxin group.

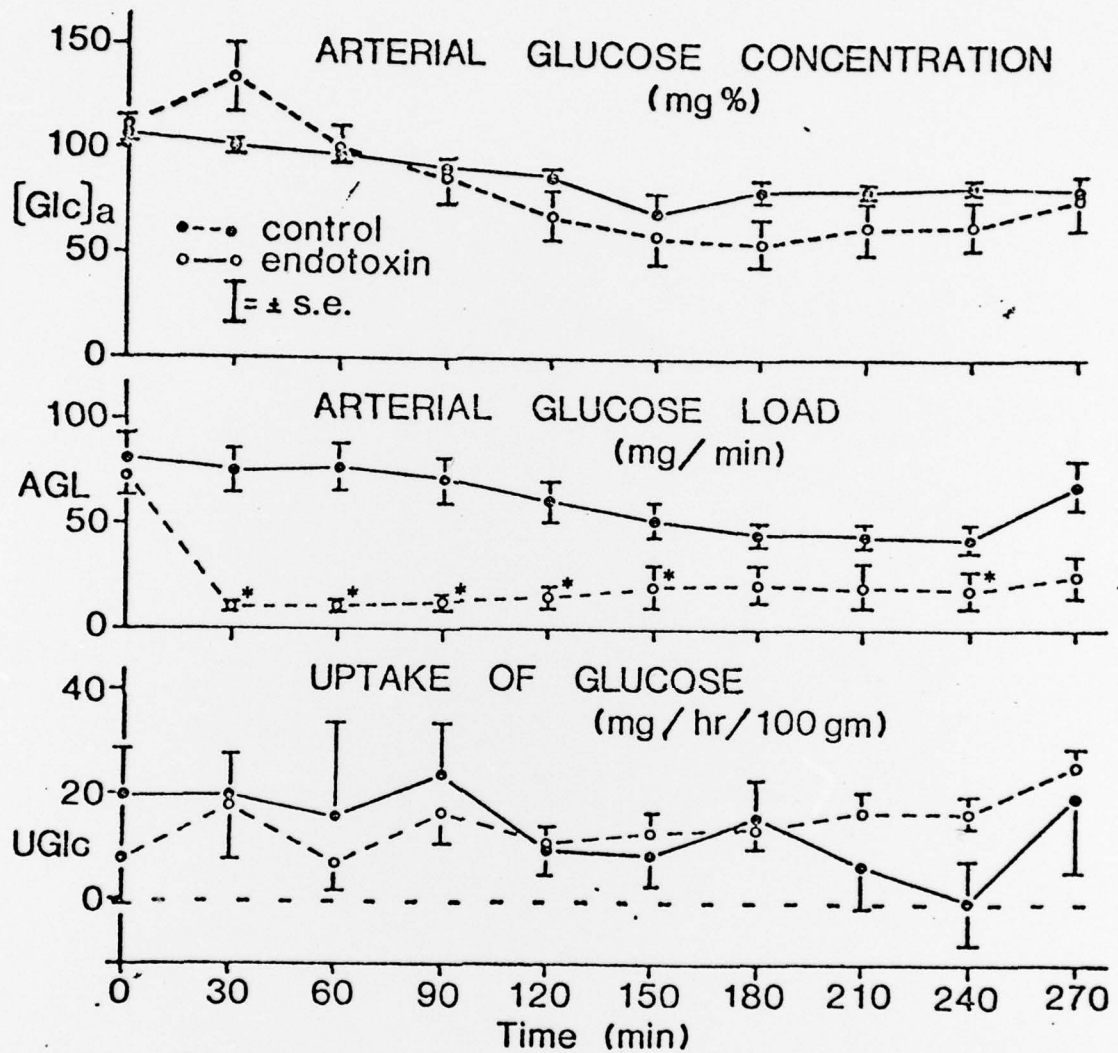


FIGURE 2. Arterial glucose concentration ([Glc]<sub>a</sub>), arterial glucose load (AGL), and uptake of glucose (UGlc) results from the innervated isolated forelimb preparations. Values are means (N = 8) ±SE. \*Significantly different from control, (p < 0.05).

TABLE 2. Changes in mean systemic arterial blood pressure (MAP), forelimb blood flow (FBF), and forelimb vascular resistance (FVR) in the innervated forelimb experiments after *E. coli* endotoxin infusion.

Parameter	Time (min)									
	0	30	60	90	120	150†	180	210	240	270††
Control (N = 8)										
MAP	137 ±9	135 ±9	138 ±8	138 ±9	130 ±7	133 ±5	129 ±6	129 ±4	128 ±3	128 ±5
FBF	12.5 ±1.6	12.1 ±1.6	12.8 ±1.6	12.4 ±1.6	11.4 ±1.7	10.4 ±1.6	9.3 ±1.3	8.9 ±1.3	8.5 ±1.0	14.3 ±2.0
FVR	2.4 ±0.5	2.4 ±0.5	2.2 ±0.4	2.3 ±0.5	2.7 ±0.7	3.0 ±0.6	3.0 ±0.6	3.2 ±0.6	2.9 ±0.4	1.8 ±0.3
Endotoxin (N = 8)										
MAP	134 ±7	59* ±10	63* ±9	56* ±9	65* ±7	74* ±6	77* ±8	77* ±9	79* ±9	77* ±9
FBF	13.8 ±1.8	1.8* ±0.3	2.0* ±0.4	2.6* ±0.7	3.5* ±0.9	4.9* ±1.0	5.3* ±1.1	4.8* ±1.3	3.9* ±0.7	5.8 ±0.6
FVR	2.3 ±0.4	7.4* ±1.2	7.2* ±0.9	6.3 ±1.3	6.6 ±2.0	5.0 ±1.6	4.0 ±1.1	5.6 ±2.1	5.4 ±1.7	2.8* ±0.9

Values are expressed as mean ±SE. MAP, mean systemic arterial pressure (mm Hg). FBF, total blood flow (ml/min/100 gm). FVR, forelimb vascular resistance (mm Hg/ml/min).

\*Significant at the  $p < 0.05$  level.

†Two endotoxin-treated animals died after 150 minutes.

††30 minutes after denervation.

### Innervated Forelimbs with Glucose Infusion

There had been a significant increase in  $UGl_c$  30 minutes after endotoxin in the denervated but not innervated forelimb. The only difference noted between the two groups which might explain the failure of the innervated limbs to show an elevated  $UGl_c$  was in the response of the  $[Gl_c]_a$  to endotoxin. Whereas the animals with the denervated limbs exhibited a significant hyperglycemia ( $171 \pm 17$  mg%--a 49% increase), the animals with the innervated limbs did not show a similar degree of elevation in the  $[Gl_c]_a$  ( $133 \pm 16$  mg%--a 20% increase). The question arises as to whether or not a definite increase in the  $UGl_c$  will occur in animals with innervated limbs if the  $[Gl_c]$  is elevated to a level corresponding to that noted in the animals with denervated limbs. Figure 3 shows the results obtained in the innervated forelimb during glucose infusion. Each parameter is shown; 1) at control "C", 2) during glucose infusion "G" with 50% glucose, 3) 15, 30, 45 and 60 min after administration of 3 mg/kg of endotoxin "E" and 4) 15 min after denervation of the limb, "D". The  $[Gl_c]_a$  was elevated from  $128 \pm$  mg% to  $189 \pm 8$  mg% ("G") by the 50% glucose infusion. The administration of endotoxin elevated the  $[Gl_c]_a$  further to  $225 \pm 13$  mg% within 15 min after the start of the infusion ("E + 15"). A significant increase in the  $UGl_c$  from points "C" to "E + 15" can be demonstrated with a paired t-test. The AGL did not rise significantly when 50% dextrose was rapidly infused ("G in Figure 3), but it declined significantly and remained low when endotoxin was administered. A significant correlation ( $P < 0.01$ ,  $\gamma = 0.538$ ) exists between the  $UGl_c$  and the  $[Gl_c]_a$  (Figure 4).

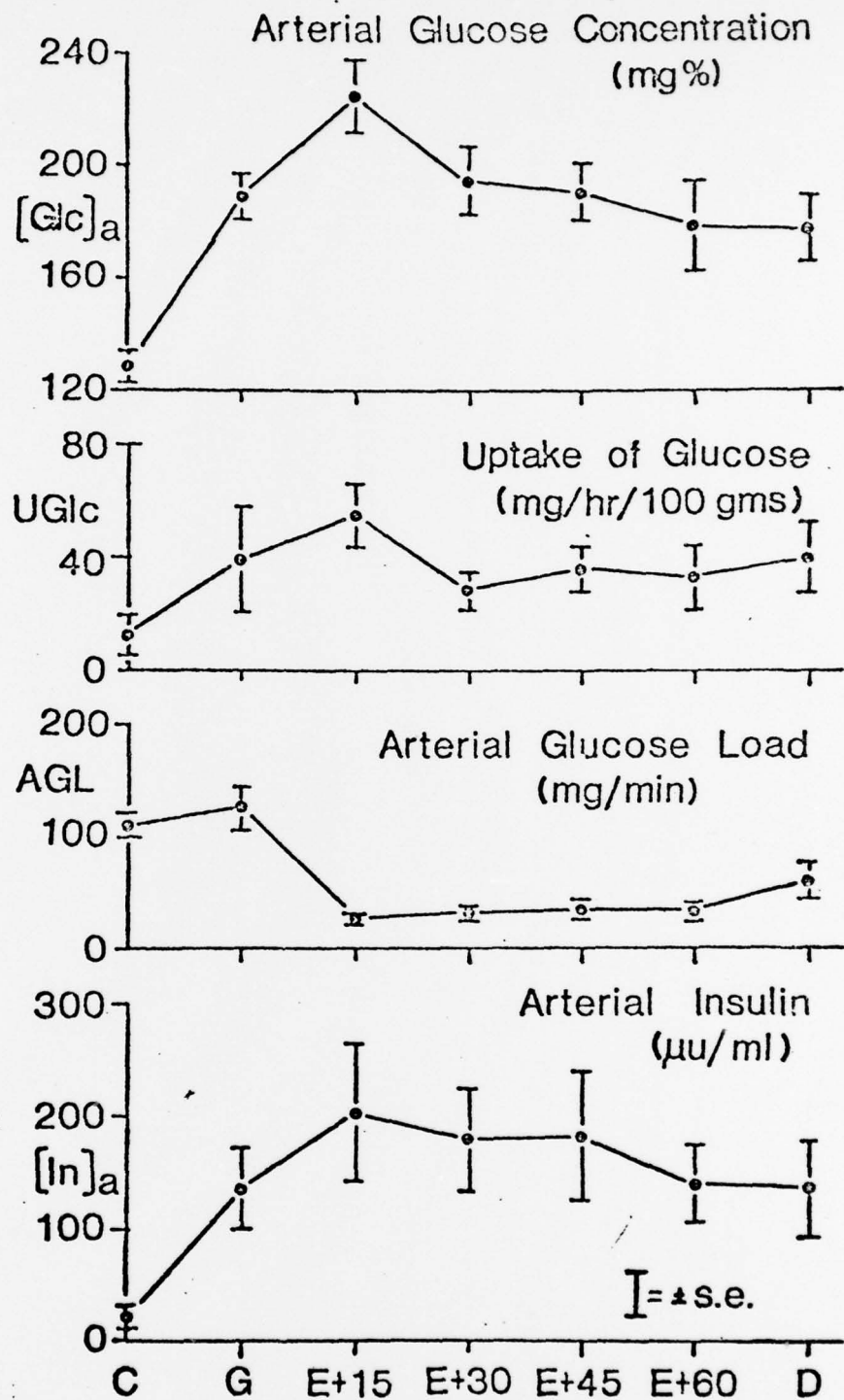


FIGURE 3. Arterial glucose concentration ( $[Glc]_a$ ), uptake of glucose (UGlc) by the isolated forelimb, arterial glucose load (AGL), and arterial plasma insulin concentration ( $[In]_a$ ) results from the additional experiments. Values are means ( $N = 6$ )  $\pm$ SE.

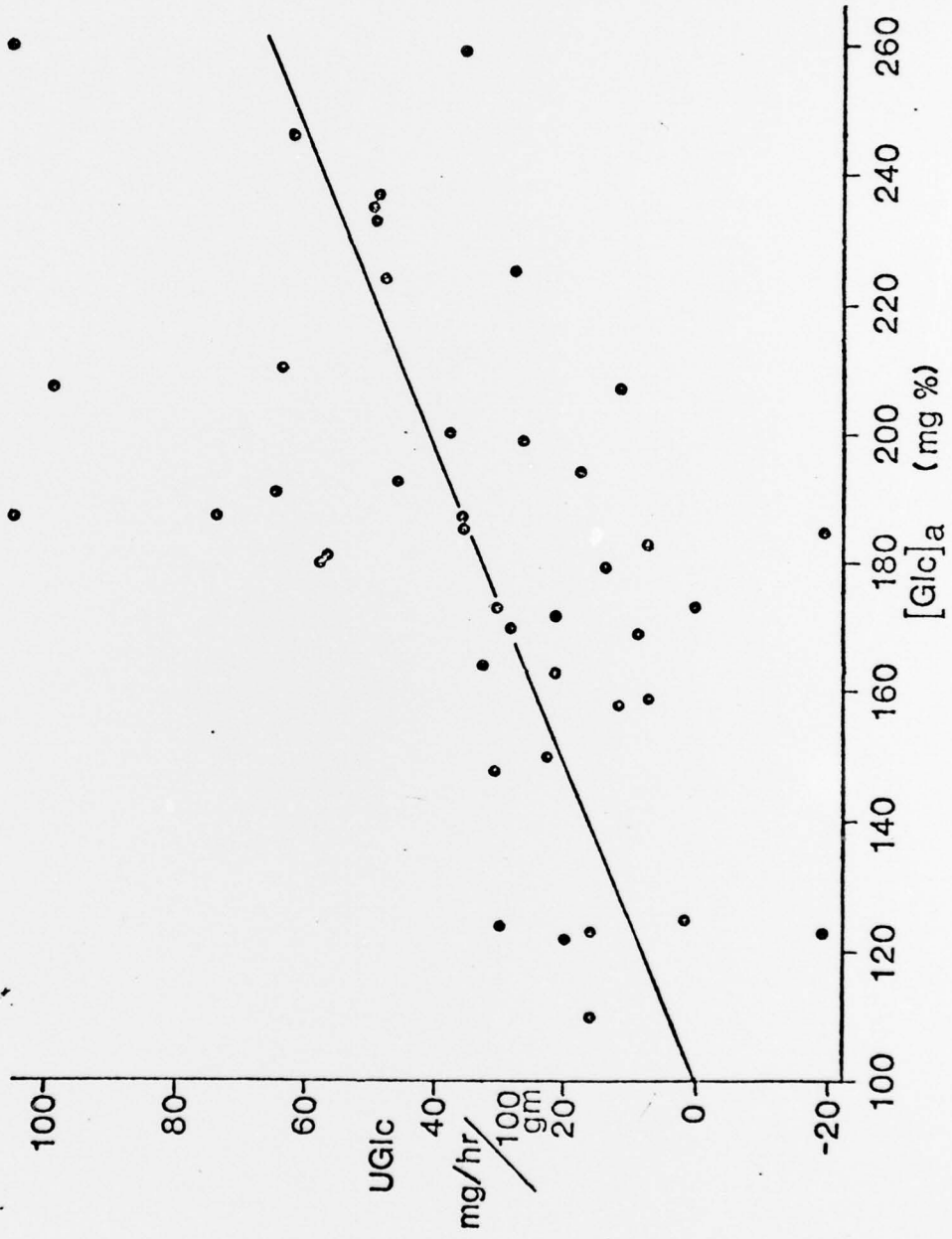


Figure 4.

## DISCUSSION

The hypothesis tested was that the forelimb uptake of glucose increases in endotoxin shock. Further, the hypoglycemia of endotoxin shock is due in part to increased utilization of glucose by peripheral tissues, especially skeletal muscle.

The results of this study show that glucose uptake by the isolated forelimb of endotoxin shocked animals is not significantly different from their controls. If the hypothesis was true, an increased glucose uptake would have occurred and would have been proportional to the degree of hypoglycemia noted in the shocked animals. Such was not the case.

When endotoxin is administered intravenously, a marked decrease in blood pressure in the dog occurs secondary to hepatosplanchnic vasoconstriction (11-15). The level of circulating catecholamines increase following a sympathoadrenal response to hypotension (16). The result is a decrease in blood flow in the isolated limb and is reflected in the arterial glucose load.

The arterial glucose load decreased significantly after endotoxin administration and remained low; yet, the uptake of glucose by the limb was not significantly different from controls. The principal factor influencing the change in the arterial glucose load was the marked decrease in limb blood flow. If the hypoperfusion caused tissue hypoxia, glucose uptake by skeletal muscle should increase. The fact that glucose uptake did not increase significantly suggests that either the low arterial glucose load prevented a significant uptake, or that tissue hypoxia did not exist in spite of a "hypoperfused" state. Denervation of the innervated preparations 240 minutes post-endotoxin produced an increase in blood flow. This would partially alleviate a hypoxic condition if one existed and also augment the arterial glucose load. However, glucose uptake was not changed.

A significant increase in glucose uptake was noted at one point (30 minutes). This response was seen only in the denervated limb experiments where a marked hyperglycemia was also noted. No corresponding increase in glucose uptake occurred in the innervated limbs where endotoxin caused only a minimal hyperglycemia. However, a significant increase in glucose uptake could be shown in the innervated limb when glucose was infused. Upon elevating blood glucose concentration, there was a slight increase in glucose uptake which was further increased 15 minutes after endotoxin administration. The glucose uptake after endotoxin was now significantly higher than control but only for a short time. There was a significant correlation between plasma glucose concentration and the uptake of glucose (Figure 4).

When plasma glucose concentration was increased there was an apparent increase in glucose uptake. In other experiments, when plasma glucose concentration is allowed to fall rapidly after being elevated, the glucose uptake became negative. That is, the concentration of glucose in venous blood became higher than that in arterial blood. It is postulated that when the arterial blood glucose is elevated, the glucose in the interstitial space in the forelimb equilibrates passively with the glucose-rich arterial plasma with an apparent increase uptake. When arterial glucose declines, glucose-rich interstitial fluid is returned to the circulation, giving rise to a venous blood glucose concentration which exceeds arterial blood glucose concentration momentarily causing an apparent negative uptake.

Skin and muscle is not the site of increased peripheral utilization of glucose in endotoxin shock as has previously been shown in hemorrhagic shock (17,18). Recent studies suggest that circulating leukocytes play a dominant role in the genesis of the hypoglycemia of endotoxin shock (17).

#### Footnotes

This investigation was supported in part by the Office of Naval Research Contract N00014-75-C-9843 and Veterans Administration Hospital, Oklahoma City, OK.

A portion of this work is contained in a dissertation submitted to the Graduate College, University of Oklahoma by Paul A. Furr in partial fulfillment of the requirements for the Ph.D. in Physiology.

A preliminary report of a portion of this work was presented at the American Physiological Society in Philadelphia, Pa., August, 1976.

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