

AD-A081 665

NORTH CAROLINA UNIV AT CHAPEL HILL

F/G 6/5

THE EFFECT OF GLUCAGON ON HEPATIC CELLULAR ENERGETICS DURING A --ETC(U)

1979

H J PROCTOR, J J WOOD, W G PALLADINO

N00014-76-C-0595

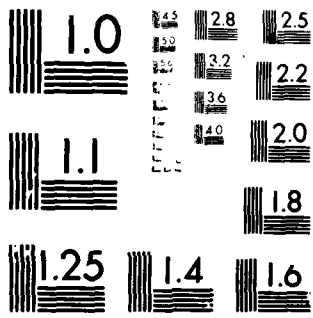
NL

UNCLASSIFIED

For 1
ALL
ADDITIONS



END
DATE
FORMED
4-80
DTIC



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

ADA081665

LEVEL

12

12

6 The Effect of Glucagon on Hepatic Cellular Energetics
During a Low Flow State

11 1979

10

H. J. Proctor, M.D., F.A.C.S.
James J. Wood, Ph.D.
William G. Palladino, M.S.

24
DTIC
ELECTE
S D

Presented at the 4th Tripartite Meeting, Oxford, England,
July, 1979.

Trauma Section and the Laboratory of Cellular Bioenergetics
Department of Surgery, University of North Carolina,
Chapel Hill, N. C., 27514

This work supported in part by ONR Contract #N00014-76-556.

15 NPM-15-1595

This document has been approved
for public release and sale; its
distribution is unlimited.

80 1 30 070

DDC FILE COPY

ABSTRACT

An investigation of the effect of glucagon on liver high energy phosphate concentration during a low flow state is presented. Two groups of dogs were subjected to a 120 minute period of hemorrhagic hypotension followed by re-infusion of shed blood plus additional normal saline to restore the central venous pressure to 10 cm H₂O. One group received in addition, intravenous glucagon. Despite a significant increase in portal flow and oxygen delivery, and a significant increase in liver glucose associated with glucagon administration, there was significant impairment in the ability of the liver to regenerate ATP. Evidence is presented to show the decreased ATP concentration was not hepatic blood flow related but was presumably due to increased catabolism.

Accession For	
NTIS GR&I	☒
DDC TAB	☐
Unannounced	☐
Justification	<i>Per</i>
By <i>[Signature]</i>	
Distribution/	
Availability Codes	
Dist	Avail and/or special
<i>A</i>	

INTRODUCTION

An increase in portal vein blood flow and pressure after glucagon infusion is now firmly documented⁽¹²⁾. This increase in flow and pressure has been attributed to arterio-venous shunting as well as a decrease in vascular resistance in the mesenteric bed^(15,5,9). As a logical consequence of this response, many authors have advocated glucagon administration to augment portal vein flow after hepatic artery ligation or major hepatic resection associated with trauma^(2,10,11,6). In addition, glucagon in large intravenous boluses has been recommended to maximize hepatic oxygenation secondary to blood loss associated with liver trauma or generalized hemorrhagic hypotension⁽¹⁶⁾. However, the influence of glucagon on portal vein blood flow during a period of sustained hypotension is less well understood.

Glucagon also has a metabolic effect on the liver by activating cyclic AMP, leading to increased gluconeogenesis and glycogenolysis⁽¹³⁾ with a concomitant increase in the need for oxygen. Mays⁽¹¹⁾ pointed out that a superimposed need for additional oxygen by glucagon administration in the presence of an already compromised hepatic circulation may severely restrict hepatocyte respiration. Fredlund, et. al.⁽³⁾ have in fact demonstrated a severe curtailment of hepatocyte respiration by glucagon administration. The net beneficial effect of the clinical use of this drug is therefore still open to question.

In view of the apparent complex hemodynamic and metabolic effects of glucagon on hepatic function, this investigation was undertaken to determine the effect of this hormone in dogs on: (a) hepatic energy metabolism and (b) portal vein blood flow, following a sustained episode

of hemorrhagic hypotension.

METHODS

Non-fasted, non-splenectomized mongrel dogs were used and handled in accordance with NIH standards for humane treatment of animals. All dogs were maintained in an anesthetized state throughout the experimental procedure with pentobarbital sodium (35 mg/kg) administered intravenously. At the start of the experiment, the dogs were intubated, connected to a Harvard ventilator, and the $FI O_2$ adjusted to achieve an arterial pO_2 of 80-100 mm Hg. Both femoral arteries and veins were then cannulated to allow the monitoring of arterial and central venous pressure, arterial blood sampling, and the infusion of blood and normal saline intravenously. A celiotomy was performed and a P.E. 50 catheter inserted into a branch of the superior mesenteric vein and the tip advanced into the portal vein to obtain portal venous blood samples. Non-cannulating electromagnetic flow probes* were then placed around the portal vein and hepatic artery.

Measurement of arterial and portal vein pO_2 ** allowed the calculation of percent O_2 saturation from the dog oxy-hemoglobin dissociation curve. Arterial and portal venous oxygen content were calculated according to the formula: [Hemoglobin*** x 1.34 x percent O_2 saturation]. Oxygen delivery to the liver was calculated as the product of oxygen content and flow, adding together the contribution of the hepatic artery and portal vein.

A liver biopsy was obtained after in situ freezing and quickly placed

*Carolina Medical Electronics, King, N. C.

**Instrumentation Laboratories Blood Gas Analyzer, model 113, Lexington, Mass.

***Instrumentation Laboratories Co-oximeter, Lexington, Mass.

in liquid nitrogen using a technique developed in this laboratory⁽¹⁴⁾. Samples thus obtained were assayed for liver ATP, ADP, inorganic phosphate (Pi), glucose, glucose-6-phosphate (G-6-P) and lactate concentrations using enzymatic, fluorometric methods according to Lowry and Passoneau⁽⁸⁾. Concentrations of inorganic phosphate and the ATP/ADP ratio were used to compute the free energy change of ATP hydrolysis (ΔG) as described by Hassinen⁽⁴⁾.

After baseline sampling, the mean arterial pressure was reduced to 50 mm Hg over a 15 minute period by arterial bleeding into a reservoir. This pressure was maintained for 90 minutes by intermittent withdrawal and re-infusion of blood, at which time the mean arterial pressure was reduced by further arterial bleeding to 35 mm Hg. This pressure was maintained for 30 minutes following which the measurements described above were repeated, and the dogs were alternately assigned to one of two treatment groups.

Group I dogs were resuscitated by re-infusing all shed blood plus additional normal saline to either restore the mean arterial pressure to baseline values or to elevate the central venous pressure to 10 cm H₂O, whichever occurred first. These conditions were maintained throughout the remainder of the study. Group II dogs were treated in an identical manner except they received in addition a 6 ug/Kg bolus of glucagon* along with the re-infusion of their shed blood, and thereafter received a continuous infusion of glucagon at a rate of 3 ug/Kg/min.

The measurements described above were repeated in both groups, 30 minutes (S + 30') and 240 minutes (S + 240') after the start of resuscitation.

*Glucagon kindly provided by Eli Lilly & Co., 307 E. McCarty St., Indianapolis, Ind., 46206

Data were averaged for each sample time and Group I contrasted with Group II using the Student t-test.

It was the authors' desire to create a workable experimental model in which adequate intravascular volume was available for any potential redistribution by glucagon, at the same time creating a state in which the animal's systemic arterial pressure was not necessarily returned to baseline values. The 120 minute period of hypotension was arrived at by trial and error, and was selected to compliment the combined surgical trauma of celiotomy and multiple liver biopsy. Although the criteria for the completion of intravascular volume replacement were as described above, the mean arterial pressure was not restored to baseline in any dog. Intravascular volume replacement was, as a result, governed solely by the central venous pressure. The failure of arterial pressure to return to the baseline value is thus not an experimental variable but results from experimental design. (Fig. 1)

RESULTS

Due to the severity of the experimental procedure, four animals expired without completing the entire six hour protocol leaving 21 animals (10 control, 11 glucagon treated) from whom data were collected at all sample times. Data from non-survivors are not included in the subsequent analysis.

(a) Effect of glucagon on portal flow

As part of the normal response to resuscitation, four of ten dogs in Group I increased their portal vein flows above their respective baseline value at (S + 30'). Glucagon administration in Group II animals was associated with a variable incidence of increased

portal-vein flow. (Seven of eleven dogs had increased portal vein flow at $S + 30'$.) When Groups I and II are contrasted, with four of ten and seven of eleven manifesting an increased portal vein flow at $S + 30'$ respectively, it is apparent that glucagon did not significantly affect the number of times increased portal blood flow was observed ($p > .1$). However, when blood flow did increase following glucagon administration, the magnitude of the increase was significantly greater ($p < .05$) than the magnitude of increase in the non-treated animals. (Fig. 2) This increase was manifested only at the $S + 30'$ sample time, presumably in response to the 6 ug/Kg bolus. The increase in portal vein flow was no longer manifested four hours after resuscitation ($S + 240'$) during which time the Group II dogs were receiving 3 ug/Kg/min. of glucagon. There was no observable effect of glucagon on hepatic artery flow at any time following administration.

(b) Effect of glucagon on liver energy metabolism

To determine if liver concentrations of adenine nucleotides and glycolytic intermediates were affected by the increased portal vein flow following glucagon administration, the data were analyzed first by excluding the four Group II animals which did not manifest an increase in portal vein flow after glucagon administration. To determine any direct, non-flow related effect of glucagon, the data from all Group I animals were utilized for comparison. The results were not significantly different ($p > .1$) whether the four animals without an increased portal vein flow were included or excluded. Accordingly, the data is presented in the form of a comparison between all Group I versus all Group II animals.

As evident in Table I, liver ATP concentrations decreased ($p < .01$) by the end of shock in both groups. Following resuscitation, the ATP concentrations improved but remained lower than control values in both groups. The ATP concentrations in Group II, however, were significantly ($p < .05$) lower than Group I at both sample times following resuscitation ($S + 30'$, $S + 240'$).

ADP and Pi concentrations remained within the normal range following resuscitation in both groups, with no significant differences between groups. The relationships between ATP and lower energy intermediates are also presented in Table I as are the ATP/ADP ratios and the ΔG values. There were no significant differences between groups for liver G-6-P, or lactate (Table II). Liver glucose was significantly ($p < .05$) increased in the glucagon treated animals at $S + 30'$.

Oxygen delivery to the liver, expressed as the combined contribution of the hepatic artery and portal vein flows, was significantly ($p < .01$) increased by glucagon administration (Table III). As already noted, hepatic arterial flow was not affected by glucagon. Oxygen content was significantly ($p < .05$) increased in portal blood at $S + 30'$, thus, the increased oxygen delivery resulted from both an increased portal vein flow and an increased oxygen content.

DISCUSSION

The major observation in this study is the significantly lower ATP values in the glucagon treated animals following resuscitation from shock. While ATP values never reached control levels during resuscitation in either group, ATP was 45% lower at ($S + 30'$) in those animals treated with glucagon as compared to the non-treated group. This difference increased

at (S + 240') to 55%. Clearly, glucagon created an adverse effect on regeneration of this high energy compound to control levels. ADP and inorganic phosphate were also lower at (S + 240') in the glucagon group but these differences were not statistically significant. No difference between the two treatment groups was found in the values of G-6-P or lactate following resuscitation.

It could be argued that the effect of glucagon was to cause arterio-venous shunting with the liver, thus excluding hepatic tissue from adequate perfusion. Despite the augmented portal vein flow, intra-hepatic redistribution of blood might also have resulted from the slightly lower arterial pressures and flows noted in Group II after re-infusion. This effect was most pronounced at (S + 240'). However, neither of these explanations are supported by the data, as the liver lactate concentrations returned toward baseline equally in both groups. The implication is that perfusion was adequate for lactate washout, and oxygen delivery was sufficient for aerobic respiration.

Grossly, the livers became dark and cyanotic during the shock period, but promptly returned to the normal red color with re-infusion of shed blood and saline, with only occasional small patches of persistent dark mottling. Care was always taken to obtain the biopsies from a normal appearing portion of the liver. The obvious gross reperfusion of the tissue further argues against intra-hepatic redistribution of blood or A-V shunting in the glucagon treated animals. These observations suggest that the mechanism by which glucagon causes a decreased hepatic ATP concentration does not appear to be flow related in view of the lack of difference noted when the data from Group II animals was analyzed with and without the four animals not experiencing

an increased portal flow after glucagon. This variable response of portal vein flow after glucagon has not been previously commented on and probably results from differences in our experimental model as compared to others^(15,5,9,18).

The rise in liver glucose noted in the study after glucagon administration is consistent with the observation that glucagon depletes liver glycogen. Inability to phosphorylate glucose does not appear to be a factor in the decreased ATP concentration in view of the normal concentrations of G-6-P present in the post-shock biopsies. The return of lactate toward normal values associated with normal and occasionally greater than normal availability of oxygen, along with the absence of significant changes in the free energy of hydrolysis, indicate that the cytochrome chain was probably not compromised in its ability to transfer electrons, and that oxygen was available in sufficient quantities as the final electron acceptor. ATP:ADP ratios were normal in both groups after resuscitation. This fact, along with the return of inorganic phosphate to control levels indicates the absence of non-specific hydrolysis of nucleotides as noted by Chaudry⁽¹⁾.

It would appear that the failure of ATP to return to baseline in Group I indicates either decreased biosynthesis or increased utilization of this metabolite, or possibly both.

Yamazaki, et. al.⁽²⁰⁾ reported a stimulation of ATP-ase activity and ATP-dependent K^+ accumulation in rat livers treated with glucagon. These studies showed a difference in the sensitivity of control and glucagon mitochondria to inhibition by antimycin, suggesting a possible hormonal effect in the Complex III region of the respiratory chain. Since the chain appears to be adequately transferring electrons, the effect might be assumed to be

in the coupling mechanism itself. Titheradge and Coore⁽¹⁷⁾ recently demonstrated that the pH - gradient across the mitochondrial membrane is increased by acute glucagon treatment. They also confirm that glucagon treatment causes a stimulation of state 3 respiration with the pyruvate-mallate couple indicating a tighter coupling between oxidation and phosphorylation. Other studies on the effect of glucagon treatment by Yamazaki⁽¹⁹⁾ on oxidative phosphorylation have shown that the hormone stimulates the rate of phosphorylation of ADP in isolated mitochondria which could also be interpreted as an increase in coupling ability.

A study by Lindberg et. al.⁽⁷⁾ on rats subjected to hemorrhagic shock demonstrated that ATP levels were not restored to normal 30 minutes after resuscitation; a finding similar to the data presented in this study. Witte et. al.⁽¹⁸⁾ found that administration of glucagon to dogs sharply increased portal oxygen delivery, as also shown in our experiments, but no improvement in hepatic tissue oxygenation was observed at high FIO_2 . The hepatic artery was previously ligated in these animals. These data suggested a rise in hepatic oxygen utilization as an explanation. This would be consistent with an increase in ATP production as reported by Yamazaki⁽¹⁹⁾. In the studies by Fredlund et. al.⁽³⁾ an elevation of intracellular hepatic enzymes after hepatic artery ligation and glucagon treatment support the concept that the hypercatabolic effect of this hormone far outstrips augmented oxygen delivery and possibly enhanced ATP production. It would thus appear that the depleted pool of ATP observed in our study, following resuscitation from severe hemorrhage, is a reflection of both an augmented production and an increased utilization of the ATP, with utilization greatly in excess of any increase in production. The results further indicate that the depleted concentration of ATP in liver

after glucagon administration was due primarily to a metabolic effect and not circulatory changes.

In light of increasing adverse experimental evidence of the effect of glucagon on hepatic energy metabolism it would seem that even in the face of an augmented portal vein blood flow and increased oxygen delivery, hepatic function is not improved by intravenous infusion of glucagon, and in fact may be adversely affected. It is felt that the routine use of this hormone in hemorrhagic shock should be discontinued and that specific criteria for its use in this context needs to be established.

REFERENCES.

1. Chaudry, I. H., Sayeed, M. M., Baue, A. E.: Alterations in adenosine nucleotides in hemorrhagic shock. *Surg Forum* 23:1-3, 1972
2. Drucker, W. R., Drapanas, T.: Trauma workshop report: liver and pancreas in injury. *J. Trauma* 10:1087-1088, 1970
3. Fredlund, P. E., Callum, B., Tibblin, S.: Influence of glucagon on the ischemic liver in the pig. *Arch Surg* 105:615-619, 1972
4. Hassinen, I. E., Hiltunen, K.: Respiratory control in isolated perfused rat heart: role of the equilibrium relations between the mitochondrial electron carriers and the adenylate system. *Biochimie et Biophysica Acta* 408:319-330, 1975
5. Jensen, H.: The effect of glucagon on pressure and oxygen saturation of splanchnic venous blood. *Am J Dg Dis* 11:447-452, 1966
6. Lewis, F. R., Lim, R. C., Blaisdell, F. W.: Hepatic artery ligation: adjunct in the management of massive hemorrhage from the liver. *J Trauma* 14:743-755, 1974
7. Lindberg, B., Haljamae, H., Jonsson, O., Pettersson, S.: Effect of glucagon and blood transfusion on liver metabolism in hemorrhagic shock. *Ann Surg* 187:103-109, 1978
8. Lowry, O. H., Passoneau, J. B.: A flexible system of enzymatic analysis. New York, 1972 Academic Press, pp 3-45
9. Madden, J. J., Ludewig, R. M., Wagensteen, S. C.: Effects of glucagon on the splanchnic and the systemic circulation. *Am J Surg* 122:85-90, 1971
10. Madding, F. G., Kennedy, P. A.: Effect of glucagon on hepatic blood flow. *JAMA* 212:482, 1970

References cont'd:

11. Mays, E. T.: The hepatic artery. Surg Gynecol Obstet 139:595-596, 1974
12. May, E. T.: Hepatic trauma. Current Problems in Surgery 13:52-53, 1976
13. Miller, L. L., Sokal, J. E., Sarcone, E. J.: Effects of glucagon and tolbutamide on glycogen in isolated perfused rat liver. Amer J Physiol 197:286-288, 1959
14. Palladino, W. G., Wood, J. J., Proctor, H. J.: Modified freeze clamp technique for tissue assay (submitted for publication).
15. Shoemaker, W. C., Van Itallie, T. B., Walker, W. F.: Measurement of hepatic glucose output and hepatic blood flow in response to glucagon. Am J Physiol 196:315-318, 1959
16. Shumer, W., Miller, B., Nichols, R. L., McDonald, G. O., Nyhus, L.: Metabolic and microcirculatory effects of glucagon in hypovolemic shock. Arch Surg 107:176-180, 1973
17. Titheradge, M. A., Coore, H. A.: Hormonal regulation of liver mitochondrial pyruvate carrier in relation to gluconeogenesis and lipogenesis. FEBS Letters 71:73-78, 1976
18. Witte, C. L., Witte, M. H., Kintner, K.: Effect of glucagon on hepatic lymph (tissue) pO_2 after ligation of the hepatic artery: an experimental study. J Trauma 18:27-33, 1978
19. Yamazaki, R. K.: Glucagon stimulation of mitochondrial respiration. J Biol Chem 250:7924-7930, 1975
20. Yamazaki, R. K., Sax, R. P., Hauser, M. A.: Glucagon stimulation of mitochondrial ATP-ase and potassium ion transport. FEBS Letters 75:295-299, 1977

LEGEND - TABLE I

Concentrations of ATP, ADP and inorganic phosphate (Pi) as well as calculated ATP:ADP ratios and free energy of hydrolysis (ΔG). Means $\pm$ SEM. Significant differences compared with corresponding control group. C = controls (n=10); G = glucagon treated (n=11) (S + 30' = 30 minutes post-resuscitation; S + 240' = 240 minutes post-resuscitation). *p<.05

TABLE I

GROUP	ATP ($\mu\text{M/g}$)	ADP ($\mu\text{M/g}$)	P _i ($\mu\text{M/g}$)	ATP/ADP	ΔG
<u>Baseline</u>					
C	2.39 $\pm$.08	0.64 $\pm$.06	1.78 $\pm$.40	3.98 $\pm$.34	-8.27 $\pm$.23
G	2.42 $\pm$.13	0.72 $\pm$.09	1.68 $\pm$.27	3.58 $\pm$.57	-7.92 $\pm$.09
<u>End Shock</u>					
C	0.72 $\pm$.14	0.71 $\pm$.10	4.90 $\pm$.95	1.00 $\pm$.20	-6.68 $\pm$.18
G	0.47 $\pm$.07	0.52 $\pm$.05	6.80 $\pm$.90	1.06 $\pm$.16	-6.50 $\pm$.14
<u>S + 30'</u>					
C	1.65 $\pm$.27	0.62 $\pm$.08	2.66 $\pm$.31	2.64 $\pm$.32	-7.60 $\pm$.10
G	0.94 $\pm$.19*	0.40 $\pm$.04	2.38 $\pm$.64	2.40 $\pm$.43	-7.49 $\pm$.25
<u>S + 240'</u>					
C	1.91 $\pm$.27	0.60 $\pm$.10	2.40 $\pm$.54	3.55 $\pm$.40	-7.95 $\pm$.19
G	0.86 $\pm$.28*	0.38 $\pm$.05	2.07 $\pm$.42	2.46 $\pm$.75	-7.52 $\pm$.21

LEGEND - TABLE II

Concentrations of glucose, glucose-6-phosphate (G-6-P) and lactate. Means $\pm$ SEM. Significant differences compared with corresponding control group. C = controls (n=10); G = glucagon treated (n=11); (S + 30' = 30 minutes post-resuscitation; S + 240' = 240 minutes post-resuscitation. *p<.05

TABLE II

GROUP	GLUCOSE ($\mu\text{M/g}$)	G-6-P ($\mu\text{M/g}$)	LACTATE ($\mu\text{M/g}$)
<u>Baseline</u>			
C	5.14 ± 0.66	$.10 \pm .03$	$1.54 \pm .36$
G	5.23 ± 0.59	$.06 \pm .02$	$2.75 \pm .58$
<u>End Shock</u>			
C	17.50 ± 4.54	$.16 \pm .03$	12.04 ± 1.43
G	18.13 ± 6.29	$.13 \pm .05$	13.78 ± 1.75
<u>S + 30'</u>			
C	$9.20 \pm 2.42^*$	$.11 \pm .03$	13.38 ± 1.94
G	45.41 ± 12.33	$.16 \pm .03$	18.72 ± 5.06
<u>S + 240'</u>			
C	3.53 ± 0.80	$.09 \pm .03$	6.41 ± 1.81
G	4.78 ± 2.02	$.07 \pm .02$	11.63 ± 3.30

LEGEND - TABLE III

Means $\pm$ SEM for O₂ delivery (cc/min) to the dog liver as well as portal vein and hepatic artery O₂ content (cc O₂/100cc blood). Significant differences compared with corresponding control groups. C = controls (n=10); G = glucagon treated (n=11). (S + 30' = 30 minutes post-resuscitation; S + 240' = 240 minutes post-resuscitation. *p<.05, ** p<.001

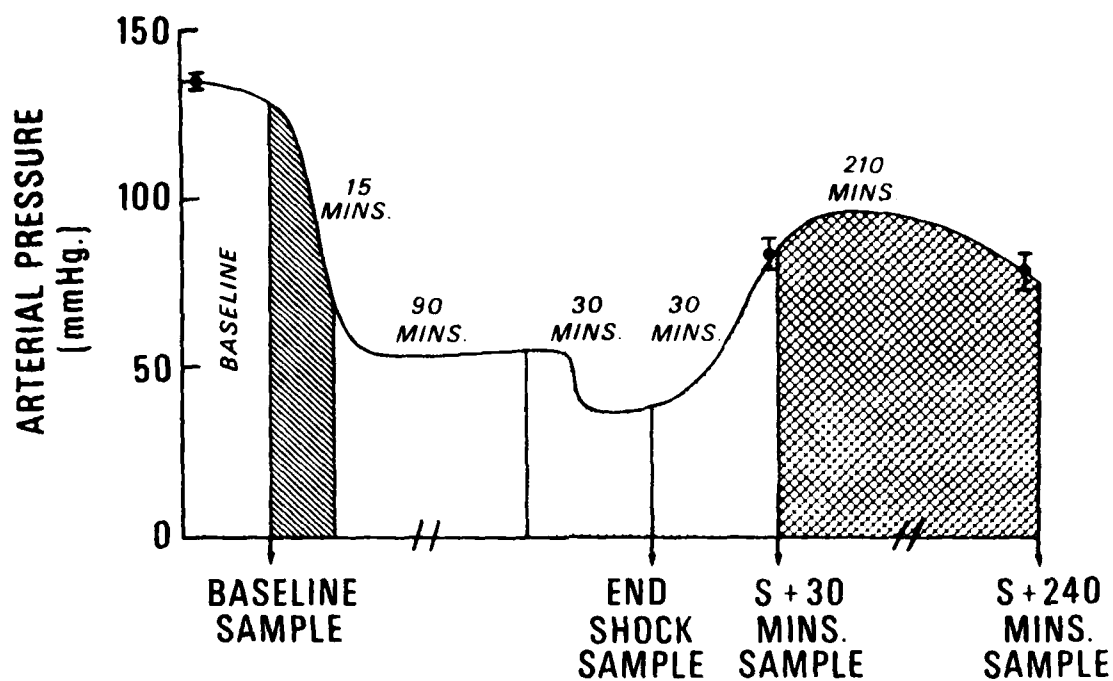
TABLE III

GROUP	O ₂ DELIVERY (cc/min)	O ₂ CONTENT Portal Vein (ccO ₂ /100cc)	O ₂ CONTENT Hepatic Artery (ccO ₂ /100cc)
<u>Baseline</u> C 6	82.0±12.9 97.8±15.4	11.6±1.2 14.6±1.2	15.5±0.8 17.1±1.0
<u>End Shock</u> C 6	12.5±1.9 27.4±9.8	10.5±1.4 9.6±1.4	11.3±2.6 9.3±3.4
<u>S + 30'</u> C 6	77.6±8.5 202.4±44.7**	14.5±0.7 16.8±1.0*	15.6±0.8 17.3±1.1
<u>S + 240'</u> C 6	53.3±11.4 61.4±12.3	12.6±1.1 14.6±0.8	11.9±2.3 15.2±2.3

LEGEND - FIGURE I

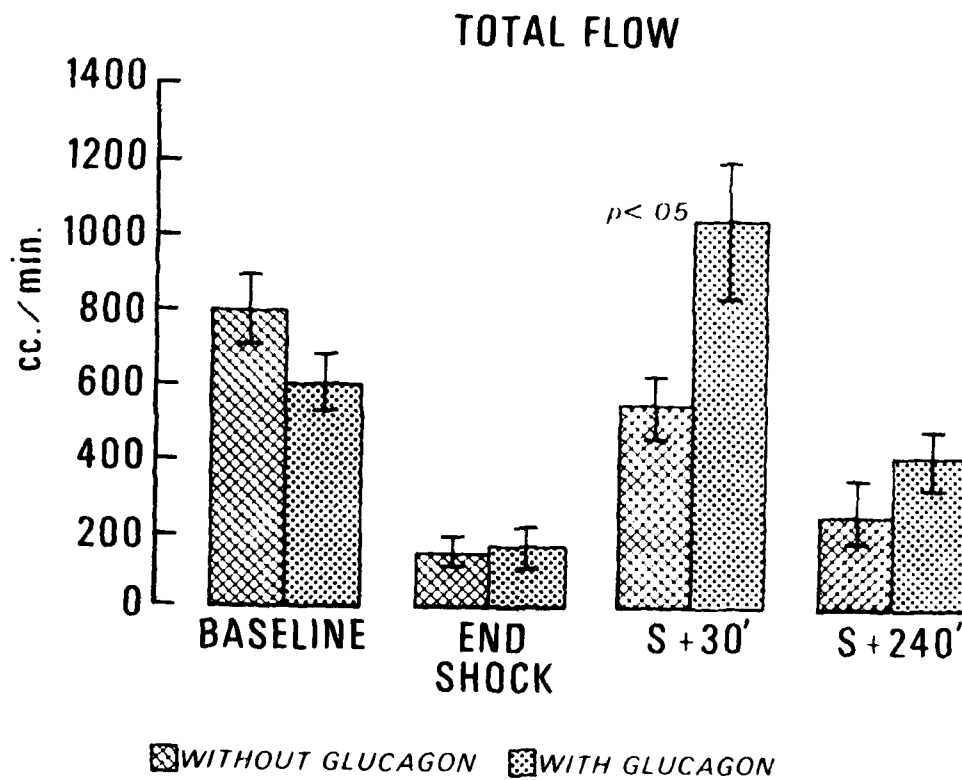
Depiction of the experimental model. Means $\pm$ SEM for the arterial pressures are shown.

EXPERIMENTAL MODEL



LEGEND - FIGURE II

Total flow (hepatic artery + portal vein) of the seven animals experiencing an increase after glucagon contracted with all Group I animals. Mean $\pm$ SEM is depicted.



ATE
LMED
—8