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Non-Suppressible Insulin-Like Activity, Glucose Hypercatabolism, and Hypoglycemia in Sepsis

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NON-SUPPRESSIBLE INSULIN-LIKE ACTIVITY,
GLUCOSE HYPERCATABOLISM, AND
HYPOGLYCEMIA IN SEPSIS

by

Rico E. Viray

A Thesis Submitted to the Faculty of the Graduate School
of Loyola University of Chicago in Partial Fulfillment
of the Requirements for the Degree of
Master of Science

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VITA

Rico E. Viray was born 30 September 1957 in Dagupan City, Philippines, the son of Teofilo C. and Cristina E. Viray.

He received his elementary education from Paco Catholic and Mater Carmeli Catholic Schools in the Philippines. In the fall of 1972, he entered John Bowne High School in Flushing, New York and was graduated in July of 1975. He was awarded the Elsbeth Kroeber Memorial Award in Biological Science and the Governor's Committee on Scholastic Achievement Citation.

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His graduate studies in the Department of Physiology, Loyola University of Chicago began in July, 1979. He has been under the tutelage of Dr. James P. Filkins and was supported by a Basic Science Fellowship.

On 28 September 1980, Rico wed Agnes S. Viray (nee Salerno).

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INTRODUCTION

Alterations in glucose metabolism have been observed to occur in a variety of infectious processes such as endotoxemia and septicemia. The metabolic response to infection is characterized by a biphasic pattern in plasma glucose levels. The hyperglycemia manifested in early endotoxemia or sepsis has been attributed to an increase in hepatic gluconeogenesis, glycogenolysis and increased availability of gluconeogenic substrates, such as alanine and lactate. This primarily catabolic response has been ascribed to an elevation in glucagon, catecholamine, glucocorticoid and growth hormone levels.

In contrast, the terminal stage of gram negative sepsis and endotoxemia is marked by a state of progressive hyperinsulinism which, if left unchecked, inevitably leads to profound hypoglycemia and death. Three basic mechanisms have been thought to contribute to this energy substrate depletion. Firstly, the increase in hepatic glycogenolysis is a transient one. The prolonged and heightened sympathetic drive exhausts the available glycogen stores early in the infectious process. Second, and perhaps more important in the progression to hypoglycemia, is the depression of hepatic gluconeogenesis. Lastly, the

increased disappearance of glucose from the body glucose pool, i.e., enhanced peripheral tissue glucose oxidation. The latter is of particular interest in this study.

It has been shown that different components of the body act as glucose sinks in the agonal stages of endotoxin and septic shock. The liver, diaphragm, spleen, epididymal fat pads and certain blood components have all been demonstrated to increase their energy expenditure during the infectious process. Work from this laboratory has shown that in endotoxemia, the sensitivity of adipose tissue to a given stimulus of insulin is elevated. Whether the same is true in experimental septic shock is a question that this study will seek to address.

Recent work from our laboratory has suggested another factor which may partially explain the increase in glucose utilization; non-suppressible insulin-like activity (NSILA). NSILA in the serum is a glycoprotein of non-pancreatic origin and is physicochemically distinct from insulin. An increase in NSILA levels has been demonstrated to occur in rats in late endotoxic shock (14). This increase was accompanied by hypoglycemia and hypoinsulinemia.

This study will also investigate the role of NSILA in the septic shock syndrome. Whether it contributes to the progression of hypoglycemia by altering tissue glucose will be ascertained.

LITERATURE REVIEW

A survey by Martin (40) in 1969 revealed a disturbing trend in the frequency of bacteremias in American hospitals. By his estimate, in 1968, 1% of hospital admissions developed bacteremia and 60% of these were due to gram negative bacteria. At least 250,000 cases of infection occurred leading to approximately 50,000 deaths. A more recent estimate by Wolff and Bennett (75) in 1974 puts the number of documented cases of gram negative sepsis at 71,000 per year with a resultant 18,000 deaths. In yet another study dealing with postoperative sepsis and total hospital infection rate, 3.9% of surgical patients developed postoperative sepsis and 13.5% was the total infection rate (72). McCabe (42) has shown that the incidence of sepsis has increased arithmetically over 2 decades until 1974.

Clearly, the frequency of sepsis has been on the rise due to the increasing proportion of elderly, chronically ill patients, increased number and complexity of surgical operations, ineffective antibiotic therapy, etc. This alarming fact has spurred the progression of ventures into the various facets of sepsis. This study will be concerned primarily with alterations in carbohydrate metabolism in the late stage of sepsis.

The metabolic response to infection is a dynamic process. It is influenced by a number of factors including the severity and duration of illness, microorganism type, and physiological status of the host. These responses may also exhibit interrelated biphasic patterns.

Generally, the early response to infection is a catabolic one. Gluconeogenesis is enhanced during sepsis by the stimulatory action of hormones and by the increased availability of gluconeogenic substrates (2). This early phase is analogous to Cuthbertson's "flow" phase (12). This catabolic or flow phase of injury or infection is characterized by an increase in the metabolic rate, body temperature, serum insulin, catecholamine, glucose, glucocorticoid, growth hormone and lactate concentrations (62). If the infectious process is severe or is unchecked and allowed to continue, an irreversible transition to a shock state occurs and death inevitably ensues. A marked hypoglycemia is characteristic of this agonal stage of infection and injury.

HYPOGLYCEMIA IN INFECTION

Terminal hypoglycemia has been demonstrated in humans and a wide variety of animals including dogs, monkeys and rats during septic and endotoxemic episodes. Berk, et al (3) observed a profound hypoglycemia (25 mg/dl) in dogs

3 hours after an LD₇₀ dose of endotoxin. Filkins and Cornell (16) elicited a drop in plasma glucose levels from a mean control of 110 to 48 mg/dl 5 hours after the administration of *S. enteritidis* endotoxin to fed rats. Berry, et al (5) injected mice with *S. typhimurium* endotoxin i.p. and hypoglycemia resulted. Intravenous injections of live microorganisms were also shown to result in a hypoglycemic crisis. Griffiths, et al (26) demonstrated a decrease in blood glucose from 90 to 70 mg/dl in dogs 4 hours following the i.v. injection of live *E. coli*. Hinshaw, et al (31) infused 5 baboons with live *E. coli* and 4 of them died in hypoglycemia. Wichterman, et al (74) using the rat caecal ligation and puncture model for sepsis showed an elevation of serum glucose levels (151 mg/dl) in early and depressed levels (20.5 mg/dl) in late sepsis. Hypoglycemia has also been reported in human septic shock (3,55). Rachwitz, et al (55) documented depressed glucose levels in septic shock patients. Similarly, Berk, et al (3) reported 50 mg/dl plasma glucose value in a patient lapsing into septic shock. The hypoglycemia appears to be associated with circulatory collapse and impending death.

In another study, Hinshaw, et al (33) injected an LD₇₀ dose (1.0-1.5 mg/kg) of *E. coli* endotoxin into the femoral veins of 11 anesthetized dogs. After 300 minutes, 8 of the

11 died in hypoglycemia while the 3 survivors maintained their plasma glucose level at 61.7 ± 16 mg/dl. A second group of 9 dogs were treated with glucose following the endotoxin challenge and all dogs became normoglycemic and survived. In baboons injected with live *E.coli*, Hinshaw, et al (31) showed that the animal's survival time was contingent on its ability to maintain a stable glucose level. McNamara, et al (44) similarly showed improved cardiovascular function in endotoxic rabbits treated with an exogenous source of glucose. Berk, et al (3) maintained that glucose was indeed necessary for the survival of an animal in endotoxemia.

MECHANISMS OF HYPOGLYCEMIA

From a metabolic point of view, three mechanisms have been proposed to contribute to the development of hypoglycemia in the septic animal: (1) depletion of hepatic glycogen stores, (2) depression of hepatic gluconeogenesis, and (3) enhanced peripheral tissue glucose oxidation.

HEPATIC GLYCOGEN DEPLETION

The initial metabolic response to a bacterial insult is increased sympathetic drive to promote hepatic glycogenolysis. Evans and Zeckwer (13) injected *E.coli* into adrenalectomized rabbits and observed the development of hypoglycemia and convulsive death. In contrast, rats with

their adrenal glands intact have been shown to go through, as previously mentioned, a biphasic pattern in blood glucose levels with an early hyperglycemia and late hypoglycemia (6,16). Groves, et al (28) reported a concurrent drop in plasma glucose levels and hepatic glycogen content in dogs injected with live *E.coli*. Berry, et al (5) found a marked carbohydrate depletion in different strains of mice injected intraperitoneally with 2 mg of *S.typhimurium* endotoxin. Accompanying the blood sugar decrease (149 ± 6 mg % to 37 ± 13 mg %) were liver (5.5 ± 0.6 to 0.21 ± 0.07 percent by weight) and muscle (0.33 ± 0.06 to 0.10 ± 0.04 percent by weight) glycogen depletion. McCallum and Berry (43) observed a drop in liver glycogen as early as 1 hour after treatment of mice with an LD_{50} dose of endotoxin. Glycogen synthase activity was also shown to be depressed in fasted endotoxic rats (490 ± 54) as compared with fasted controls (826 ± 83). Filkins and Cornell (16), likewise, demonstrated liver glycogen to fall in both fed and fasted endotoxic rats. Hypoglycemia and glycogen depletion were most prominent 300 minutes post injection. Clearly, the decreased inflow of glucose derived from glycogen into the total body glucose pool plays an important part in the development of hypoglycemia.

The gluconeogenic capacity of the liver is also impaired in infection and injury. McCallum and Berry (43) evaluated the gluconeogenic ability of the endotoxic mice's liver through the incorporation of the ^{14}C label from alanine - U - ^{14}C into blood glucose. The total amount of ^{14}C - label incorporated from both alanine - U - ^{14}C and pyruvate - 2 - ^{14}C into blood glucose was significantly reduced by endotoxin to approximately 70% of control values 12 hours post injection. *In vitro* analysis on the effect of endotoxin on gluconeogenesis in isolated normal hepatocytes showed no significant impairment of function (16). If the isolated hepatocytes were harvested from endotoxin donors, however, a pointed reduction in glucose production in the presence of gluconeogenic substrates is seen (16). Wannemacher, et al (71) in an extensive study of glucose and alanine metabolism during bacterial infections in rats and rhesus monkeys showed glucose formation from gluconeogenic substrates to be reduced. Perfused rat livers during acute (24 hours after injection of 10^4 organisms) and agonal (24 hours after 10^7) stages of *S.pneumoniae*, infection were able to produce only 12 ± 1.8 nmol glucose/g liver/min in the presence of 10 mM alanine as compared with a control value of 183 ± 20 nmol glucose/g liver/min (71). Glucose formation from 10 mM pyruvate by isolated hepato-

cytes from infected donor rats was shown to be reduced 40 hours after the initial insult. There was no significant change after 24 hours. Evidently, the decreased hepatic gluconeogenic capacity is most pronounced during the agonal stage of the infectious process.

ENHANCED PERIPHERAL TISSUE GLUCOSE OXIDATION

In addition to the impairment of input into the blood glucose pool, there is also a derangement in the outflow, i.e. glucose utilization. This is the least understood of the three aforementioned mechanisms and is the area of inquiry in this study. Kinney (37) provided evidence to show the greater glucose disappearance, i.e. increased glucose turnover rate, in septic shock. Long, et al (39) substantiated this finding by demonstrating an increase in glucose oxidation in septic patients. Ryan, et al (63) induced general peritonitis in rats through caecal ligation and found a 3-fold increase in the adipose tissue glucose oxidation of septic fasted rats as compared with normal fasted controls. The septic rat's adipose tissue pyruvate dehydrogenase was significantly elevated with respect to the fasted control group. They did not show the same relationship to exist in muscle tissue. The rate of conversion of U - ^{14}C glucose to $^{14}\text{CO}_2$ and labelled glycogen by incubated hemidiaphragms was not significantly different

between the fasted septic and control groups (63). They concluded that muscle was preferentially combusting its own amino acids to increase the availability of glucose to obligate organs. Filkins and Figlewicz (17) were able to show, however, enhanced glucose oxidation in endotoxic hemidiaphragm, liver, spleen and epididymal fat pad. The difference may perhaps be attributed to the stage of shock at which the determinations were made. The hypoglycemia associated with the agonal stages of sepsis was not reported by Ryan and coworkers (63). Wichterman, et al (74) showed this to be the case in peritonitis. There was no rise in soleus muscle glucose uptake in early sepsis (6.9 ± 0.5 umoles glucose/g/hr) vs. control (6.6 ± 0.4 umoles/g/hr). Rats in late sepsis were, however, characterized by a profound hypoglycemia (20.5 ± 4.2 mg/dl), hypoinsulinemia (21 ± 5 uU/ml) and increased glucose uptake (9.7 ± 0.6 umoles glucose/g/hr). Wannemacher, et al (71) injected rats with *S.pneumoniae* and *F.tularensis* and measured the oxidation of 1 - ^{14}C - glucose and 6 - ^{14}C - glucose. Since glucose labelled at carbon 1 measures both the pentose phosphate shunt and Krebs cycle oxidation rates and glucose labelled at carbon 6 measures only Krebs cycle activity, it was possible to gauge the effects of infection via the pentose phosphate shunt. There was an increase in the oxidation of both labelled glucoses with the

pentose phosphate shunt registering a greater increase. Thus, Wannemacher and coworkers have suggested that the increase in energy utilization may be due to the increased activity of phagocytic cells which derive their energy from the oxidation of glucose through the pentose phosphate shunt. Recent studies have shown that components of the blood itself do increase their glucose utilization in endotoxin shock (32,36,71). Hinshaw, et al (30) have suggested that white blood cells are the components acting as glucose sinks in shock.

The majority of the evidence suggests that there is a hypercatabolism of glucose by peripheral tissues. Filkins and Figlewicz (17) suggested that this may partly be due to altered tissue responsiveness to insulin and/or insulin-like substances. They showed that epididymal fat pads from endotoxic rats had increased basal glucose oxidation and, in the presence of a suprastimulatory dose *in vitro*, marked responsiveness to insulin. Ryan, et al (63) showed heightened adipose tissue pyruvate dehydrogenase activity and glucose oxidation in the epididymal fat pads of rats. This increase may be attributed to the high levels of circulating insulin they found to be present in the septic animal. The agonal stage of shock is, however characterized by hypoinsulinemia and hypercatabolism of glucose. It would be a worthwhile venture to investigate

whether the altered responsiveness described by Filkins and Figlewicz is evident in an experimental model of general peritonitis.

In addition, there has been growing evidence that insulin-like substances may mediate the increased glucose oxidation. Berry (4) has shown that endotoxin stimulates cellular glycolysis in a fashion similar to insulin. Eviscerated rats subjected to hemorrhagic shock have been shown to deplete the glucose pool in the absence of insulin (61). Filkins (15) has shown that macrophage secretory products induce the hypercatabolism of epididymal fat pads, *in vitro*. Nies, et al (46) have reported kinin release in endotoxic monkeys and Haberland (29) has shown that kinins have insulin-like effects on glucose transport. It is conceivable that, in shock where vascular permeability is compromised, these products leak from the capillaries into the extracellular space and stimulate glucose transport.

One such entity is non-suppressible insulin-like activity. In 1941, Gellhorn, Feldman and Allen showed a significant hypoglycemic effect to occur in hypophysectomized adrenodemedullated rats following the injection of 1 ml of human blood drawn 2½ hours after a midday meal (22). This was the first demonstration of insulin-like activity (ILA) in human blood. Insulin-like activity or insulin

effect was considered to be any effect obtained over and above that produced by insulin-free solutions, such as buffered saline, on both *in vitro* and *in vivo* systems (47). Characterization of plasma ILA progressed after the development of more sensitive biological assay methods. Martin, et al (41) and Renold, et al (59) developed an isolated rat adipose tissue assay, *in vitro*, and Gemmill (23) and Gemmill and Hamman (24) showed the feasibility of an isolated rat diaphragm bio-assay. The conversion of 1 - ^{14}C - glucose to $^{14}\text{CO}_2$ by rat adipose tissue was used as the metabolic index of blood ILA. Glucose uptake (67), glucose consumption and glycogen deposition (23) and net gas exchange were likewise employed.

The sensitivity, specificity and accuracy of these bio-assay systems varied. Estimates of plasma ILA in normal fasting man ranged from 50-350 uU/ml in a study by Martin, et al (41) using the *in vitro* adipose tissue assay. Somewhat lower range of 30-80 uU/ml was obtained through the isolated rat diaphragm method (27,56,70). The consistently lower value for blood insulin concentration obtained through the muscle bio-assay led numerous investigators to suggest a greater sensitivity to insulin-like substances by adipose tissue.

The introduction by Yalow and Berson (76) of a radio-

immunoassay specific for insulin yielded a more precise serum insulin content of 20 uU/ml in the normal fasting subject.

A survey of the estimated values of blood insulin levels reveals a marked discrepancy to exist between bioassayable insulin-like activity (ILA) and immunologically determined immunoreactive insulin (IRI). The consensus was that IRI was not identical to ILA. It was believed that ILA represented the cumulative biological effect of insulin and other physicochemically distinct substances that mimic its actions.

In 1958, Leonards (38) demonstrated that human serum ILA was only partially suppressed, if at all, by Guinea Pig anti Porcine Insulin Serum (GPAIS). GPAIS is a specific insulin inactivator which, when added *in vitro* to an adipose tissue or muscle preparation, blocks the stimulatory action of insulin on glucose metabolism. Human serum ILA was totally suppressed with GPAIS in an isolated diaphragm preparation (69), whereas only a partial suppression of ILA was achieved in an adipose tissue assay (19). Froesch, et al (19) referred to the latter as "non-suppressible insulin-like activity" or NSILA. It was also called "atypical insulin" by Samaan, et al (65) and "bound" insulin by Antoniadis (1). The fraction of serum ILA that was inactivated by GPAIS was called suppressible ILA, typical and

free insulin (1,19,65). The relative ratio of NSILA to suppressible ILA in serum was shown to be 93:7. Only 7% of total serum ILA was suppressible with anti-insulin serum in normal fasting subjects (19).

SUPPRESSIBLE INSULIN-LIKE ACTIVITY

Suppressible ILA in normal fasting humans was shown by Froesch, et al (19) to be approximately 13 uU/ml. This basal value rose one hour after an oral glucose load to 100 uU/ml suggesting a direct relationship between suppressible ILA and plasma glucose level. Suppressible ILA was also shown to increase following i.v. administration of glucose (25) or crystalline insulin (19) into normal subjects. Elevated levels of suppressible ILA were found in patients with active B islet cell adenomas (47) whereas pancreatectomized dogs exhibited none at all (68). Were suppressible ILA and insulin one and the same as suggested by the preceding evidence? A study by Froesch, et al (19) found a good correlation to exist between suppressible ILA and IRI values in diabetic patients to suggest that identity does exist. Sonksen, et al (66) in a study of a large pool of sera, showed a significant correlation ($r = 0.77$, p less than .001) between suppressible ILA and insulin measured by immunoassay. They also showed no correlation ($r = 0.25$, p greater than .05) between NSILA

and immunoreactive insulin. As a result, it is now generally accepted that bio-assayable suppressible ILA and IRI are indeed equivalent entities.

NON-SUPPRESSIBLE INSULIN-LIKE ACTIVITY

Suppressible ILA, however, accounted for less than 10% of the total bio-assayable ILA. Non-suppressible insulin-like activity (NSILA), whose role in septic shock hypoglycemia is of interest to us, represents by far, the majority. Like insulin, NSILA stimulated glucose uptake and utilization in both adipose and muscle tissue. Unlike insulin and suppressible ILA, however, NSILA levels were independent of blood glucose levels and were shown not to be elevated in patients with diabetes or organic hyperinsulinism (47). Perturbation of the B-cell system by the addition of mannoheptulose (20) produced no alterations in NSILA although acute changes in plasma glucose occurred. Oral or i.v. administration of insulin also did not result in a change in NSILA levels (47).

NSILA levels were shown to increase under conditions of heightened energy demands. Electrically induced muscular hindlimb contraction of 30-minute duration resulted in a marked increase in leg lymph NSILA (57). Filkins (14) has shown elevated NSILA in rats during late endotoxemia. This work suggested that the escalation of NSILA may be respon-

sible for the hyperinsulinemic state which leads to lethal hypoglycemia. Burn or surgical trauma (52), non B-cell tumor hypoglycemia, such as intra-abdominal fibrosarcoma (50) and retroperitoneal sarcoma (54) also exhibited elevated NSILA. Froesch, et al (18) have suggested that the physiological import of serum NSILA may be manifested only during conditions of injury and inflammation.

Non-suppressible ILA was shown to be a heterogenous entity (35). Gel chromatography of NSILA yielded two protein fractions: a main fraction with an estimated molecular weight of about 100,000 daltons and a minor one (5% of total) with an estimated molecular weight range of 6,000 - 10,000. Only the minor fraction was soluble in acid ethanol, hence it was called NSILA-s. The majority of the serum NSILA, about 80 - 90%, was precipitated by ethanol, and this fraction was called NSILA-p. This rather clear distinction has since been questioned but, what is clear is that serum contains several proteins possessing insulin-like properties. These include in addition to NSILA-s, small molecular weight proteins such as somatomedins and nerve growth factors. Poffenbarger, et al (51) have also shown a large molecular weight protein (88,000) with non-suppressible insulin-like activity. He called this "non-suppressible insulin-like protein" or NSILP. It has not been determined whether Poffenbarger's NSILP corre-

sponds to Jakob's precipitable large molecular weight fraction.

NSILA-s

NSILA-s was shown to be a heat-resistant protein that was inactivated by base, cysteine and glutathione (7). This substance is tightly bound to a carrier protein and therefore normally inactive in serum. The active moiety dissociates from the complex under acidic conditions. Rinderknecht and Humbel (60) were subsequently able to differentiate the NSILA-s fraction into two polypeptides of like molecular weight (7500). Both protein units exhibited non-suppressible insulin-like activity. The amino acid sequence of both polypeptides resembled that of proinsulin (34). Out of 73 amino acid positions, 45 (62%) were identical in the two polypeptides (34). Plasma levels of these two isomers were shown, to a general degree, to be under the control of growth hormone (34). Because of their growth promoting abilities, growth hormone dependency (somatomedin like), and homology to insulin structure and action, these polypeptides were termed Insulin Growth Factor I and II, i.e., IGF I and IGF II (60).

NSILA-s has been shown to stimulate all aspects of glucose metabolism in much the same manner as insulin. The dose response curves of NSILA-s on glucose uptake and net

gas exchange were shown to be parallel to those of insulin (21). Furthermore, their effects were additive. A few important differences do exist between NSILA-s and insulin. Unlike insulin, NSILA-s or for that matter any of the serum proteins possessing insulin-like bioactivity are, for the most part, not produced or stored in abundance in a specific organ. NSILA is not of pancreatic origin (53). Furthermore, NSILA-s has a greater binding affinity to muscle (49) and is not inactivated by the liver (49). Oelz, et al (49) injected NSILA-s and insulin intravenously in separate experiments and found them both to result in a marked hypoglycemia. The duration of NSILA-s' *in vivo* effect, however, outlasted that of insulin. The liver's inability to clear NSILA-s from the blood and its greater binding affinity contributed, no doubt, to the longer duration.

Present evidence suggests that IGF I and IGF II (NSILA-s) do not play a role in the normal regulation of glucose homeostasis. Rather they seem to be correlated with conditions that are characterized by increased energy expenditure, as in diseased states. Increased levels of NSILA-s have been shown in extrapancreatic neoplasms with severe hypoglycemia (8). It is conceivable, therefore, that NSILA-s, although a minor fraction, plays an important role in the provision of energy substrates to the body's cells under abnormal conditions.

The main fraction of serum NSILA is NSILA-p. This large molecular weight substance is not interconvertible with the smaller NSILA-s. NSILA-p is a heat labile substance that denatures at temperatures greater than 80°C (35). It mimics all of insulin's action except on glycogenolysis (48). Dose response curves of NSILA-p on net gas exchange and glucose transport, like NSILA-s, were parallel and additive to insulin.

Poffenbarger (51) isolated an 88,000 molecular weight glycoprotein consisting of two non-identical chains of approximately 42,000 and 46,000 daltons. This "non-suppressible insulin-like protein" or NSILP may account for a major portion of serum NSILA. It is probably similar, if not identical, to the precipitable large molecular weight fraction, NSILA-p, isolated by Jakob, et al (35). Conclusive evidence to the contrary has not been forthcoming.

Serum ILA under normal conditions has been shown to behave as if it were entirely composed of macromolecular substances. Rasio, et al (58) were not able to recover serum ILA in similar concentrations in lymph, whereas serum and lymph IRI were similar. This behavior may be attributed to the large size and relative proportion of the NSILA-p molecule in the serum. Evidently, under normal

situations, NSILP and other large molecular weight substances are restricted to the vascular space.

It is a well known fact that cardiovascular and metabolic inadequacies are manifested in conditions of infection, injury and inflammation (11). It is under these conditions of altered vascular function, such as increased capillary permeability, that Froesch, et al (18) postulated NSILA to cross the capillary membrane and come into contact with local tissue. This would, in effect, provide additional glucose to the cells in times of urgent need.

One of the foci of this paper is to assess the role, if any, of these substances in the blood that display non-suppressible insulin-like activity, in the hypoglycemia seen in the terminal stages of septic shock. Encouraging evidence has already been gathered from this laboratory implicating an increase in total NSILA levels in late endotoxemia (14).

MATERIALS AND METHODS

ANIMALS

Healthy male rats of the Holtzman strain (Holtzman Company, Madison, Wi.) weighing 270-370 gm were used. They were kept on a diet of Purina Rat Chow (Ralston Purina, St. Louis, Mo.) and water for at least a week prior to the study. They were acclimated to an ambient temperature of 22°C (71.6°F) and a 12-hour light-dark cycle (7:00 A.M. - 7:00 P.M., CST). All rats were fasted overnight prior to experimentation, unless otherwise mentioned. Water was provided ad libitum throughout.

SEPSIS MODEL: CAECAL LIGATION AND PUNCTURE PROCEDURE

The method introduced by Wichterman, et al (73) for the induction of peritonitis in laboratory rats as a model of sepsis was adopted and modified for the purposes of this study.

Generally, unless otherwise mentioned, the surgical procedure was performed between 8:00 and 10:00 A.M. on the day of the study. A midline laparotomy, approximately 1-2 cm in length, was done under ether anesthesia. The caecum was exposed and isolated from the abdominal cavity. Ligation of the caecum was performed just below

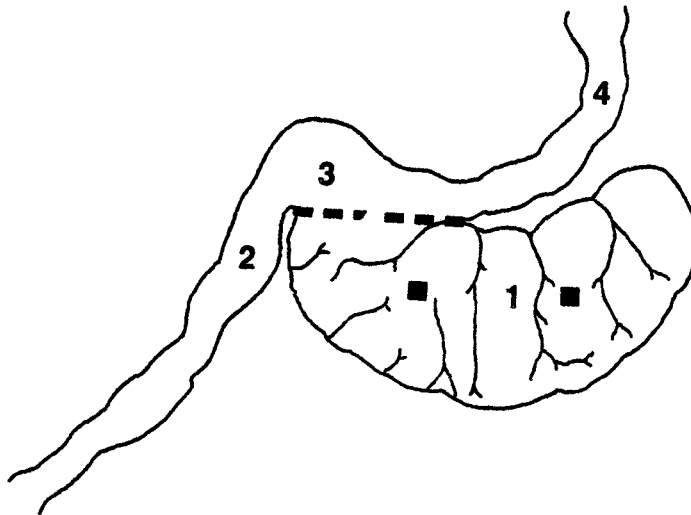
the ileocaecal junction with a 3-0 silk ligature (Figure 1) (73). Intestinal continuity was later ascertained during post-mortem examination by the unimpeded aboral flow of an opaque dye originally injected into a region of the ileum above the tie. The caecum was punctured twice, through and through, with an 18-gauge needle. To ensure patency of the perforation and hence, to hasten the infectious process, caecal contents were expressed into the peritoneal cavity before closure of the incision. The abdominal muscle layers were sutured closed whereas Michel wound clips were employed to rejoin the outer skin layer.

MEASUREMENTS

The rats were divided into three groups: (1) rats whose caeca were ligated and punctured (CLP), as described above, (2) rats whose caeca were isolated and replaced without being tied or perforated (SHAM), and lastly (3) control rats who were fasted but not subjected to surgery (FC).

LETHALITY

CLP rats were sacrificed by decapitation after their righting reflex was judged to be lost, i.e., they would lie in the supine position without objection. The loss of this reflex was used as an indication of the onset of septic shock. SHAM and FC animals were sacrificed at the same



- 1 Caecum**
- 2 Colon**
- 3 Ileo-caecal junction**
- 4 Ileum**
- - Ligation site**
- Puncture site**

FIGURE 1. Site of caecal ligation and puncture.

time as the septic rats to serve as time matched controls.

CLP rats that were alive 72 hours post operation were considered as survivors in preliminary studies. In successive experiments, however, the mortality rate was determined 16 hours post surgery. Most septic rats were in the throes of death at this time.

Post-mortem examination agreed with the description provided by Wichterman, et al (73) of a fluid filled abdominal cavity, engorged and gangrenous caecum, patent caecal perforations and no visible presence of stool in the abdomen. Cultures done by this group showed a myriad group of microorganisms such as *Escherichia coli* and *Proteus mirabilis* flourishing in the peritoneal fluid (74). No effort was made to identify such organisms in this study. A qualitative determination of bacterial presence in blood was, however, performed and resulted in a positive culture from the septic animals.

PLASMA GLUCOSE

In the early phase of the study, plasma glucose levels were measured prior to surgery and at 8-hour intervals, thereafter, until the rats were sacrificed. This group of animals were fasted in the morning (8:00 A.M.) and underwent surgery in the afternoon (4:00 P.M.). This

procedure was identical to the sepsis model of Wichterman, et al (73). This set of experiments sought to determine the lethality of the model and the progression of blood glucose disturbances. In subsequent studies, however, only presurgical and terminal levels of plasma glucose were measured, as the emphasis of the study focused on glucose dyshomeostasis during the agonal stages of septic shock. Blood was collected prior to surgery through tail bleeds under ether anesthesia. At the time of death, however, blood was obtained by decapitation.

GLUCOSE ANALYZER

Blood samples were collected in 250 ul heparinized micro-centrifuged tubes (Beckman Co., Lincolnwood, Il.) over ice and centrifuged. Plasma glucose values, expressed in mg of glucose per dl of blood or mg%, were obtained directly from a glucose analyzer (Yellow Springs Instrument Model 23A, Yellow Springs, Oh.).

SERUM IRI

Blood samples were collected in centrifuge tubes over ice at the same time points already mentioned and allowed to clot. Subsequent centrifugation (6,000 RPM, IEC PR-J centrifuge) allowed the separation of serum from the formed elements. Sera was stored at -20°C until radioimmunoassay was done.

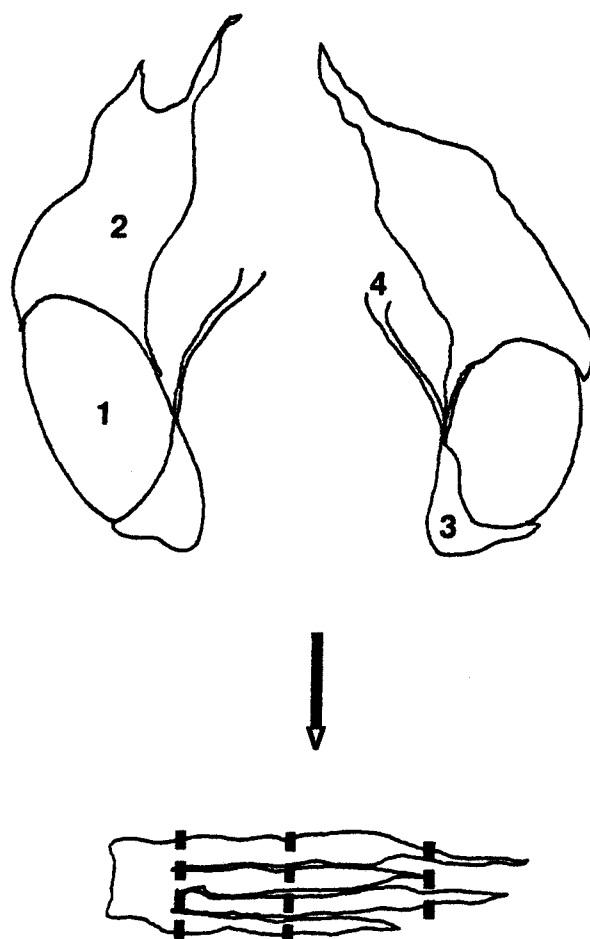
Phadebas Insulin Test (Pharmacia Diagnostics, Sweden) is a radioimmunoassay based on the Sephadex solid phase technique. The insulin in an unknown sample is allowed to compete with a fixed amount of an ^{125}I -labelled insulin for the binding sites on highly specific insulin antibodies. Bound and free insulin are separated by centrifugation and washing. The radioactivity of the bound complex is measured in a gamma counter (1185 Series, Nuclear Chicago). The amount of bound radioactivity is inversely proportional to the amount of insulin present in the sample. Serum IRI values are expressed as uU of Insulin per ml of blood.

IN VITRO DETERMINATION OF GLUCOSE OXIDATION

Glucose metabolism by the rats' epididymal fat pads (EFP) was determined at the time of sacrifice for all three groups: CLP, SHAM and FC. Criteria used for sacrifice of the septic rat was loss of its righting reflex, as indicated earlier.

ADIPOSE TISSUE PREPARATIONS

Subsequent to sacrifice by decapitation, blood was collected for serum IRI, PG and NSILA determinations. The peripheral segments of the rats' epididymal fat pads (EFP) (Figure 2) were quickly excised and placed into a 30 ml



- 1 Right testis**
- 2 Fat pad**
- 3 Cauda epididymis**
- 4 Vas deferens**
- Sections**

FIGURE 2. Peripheral segments of rat epididymal fat pads used in the segmental bioassay for basal glucose oxidation, insulin responsiveness, and non-suppressible insulin-like activity (NSILA).

beaker filled with 0.9% saline solution kept at room temperature. These EFP portions (approximate individual weight = 500-600 mg) were reduced to several 75-125 mg sections and pooled randomly in a petri dish containing physiological saline (53). All EFP segments, thereafter, were blotted and weighed before being deposited into metabolic (Saba) flasks. Two to three portions of adipose tissue were used per incubation vessel to bring the accumulated tissue wet weight to about 200 mg (180-225 mg range). The entire process, outlined above, from the resection of the tissues to their placement into the flasks, took no more than 15 minutes.

CO₂ TRAPPING APPARATUS

Shortly prior to the placement of the EFP segments into the Saba flasks, 5 ml of an equilibrated (95:5 O₂/CO₂ mixture, pH = 7.4) solution of Krebs Ringer Bicarbonate (KRB) buffer (100 ml 0.9% NaCl; 4 ml 1.15% KCl; 3 ml 1.22% CaCl₂; 1 ml 2.11% KH₂PO₄; 1 ml 3.82% MgSO₄·7H₂O; 21 ml 1.3% NaHCO₃) was added. In addition, 100 mg of D-glucose (Malinckrodt, St. Louis, Mo.) and 50 ul of U-¹⁴C-D-glucose (Amersham, Arlington Hts., Il.) were combined with the original KRB solution to bring the final glucose concentration and specific activity in each flask to 1 mg/ml and 0.5 uCi/ml, respectively.

The flasks containing the incubation media were kept

at room temperature until after the addition of the EFP segments and a second aeration of the preparation with a 95% O₂: 5% CO₂ mixture for 1 minute.

The *in vitro* method of Saba and Diluzio (64) was used for the collection of expired CO₂. The EFP segments were incubated in capped 50 ml Saba flasks (Kontes Glass Co., Vineland, N. J.) for 60 minutes at 37°C in a Dubnoff metabolic shaker at a rate of 120 cpm.

At the end of the incubation period, scintillation vials (Wheaton Scientific, Millville, N. J.) with 24 mm cap diameters were fitted into Teflon adapters which, in turn, screwed on to the sidearm of the incubation flask. To trap the released ¹⁴CO₂, strips of Whatman No. 52 filter paper saturated with 0.3 ml of Hyamine hydroxide (1.0 M in Methanol, Amersham/Searle) were placed earlier in each scintillation vial. The reaction was stopped and the ¹⁴CO₂ released by the addition of 1 ml of 62.5% citric acid solution. Recovery of ¹⁴CO₂ was allowed to proceed for 15 minutes after which time, 15 ml of liquid scintillation fluor (1 L toluene; 4 gm PPO; 0.1 gm POPOP) was added to each vial. It has since been determined that a correction factor is necessary to calculate the total evolved ¹⁴CO₂ from a 15-minute collection period. Each vial was subsequently counted in a liquid scintillation counter (ISOCAP 300, Nuclear Chicago) for 20 minutes.

Data was expressed as disintegrations per minute (DPM) of $^{14}\text{CO}_2$ recovered per gram wet weight adipose tissue per hour of incubation. A computer program was used to compensate for a possible decrease in counting efficiency due to water evaporation. This program constructed a sample channels ratio vs. efficiency curve based upon a C-14 standard quench set count rate (Amersham/Searle, Arlington Hts., Il.).

Means, standard errors and analysis of variance between groups were determined using standard computer programs.

BASAL GLUCOSE OXIDATION

Procedure followed was as outlined above for *in vitro* determination of glucose oxidation.

INSULIN RESPONSIVENESS

Insulin stimulated glucose oxidation by the EFP segments from septic, sham and fasted control rats was assessed and compared at the time of death. The segmental EFP assay was followed except for the addition of a supra-stimulatory concentration of insulin (1 $\mu\text{u}/\text{ml}$). Insulin solution was prepared fresh (47.3 μg crystalline insulin

dissolved in 5 ml 0.9% NaCl) during the day of the experiment in siliconized glassware. One hundred ul of this solution was added to each flask.

Duplicate companion flasks without insulin were run with a like number of insulin stimulated vessels to serve as paired controls for each animal in any given group.

Data was expressed as DPM $^{14}\text{CO}_2$ /gm wet weight/hr incubation. A comparison of the glucose metabolism stimulated by insulin was made across the three groups.

NON-SUPPRESSIBLE INSULIN-LIKE ACTIVITY

The fraction of serum insulin-like activity (ILA) that does not lose its activity when treated with anti insulin serum is termed as non-suppressible ILA (NSILA) (19). Serum was collected from septic, sham and fasted control rats after sacrifice by decapitation. It was oftentimes necessary to pool the rat sera, especially for the septic group to attain the desired 1 ml of sera per 5 ml of incubation media ratio. The accumulated sera was stored at a temperature of -20°C until needed.

The assay called for the preincubation of 1 ml of the collected sera with 200 ul of guinea pig anti porcine insulin serum, GPAIS (Miles Laboratories, Elkhardt, Ind.)

for 1 hour at 37°C. Following this preincubation period, glucose oxidation was assayed as previously described with segmented EFP from fed donor rats (wt. range: 325-350 gm).

The constituents of the incubation media were varied to ascertain total ILA, NSILA and suppressible ILA. Total ILA was measured by withholding the addition of GPAIS to the KRB-rat serum solution. NSILA was measured by incubating EFP segments in flasks containing 5 ml KRB, 1 ml rat sera and 200 ul of GPAIS solution. Suppressible ILA was estimated to be the difference between the total ILA and NSILA.

The immunological ILA or immunoreactive insulin (IRI) was also measured in these experiments by the Phadebas method.

RESULTS

LETHALITY

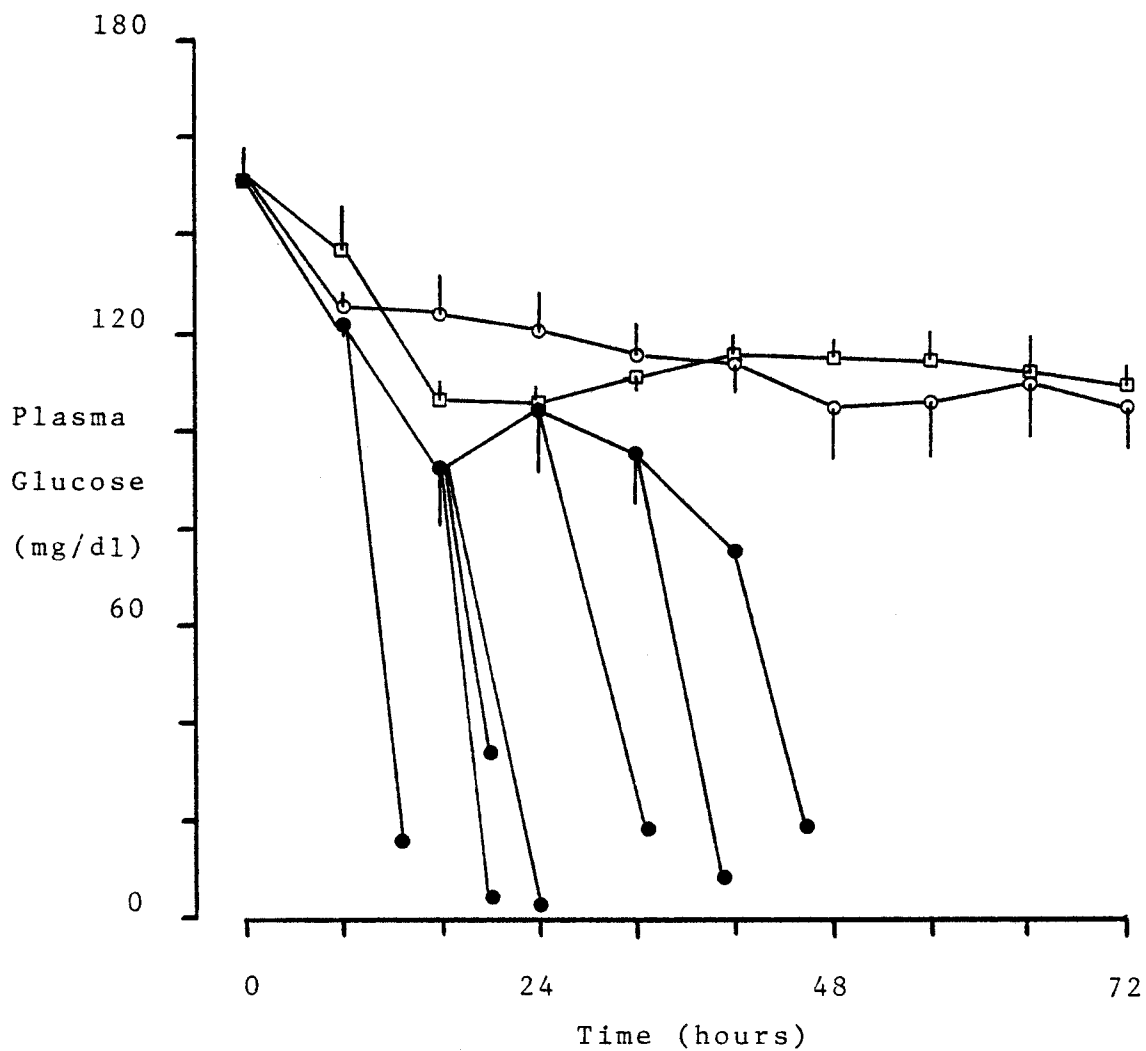
The preliminary experiments in this study mimicked the caecal ligation and puncture method of Wichterman, et al (73) to induce general peritonitis. Surgery was performed in the afternoon following an 8-hour morning fast. Subsequent to the operation a 5 ml/100 gm wt. bolus of 0.9% NaCl was injected intramuscularly. This procedure resulted in a 58.3% (7/12) mortality rate 72 hours post operation. A second group of control rats (SHAM) showed 100% (N = 7) survival rate 72 hours after surgery. This group of rats underwent surgery with caecal manipulation but no ligation or puncture.

A modification of Wichterman, et al's (74) procedure, i.e., morning surgery following an overnight fast, enlarged puncture holes, expression of caecal contents prior to wound closure and non injection of the 0.9% saline solution resulted in a hastening of the pathological process. Sixteen hours after surgery, 67% (35/52) of caecal ligated and punctured (CLP) rats had expired. SHAM (N = 26) and fasted control (FC) (N = 24) rats had no lethality. A 100% mortality rate was approached 48 hours after the surgery for the septic group.

PLASMA GLUCOSE AND SERUM INSULIN

In the earlier experiments, two groups of rats (SHAM and CLP) were studied. Blood samples were taken 1 hour prior to the operation and at 8-hour intervals thereafter until the time of death or sacrifice at 72 hours, whichever came first. Figure 3 maps out the plasma glucose pattern in SHAM, CLP survivors (CLP-s) and CLP non survivors (CLP-ns). The SHAM and CLP-s group of rats maintained their plasma glucose at normoglycemic levels of 100 - 120 mg/dl whereas the CLP-ns rats exhibited plummeting blood glucose values. Figure 4 illustrates the terminal glucose values for the aforementioned three groups. The CLP-ns rats demonstrated a severe hypoglycemia (14.5 ± 4.0 mg/dl) upon death. In contrast CLP-s and control SHAM rats were at normal fasting levels of 103.7 ± 6.9 and 108.7 ± 3.7 , respectively.

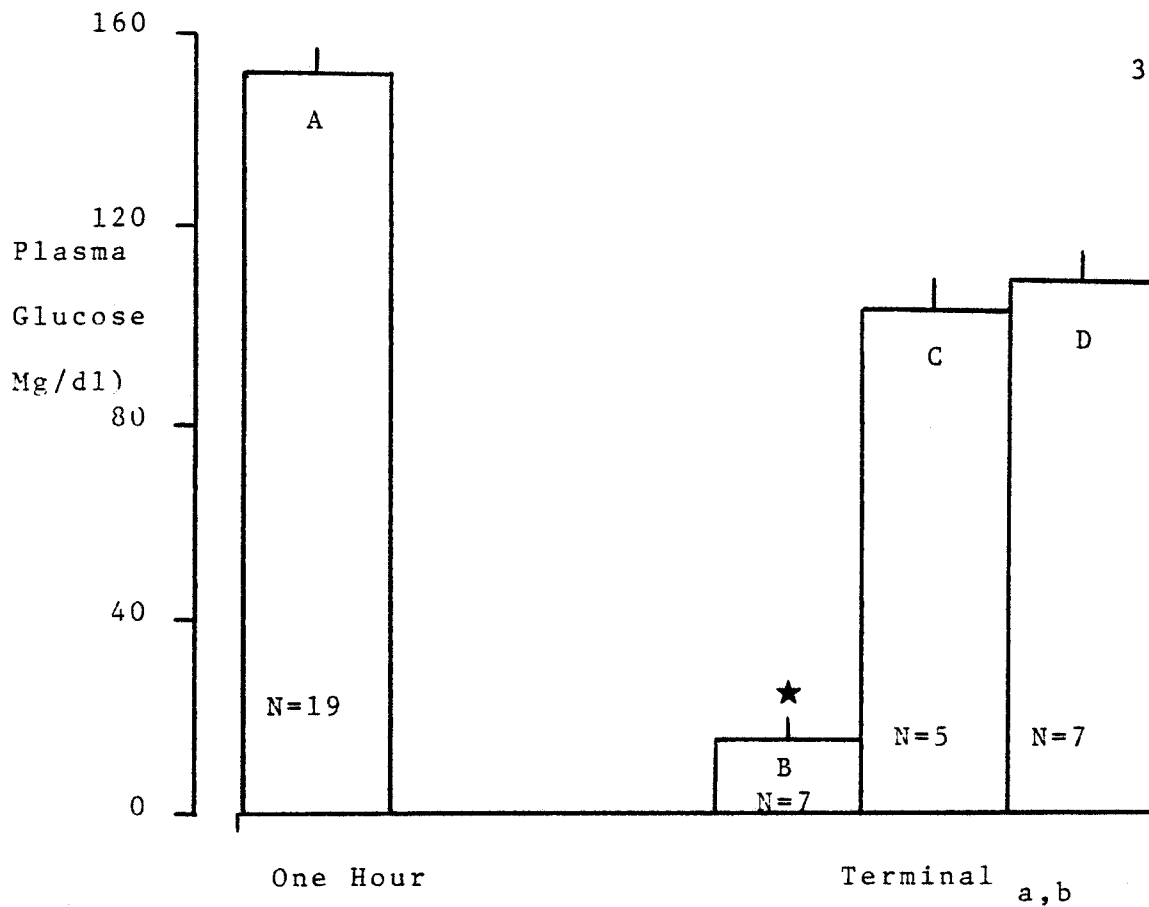
Subsequent experiments employed the modification of Wichterman, et al's (73) sepsis model. A series of experiments was done to measure plasma glucose levels and serum insulin content 1 hour prior to surgery and at the time of death or sacrifice. Three groups were studied: (1) CLP; (2) SHAM; (3) FC. As before, the CLP rats were hypoglycemic (35.9 ± 2.7 mg/dl) at death whereas both FC and SHAM control groups were normoglycemic at 100 ± 1.6 mg/dl



- - Surgical control (SHAM) N=7
- - Septic survivor (CLP-s) N=5
- - Septic non-survivor (CLP-ns) N=7

FIGURE 3. Plasma glucose levels of sham rats and progression to hypoglycemia of septic rats.

See Appendix A for numerical data.



Pre-Operation

A - All rats one hour prior to surgery

B - Septic non-survivors (CLP-ns)

C - Septic survivors (CLP-s)

D - Surgical control (SHAM)

FIGURE 4. Presurgical and terminal plasma glucose levels in control and septic (afternoon surgery) rats.

^a Blood samples for septic non-survivors (CLP-ns) were taken at the time of death;

^b Blood samples for septic survivors (CLP-s) and sham operated controls (SHAM) were collected 72 hours post operation.

Data expressed as MEAN \pm S.E.M.

* CLP-ns vs. CLP-s and CLP-ns vs. SHAM. All significant comparisons at p less than 0.05.

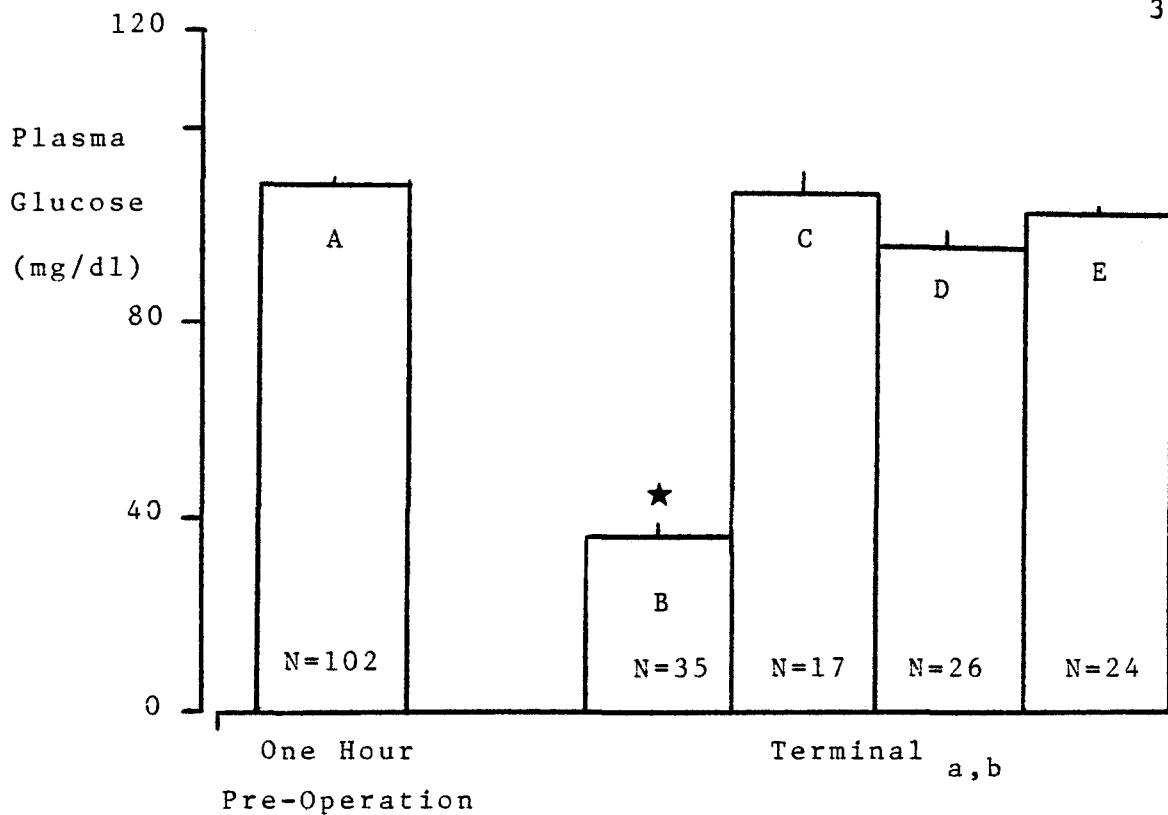
See Appendix A for numerical data.

and 94.3 ± 3.9 mg/dl, respectively (Figure 5). Ligated and punctured rats that were alive 16 hours after surgery, likewise, demonstrated not significantly different blood glucose value of 105.9 ± 4.9 mg/dl.

Terminal serum immunoreactive insulin (IRI) content for the three groups; FC, SHAM and CLP did not vary from each other (Figure 6). No significant difference was found to exist between groups at the same time point. There was a difference, however, between the presurgical and terminal IRI levels. The significance of this will be discussed later.

BASAL GLUCOSE OXIDATION

Peripheral tissue glucose oxidation was assessed for all three groups: FC, SHAM and CLP. Table 1 illustrates the hypoglycemia and enhanced adipose tissue glucose utilization in the agonal stages of sepsis. The oxidation of ^{14}C - glucose to $^{14}\text{CO}_2$ by the septic rat's epididymal fat pads was significantly greater than either SHAM or FC control group (56.3 ± 6.4 vs. 35.0 ± 3.3 or 24.5 ± 2.4 10^3 $^{14}\text{CO}_2$ dpm/g/hr) *in vitro*. Although the measured glucose oxidation value for the SHAM group was greater than the unoperated rats (FC), analysis of variance showed no significant difference to exist.



- A - All rats prior to surgery
 B - Septic non-survivors (CLP-ns)
 C - Septic survivors (CLP-s)
 D - Surgical control (SHAM)
 E - Fasted control (FC)

FIGURE 5. Presurgical and terminal plasma glucose levels in control and septic (morning surgery) rats.

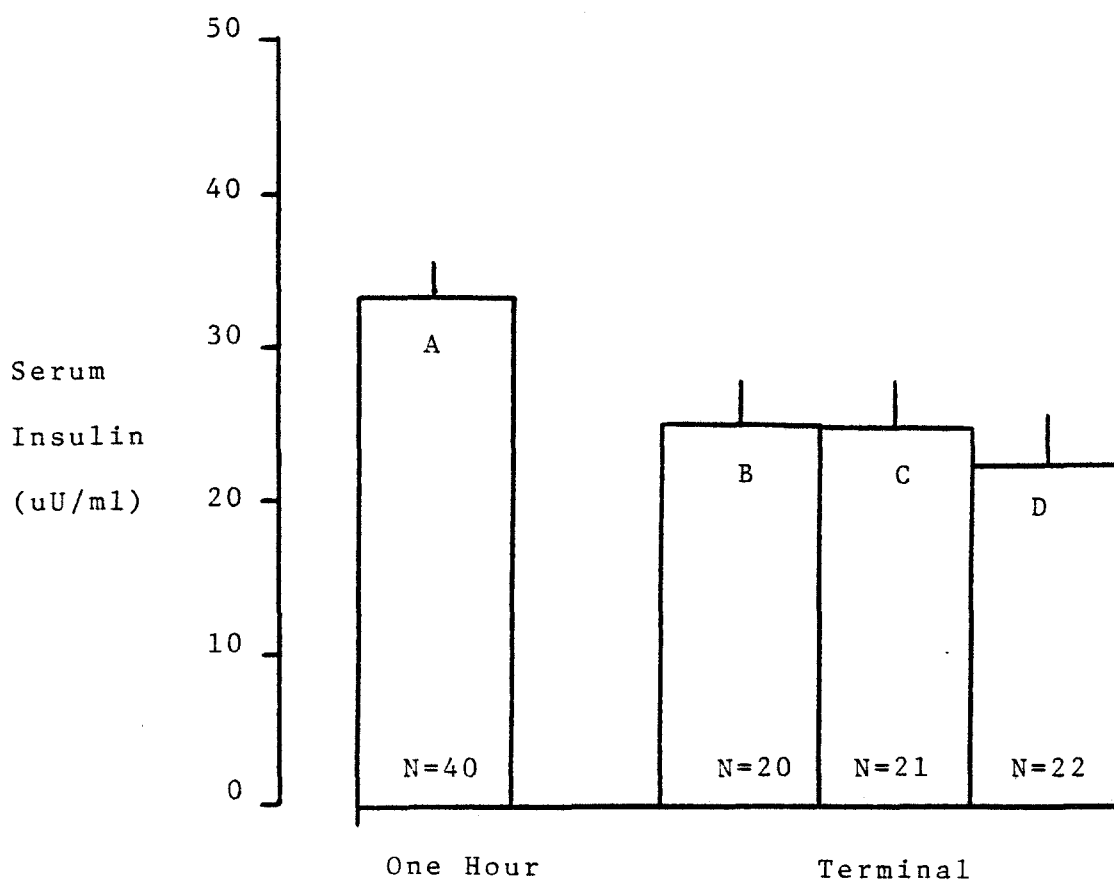
^a SHAM and fasted control (FC) group blood samples were taken after simultaneous sacrifice with dying septic rats.

^b CLP survivor plasma glucose levels taken 16 hours post operation.

Data expressed as MEAN \pm S.E.M.

* Significant difference at p less than 0.05.

See Appendix B for numerical data.



Pre-Operation

- A - All rats one hour prior to surgery
- B - Septic non-survivor (CLP-ns) group
- C - Surgical control (SHAM) group
- D - Fasted control (FC) group

FIGURE 6. Presurgical and terminal serum immuno-reactive insulin (IRI) concentration in control and septic (morning surgery) rats.

Data expressed as MEAN \pm S.E.M.

No significant differences between groups at p greater than 0.05.

See Appendix C for numerical data.

TABLE 1

IN VITRO BASAL GLUCOSE OXIDATION BY SEPTIC
AND CONTROL EPIDIDYMAL FAT PADS

Groups	Plasma Glucose (mg/dl)	Glucose Oxidation (10^3 $^{14}\text{CO}_2$ dpm/g/hr)
1. CLP N = 32	40.2 ± 3.0 ^a	56.3 ± 6.4 ^a
2. SHAM N = 33	97 ± 2.0	35.0 ± 3.3
3. FC N = 32	96.5 ± 2.5	24.5 ± 2.4

Data expressed as MEAN ± S.E.M.

^a CLP vs. SHAM, CLP vs. FC. Significant comparisons.

ANOVA at p less than 0.05.

INSULIN RESPONSIVENESS

Filkins and Figlewicz (17) had shown that the adipose tissue of endotoxic rats was hyperresponsive to a given stimulus of insulin *in vitro*. They suggested this as a possible mechanism for the enhanced peripheral tissue glucose oxidation. The responsiveness of the septic rat's epididymal fat pads was assessed in this study by the addition of a supra stimulatory concentration of insulin (1 mU/ml) *in vitro*. As mentioned earlier, the basal glucose oxidation, *in vitro*, of adipose tissue excised from septic rats catabolized glucose at a higher rate than either control group (42.1 ± 4.2 vs. 23.9 ± 2.0 , $17.8 \pm 1.9 \times 10^3 \text{ }^{14}\text{CO}_2 \text{ dpm/g/hr}$). Table 2 also shows that upon addition of 1 mU/ml of insulin into the incubation media, the septic rats' fat pads were further stimulated to a greater value of glucose oxidation than either SHAM or FC group. Statistical analysis showed these differences to be significant.

NON-SUPPRESSIBLE INSULIN-LIKE ACTIVITY

Along with the aberration of tissue responsiveness, recent work from our laboratory has shown the possibility of non-suppressible insulin-like activity (NSILA) as an important factor in glucose homeostasis in infectious states (14). A modification of Poffenbarger's segmental

TABLE 2
EFFECT OF INSULIN, *IN VITRO*, ON SEPTIC AND CONTROL
EPIDIDYMAL FAT PAD GLUCOSE OXIDATION

Groups	PG ^b (mg/dl)	Glucose Oxidation (10 ³ ¹⁴ C ₂ dpm/g/hr)	
		Basal ^c (0 mU/ml)	Stimulated (1 mU/ml) ^c
1. CLP	52.6 ± 6.9 ^a (18)	42.1 ± 4.2 ^a (18)	104.7 ± 11.8 ^a (18)
2. SHAM	90.3 ± 1.8 (14)	23.9 ± 2.0 (16)	71.8 ± 11.0 (15)
3. FC	93.7 ± 3.42 (17)	17.8 ± 1.9 (16)	56.6 ± 4.9 (15)

Data expressed as MEAN ± S.E.M.

^a CLP vs. SHAM, CLP vs. FC. All significant comparisons.

ANOVA at p less than 0.05.

^b PG: Plasma Glucose

^c Insulin concentration

bioassay for NSILA was employed in this study. The epididymal fat pads from fed donor rats were incubated under three separate conditions: (1) in Kreb's Ringer bicarbonate buffer (KRB) alone, (2) KRB + test serum and lastly, (3) KRB + test serum + Guinea Pig Anti Insulin Serum (GPAIS). The first group served as control, and the second and third were for assessing total ILA and NSILA, respectively. The presence of CLP, SHAM or FC sera in the media significantly elevated the fat pad glucose oxidation (Table 3). This indicated the presence of insulin and insulin-like substances in the sera of all three rat groups. IRI was measured and no significant difference was found between the septic and control groups. When the GPAIS was added along with serum into the media to assess NSILA, CLP sera demonstrated a greater level of activity than SHAM or FC sera. Glucose oxidation did not vary in the presence or absence of GPAIS in either CLP or FC group. There was a marked reduction, however, if GPAIS was added to a preparation with sera from SHAM rats. The relevance of these observations will be discussed in the next section.

TABLE 3
 COMPARISON OF NON-SUPPRESSIBLE INSULIN-LIKE ACTIVITY
 IN THE SERA OF SEPTIC AND CONTROL RATS

GROUPS	PG ^d	IRI ^{b,d} (uU/ml)	Glucose Oxidation ^c (10 ³ ¹⁴ CO ₂ dpm/g/hr)		
			KRB only	KRB + serum	KRE + SERUM + GPAIS
1 CLP	32.8 ± 3.6 (8)	18.8 ± 2.0 (10)		84.1 ± 9.9 (7)	88.5 ± 8.0 ^a (7)
			38.2 ± 1.5 (63)		
2 SHAM	90.6 ± 3.3 (12)	15.6 ± 1.3 (10)		90.0 ± 13.5 (11)	67.8 ± 5.2 ^a (26)
3 FC	92.4 ± 4.0 (10)	14.4 ± 1.1 (10)		48.3 ± 6.0 (11)	50.8 ± 4.0 ^a (28)

Data expressed as MEAN ± S.E.M.

^a CLP vs. SHAM, CLP vs. FC, SHAM vs. FC. All significant comparisons ANOVA at p less than 0.05.

^b No significant difference in IRI, p greater than 0.05.

^c Numbers in parentheses denote number of assays run.

^d Numbers in parentheses denote number of animals

DISCUSSION

SEPSIS MODEL EFFICACY

The effectiveness of the caecal ligation and puncture (CLP) model to induce peritonitis was assessed through the lethality rate and incidence of hypoglycemia. As stated earlier, 67% of CLP rats died 16 hours post surgery. This was comparable to a 75% (138/184) mortality rate reported by Wichterman, Baue and Chaudry (73) for similarly treated rats (caecal ligation + 2 punctures with an 18-gauge needle) after 24 hours. In the present modification of their model, the majority of the animals died 8 - 16 hours following the surgery. This model is significantly more rapid and lethal than the model Wichterman, et al (73) first proposed.

PLASMA GLUCOSE AND SERUM INSULIN LEVELS

As illustrated by both Figures 4 and 5, the septic rats died in a state of metabolic decompensation, viz., hypoglycemia. Although CLP (caecal ligation and puncture) rats that underwent morning surgery showed a less severe hypoglycemia (35.9 ± 2.7 mg/dl) than their afternoon counterpart (14.5 ± 4.0), the former was the method of choice because of sampling ease since the bulk of deaths (67%) (35/52) oc-

occurred within a narrow time period (8 - 16 hours post surgery). The measurement of low blood glucose agrees well with previous works in the literature for rats in the agonal or hypodynamic stages of endotoxemia and sepsis (14,74). It can also be seen from Figures 3, 4 and 5 that the survival of an animal was reflected by his ability to maintain a status of normoglycemia. These data concur with work by Hinshaw, et al (31) and Filkins and Cornell (16).

Figure 6 illustrates that no significant difference existed between the three groups of animals (CLP, SHAM and FC) in serum insulin content at the time of death. There was, however, a difference in insulin levels when the terminal readings for the three groups of animals were compared to pre-surgical levels. This difference may be due to the slightly higher level of plasma glucose before surgery and also to the blood sampling method under ether anesthesia. Furthermore, pre-surgical glucose and insulin levels were taken at a point closer in time to the initial withholding of food than terminal samples.

This finding of no significant difference between control (SHAM and FC) and septic (CLP) rats' insulin content does not fully agree with previous work by Ryan, et al (16) and Filkins (14). Ryan, et al (16) found 72 hours septic fasted rats to have a threefold higher level of insulin

than 72-hour fasted control rats. A recent study by Filkins (14) showed the opposite to be true in late endotoxic shock. He showed a hypoinsulinemia (9.2 ± 3.8 uU/ml vs. control value of 36 ± 6) to occur prior to the animal's death. The difference in measured insulin concentration between this study and those previously mentioned may be due to a number of factors such as: (1) mode of induction of infection (caecal ligation vs. caecal ligation and puncture vs. endotoxin injection), and (2) stage of progression of the infectious process. Ryan, et al (63) showed no hypoglycemia to exist in their study, whereas Filkins (14) demonstrated blood glucose levels of 41 ± 8 mg/dl. It seems evident, therefore, that Ryan, et al's results were taken prior to metabolic decompensation whereas this study's results and Filkins (14) were taken during the hypoglycemic depression. Wichterman, et al (74) measured insulin content to be 21 ± 5 uU/ml in rats in late sepsis and this agrees well with this study's insulin quantitation.

ENHANCED PERIPHERAL TISSUE GLUCOSE OXIDATION

Numerous studies in the past have repeatedly shown the hypercatabolism of glucose during the infectious process (17,63,71). This increased utilization of glucose appears early on in endotoxicosis as a result of hyperglycemia and associated hyperinsulinemia (6). Ryan, et al

(63) has similarly shown this to occur in the fat pads of septic rats. The hypercatabolism of glucose continues, however, until the later stages of endotoxic or septic shock (17,32,71). The result of this study provides further evidence for the hypoglycemic action of increased glucose utilization. Table 1 shows the markedly greater oxidation of ^{14}C - glucose to $^{14}\text{CO}_2$ and associated hypoglycemia in septic animals.

Two mechanisms for this hypercatabolism suggested by Filkins and Figlewicz (17) and Filkins (14) were investigated further in this study; (1) tissue hyperresponsiveness to insulin and (2) non-suppressible insulin-like activity.

INSULIN RESPONSIVENESS

The responsiveness of the septic rats' adipose tissue was ascertained, *in vitro*, by the addition of a suprastimulatory dose (1 mU/ml) of insulin to the incubation media. Table 2 shows that the added insulin stimulus drives the septic (CLP), sham operated (SHAM) and fasted control (FC) rats' fat pads to approximately three times the basal level. Insulin further stimulated the already hypercatabolic septic rats' fat pads such that, empirically, both basal and stimulated glucose oxidation rates for the CLP group were significantly greater than SHAM or FC. This in itself does not indicate an altered sensitivity by the septic rats'

adipose tissue to insulin. These results, however, agree with the findings of Filkins and Figlewicz (17) of increased adipose tissue insulin responsiveness in endotoxic rats. One of the criteria satisfied to imply an alteration in insulin responsivity has been the antagonism of insulin action by ATP. Chang and Cuatrecasas (9) had shown that the addition of 10^{-4} and 10^{-5} M ATP to the incubation media decreased the responsiveness of isolated adipocytes to insulin. This *in vitro* experiment was not done in this study, however, an *in vivo* study by Chaudry, et al (10) had already shown, using the same experimental model of sepsis, the prevention of hypoglycemia and increased survivability following the administration of ATJ-MgCl₂ and glucose. It is not unreasonable, therefore, to cautiously suggest that adipose tissue insulin responsiveness is altered in septic as well as endotoxic shock.

NON-SUPPRESSIBLE INSULIN-LIKE ACTIVITY (NSILA)

Serum contains several proteins possessing insulin-like properties. One of these entities, non-suppressible insulin-like activity (NSILA) is of interest in this study since Filkins (14) showed increased levels of NSILA associated with the terminal hypoglycemia in endotoxemia. The NSILA assay employed in this study did not make any distinction between soluble (NSILA-s) and precipitable (NSILA-p) portions.

Table 3 illustrates significant insulin-like activity (ILA) in serum after its addition to the incubation media. Total serum ILA may be gauged by comparing glucose oxidation rates between assays in the presence and absence of serum in the incubation media. As Table 3 shows, sera from rats that underwent surgery, CLP and SHAM, exhibited greater total ILA activity than fasted and hydrated (FC) rats. This may be attributed to the slightly higher values of immunoreactive insulin (IRI) and NSILA in the former two groups. Statistical testing shows, however, no significant difference to exist in insulin content. One notices that these insulin values are lower than what was previously reported in this study. This discrepancy is perhaps due to variation within this strain since all the animals in this shipment demonstrated lower serum insulin content.

All three groups of rats exhibited non-suppressible insulin-like activity as illustrated by the increase in glucose oxidation despite the presence of Guinea Pig Anti Porcine Insulin Serum (GPAIS) in the media. The level of NSILA, in descending order, is as follows: CLP, SHAM and FC. These results support Filkins' finding of increased NSILA levels in the late stages of endotoxic shock (14). NSILA has been associated with hypoglycemia in various disease processes (8,45). Froesch, et al (47) have suggested a

probable role for NSILA in the provision of energy substrates in conditions of vascular collapse. Since sepsis and endotoxemia both involve vascular as well as metabolic lesions, it is a plausible supposition that NSILA as well as altered tissue insulin responsiveness play a deciding role in the progression to hypoglycemia. The hypothesis extended here is that the hypoglycemia seen in the agonal stages of sepsis may, in part, be attributed to an increase in the level of substances in the blood and interstitial spaces which are physicochemically distinct, yet bioactively similar to insulin.

SUMMARY OF CONCLUSIONS

1. Induction of septic shock through caecal ligation and puncture is a sufficiently lethal and appropriate model for metabolic studies in infectious processes.
2. Late stage of sepsis was characterized by hypoglycemia. Normal blood glucose level is correlated to the survival of an animal after a septic episode.
3. There was no demonstrable difference in the serum insulin concentration of septic, sham operated and fasted control rats at the time of death.
4. There was a significantly higher rate of glucose oxidation in septic adipose tissue as compared to sham operated and fasted control rats.
5. Addition of insulin, *in vitro*, equally led to a threefold increase in glucose oxidation rates by the fat pads from all three groups.
6. Increased level of NSILA in septic rats as compared to both control groups suggest a prominent role for NSILA in the development of hypoglycemia.

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APPENDIX A

APPENDIX A

PRESURGICAL AND TERMINAL PLASMA GLUCOSE LEVELS IN
CONTROL AND SEPTIC (AFTERNOON SURGERY) RATS

Groups	Plasma Glucose (mg/dl)	
	All Rats One Hour Preoperation	Terminal
1. CLP-ns (N=7)		14.5 ± 4.0
2. CLP-s (n=5)	151.5 ± 5.7 (N=19)	103.7 ± 6.9
3. SHAM (N=7)		108.7 ± 3.7

Data expressed as MEAN ± S.E.M.

CLP-ns : Septic non-survivor group

CLP-s : Septic survivor group

SHAM : Surgical control group

^a CLP-ns vs. CLP-s and CLP-ns vs. SHAM : Significant comparisons through analysis of variance, p less than 0.05.

APPENDIX B

APPENDIX B

PRESURGICAL AND TERMINAL BLOOD GLUCOSE LEVELS
IN CONTROL AND SEPTIC (MORNING SURGERY) RATS

Groups	Plasma Glucose	
	All Rats One Hour Preoperation	Terminal
1. CLP-ns (N=35)		35.9 ± 2.7 ^a
2. CLP-s (N=17)	108.6 ± 1.03	105.9 ± 4.9
3. SHAM (N=26)	(N=102)	94.3 ± 3.9
4. FC (N=24)		101.0 ± 1.6

Data expressed as MEAN ± S.E.M.

FC : Fasted control group

^a CLP-ns vs. CLP-s, CLP-ns vs. SHAM, CLP-ns vs.

FC : All significant comparisons through

ANOVA, p less than 0.05.

APPENDIX C

APPENDIX C

PRESURGICAL AND TERMINAL SERUM IMMUNOREACTIVE
 INSULIN CONCENTRATION IN SEPTIC (MORNING
 SURGERY) NON-SURVIVOR AND CONTROL RATS

Groups	Serum Immunoreactive Insulin (uU/ml)	
	All Rats One Hour Preoperation	Terminal
1. CLP		24.7 ± 2.5
2. SHAM	33.3 ± 2.1 (N=40)	24.5 ± 2.7 (N=21)
3. FC		22.1 ± 2.4 (N=22)

Data expressed as MEAN ± S.E.M.

CLP : Septic non-survivor group.

No significant difference between groups.

Anova less than 0.05.

APPROVAL SHEET

The thesis submitted by Rico E. Viray has been read and approved by the following committee:

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The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

Nov 30 1981
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