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RECOVERY PATTERNS AND LETHAL MANIFESTATIONS OF
LIVE E. COLI ORGANISM SHOCK

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INTRODUCTION

There is a growing recognition of the fact that no really effective, reproducible means of reversing the adverse effects of experimental endotoxin shock has been developed. A great number of claims for successful pre- or post-treatment have been described but have been rather uniformly unacceptable (20). There is a serious concern regarding the failure to develop an effective treatment of septic shock in clinical medicine, and the development of a more relevant animal shock model would greatly aid in elucidating clinical problems observed in the human patient (1,4,5,18,24,27,29).

A primary purpose of research in this laboratory has been to determine the effects of intravenous injections of living E. coli organisms in dogs and monkeys and compare them with responses produced by endotoxin. In contrast to findings in dogs given endotoxin (9,11,19,26), results with live E. coli organism injection suggest that factors other than hepatic pooling and peripheral vasoconstriction may be responsible for the decrease in cardiac output and subsequent deterioration in shock (7,12). The sustained decrease in total peripheral resistance in dogs administered live organisms (12) correlates well with recent clinical reports of septic shock (18,29). Renal vasoconstriction, decreased renal blood flow and urine flow, regularly observed after lethal injections of endotoxin in dogs (6,10,13) were replaced in half the animals given live E. coli organisms with renal hyperemia, decreased renal resistance and increased urine flow (12).

Other basic problems arise concerning the development of a more clinically relevant animal shock model and these have to do with the length

of the experiments and the type of anesthesia administered. Objections may be raised that the great majority of experimental reports have been confined to a relatively short time for measurements in an animal depressed by an anesthetic (20,23).

The purpose of the present study was to develop a more clinically applicable animal shock model by withholding anesthetics while administering live E. coli organisms at a dosage producing the degree of lethality seen in clinical medicine and to study the animals in an unrestrained condition during a substantially extended post-shock period. Results from these experiments are thought to be of promise in regard to more closely approximating the manifestations of clinical shock in man.

METHODS AND MATERIALS

Experiments were carried out on twenty-nine adult mongrel dogs of either sex. Animals were vaccinated against distemper, treated for intestinal parasites and allowed to stabilize for a two-week period prior to surgery. Animals were intravenously anesthetized with sodium pentobarbital, 30 mg/kg, and a 4-6 cm incision was made in the right side of the neck medial to the jugular vein. Fascia and muscles were spread to expose the carotid artery which was lifted out and tied off distally. The artery was cannulated with silastic tubing, the diameter and length varied to fit the sizes of the individual vessels. The cannula was pre-measured and marked with a collar to reach the descending aorta. Blood moving downstream from the tip of the catheter prevented clot formation within the tubing. After the cannula was inserted into the vessel, it was anchored to the artery with ties placed on either side of the collar and secured to the tie used to close off the

distal end of the artery. The incision was sutured and the cannula was maintained patent with minute daily flushings of saline and heparin. The dogs were given a minimum three-day recovery period prior to live E. coli organism injections.

A special restraining device with sling supports was made to hold the animals in a comfortable upright position for an initial two-hour study. The animal was placed in the restraint and a blood sample was taken for control determinations of pH, P_{CO_2} , P_{O_2} , serum glutamic oxalacetic transaminase (SGPT), white blood cell (WBC) counts, and hematocrit. Control rectal temperature, mean systemic arterial pressure (MSAP) and heart rate were also taken at this time. Blood gases and pH were determined by use of the I. L. Ultra Micro pH and Blood Gas Analyzing System (Instrumentation Laboratory, Inc., Watertown, Mass.). Colormetric assays for the determination of SGPT and SGOT were performed by the modified method of Reitman and Frankel (21). With this procedure, keto acids react with dinitrophenyl hydrazine to form the keto acid hydrazones which, on the addition of sodium hydroxide, yield an intense brownish color read on a colorimeter or photometer. Prepared solutions of aspartic and alpha ketoglutaric acid, alanine and alpha ketoglutaric acid, 2,4,-dinitrophenyl hydrazine and pyruvic acid standard were obtained from Dade Reagents, Inc. Using a Thomas White cell pipette, a 1:20 dilution was made of whole blood and 1% acetic acid which was then put into a Neubauer counting chamber. Using the 10X power on the microscope, four 1 mm square areas were counted and the number of cells multiplied by 50 gave the white cell count/cmm. Blood smears were made at pre-determined intervals, air dried and stained by the

Wright's staining method. Packed cell volume of the blood was obtained by using the Guest-Weichselbaum micro hematocrit determination. Whole blood was put in heparinized capillary tubes, sealed with a special plastic putty, and placed in a centrifuge for 5 minutes at 10,000 RPM.

Both the MSAP and heart rate were taken by connecting tubing to the indwelling catheter surgically placed in the carotid artery. Pressures were recorded with a P23DC pressure transducer attached to a Sanborn direct writing recorder.

The blood sample designations were (a) pre E. coli, (b) +30 minutes post E. coli, (c) +2 hours post E. coli, and (d) daily thereafter. MSAP was monitored constantly and a heart rate was taken every 15 minutes for the initial 2-hour period. The dose of organisms utilized was an approximate LD₆₀. Surviving dogs were brought out of the cages each day and, after a blood sample was withdrawn from the artery, they were monitored for 15 minutes for MSAP and heart rate. Dogs surviving eight days were sacrificed.

Statistics were carried out utilizing a modified Student's T test. All values equal to or less than p0.05 were considered significant.

E. coli of the Dunwald Strain obtained from swine, type B15:0125 was maintained on trypticase soy agar slants (TSA), transferred weekly, incubated and held in the refrigerator. To prepare the culture, 2-3 ml of TS broth from the stock culture was inoculated and broth was incubated 4-6 hours until cloudy in appearance. TSA slants were inoculated by dipping a sterile swab in broth and streaking entire surface of slant and incubated overnight (18 hours). To harvest the cells for the inoculum, sterile saline was poured into the TSA slant and surface growth was carefully

removed with a sterile swab. The suspension was poured into a sterile cap tube and centrifuged. After pouring off the supernatant fluid, the cells were resuspended in sterile saline and the density of the suspension was estimated on a B and L Spectro 20 and adjusted to 7% T. A colony count was done on each day's inoculum to determine the viable organism count per milliliter.

Some experiments were carried out using a tranquilizing agent (15) (Innovar Vet); however, results showed that no measured parameters varied differently in either Innovar-treated or totally unanesthetized dogs. Animals were intravenously injected with 0.20 - 0.25 ml/kg live E. coli organisms. Counts ranged between $2-4 \times 10^9$ organisms/ml.

RESULTS

Figures 1, 2 and 3 show results from 29 animals intravenously administered live E. coli organisms. Dogs are divided into survivors (N = 12) and non-survivors (N = 17), and data is graphed with the two groups expressed separately. Non-survivors died within the first twenty-four hours, while survivors lived beyond the seventh day. Daily measurements were not carried out in every surviving animal due primarily to technical problems.

Figure 1 presents changes in pH and mean systemic arterial pressure (MSAP). Both surviving and non-surviving animals showed significant decreases in pH for the first two hours ($p < 0.05$) while non-survivors had dropped to a significantly lower pH than the survivors at two hours ($p < 0.01$). Three days later, mean pH of survivors was statistically indistinguishable from the pre-injected value. MSAP fell to markedly low values at 30 and 120 minutes in non-survivors ($p < 0.01$). MSAP was significantly depressed below pre-injection values in surviving animals, from 120 minutes to 7-8 days ($p < 0.05$). In

addition, MSAP at 30 and 120 minutes in the non-survivor group was notably less than the survivors ($p < 0.05$).

Figure 2 illustrates changes in hematocrit and white blood cell count (WBC) in 29 animals intravenously injected with live E. coli organisms. Dying animals exhibited an average hematocrit rise from 35 to 54 per cent ($p < 0.05$), which was greater than the surviving group at 120 minutes ($p < 0.05$). The hematocrit was increased above control at two days ($p = 0.05$) and fell below control by 7-8 days ($p = 0.05$) in surviving animals.

Figure 2 indicates that there were marked decreases in WBC count from about 15-18,000 to approximately 2000/cmm within 30 minutes post injection in both groups ($p < 0.01$) remaining low by two hours ($p < 0.01$). In the surviving animals, however, WBC count rose to exceedingly high values, reaching a peak of approximately 49,000 ($p < 0.01$) by two days, falling to 38,000 ($p < 0.01$) in three days, 21,000 ($p < 0.05$) in 4-6 days, and to control (about 14,000) by 7-8 days.

The white cell count decreasing by 30 minutes was due to segmented neutrophils leaving the circulation and therefore the lymphocytes assumed a greater percentage of the total white cell count at that time. On the second day, the large increase in white count was due to the presence of both immature and mature segmented neutrophils.

Table 1 shows a correlation of nucleated red cells and hematocrit in five survivors and six non-survivors, all administered live E. coli organisms. There was a positive correlation between the two parameters; it is seen that the marked increases in number of nucleated red cells and hematocrits occur at 120 minutes post-injection.

Changes in serum glutamic oxalacetic transaminase (SGOT) are reported in Figure 3 and indicate an increased release of this enzyme within two hours in both survivors and non-survivors ($p < 0.01$) by 120 minutes. SGOT values of the non-survivor group were higher ($p < 0.05$) than the survivor animals at all points. Even in survivors, SGOT remained significantly elevated above control by 3 days after injection. Figure 3 also shows similar increases in SGPT, however, the peak value occurred at two days ($p < 0.01$) which remained above control levels ($p < 0.05$, < 0.01) for the entire eight-day survival period.

Table II shows changes in heart rate, PO_2 , PCO_2 and rectal temperature following the intravenous injection of live E. coli organisms in dogs. The only significant change in heart rate was the tachycardia observed in both groups at two hours ($p < 0.05$). Arterial PO_2 increased in both survivors and non-survivors during the first day of E. coli injection ($p < 0.01$, < 0.05) while arterial PCO_2 decreased at 30 minutes and 120 minutes in non-survivors and survivors ($p < 0.01$). Rectal temperature remained constant during the entire post-injection period in both survivors and non-survivors.

DISCUSSION

The primary purpose of this investigation was to develop an experimental septic shock animal model which was more meaningful in terms of its clinical relevance. Three major objections of earlier studies have been circumvented in the present experiments in that anesthetics were avoided, shock was induced by controlled vascular invasion of living E. coli organisms rather than endotoxin, and measurements of parameters were extended for a lengthy chronic observation period.

The presently developed protocol clearly moves closer to the clinical entity of septic shock, and should lend itself favorably to long-term therapeutic studies. Of major assistance in developing a successful chronic animal model was the use of an indwelling arterial catheter and a restraining device which permitted animals to remain comfortable yet secure.

Results from these experiments suggest that acidosis and systemic hypotension are correlated with lethality. Surviving animals showed a mild degree of acidosis which was eventually reversed and a slight lowering of mean systemic arterial pressure. In contrast to previous results with endotoxin (19,26), changes in arterial pressure occurred only gradually. It is proposed that this manner of achieving a progressive development of systemic hypotension is more characteristic of the patient's manner of developing septic shock. Changes in white cell counts were biphasic; prominent and rapidly induced leucopenia was followed by leucocytosis on the second day in the survivors. The delayed increase in cell count may be comparable to that seen in patients, since early clinical measurements appear to be lacking. Atkins, in rabbit experiments (2), observed marked leukopenia within ten minutes followed eventually by some degree of leukocytosis involving largely immature granulocytic forms. This was described also by Bennett and Cluff (3) and Lillehei and MacLean (17). In animal experiments, although specific quantitative data is lacking. Weil and others (25) reported the return of white cell numbers from lowered values, within three hours. Hall and Gold (8) and Weil and Spink (28) reported leukocytosis in patients in bacteremic shock while information relating to leukopenia appears to be lacking. Results from the present study

demonstrating large increases in immature nucleated red blood cells by two hours after E. coli injection appears to be a very significant observation and may explain, in part, the ability of the animals to support a higher P_{O_2} as observed in these experiments. The significance of a correlation between the elevation of hematocrit and increases in numbers of immature red blood cells remains to be investigated.

Increases in P_{O_2} and decreases in P_{CO_2} in the early post-injection period (30-120 minutes) in all animals suggests stimulation of the respiratory center and occurred even in the absence of systemic hypotension. Elevations of serum levels of transaminases observed in the present study are indicative of both early and sustained injury or depression of function in cardiac or hepatic tissue. Similar increases have been reported in several disease states (15,16,22). Hematocrit changes were only correlated with impending death in instances in which extreme increases were noted two hours post-injection. Recovery of the hematocrit was slow in surviving animals and possibly represents a diminished stimulation of factors bringing about an increase in RBC concentration or recovery of fluid lost during the early stages of shock.

SUMMARY

There is serious concern regarding the failure to develop an effective treatment of septic shock in clinical medicine. The primary purpose of this investigation was to develop an experimental animal septic shock model which would be more meaningful in terms of its clinical relevance. Shock was induced by intravenous injection of an LD_{60} live E. coli organism suspension in unrestrained unanesthetized catheterized dogs with measurements

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of parameters extended for a lengthy chronic observation period. Results of the study suggest that acidosis and systemic hypotension are correlated with lethality. Changes in white cell counts were biphasic; prominent and rapidly induced leucopenia was followed by leucocytosis by the second day in survivors. The large increase in white cell count was due to the presence of both immature and mature segmented neutrophils. Large increases in immature nucleated red blood cells observed two hours after injection of organisms were correlated with increasing hematocrits. Early marked and sustained elevations in serum levels of transaminases were observed and considered indicative of injury or depression of function of cardiac or hepatic tissue. Rapid, large increases in hematocrit were correlated with early death, and recovery of hematocrit in surviving animals, was slow, extending through the entire survival period. The presently developed experimental protocol clearly moves closer to the clinical entity of septic shock and should lend itself favorably to long-term therapeutic investigations.

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TABLE I
CORRELATION OF NUCLEATED RED BLOOD CELLS AND
HEMATOCRIT IN ANIMALS ADMINISTERED LIVE E. COLI ORGANISMS

NUCLEATED RED CELLS (NUMBER/100 WBC COUNTED)											
ANIMAL NO.	SURVIVORS			DAYS POST-INJECTION				ANIMAL NO.	NON-SURVIVORS		
	MINUTES POST-INJECTION								MINUTES POST-INJECTION		
	<u>0</u>	<u>+30</u>	<u>+120</u>	<u>2</u>	<u>3</u>	<u>4-6</u>	<u>7-8</u>		<u>0</u>	<u>+30</u>	<u>+120</u>
1	0	28	57	1	0	0	0	6	0	16	16
2	0	0	38	0	0	0	0	7	0	20	180
3	0	0	14	1	2	0	1	8	0	0	11
4	0	0	25	2	0	0	0	9	0	0	40
5	2	24	130	2	0	0	0	10	0	4	41
	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u>11</u>	<u>0</u>	<u>30</u>	<u>205</u>
MEAN	0.4	10.4	54.0	1.2	0.4	0	0.2	MEAN	0	11.7	82.2

HEMATOCRITS											
ANIMAL NO.	SURVIVORS			DAYS POST-INJECTION				ANIMAL NO.	NON-SURVIVORS		
	MINUTES POST-INJECTION								MINUTES POST-INJECTION		
	<u>0</u>	<u>+30</u>	<u>+120</u>	<u>2</u>	<u>3</u>	<u>4-6</u>	<u>7-8</u>		<u>0</u>	<u>+30</u>	<u>+120</u>
1	30	33	33.5	31.5	33.5	33	24.5	6	37	45	66.5
2	42	42	51	46.5	45.5	37.	38.5	7	35	44.5	54.5
3	40	40	40.5	40	39.5	34.5	33.5	8	36.5	38	45
4	35	39	43	35	34.5	32	28.5	9	32	38.5	59.5
5	43.5	43.5	46	41.5	---	---	---	10	32.5	44	59
	---	---	---	---	---	---	---	<u>11</u>	<u>30</u>	<u>30</u>	<u>40</u>
MEAN	38.1	39.5	42.8	38.9	38.3	34.3	31.3	MEAN	33.8	40.0	54.1

TABLE II
CHANGES IN HEART RATE, PO_2 , PCO_2 , AND RECTAL TEMPERATURE IN DOGS

ADMINISTERED LIVE E. COLI ORGANISMS (MEAN $\pm$ S.E.)*

	HEART RATE (beats/min)						
	MINUTES POST-INJECTION			2	DAYS POST-INJECTION		
	0	+30	+120		3	4-6	7,8
Non-Survivor	112 $\pm$ 7	96 $\pm$ 8	149 $\pm$ 19				
P =		NS†	0.05				
N =	17	16	10				
Survivor	109 $\pm$ 8	120 $\pm$ 16	155 $\pm$ 15	132 $\pm$ 8	114 $\pm$ 8	125 $\pm$ 8	116 $\pm$ 8
P =		NS	<0.05	NS	NS	NS	NS
N =	12	11	12	11	9	11	10

	ARTERIAL PO_2 (mm Hg)						
	MINUTES POST-INJECTION			2	DAYS POST-INJECTION		
	0	+30	+120		3	4-6	7,8
Non-Survivor	72 $\pm$ 1	86 $\pm$ 2	87 $\pm$ 5				
P =		<0.01	<0.01				
N =	12	12	12				
Survivor	75 $\pm$ 3	82 $\pm$ 3	83 $\pm$ 2	67 $\pm$ 2	72 $\pm$ 1	69 $\pm$ 1	67 $\pm$ 2
P =		<0.05	<0.05	NS	NS	0.05	0.05
N =	12	12	12	11	9	11	8

	ARTERIAL PCO_2 (mm Hg)						
	MINUTES POST-INJECTION			2	DAYS POST-INJECTION		
	0	+30	+120		3	4-6	7,8
Non-Survivor	31 $\pm$ 1	25 $\pm$ 2	22 $\pm$ 2				
P =		<0.01	<0.01				
N =	16	16	11				
Survivor	31 $\pm$ 1	29 $\pm$ 2	25 $\pm$ 1	32 $\pm$ 1	31 $\pm$ 1	31 $\pm$ 1	31 $\pm$ 1
P =		NS	<0.01	NS	NS	NS	NS
N =	12	12	12	11	9	11	8

	RECTAL TEMPERATURE ($^{\circ}C$)						
	MINUTES POST-INJECTION			2	DAYS POST-INJECTION		
	0	+30	+120		3	4-6	7,8
Non-Survivor	39.4 $\pm$ 0.2	39.4 $\pm$ 0.3	39.6 $\pm$ 0.5				
N =	17	17	10				
Survivor	39.1 $\pm$ 0.2	39.3 $\pm$ 0.3	39.9 $\pm$ 0.5	39.3 $\pm$ 0.1	39.0 $\pm$ 0.2	39.7 $\pm$ 0.3	39.6 $\pm$ 0.2
N =	12	12	10	11	9	9	9

*See text for statements of statistical significance.

†NS = Not Significant

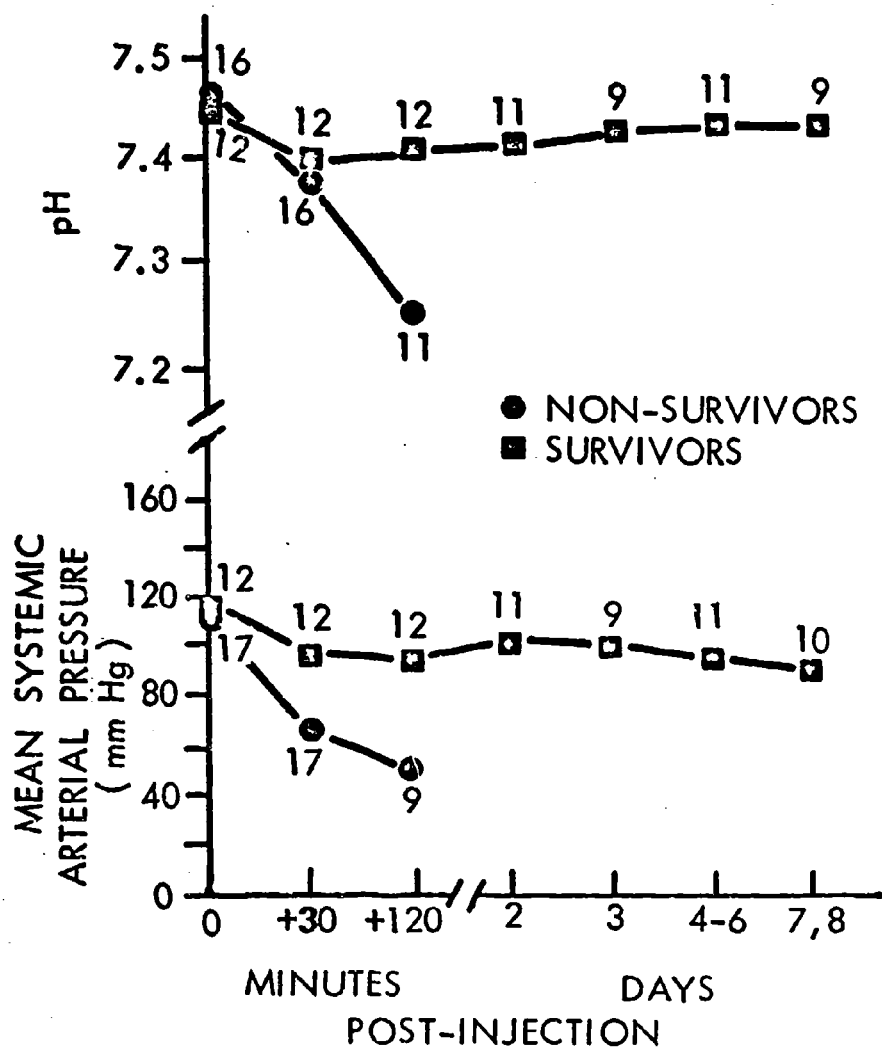


Figure 1

Effects of intravenous injection of living *E. coli* organisms on pH and mean systemic arterial pressure in dogs (approximate dose *E. coli*, 10^9 organisms/kg). (Mean values, total of 29 dogs) (injection at zero time). (See text for levels of statistical significance.)

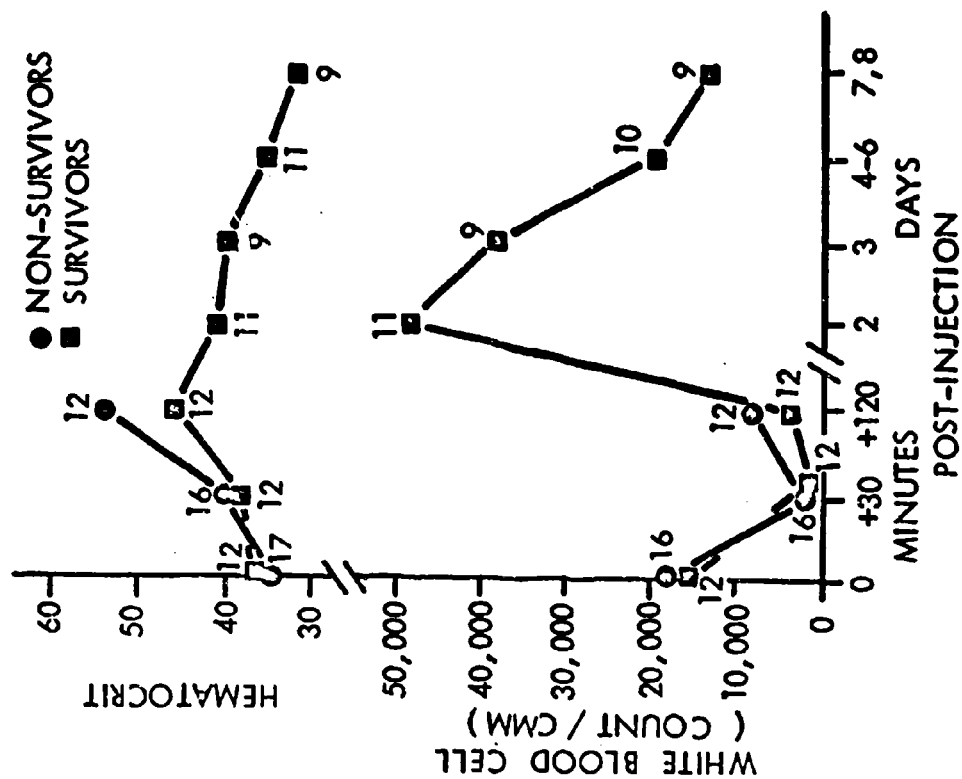


Figure 2

Effect of live *E. coli* on hematocrit and white blood cell counts in dogs (mean values, 20 dogs) (approximate dose *E. coli* 10^9 organisms/kg). (Injection at zero time.) (See text for statement of statistical significance.)

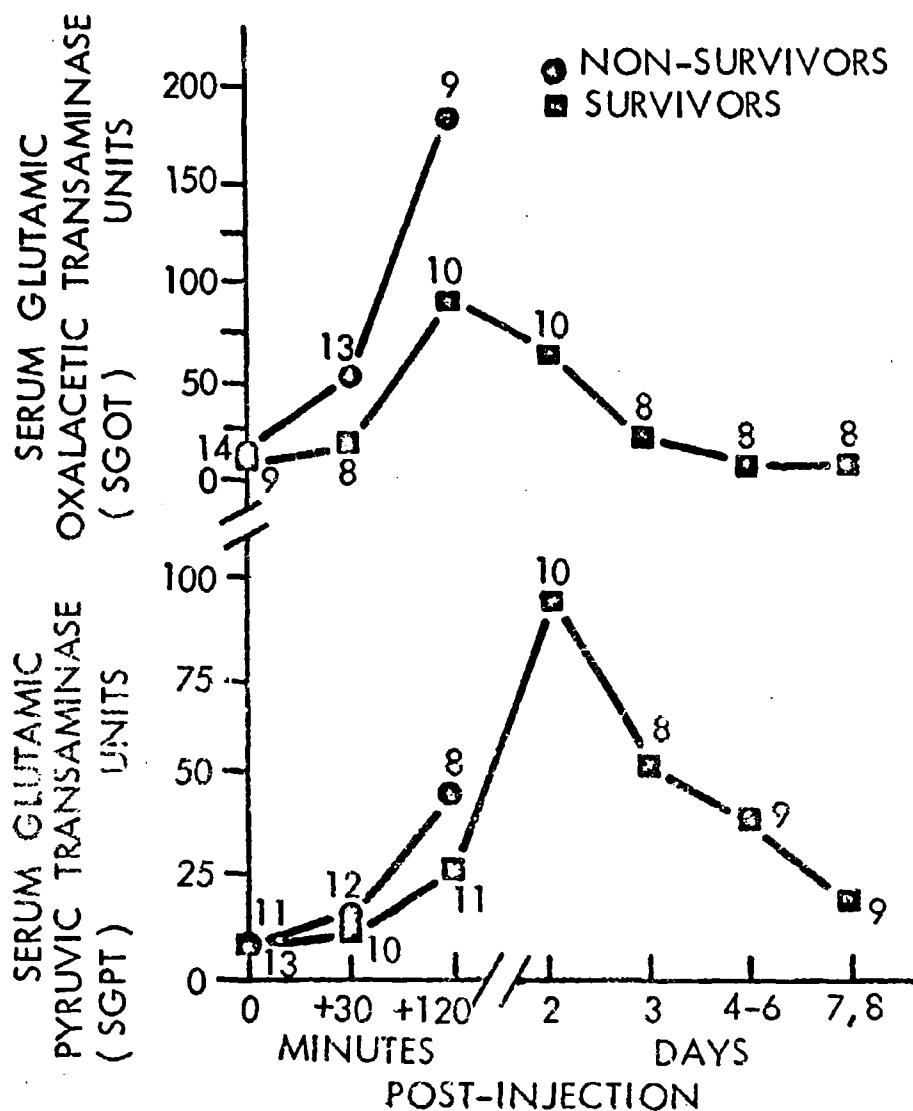


Figure 3

Changes in SGOT and SGPT in dogs induced by intravenous administration of live *E. coli* organisms (mean values, total of 28 dogs) (approximate dose *E. coli*, 10^9 organisms/kg). (Injection at zero time.) (See text for determinations of statistical significance.)

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13. ABSTRACT			
There is a growing recognition of the fact that no really effective, reproducible means of reversing the adverse effects of experimental endotoxin shock has been developed. A great number of claims for successful pre- or post-treatment have been described but have been rather uniformly unacceptable. There is a serious concern regarding the failure to develop an effective treatment of septic shock in clinical medicine, and the development of a more relevant animal shock model would greatly aid in elucidating clinical problems observed in the human patient. A primary purpose of research in this laboratory has been to determine the effects of intravenous injections of living <u>E. coli</u> organisms in dogs and monkeys and compare them with responses produced by endotoxin. In contrast to findings in dogs given endotoxin, results with live <u>E. coli</u> organism injection suggest that factors other than hepatic pooling and peripheral resistance in dogs administered live organisms correlates well with recent clinical reports of septic shock. Renal vasoconstriction, decreased renal blood flow and urine flow, regularly observed after lethal injections of endotoxin in dogs were replaced in half the animals given <u>E. coli</u> organisms with renal hyperemia, decreased renal resistance and increased urine flow. Other basic problems arise concerning the development of a more clinically relevant animal shock model and these have to do with the length of the experiments and the type of anesthesia administered. Objections may be raised that the great majority of experimental reports have been confined to a relatively short time for measurements in an animal depressed by an anesthetic. The purpose of the present study was to develop a more clinically applicable animal shock model by withholding anesthetics while administering live <u>E. coli</u> organisms at a dosage producing the degree of lethality seen in clinical			

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ABSTRACT (CONT)

medicine and to study the animals in an unrestrained condition during a substantially extended post-shock period. Results from these experiments are thought to be of promise in regard to more closely approximating the manifestations of clinical shock in man.