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Oklahoma University

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PATHOPHYSIOLOGICAL RESPONSE OF THE CANINE SPECIES IN *ESCHERICHIA COLI* ENDOTOXEMIA

James A. Shmidl and Donald D. Holmes

Technical Report No. 77
University of Oklahoma Health Sciences Center ONR Contract

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13. ABSTRACT

This study consisted of inducing septicemia in a group of 9 dogs by the i.v. injection of live E. coli organisms and comparing their pathophysiological alterations to a group of 6 control animals. The study was carried out over a period of 12 hrs. The physiological parameters of mean systemic arterial pressure, heart rate, hematocrit, pH, and serum glutamic oxalacetic transaminase were compared at the zero time, +4, +8 and +12 hour times. Studies of the lung for assay of pathological changes by use of the light microscope were completed at the end of the study, which also included the Mallory's Phosphotungstic Acid Hematoxylin technique for fibrin. The results from the study are: (1) Septicemic dogs had a highly significant drop in mean systemic arterial pressure at the +4 hr period which remained statistically significant at 12 hrs. (2) A marked increase in heart rate was observed at both 8 and 12 hrs in the septicemic dogs. (3) The increase in hematocrit for the septicemic dogs was highly significant at 4, 8 and 12 hrs. (4) The septicemid dogs had a notable drop in pH at the 4 hr time period. (5) The increases in serum glutamic oxalacetic transaminase were significant at 4 and 8 hrs for the septicemic dogs. (6) The differences in lactate readings were significant at 4, 8 and 12 hrs. (7) Using an arbitrary grading system, the hematoxylin and eosin stained lung tissues from the septicemic dogs were generally more severely affected than those of the controls. The pathological observations supporting this statement included a more severe thickening of the alveolar walls, more diffuse atelectasis and more severe intra-alveolar hemorrhage. (8) The Mallory's Phosphotungstic Acid Hematoxylin stained tissues failed to reveal any significant amounts of fibrin in either septicemic or control animals at the light microscopic level of observation. These findings indicate that induced septicemia in a 12 hr study model produces significant physiological alterations and observable morphological changes of the lung.

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Sepsis is surpassed in frequency only by hemorrhage and myocardial infarction as a cause of clinical shock (1). The most common offending organisms are gram-negative bacteria, although septic shock may occasionally occur after infection with fungi, rickettsiae, viruses and gram-positive bacteria. The infections with gram-negative bacilli are increasing in frequency and at present constitute the most commonly encountered life-threatening infections in patients in general hospitals. The mortality in patients in whom shock develops as a consequence of the gram-negative bacteremia ranges from 50-80% (1). There is a serious concern regarding the failure to develop an effective treatment in clinical medicine, and Hinshaw et al. (2) stated that there is increasing evidence that gram-negative septicemia remains a serious threat in surgical practice today.

The pathophysiological effects of short-term septicemia and shock have been well documented in previous studies (3,4,5,6). The purpose of this research was to induce septicemia in a group of dogs with the use of live Escherichia coli organisms and extend the study over a 12-hour period of time. This period of time was in contrast to most previous studies which were conducted over a shorter time span. The live organisms were used rather than purified endotoxin so as to more closely approximate the clinical entity as seen in the human patient. In summation, there was a correlation of hemodynamic measurements, clinical pathological alterations and morphological changes of the lung during a 12-hour study of septicemic animals.

MATERIALS AND METHODS

The Escherichia coli organism used in the experiment was prepared as previously described (2). Counts ranged between 2 - 4 X 10^9 organisms/ml and a concentration was established which was lethal in 50% of animals receiving 0.7 ml of inoculum per kg of body weight.

Fifteen adult mongrel dogs (Canis familiaris) with a weight range of 12.0 to 18.4 kg were used in this study. No conditioning procedures were performed on the experimental subjects. Each dog was intravenously anesthetized with 30 mg/kg of sodium pentobarbital. During each experiment, supplementary sodium pentobarbital was administered when the animal showed evidence of rousing. An endotracheal tube was inserted to simplify breathing.

The animals were then placed in a supine position. The femoral artery was located and cannulated with Silastic tubing. This tubing was advanced to the diaphragmatic level of the abdominal aorta so as to record systemic arterial pressure and heart rate. The femoral vein was also incised and Silastic tubing inserted. This tubing was used to make injections and to obtain venous blood samples.

By monitoring with a rectal thermometer, the body temperatures of the animals were maintained with small fluctuations by the use of heating pads.

Septicemia was produced in 9 dogs by the injection of the live E. coli organisms into a femoral venous catheter. The 6 control dogs received a comparable amount of sterile saline. These injections were completed in approximately a one-minute period of time.

Blood samples for determination of pH, hematocrit, serum glutamic oxalacetic transaminase and lactates were drawn from the venous catheter

prior to injection and at 4, 8, and 12 hours post-injection of organisms.

Physiological determinations for systemic arterial pressure and heart rate were recorded prior to injection and at hourly intervals until the 12-hour post-injection time. These readings were recorded on a four-channel Sanborn recorder.

After the 12-hour study was completed the animals were sacrified by rapid administration of concentrated sodium pentobarbital solution intravenously. Following euthanasia, an autopsy was performed and a section of the diaphragmatic lobe from the lung of each animal was obtained and fixed in 10% neutral buffered formalin. After proper fixation the tissues were processed and sectioned. This was followed with the routine hematoxylin and eosin stains as well as the Mallory's Phosphotungstic Acid Hematoxylin technique (7) for detection of fibrin.

RESULTS

Graph Figures 1, 2, 3, 4, and 6 show results from nine animals to which live E. coli organisms were administered. Also shown are the 6 animals which are designated as controls. The figures are graphed as change from control (zero time) values and are plotted at +4, +8 and +12 hour intervals.

Figure 1 presents changes in mean systemic arterial pressure (SAP) and is expressed in mm of Hg. At zero time all anesthetized animals (N = 15) had a mean SAP of 130 mm Hg. After 5 hours the difference in SAP between the control and septicemic animals was highly significant ($p < 0.01$). The mean drop in SAP was -2.83 for the control as compared to -25.00 for the animals given the E. coli. At the +8 hour recording, the differences in SAP were not significant, but were significant ($p < 0.05$) again at +12 hours. The mean changes from control time at the

12 hour interval were -32.14 for septicemic models and -13.83 for control animals.

Figure 2 illustrates changes in the heart rate and is expressed in beats per minute. At +4 hour readings the differences in heart rate between control and septicemic animals were not significant, but were significant ($p < 0.05$) at both +8 and +12 hour readings. At +8 hours the animals given E. coli had a mean increase in heart rate of +32.25 while the control animals had a decrease of -13.00. Twelve hour readings were +29.14 for septicemic animals and -16.00 for controls.

Illustration of the hematocrit (Hct) changes expressed in % packed cell volume are shown in Figure 3. The differences between control and septicemic animals in hematocrit readings were highly significant ($p < 0.01$) at all three recording periods. At the +4 hour reading the mean hematocrit increased +14.00 units for dogs given E. coli while it increased +2.67 units for control dogs. After 8 hours the mean reading was +13.13 for septicemic animals compared to +1.00 for controls. The 12 hour readings were +13.29 and +1.16, respectively.

Figure 4 presents the data collected as to pH changes in the venous blood. At +4 hours the readings were significantly different ($p < 0.05$) between septicemic and control animals as the mean pH dropped -.022 in septicemic animals and was raised +.03 in controls. At the 8 hour interval the pH of the septicemic animals regained its original zero hour reading +.01 while the control animals leveled to a +.01. At 12 hours the animals given E. coli continued to raise to +.02 and the controls to +.03. Thus the differences between the groups were not significant at either 8 or 12 hours in relation to pH readings.

In Figure 5, the changes in the release of the enzyme, serum glutamic oxalacetic transaminase (SGOT) are illustrated. At the 4 hour interval the differences in the release of SGOT were significant ($p < 0.05$) with a mean reading of +210.63 International Units for the septicemic animals and a -.82 reading for controls. After 8 hours the differences were still significant ($p < 0.05$) with the septicemic animals now having a mean reading of +320.05 and control animals showing -.55. The differences in SGOT failed to be significant at the 12 hour interval due to large individual variations in the experimental group.

Figure 6 presents changes in lactates and is expressed in mg %. At the zero hour the mean lactate readings for the control and septicemic animals were 11.64 and 11.13 mg %, respectively. The differences between control and septicemic animals in lactate readings were significant ($p < 0.05$) at all three recording periods. At the +4 hour reading the mean lactate reading increased to 19.31 for the dogs given E. coli while it decreased to 10.28 for the control animals. After 8 hours the lactates were 16.15 for the septicemic animals and 6.76 for the controls. The 12 hour readings were 13.68 and 5.84 respectively.

In Table 1, an arbitrary grading system is presented for the purpose of evaluating the H and E stained sections of lung tissue for pathological purposes. The lung tissues were obtained at the conclusion of the 12 hour study period and the evaluation made by light microscopy. The table presents four grades of changes with Grade 1 representing very mild pathology and being essentially normal. Grade 2 is for tissues presenting a slight thickening of alveolar walls, focal atelectasis, moderate congestion with some intra-alveolar hemorrhage and edema. Grade 3 lung tissue represents moderate thickening of alveolar walls, more diffuse

TABLE 1

LIGHT MICROSCOPE LUNG GRADING SYSTEM

Grade 1:	Very mild changes, essentially normal.
Grade 2:	Slight thickening of alveolar walls, focal atelectasis, moderate congestion, some intra-alveolar hemorrhage and edema.
Grade 3:	Moderate thickening of alveolar walls, more diffuse atelectasis, severe congestion, moderate intra-alveolar hemorrhage and edema.
Grade 4:	Severe thickening of alveolar walls, very diffuse atelectasis, severe congestion, intra-alveolar hemorrhage and edema.

atelectasis, severe congestion with moderate intra-alveolar hemorrhage and edema. Grade 4 is reserved for the severe pathological changes with severe thickening of alveolar walls, very diffuse atelectasis, severe congestion, hemorrhage and edema seen.

Table 2 presents the evaluation of the lung tissues of the experimental animals using Table 1 as the guideline for the pathological classification. Of the control animals the Grade of 2 is assigned to 4 animals representing slight pathological changes. The other two control animals are judged to be Grade 3 denoting moderate changes. Of the septicemic animals only one showed slight Grade 2 changes and 5 animals were classified as the moderate Grade 3. Three of the septicemic models were placed in the Grade 4 class representing very severe pathological changes.

All lung tissues were stained by the Mallory's Phosphotungstic Acid Hematoxylin technique and the observation for fibrin was negative except for Septicemic Dog Number 13. This one animal displayed a very minute amount of fibrin present and it is considered of no real significance due to the small quantity detected.

DISCUSSION

Physiological results from this study are in general agreement with previous reports (8,9,10,11). Mean systemic arterial pressure as reported in this experiment shows a highly significant hypotension which was continuing to drop at time of termination of study. This is in agreement with Hinshaw et al. (2) who described a decreasing mean systemic arterial pressure when using live organisms. Motsay et al. (8) attributed this hypotension to stagnation and shunting of blood. This present study reveals a marked increase ($p < 0.05$) in heart rate as did Buckerg et al.

TABLE 2

EVALUATION OF EXPERIMENTAL ANIMALS USING THE LIGHT MICROSCOPE LUNG GRADING SYSTEM

Control Dogs		Septicemic Dogs	
Experimental Number	Grade	Experimental Number	Grade
4	2	1	2
6	3	2	4
8	2	3	3
10	2	5	3
12	2	7	3
14	3	9	3
		11	4
		13	3
		15	4
Average	2.33	Average	3.22

(12) note a tachycardia in his 5-hour study with primates. Guenter et al. (9) did not have a significant increase in heart rate in a 4-hour monkey study. The increase in hematocrit was highly significant in this study and continued to be rising slightly at the termination. Hinshaw et al. (10) also observed a marked increase in hematocrit in his non-surviving dogs. The pH values derived from this study were significantly acidic at +4 hours, but then continued to be rising slightly at the time of sacrificing the models. Hinshaw et al. (10) found the pH to show significant decreases until death, but Blair (11) considered lactacidemia to be a much better indicator of degree of shock as he found the pH of many human patients to be normal as late as immediately before death. Extreme elevations in serum levels of glutamic oxalacetic transaminase were observed in this study and this was considered indicative of injury to the vital tissues of the body. These enzyme changes correlated well with Hinshaw's et al. (10) reports on his non-surviving dogs.

One factor which must be strongly considered in evaluating the physiological alterations is that of anesthetic effect. Soma (13) reports that the hemodynamic changes produced by pentobarbital are conflicting and thought due to experimental model and dose. In the dog he reported an initial fall in mean blood pressure followed by a rise to control or above in 30-60 min. Also he reported an increase in heart rate which remained elevated throughout the duration of anesthetic. These general effects of pentobarbital must be considered.

Considering the severity of the physiological parameters recorded in this study it would be assumed that with exceptions the majority of the experimental models were deteriorating and death would have been occurring if sacrificing had not been accomplished.

The morphological lung pathology changes observed in the septicemic animals were compatible with those reported by Pool et al. (5) in his primate study in which death occurred at 5-19 hours after injection of live organisms. Those findings consisted of intra-alveolar hemorrhage, thickening of alveolar walls and atelectasis. Findings of this present study were also similar to light microscopy results reported by Coalson et al. (6) in a 4-hour primate experiment. Those changes were extreme atelectasis, focal intra-alveolar hemorrhage, pulmonary edema and very cellular alveolar walls with no fibrin present. Groves et al. (14) had reported fibrin observed with the light microscope in his 24-hour study, but none at the 6 hour level. This present study showed a minute amount of fibrin in one septicemic animal at the end of 12 hours so there is a possibility of being in agreement with Groves et al. (14) if longer study periods were undertaken. The moderate to severe congestion observed in control and septicemic animals is attributed to immobilization and positioning due to prolonged anesthesia. In conclusion, the lung changes observed in this study would be in agreement with Kux and Massion (15) who state that respiratory insufficiency is recognized with increasing frequency as an important cause of death in patients suffering from septic shock.

SUMMARY

This study consisted of inducing septicemia in a group of 9 dogs by the intravenous injection of live E. coli organisms and comparing their pathophysiological alterations to a group of 6 control animals. The study was carried out over a period of 12 hours. The physiological parameters of mean systemic arterial pressure, heart rate, hematocrit, pH, and serum glutamic oxalacetic transaminase were compared at the

zero time, +4, +8 and +12 hour times. Studies of the lung for assay of pathological changes by use of the light microscope were completed at the end of the study, which also included the Mallory's Phosphotungstic Acid Hematoxylin technique for fibrin. The results from the study are:

1. Septicemic dogs had a highly significant drop in mean systemic arterial pressure at the +4 hour period which remained statistically significant at 12 hours.

2. A marked increase in heart rate ($p < 0.05$) was observed at both 8 and 12 hours in the septicemic dogs.

3. The increase in hematocrit for the septicemic dogs was highly significant at 4, 8 and 12 hours.

4. The septicemic dogs had a notable drop in pH ($p < 0.05$) at the 4 hour time period.

5. The increases in serum glutamic oxalacetic transaminase were significant at 4 and 8 hours for the septicemic dogs.

6. The differences in lactate readings were significant at 4, 8 and 12 hours.

7. Using an arbitrary grading system, the hematoxylin and eosin stained lung tissues from the septicemic dogs were generally more severely affected than those of the controls. The pathological observations supporting this statement included a more severe thickening of the alveolar walls, more diffuse atelectasis and more severe intra-alveolar hemorrhage.

8. The Mallory's Phosphotungstic Acid Hematoxylin stained tissues failed to reveal any significant amounts of fibrin in either septicemic or control animals at the light microscopic level of observation.

These findings indicate that induced septicemia in a 12 hour study model produces significant physiological alterations and observable morphological changes of the lung.

9. Guenter, C. A., Fiorica, V. and Hinshaw, L. B. Cardiorespiratory and metabolic responses to live E. coli and endotoxin in the monkey. J. Appl. Physiol. 26: 780, 1969.

10. Hinshaw, L. B., Mathis, M. C., Nanaeto, J. A. and Holmes, D. D. Recovery patterns and lethal manifestations of live E. coli organism shock. J. Trauma 10: 787, 1970.

11. Blair, E. Acid-base balance in bacteremic shock. Arch. Int. Med. 127: 731, 1971.

12. Buckerg, G., Cohn, J. and Darling, C. Escherichia coli bacteremic shock in conscious baboons. Ann. Surg. 173: 122, 1971.

13. Soma, L. R. Textbook of Veterinary Anesthesia. 1st Edition, Williams and Wilkins, Baltimore, Maryland, 1971.

14. Groves, A., Griffiths, J., Leung, F. and Naiman, S. Fibrin thrombi in the pulmonary microcirculation of dogs with gram-negative bacteremia. Surg. Gynec. Obstet. 134: 433, 1972.

15. Kux, M., and Massion, W. H. Effects of E. coli organisms on hemodynamics of the dog lung. Ann. Surg. 173: 116, 1971.

FIGURE LEGENDS

Figure 1. Effects of intravenous injection of live E. coli organisms on mean systemic arterial pressure (SAP). Expressed in units of mm of Hg and graphed as change from control (zero time) values.

Figure 2. Effects of intravenous injection of live E. coli organisms on heart rate. Expressed in beats per minute and graphed as change from control (zero time) values.

Figure 3. Effects of intravenous injection of live E. coli organisms on hematocrit (Hct). Expressed in per cent packed cell volume and graphed as change from control (zero time) values.

Figure 4. Effects of intravenous injection of live E. coli organisms on blood pH. Graphed as change from control (zero time) values.

Figure 5. Effects of intravenous injection of live E. coli organisms on serum glutamic oxalacetic transaminase (SGOT). Expressed in international units and graphed as change from control (zero time) values.

Figure 6. Effects of intravenous injection of live E. coli organisms on lactates. Expressed in mg per cent and graphed as change from control (zero time) values.

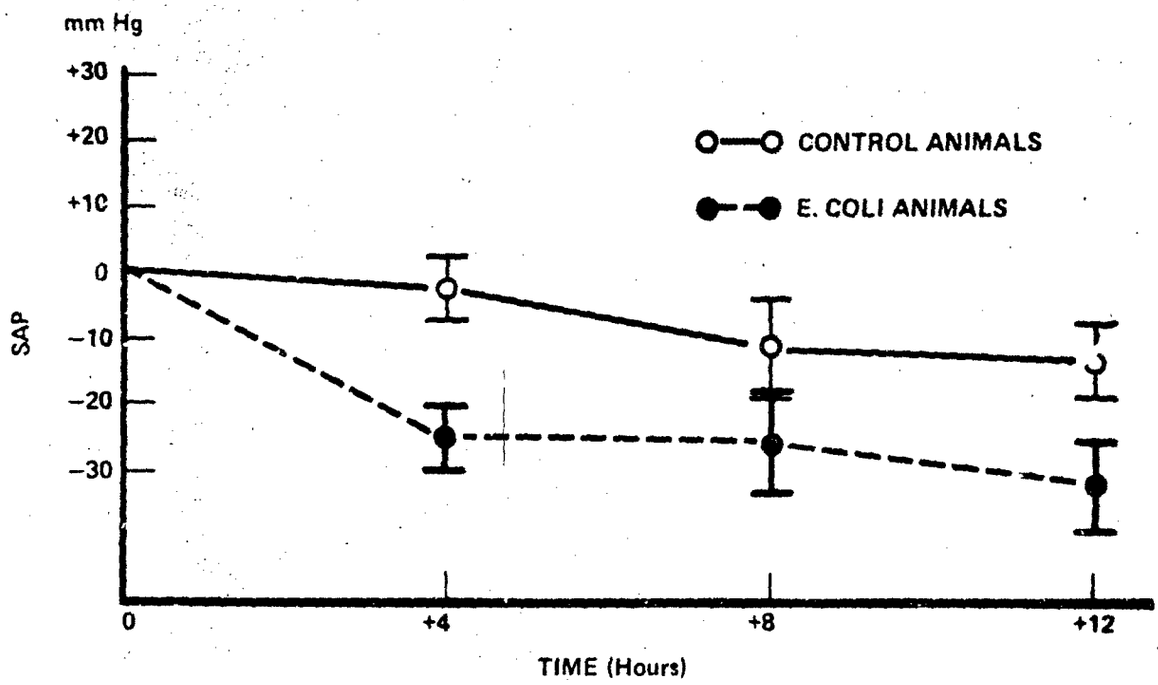
mm Hg
+30
+20
+10
0
−10
−20
−30
SAP
CONTROL ANIMALS
E. COLI ANIMALS
0
+4
+8
+12
TIME (Hours)

FIGURE 1

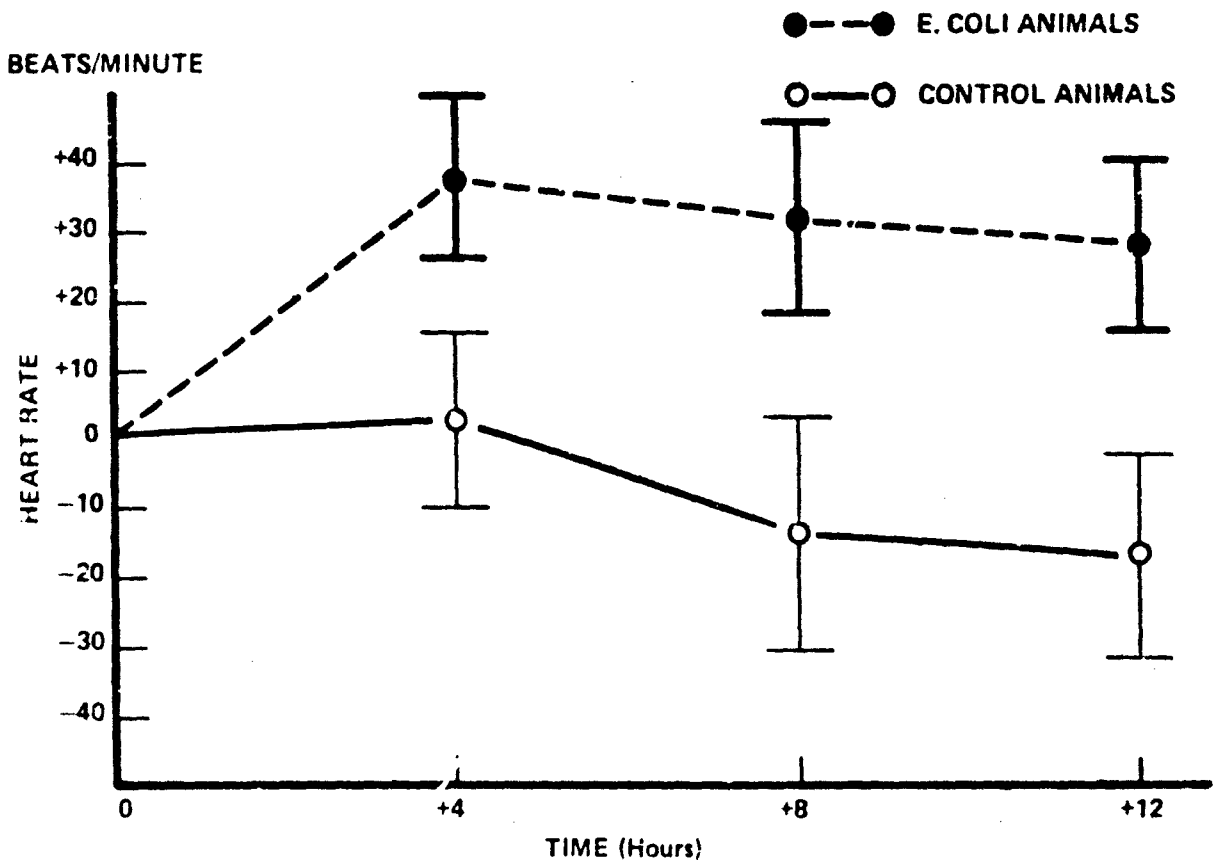
E. COLI ANIMALS
CONTROL ANIMALS
BEATS/MINUTE
HEART RATE
+40
+30
+20
+10
0
−10
−20
−30
−40
0
+4
+8
+12
TIME (Hours)

FIGURE 2

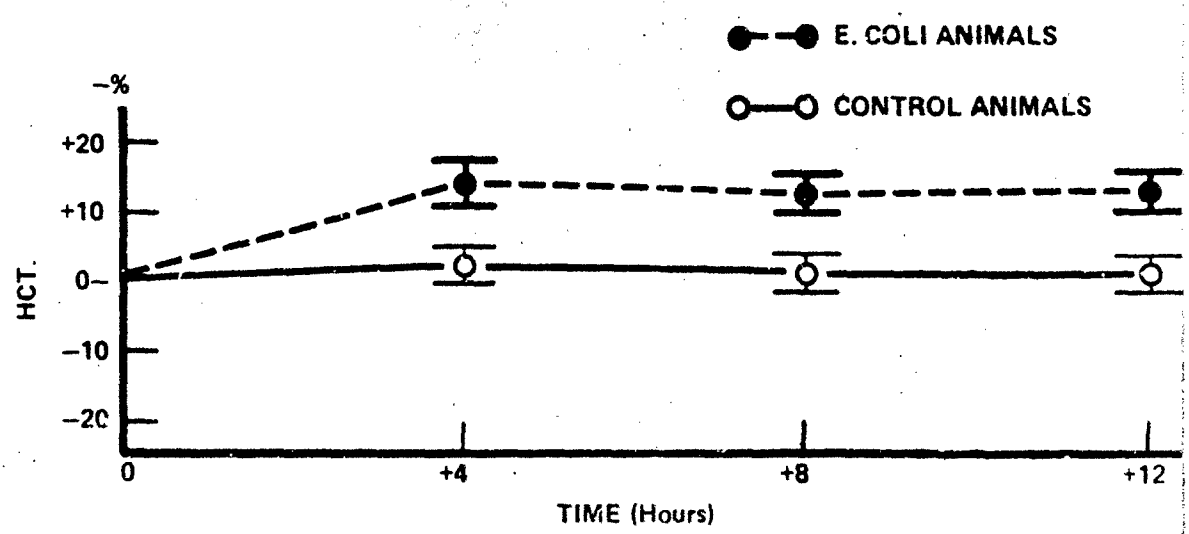
E. COLI ANIMALS
CONTROL ANIMALS
-%
+20
+10
0
-10
-20
HCT.
0
+4
+8
+12
TIME (Hours)

FIGURE 3

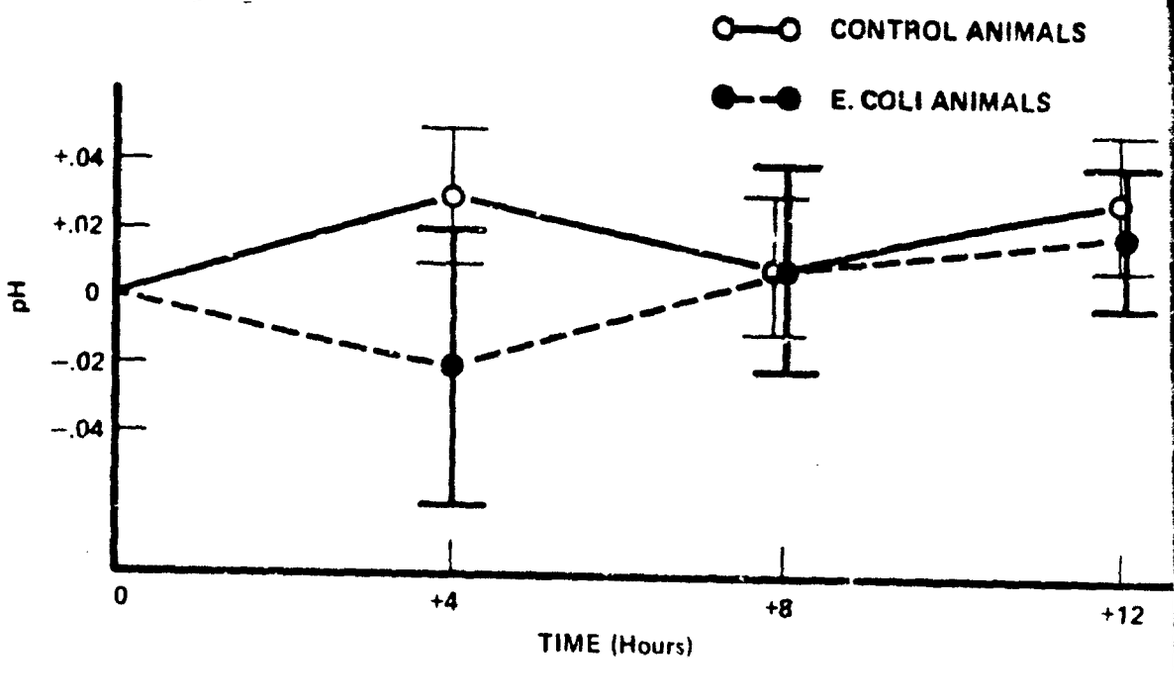
CONTROL ANIMALS
E. COLI ANIMALS
+.04
+.02
0
-.02
-.04
pH
0
+4
+8
+12
TIME (Hours)

FIGURE 4

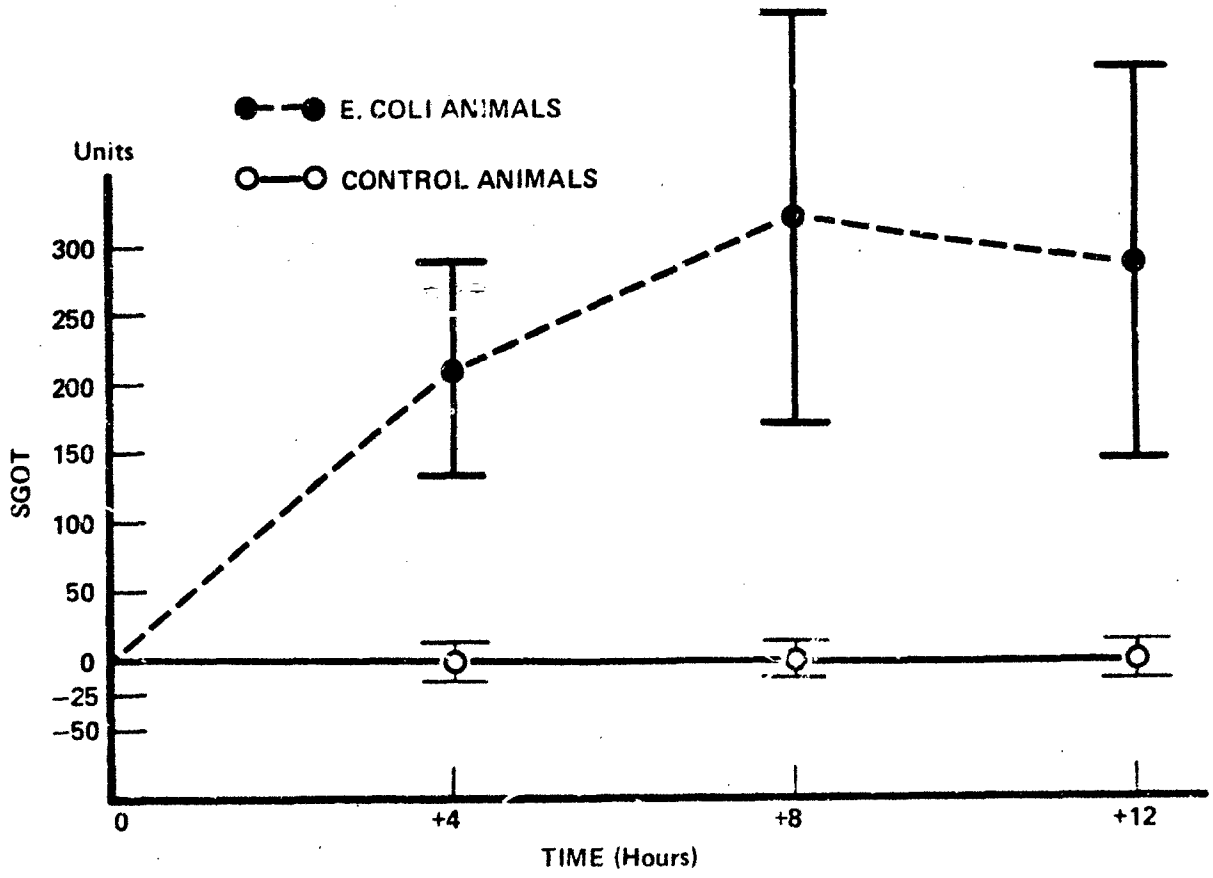
E. COLI ANIMALS
CONTROL ANIMALS
Units
SGOT
300
250
200
150
100
50
0
−25
−50
0
+4
+8
+12
TIME (Hours)

FIGURE 5

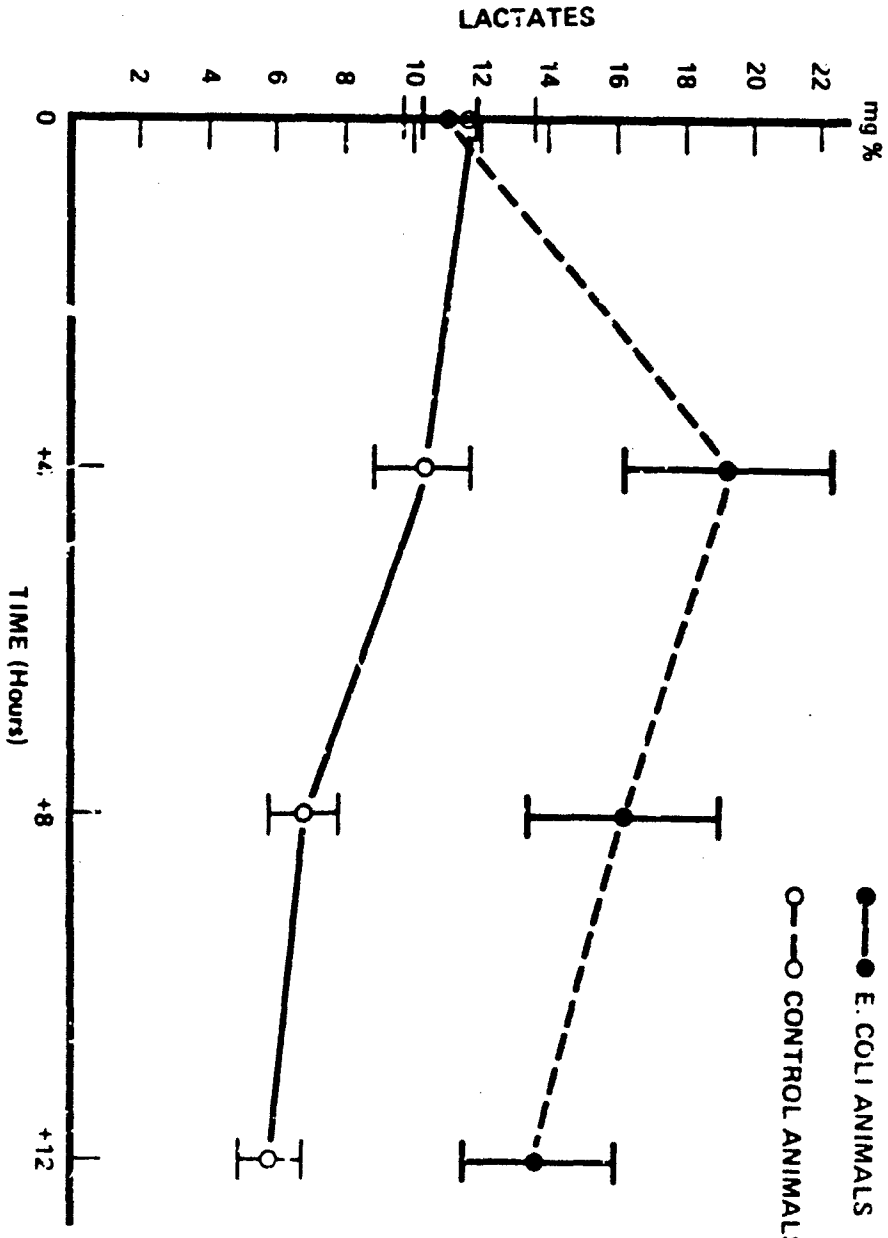
LACTATES
mg %
22
20
18
16
14
12
10
8
6
4
2
0
+4
+8
+12
TIME (Hours)
E. COLI ANIMALS
CONTROL ANIMALS

FIGURE 6