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(6) BACTERIAL INVASION OF THE LIVER DURING THE COURSE OF HEATSTROKE IN THE DOG HEATSTROKE MODEL,

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Abstract

The question of septicemia and endotoxemia as an etiologic or complicating factor of heatstroke has been alluded to in clinical settings but not explored under experimental conditions. To this end, using an anesthetized dog heatstroke model, liver tissue sections were taken under aseptic conditions for microbial culture at the death or sacrifice of the heatstroked animal. Seventy-eight percent contained species of clostridium, lactobacillus, alpha streptococcus, staphylococcus, or E. coli. Microbial cultures of liver tissue from conditioned* unheated dogs demonstrated a 50% incidence of microbial growth, all of which were clostridium. The remaining liver tissues of both heated and unheated animals, and samples of portal, hepatic, vena caval, and jugular blood taken from a series of animals at intervals during the heating and subsequent monitoring periods did not demonstrate microbial growth.

* All dogs were conditioned as laboratory animals before arrival at this Institute by Flow Research Animals of Dublin, Virginia. Details furnished upon request.

A

Introduction

Heatstroke is one of the oldest of recorded diseases, as evidenced in Hebrew, Roman, and Chinese literature (1,2,3). It remains a concern for those planning medical care for large population movements such as the 1968 pilgrimage to Mecca (4) or for military and athletic training (5,6).

The flow of clinical events in heatstroke parallels those found in shock (7,8). Septicemia or endotoxemia has been implicated as a contributing agent in a variety of shock states (9,10,11). The occurrence of septicemia or endotoxemia with heatstroke has been demonstrated in a limited number of isolated clinical cases (12,13). However, there is no data in the experimental literature linking sepsis or endotoxemia with the etiology of heatstroke. This study attempts to define the patterns of sepsis occurring with experimental heatstroke in anesthetized mongrel dogs.

Materials and Methods

Utilizing an anesthetized dog heatstroke model, described elsewhere (14), a total of 24 anesthetized mongrel dogs (Nembutal®, 50 mgm/kgm, i.v.) were heated with a water blanket over a one to one-and-one-half hour period to a rectal temperature range of 43.0°C - 43.4°C. This has been described as the critical temperature range for inducing heatstroke in dogs (14,15). Under anesthesia, each heatstroked dog was allowed to passively cool at room air temperature (27°C) until death, or until 18 hours had elapsed from the point of maximum rectal temperature at which time surviving dogs were sacrificed with i.v. KCl. All animals included in this study lived a minimum of 2 hours post heatstroke.

After induction of anesthesia, but prior to heating, all dogs were instrumented with a thermister probe (YSI) inserted per rectum 10 to 12 cm. Via an aseptically prepared and draped cutdown the external jugular vein was isolated and ligated and an Intracath® was inserted to the level of the right atrium.

Nineteen of the twenty-four dogs experienced no other instrumentation or manipulation until death or sacrifice, when 1 cc of jugular blood was obtained for culture. The abdomens were then shaved, prepared for autopsy using a Betadine and alcohol scrub, and covered with a sterile surgical drape. Under sterile operating room conditions a ventral midline incision was made and approximately 3 grams of right liver lobe were removed for culture.

One week prior to utilization, the remaining five dogs had chronic catheters aseptically placed in the hepatic vein, portal vein, and inferior vena cava via a large midline incision. Each catheter was then exteriorized through the right flank, heparinized, tied off, and buried subcutaneiously until the day of the study. The day of utilization, after anesthesia and normal instrumentation were achieved, the catheters were aseptically exteriorized via small percutaneous incisions. None of these animals received post-operative antibiotic therapy for a minimum of one week prior to heatstroke. 1 cc blood samples were obtained from all catheters after anesthesia administration but before initiation of heating, at 42°C rectal temperature, at maximum rectal temperature, at 5 minute intervals for one hour, and every 1/2 hour thereafter until the death or sacrifice of the animal. No liver samples were obtained for microbial culture from this group.

An additional 6 conditioned unheated dogs were anesthetized with Nembutal and sacrificed with i.v. KCl. Liver and jugular blood samples were obtained for culture as described above.

All blood and tissue samples were incubated at 37°C for 72 hours under anaerobic conditions using thioglycolate medium in a Gas-Pac® anaerobic system and under aerobic conditions using brain-heart infusion broth. Transfers from broths were made to brain-heart infusion agar plates under both aerobic and anaerobic (Gas-Pac®) conditions. Isolates

were then transferred to B.H.I. agar slants and sent to Leary Laboratories, Inc. of Boston, Massachusetts, for identification. Results from Leary were later verified using an API 20 enteric system (Fischer Products).

Results

Of liver tissue obtained for microbial culture from 19 heatstroked dogs, 15 (78%) were infected with species of clostridium, lactobacillus, alpha streptococcus, staphylococcus, or E. coli (Fig.1). E. coli was the predominent organism being present in 66% of the liver samples demonstrating microbial growth. Incidences of multiple microbe isolation were common (50%). Of the liver tissue obtained for microbial culture from 6 conditioned, unheated dogs, 3 samples (50%) demonstrated microbial growth and these were entirely of clostridium (Fig. 2). The incidence of isolation of clostridium from heated and unheated liver samples was the same (50%).

Blood samples from hepatic, portal, vena caval and jugular veins from a series of five heatstroked dogs invariably failed to demonstrate microbial growth.

Discussion

There appears to be general agreement among investigators that bacteria may be cultured from normal canine liver (16,17). The data presented are consistent with these observations, demonstrating a 50% incidence of clostridia isolates from unheated canine livers. The predominent species shift of hepatic flora to E. coli with heat stress

suggests contamination with gut organisms via the portal system, since other models demonstrate portal bacteremia and/or endotoxemia with stress (13,18,19). However blood taken from hepatic, vena caval, and portal veins was sterile during and after the heating process and up to the death or sacrifice of the animal. If in fact this shift represents an invasive process and the portal vein is not the prime vehicle for transport of gut bacteria to the liver with heat stress, then alternate means of dissemination such as transperitoneal migration must be considered. Regardless of the specific route by which bacteria gain access to the liver, the occurrence of gram negative hepatic sepsis raises the question of gram negative or endotoxic shock as contributing factors in the etiology of heatstroke, particularly since antibiotic and cathartic therapy administered prior to heatstroke in dogs significantly increases survival rates (20). Additional models should be sought for evaluation of this question since the inherent characteristics of canine splanchnic circulation render the dog particularly susceptable to endotoxemia (21,22).

As an additional consideration, it is well documented that heat stress results in hepatic dysfunction (23,24). The combination of heat stress and hepatic sepsis could conceivably decrease the liver's capacity to reduce the variety of toxic factors which may occur in shock (25,26,27), contributing to the spiral of events which frequently results in heatstroke deaths.

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Figure Legend

Fig. 1. This table indicates the organisms isolated from the livers of the fifteen heatstroked dogs which were positive upon culture.

Fig. 2. This table demonstrates any organisms isolated from the livers of the control group of healthy unheated dogs.

"In conducting the research described in this report, the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care', as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences - National Research Council."

"The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense."

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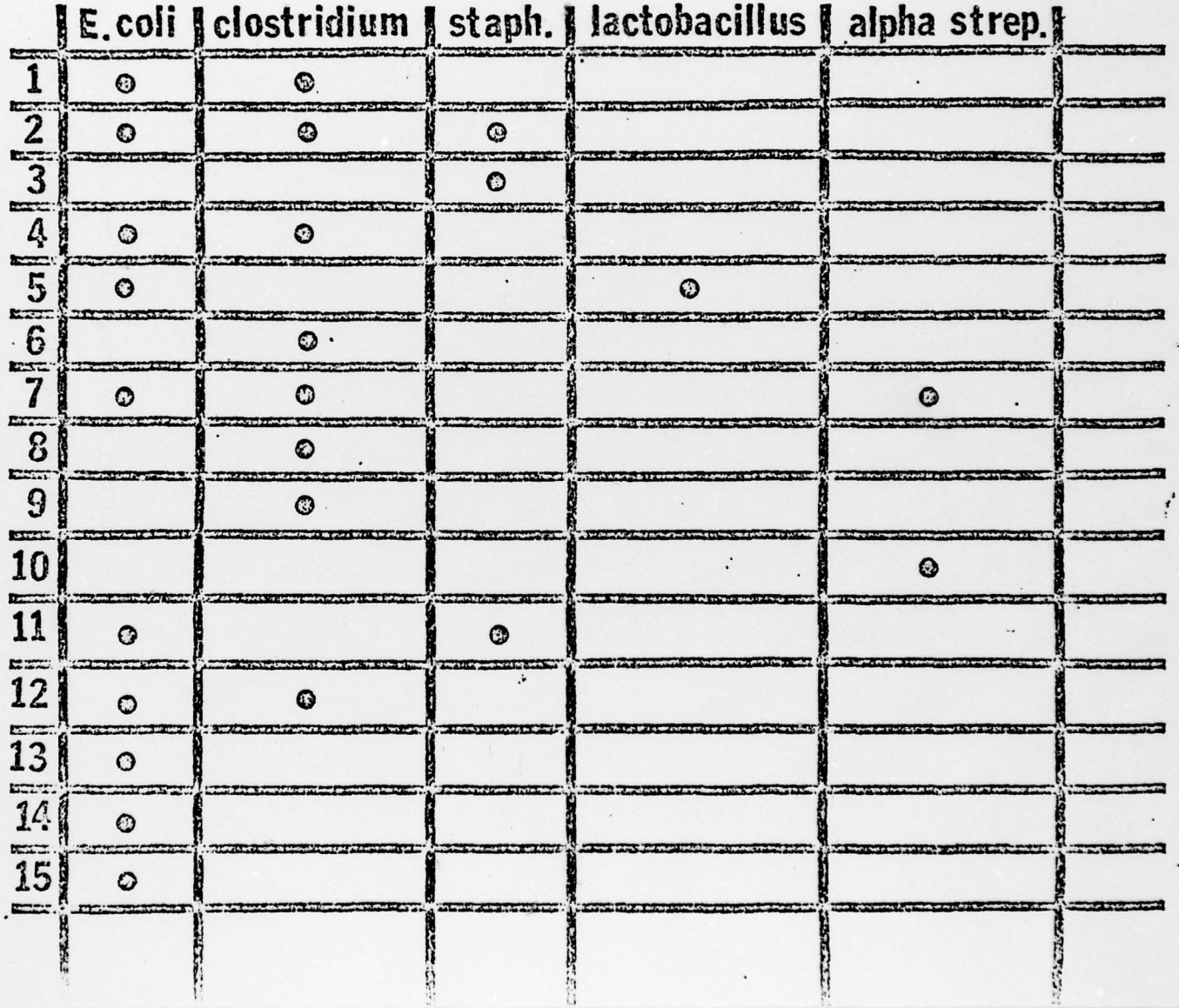
HEATED DOGS



DOG NUMBER

Fig 1

UNHEATED DOGS

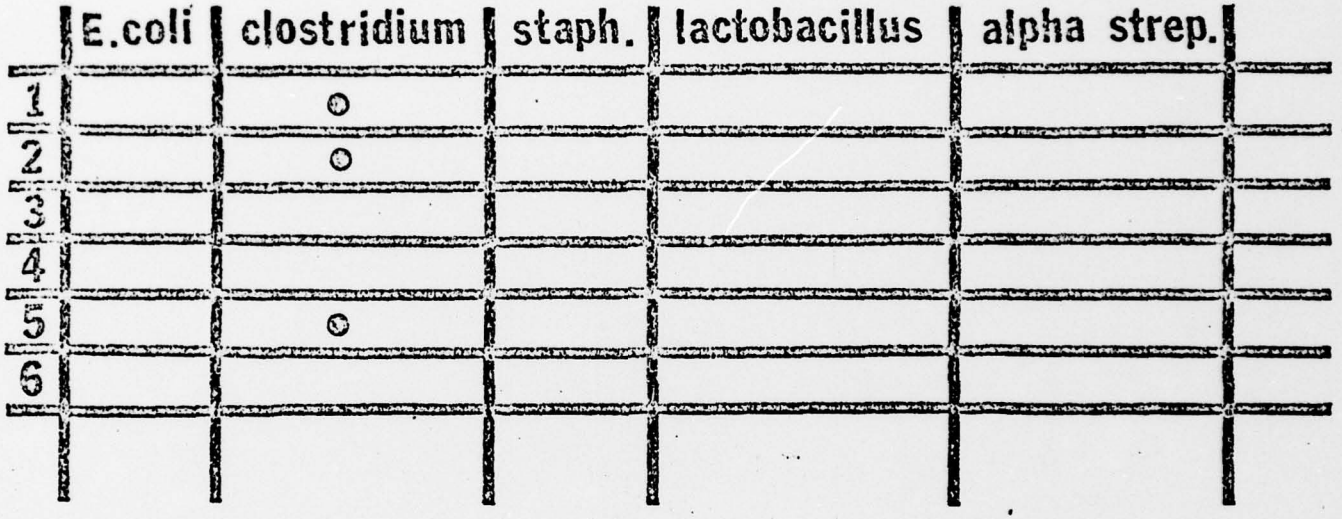


Fig 2

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