EFFECTS OF THERMAL STRESS AND EPINEPHRINE ON UTERINE BLOOD FLOW IN EWES

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SUMMARY

Four multiparous ovariectomized ewes, fitted with electromagnetic blood flow transducers around one mid-uterine artery, were utilized in two latin square design experiments to evaluate interrelationships of thermal stress and epinephrine on an increase in uterine blood flow (UBF) induced by estradiol. Estradiol (20 µg) was injected into the femoral vein and the subsequent induced increase in UBF was evaluated in response to thermal stress (21 vs 32 °C; Experiment I) and the interaction of thermal stress with epinephrine (Experiment II). Thermal stress reduced the estradiol-induced increase in UBF (ml/min) by 37% and a combination of thermal stress plus epinephrine infusion (.20 µg/kg body weight/min for 150 min) reduced UBF by 55%. Uterine blood flow is sensitive both to thermal stress and epinephrine.

(Key Words: Sheep, Uterine Blood Flow, Thermal Stress, Estradiol, Epinephrine.)

INTRODUCTION

It is well documented that thermal stress is detrimental to reproductive processes in domestic animals (Hafez, 1965; Viñicent, 1972; Thatcher, 1974). Although thermal stress has been associated with changes in reproductive behavior (Branton et al., 1957; Gängwar et al., 1965), increased temperature of the reproductive tract (Burfening and Ulberg, 1968; Gwazdauskas et al., 1973) and altered hormonal balance (Madan and Johnson, 1973; Miller and Alliston, 1973; Gwazdauskas et al., 1974b; Stott and Wiersma, 1974), there still is no clear understanding of the mechanisms by which thermal stress exerts its negative effects on fertility in domestic animals. One possibility is a decrease in blood flow to the reproductive tract which prejudices zygote development in its early stages (Alliston and Ulberg, 1961; Dutt, 1963). Uterine blood flow (UBF) is a major dissipator of uterine metabolic heat (Abrams et al., 1971; Gwazdauskas et al., 1974a), and a source of nutrients, oxygen and water for the developing embryo (Senger et al., 1967; Bazer et al., 1969; Barron, 1970). Estrogens and progesterone appear to be the principal hormones associated with regulation of UBF (Markee, 1940; Huckabee et al., 1970; Greiss and Anderson, 1970; Killam et al., 1973; Caton et al., 1974). Gwazdauskas (1974) suggested that uterine blood flow may be reduced during thermal stress for uterine temperature increased at a greater rate than arterial blood temperature during periods of thermal stress. Leduc (1972) observed a 46% reduction in placental blood flow in rabbits which was associated with an increase in number of runts and dead fetuses during the warm summer (27 °C) in England. Recently, Oakes et al. (1976) detected a decrease in UBF (25 to 48%) in response to induced hyperthermia of pregnant ewes. Associated with acute thermal stress under environmentally controlled conditions is an increase in blood plasma catecholamines of cattle (Alvarez and Johnson, 1973). Several investigators have reported that epinephrine, due to its vasoconstrictor effects, reduces uterine blood flow (Robson and Schild, 1938; Greiss, 1963; Abrams et al., 1971; Leduc, 1972; Greiss, 1972; Barton et al., 1974; Rosenfeld et al., 1976).

Objectives of the present experiments were...
to study effects of thermal stress and epinephrine infusion on the increase in UBF that followed an intravenous injection of estradiol-17β (E₂) into ovariectomized ewes.

**EXPERIMENTAL PROCEDURE**

An electromagnetic flow meter (Narcomatic Model RT-500, Narco Bio-Systems Inc.), receiving an electrical input from a flow transducer probe (C and C Associates) chronically implanted around one mid-uterine artery at random, was used to measure UBF of ewes. Continuous recordings were monitored with a physiograph (Desk Model DMP-4B, Narco Bio-Systems Inc.). The principle of operation of the flow transducer probe is based on Faraday's law of magnetic induction (Kramer et al., 1963).

Four blood flow transducers (internal diameters of 2.5, 3.0, 3.0 and 3.5 mm) were calibrated in vitro before being implanted into four ewes. The in vitro calibration system was carried out by infusing ewe blood through a segment of artery from a ewe with the blood flow transducer around the vessel and submerged in isotonic saline (.9% NaCl). Rate of flow was controlled by air pressure, and direct measurement of blood flow into a graduated cylinder was compared with the flowmeter display. A calibration factor for each probe was selected based on a linear response over a range in flow from 5 to 500 ml/min. The calibration factor for each probe was set on the flowmeter for each recording period in subsequent in vivo experiments.

The relationship between blood flow (ml/min as measured with stopwatch and graduated cylinder; X) and electromagnetic flowmeter display (ml/min; Y) was linear. Overall differences between the two measurements was less than 3% (table 1). Killam et al. (1973) utilizing a similar in vitro system to calibrate blood flow transducers observed a linear response with a difference of less than 10% between actual flow and recorded transducer flow. Several investigators have used electromagnetic flowmeter systems successfully for chronic UBF measurements (Assali et al., 1959; Greiss and Anderson, 1969; Anderson and Hackshaw, 1974; Oakes et al., 1976).

Four cycling multiparous mixed-breed ewes, after several days of adaptation to the laboratory environment, were ovariectomized and prepared with a blood flow transducer and catheters. Feed but not water was withheld 24 hr prior to surgery and ewes were anesthetized with oxygen and methoxyflurane (Metafane, Pitman-Moore). The reproductive tract was exposed through a midline incision extending approximately 15 cm forward from the anterior margin of the mammary gland. Both ovaries were removed. A small incision was made into the anterior leaf of the broad ligament parallel to the middle uterine artery. A segment of this artery, proximal to its first bifurcation, was exposed and the adventitia removed from a 10 mm segment. The flow transducer head was placed around the artery and fixed within the broad ligament with a series of silk sutures (#1; Sutupak, Ethicon, Inc.).

Polyvinyl catheters were introduced into the femoral artery and vein, and they were threaded cephalad a distance sufficient to bring their tips into a common iliac artery and inferior vena cava, respectively (Meschia et al., 1959). The free end-wires of the flow transducer and polyvinyl catheters were exteriorized through the abdominal wall of the flank area and placed into a canvas pouch attached to the ewe with two steel pins. Following surgery ewes received three daily intramuscular injections of penicillin-streptomycin (5 cc per day, Combiotic; Pfizer). Experiments were started on day four after surgery at which time the animals appeared to have recovered from surgical stress.

**TABLE 1. CALIBRATION IN VITRO OF BLOOD FLOW TRANSDUCERS**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>2.5</th>
<th>3.0</th>
<th>3.0</th>
<th>3.5</th>
</tr>
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<tr>
<td>Regression coefficient</td>
<td>1.01</td>
<td>1.05</td>
<td>.98</td>
<td>1.01</td>
</tr>
<tr>
<td>Intercept</td>
<td>.02</td>
<td>-1.55</td>
<td>2.03</td>
<td>-12</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>.99</td>
<td>.99</td>
<td>.99</td>
<td>.99</td>
</tr>
<tr>
<td>Percent difference</td>
<td>1.00</td>
<td>3.00</td>
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<td>1.00</td>
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<tr>
<td>Calibration factor</td>
<td>105</td>
<td>160</td>
<td>140</td>
<td>170</td>
</tr>
</tbody>
</table>

*Regression of electromagnetic flowmeter display (Y; ml/min) on blood flow (X; ml/min).*
since stable recordings of UBF were being obtained. During experimental days ewes were kept in small wooden crates (1.18 x .4 x .75 m) which permitted ewes to stand or lay down. When not on experiment they were maintained in a larger room within the laboratory. Feed (Purina Chops and pangola grass hay) and water were offered ad libitum.

In Experiment I, four treatments were administered: I = ewes maintained in the laboratory from 2400 to 1730 hr at 21.8 ± .34 C and 56.9 ± 1.1 relative humidity (RH); II = ewes in the laboratory from 2400 to 1100 hr (21.8 C), then in an environmental chamber (Forma Scientific, Inc.) until 1730 hr (32.5 C); III = ewes in the chamber (32.5 C) from 2400 to 1100 hr, then in the laboratory until 1730 hr (21.8 C); IV = ewes in the chamber from 2400 to 1730 hr at 32.5 ± 1.1 C and 78.3 ± .32% RH. Ewes were assigned to treatments in a 4 x 4 latin square design balanced for residual effects (table 2). Each ewe received one of the four treatments on each of 4 experimental days. Consequently, all ewes and treatments were represented on each experiment day. A recovery day was allowed between each experimental day. Uterine blood flow was recorded continuously from 0800 to 1730 hr, and 20 μg of estradiol (Progynon, Schering Corp., Bloomfield, NJ) were injected through the polyvinyl catheter into the femoral vein at 0900 hr to increase uterine blood flow. Estradiol was prepared fresh each day by dilution from the parent suspension in which 20 μg were dissolved in 2 ml volume of 10% ethanol and 90% isotonic saline. Rectal temperatures and respiration rates were measured at 0800 and 1800 hour. Rationale of the intermittent thermal stresses (II and III) was to determine if temperature changes altered UBF increases induced by E₂ during the early response phase (III) or during the peak and maintenance phases (II), in contrast to constant temperature exposures during the experimental period (I and IV).

The same ewes were utilized in Experiment II, but only after 12 days had elapsed to avoid residual effects from estradiol given in Experiment I. In Experiment II ewes were maintained either in the laboratory (21.7 ± .53 C; 50.6 ± 3% RH) or in the environmental chamber (32.4 ± 27 C; 82.4 ± .97% RH) from 2400 to 1230 hr and received an injection of estradiol (20 μg) into the femoral vein at 0630 hr. In the same design as Experiment I, ewes received the following four treatments: A = ewes maintained in the laboratory (21.7 C) and infused for 150 min with isotonic saline; B = ewes maintained in the laboratory and infused for 150 min with epinephrine (.20 μg/kg/body weight/min); C = ewes maintained in the environmental chamber (32.4 C) and infused for 150 min with isotonic saline; D = ewes maintained in the chamber and infused for 150 min with epinephrine. Saline and epinephrine infusions began 45 min after estradiol (E₂) injection or at 0715 hr, and UBF was recorded continuously beginning at 0615 hour. Epinephrine (adrenaline chloride 1:1000; Parke Davis) or .9% NaCl were infused into the femoral vein catheter with a Harvard infusion pump (model 600-954) at an infusion rate of .051 ml/min. Epinephrine was prepared fresh each day at a concentration of 4 μg/ml in sterile nonpyrogenic saline (.9% NaCl).

Other physiological characteristics which included rectal temperature, respiration rate (measured by counting flank movements for .5 min), hematocrit % (International micro-capillary, Centrifuge, Model MB), plasma protein % (Hitachi hand protein refractometer, National Instruments Co.) and blood pressure (mercury manometer) were measured after the infusion at approximately 0945 to 1000 hours. Blood pressure was recorded through the polyvinyl catheter in the femoral artery. At termination of the experiment, placement of electromagnetic flow transducers was verified at autopsy and transducers recalibrated in vitro.

Uterine blood flow readings (ml/min) were taken from the recording paper every 1 to 2 min depending upon variability of tracings. A 15-min average was considered as a single observation for statistical analyses to characterize time trends. Uterine blood flow responses and other physiological measurements were analyzed by the method of least squares.

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**TABLE 2. EXPERIMENTAL DESIGN FOR EXPERIMENT I**

<table>
<thead>
<tr>
<th>Days after surgery</th>
<th>Ewe number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>II*</td>
</tr>
<tr>
<td>2</td>
<td>III</td>
</tr>
<tr>
<td>3</td>
<td>IV</td>
</tr>
<tr>
<td>4</td>
<td>I</td>
</tr>
<tr>
<td>6</td>
<td>III</td>
</tr>
<tr>
<td>7</td>
<td>IV</td>
</tr>
<tr>
<td>8</td>
<td>I</td>
</tr>
<tr>
<td>9</td>
<td>II</td>
</tr>
<tr>
<td>10</td>
<td>III</td>
</tr>
</tbody>
</table>

*Roman numerals indicate treatments described in text.*
RESULTS AND DISCUSSION

Environmental treatments in Experiment I resulted in graded thermal stress responses as reflected by differences among treatments in rectal temperatures and respiration rates. Average rectal temperatures (°C) and respiration rates (resp./min) were lower (P<.05) for comparisons I vs II, III and IV (38.9 vs 39.2, 39.3 and 39.5°C; and 23 vs 66, 63 and 91 resp./min); error mean squares (22 df) were .1595 and 1432.

Respiration rate for ewes in treatment IV was higher (P<.05) than for those in treatments II and III combined (91 vs 66 and 63 resp./min). Within 30 to 60 min after first exposure to thermal stress in discontinuous (II or III) or continuous treatment (IV), ewes increased their respiration rate, started panting, had a tendency to lie down and did not eat. Ames et al. (1971) detected similar changes in ewes exposed to 35°C. Thwaites (1969) reported that ewes exposed to a moderate thermal stress similar to that of the present experiment had elevations in rectal temperatures, respiration rates and embryo mortality compared to control ewes. Consequently, our treatment protocol mimicked a thermal stress known to suppress reproductive rate in ewes.

Uterine blood flow was analyzed considering variability due to ewes (n = 4), experimental days after surgery (4, 6, 8, 10), treatment (I, II, III, IV) and time after E2 injection as a continuous independent variable. There were differences in UBF associated with ewes, days after surgery, treatments, and time up to the seventh order regression. Mean UBF (ml/min) response to E2 injection was lower (P<.05) on day 4 than days 6, 8 and 9 after surgery (41 vs 48, 58 and 50 ml/min). In ovariectomized ewes chronically fitted with electromagnetic flow transducers, Killam et al. (1973) also observed a lower UBF in response to E2 (1 μg/kg) during the first postoperative day.

Ewes in the laboratory environment (I) at 21°C had higher (P<.05) UBF in response to E2 than ewes exposed to a discontinuous thermal stress (II, III) or continuous stress (IV; table 3). Ewes that started either inside the environmental chamber or in the laboratory at 2400 hr and were switched at 1100 hr (II and III) had intermediate UBF means that were lower than control ewes (I) and higher (P<.05) than the response to continuous stress (IV). Overall difference in UBF for comparison of ewes in treatments I vs II, III was 24%, whereas the difference between ewes in treatments I and IV was 37%. It appears that thermal stress reduced the usual response of UBF to injection of estradiol. These differences among treatments were reflected as well by comparisons of peak flows (table 3). Peak flows were observed from 2.25 to 2.75 hr after E2 injection.

Tests for heterogeneity of regression of treatment UBF response curves indicated that treatment responses were not parallel. The orthogonal contrast of UBF I vs. II, III and IV was significant (P<.005). Regression curves for treatments I and IV are illustrated in figure 1. Curves representing UBF for ewes in the other two treatments (II and III) were intermediate with trends similar to treatment IV. A consistent delay of about 45 min was observed in each of the treatments between the time of E2 injection and onset of UBF rise. A plateau of maximum UBF was maintained for approximately 60 to 90 min, then UBF declined 50% when recordings were stopped at 1730 hr or 8.5 hr after E2 injection. Differences in UBF among

<table>
<thead>
<tr>
<th>TABLE 3. LEAST SQUARES MEANS FOR UTERINE BLOOD FLOW (UBF) AND PEAK FLOW IN EXPERIMENT I</th>
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</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>I (21°C)</td>
</tr>
<tr>
<td>II (21-32°C)</td>
</tr>
<tr>
<td>III (32-21°C)</td>
</tr>
<tr>
<td>IV (32°C)</td>
</tr>
<tr>
<td>aMean UBF (ml/min) for 8.5 hr after E2 (20 μg) injection.</td>
</tr>
<tr>
<td>b, c, dMeans not followed by the same superscripts differ (P&lt;.05). Orthogonal contrasts were I vs II, III, IV: II, III vs IV; II vs III. Residual mean square = 209.3 (500 df).</td>
</tr>
</tbody>
</table>
ewes (P<.01) were characterized by differences in magnitude of the UBF increase after E2 injection. These differences may be related to ovarian follicular and luteal characteristics at time of ovariectomy, size of uterus, parity number, and location of probe on the artery. However, all treatment comparisons were on a within ewe basis.

In pregnant ewes, Oakes et al. (1976) with a more intense thermal stress (42 C and 70% RH) for only 60 min, found that hyperthermia with respiratory alkalosis decreased UBF by 48% and umbilical blood flow by 30%. Hyperthermia without respiratory alkalosis decreased UBF by 25% with no apparent change in umbilical blood flow. The decrease in UBF was associated with the effect of hyperthermia increasing uterine vascular resistance. The decrease in UBF due to thermal stress may account for the decrease in placental and lamb birth weights observed by Alexander and Williams (1971). After studying the influence of maternal nutrition and high temperature on growth and body proportions of Merino fetuses, Cartwright and Thwaites (1976) concluded that adverse effects of high temperature arose from an extreme form of fetal undernutrition, which may be due to a decreased UBF.

In the present study rhythmic oscillations in UBF were observed every 2 to 3 min in ewes exposed to thermal stress (figure 2). These oscillations may have been associated with changes in myometrial activity and/or smooth muscle contractions of the uterine arterial wall due to hyperthermia. Increased secretion of catecholamines in response to thermal stress (Alvarez and Johnson, 1973) may affect myometrial activity and/or uterine vascular resistance. Alteration of spontaneous myometrial activity of the human uterus in response to epinephrine and norepinephrine is characterized by an increased frequency of contractions (Stander and Barden, 1970). The lower, characteristic oscillations of UBF in response to thermal stress of Experiment I led us to design a second experiment to test the interaction of thermal stress and epinephrine on UBF.

Physiological, blood and UBF data for treatments in Experiment II are presented in tables 4 and 5. As in Experiment I, rectal temperatures and respiration/min were lower (P<.01) for ewes under laboratory conditions (treatments A and B) than ewes exposed to thermal stress (treatments C and D; table 4). An interaction between environmental temperature and epinephrine on rectal temperature was detected (P<.01). Rectal temperatures of ewes in the environmental chamber at 32.4 C and infused with epinephrine (treatment D) were higher than for thermal stressed ewes without epinephrine (treatment C). It is known that exogenous epinephrine enhances calorigenic functions such as increasing metabolic rate, oxygen consumption and glycogenolysis within the liver (Hsich and Carlson, 1957; Guyton, 1971). In heat stressed sheep Ames et al. (1971) observed a 27% increase in oxygen consumption. The slightly higher RH% (82 vs 78) recorded in the environmental chamber for Experiment II, as compared to Experiment I,
TABLE 4. PHYSIOLOGICAL AND BLOOD RESPONSES IN EXPERIMENT II

<table>
<thead>
<tr>
<th>Treatment means</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectal temperature (C)</td>
<td>38.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Respirations/minute</td>
<td>33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>137&lt;sup&gt;b&lt;/sup&gt;</td>
<td>122&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>23.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma protein (%)</td>
<td>6.2</td>
<td>6.4</td>
<td>6.2</td>
<td>6.3</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>71</td>
<td>71</td>
<td>76</td>
<td>72</td>
</tr>
</tbody>
</table>

Residual mean squares<sup>d</sup> = 0.01

<sup>a,b,c</sup>Means not followed by the same superscripts differ (P<.05). Orthogonal contrasts were AB vs CD, A vs B, C vs D.

<sup>d</sup>Associated with 6 df.

may account for the higher respiration rates of ewes (130 vs 91). The respiratory response of sheep to a high ambient temperature increases when RH% is elevated (Bligh, 1963).

Thermal stressed ewes (C, D) had higher (P<.05) hematocrit values (table 4) than non-heat stressed (A, B) animals (25.9 vs 23.9%). Ames et al. (1971) found a positive correlation between evaporative water loss and respiration rate in heat stressed sheep. Likewise a higher rate of water turnover with rising ambient temperature (18 C to 32 C) has been observed in sheep (Kamal et al., 1972). It is possible therefore that thermal stress in our experiment may have caused a transitory state of dehydration since ewes refused or drank little water during heat stress periods. No changes in blood pressure associated with this dose of epinephrine (.20 µg/kg/min) or with thermal stress were detected in the present experiment. This agreed with results of other investigators (Hales, 1973; Rosenfeld et al., 1976; Oakes et al., 1976).

Least squares means for UBF (ml/min) were different among treatments (table 5). An interaction between thermal stress and epinephrine was detected (P<.01). The gradual decline from A to D verified the decrease in UBF due to heat stress in Experiment I, and agreed with other research (Robson and Schild, 1938; Greiss, 1963, 1972; Abrams et al., 1971; Leduc, 1972; Barton et al., 1974; Rosenfeld et al., 1976) which demonstrated that exogenous epinephrine decreased UBF. Treatment with 32 C plus epinephrine infusion (D) reduced UBF by 55%. Treatment response curves were heterogeneous among treatments (P<.01; figure 3). A faster rise of UBF after E<sub>2</sub> injection was observed in treatments A and B than in treatments C and D. In both experiments I and II rhythmic oscillations were observed in thermal stress treatments.

In summary, increase of UBF in response to estrogen appears to be modified by heat stress and this effect is intensified by epinephrine.

TABLE 5. LEAST SQUARES MEANS FOR UTERINE BLOOD FLOW AND PEAK FLOW IN EXPERIMENT II

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Peak flow</th>
<th>Time of peak (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74</td>
<td>3.50</td>
</tr>
<tr>
<td>B</td>
<td>45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>62</td>
<td>4.50</td>
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<tr>
<td>C</td>
<td>36&lt;sup&gt;d&lt;/sup&gt;</td>
<td>52</td>
<td>4.50</td>
</tr>
<tr>
<td>D</td>
<td>23&lt;sup&gt;e&lt;/sup&gt;</td>
<td>33</td>
<td>4.75</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean UBF (ml/min) for 6 hr after E<sub>2</sub> (20 µg) injection.

<sup>b,c,d,e</sup>Means not followed by the same superscripts differ (P<.05). Orthogonal contrasts were A vs BCD, B vs CD, C vs D. Residual mean square = 194.9 (344 df).
infusion. Decrease in UBF response to thermal stress may be a factor contributing to low fertility in domestic animals. A decrease in UBF might increase uterine temperature and perhaps affect availability of water and nutrients to the developing embryo. Uterine blood flow changes in normal cycling and early pregnant ewes in response to thermal stress need to be evaluated.

LITERATURE CITED


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