HYPOTENSION IN ACUTE BABESIA BOVIS
(— B. ARGENTINA) INFECTIONS OF SPLENECTOMIZED CALVES

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INTRODUCTION

In recent years the importance of inflammatory agents in intravascular protozoan diseases characterized by circulatory disturbances has been recognized by various workers including Goodwin (1970) and Onabanjo and Maegraith (1970a). Evidence suggests that plasma kinin and its precursor enzyme, kallikrein, are involved in the disturbance of function in these diseases. Increased levels of vasoactive peptides were first described in blood and urine from mice and rats infected with Babesia rodhaini and in urine of mice infected with Trypanosoma rhodesiense, Plasmodium berghei, Streptococcus pyogenes and Rift Valley Fever virus by Goodwin and Richards (1960). Elevated plasma kinin levels, with a concurrent fall in plasma kininogen, have also been observed in P. knowlesi infected monkeys (Tella and Maegraith, 1962, 1963, 1966), in T. brucei infected rabbits and T. rhodesiense infected cattle and humans (Boreham, 1966, 1968a,b, 1970), and in P. berghei infected mice (Ohtomo and Katori, 1972). The precursor enzyme kallikrein has also been implicated in the inflammatory process of P. knowlesi infected monkeys (Onabanjo and Maegraith, 1970a,b,c,d) and in B. argentina infected cattle (Wright, 1973, 1975; Wright and Kerr, 1975; Wright and Mahoney, 1974), as well as in human shock and trauma (Attar, McLaughlin, Hanashiro and Cowley, 1971).

Plasma kallikrein and kinin are vasodilators which enhance capillary permeability, and the injection of small amounts of these substances results in a profound hypotension (Senior, 1975). A hypotensive state has been recorded in a limited number of protozoan infections. Skirrow, Chonsuphajaisiddhi and Maegraith (1964) reported that arterial pressure in terminal P. knowlesi infections fell from 91 mmHg (normal) to 62 mmHg. In a parallel experiment, monkeys in which traumatic shock of the gut occurred had a 65 per cent fall in arterial pressure and it was concluded that common mechanisms were involved. Ohtomo and Katori (1972) found significant decreases in mean systemic arterial blood pressure in mice severely affected by P. berghei infection. Recently
Boreham and Wright (1976) reported significant hypotension in rabbits chronically infected with *T. brucei* and in normal rabbits in which profound hypotension was induced with *T. brucei* immune complexes.

The present study reports changes in blood pressure, plasma kallikrein and packed cell volume (PCV) in 12 calves acutely infected with *B. argentina*. These changes indicate that a hypotensive shock syndrome is characteristic of the disease and that kinins and kallikrein play a significant role in its production.

**MATERIALS AND METHODS**

*Animals.* *Bos taurus* calves, 3-months-old, of mixed breeds and sexes were purchased from a tick-free zone and maintained under tick-free conditions at the laboratory. Calves were splenectomized 1 to 2 weeks prior to use.

*Parasites.* It is now accepted that the Australian parasite known as *B. argentina* is identical with *B. bovis* (Hoyte, 1976).

Calves were infected with $1 \times 10^7$ *B. bovis* Lismore strain intravenously on day 0. The strain was freshly passaged by ticks to a donor animal from which the inoculum was obtained.

*Experimental procedure.* Jugular blood was collected daily for estimation of PCV, kallikrein, and percentage parasitaemia. The animals were randomized for blood pressure measurements, 4 being examined on day 3 and again on day 7, a second group of 4 on days 6 and 8, and a last group of 4 on day 9.

1. **Blood pressure measurement.** Conscious calves (both infected and control) were immobilized in a "Spinroll" crush similar to that described for the preparation of tick fever vaccine by the Department of Primary Industries, Queensland (Anon, 1968). This enabled relatively small animals to be restrained in lateral recumbency. The neck was shaved and the region of the jugular vein was infiltrated with 1 per cent xylocaine. The animal was blindfolded throughout.

   The carotid artery and jugular vein were exteriorized surgically with aseptic technique. A sterile vinyl cannula (1.5 mm o.d., 1.0 mm i.d.) were inserted into the jugular vein. Pressures in the right atrium, right ventricle, pulmonary artery and ascending vena cava were measured from this cannula. A slight bend in the end of the cannula was necessary for easier cannulation of the right ventricle and pulmonary artery. Side-pressure measures were used throughout. The carotid artery was punctured with an 18-gauge hypodermic needle and the side-pressure reading measured directly. A Statham UC-4 blood pressure transducer was used for all pressure measurements which were recorded on a Varian Techtron model 135-A rectilinear pen recorder. Both the carotid artery and jugular vein incisions were repaired with purse string sutures after the cannula had been removed. These vessels were functional until the time of death. The left vessels were used on the first day and a similar procedure was used on the right side vessels for the subsequent measurements (where applicable). Skin was repaired with mattress sutures.

2. **Haematological parameters.** The packed cell volume (PCV) was determined according to the technique of Dacie and Lewis (1968). Parasitaemias were calculated by the thick jugular blood film technique of Mahoney and Saal (1961). Plasma pre-kallikrein and activated kallikrein levels were measured by the *α* Tosyl-L-arginine methyl ester (TAME) hydrolysis technique described by Wright and Mahoney (1974).

**Statistical analysis.** Two analyses were made. In one, the effect of treatment was estimated first and then the effect of time elapsed (3, 6, 7, 8 or 9 days) was estimated with 4 degrees of freedom for treated animals. In the other analysis, orthogonal polynomials were fitted to time since treatment, the control animals being treated as zero time. In most cases, where there were significant effects, the linear time effect adequately described the data.
RESULTS

The PCV began to fall on day 3 and fell slowly until day 6. From days 7 to 9 the PCV fell rapidly (Fig. 1). The terminal rapid fall corresponded with a parasitaemia in excess of 1 per cent and the appearance of haemoglobinaemia and haemoglobinuria. During the last 2 to 3 days of infection animals were markedly anorexic, ataxic and showed increasing hyperpnoea and pulmonary distress. Terminally, many animals were recumbent and some showed signs of cerebral involvement including limb paddling and inco-ordination. Posterior leg weakness was a common sign terminally. Four animals died on day 8, the remainder on day 9. Parasites were first detected on day 2 when approximately $10^9$ parasites per animal were present.

![Fig. 1](image)

Fig. 1. (a) Mean and standard error (s.e.) of packed cell volume of 12 calves infected with *Babesia argentina* on day 0. (b) Mean and s.e. of activated kallikrein and prekallikrein of 12 calves infected with *Babesia argentina* on day 0. Kallikrein (---), prekallikrein (——).

Plasma kallikrein activation commenced on days 2 to 3 and reached maximum levels on days 6 to 7. Both pre- and activated kallikrein levels declined terminally (Fig. 1).

Carotid arterial systolic pressure had fallen to 65 per cent of normal by day 3 and fell continuously to 31-5 per cent of normal by day 9. Although highly significant ($P < 0.001$), this data was not adequately fitted by a simple polynomial. The diastolic pressure also fell continuously, being 65 per cent of
Fig. 2. Means and s.e. of carotid and pulmonary artery systolic and diastolic pressures of 5 control calves (plotted as day 0) and of groups of 4 infected calves on days 3, 6, 7, 8, 9. Systolic pressure carotid artery (---); diastolic pressure carotid artery (-----); systolic pressure pulmonary artery (--- ---); diastolic pressure pulmonary artery (....).

**TABLE 1**

**MEANS AND ESTIMATED POLYNOMIAL FIT (WHERE APPLICABLE) OF VASCULAR PRESSURES IN CALVES INFECTED WITH *Babesia bovis***

<table>
<thead>
<tr>
<th>Day</th>
<th>Respiration rate/min</th>
<th>Right atrium systolic pressure (mm Hg)</th>
<th>Right atrium diastolic pressure (mm Hg)</th>
<th>Right ventricle systolic pressure (mm Hg)</th>
<th>Ascending vena cava systolic pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Linear fit</td>
<td>Mean</td>
<td>Linear fit</td>
<td>Mean</td>
</tr>
<tr>
<td>0</td>
<td>39.0</td>
<td>36.3</td>
<td>10.0</td>
<td>9.6</td>
<td>1.4</td>
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<td>1</td>
<td>43.5</td>
<td>51.5</td>
<td>8.0</td>
<td>8.3</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
<td>67.0</td>
<td>66.7</td>
<td>6.0</td>
<td>6.9</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>77.0</td>
<td>76.8</td>
<td>8.0</td>
<td>6.0</td>
<td>1.3</td>
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<td>79.0</td>
<td>81.9</td>
<td>5.0</td>
<td>5.6</td>
<td>4.7</td>
</tr>
<tr>
<td>5</td>
<td>85.0</td>
<td>81.9</td>
<td>5.0</td>
<td>5.6</td>
<td>4.7</td>
</tr>
</tbody>
</table>

* Figure in brackets is for the standard error of differences with day 0.
Each mean is based on 4 animals.
HYPOTENSION IN BABESIOSIS IN CALVES

normal on day 3 and 28.5 per cent on day 9 (Fig. 2). This data was highly significant \( P < 0.001 \) and was well fitted by a simple polynomial. The pulmonary arterial systolic pressure fell continuously, being 46.7 per cent of normal on day 3 and 28.5 per cent of normal on day 9 (Fig. 2). This was highly significant \( P < 0.001 \). The pulmonary artery diastolic pressure also fell significantly \( P < 0.001 \) (Fig. 2). The ascending vena cava diastolic pressure did not alter significantly. The heart rate remained relatively unaltered throughout the infection. Data for respiration rate, right atrial diastolic and systolic pressure, right ventricle systolic pressure and ascending vena cava systolic pressure are detailed in Table 1.

DISCUSSION

The data obtained in this experiment agree with those in acute \( P. knowlesi \) infected monkeys (Skirrow et al., 1964), acute \( P. berghei \) infected mice (Ohtomo and Katori, 1972) and chronic \( T. brucei \) infected rabbits and are a common feature of numerous acute protozoan, bacterial and viral infections and of traumatic shock (Skirrow et al., 1964). These changes include hypotension, sluggish peripheral circulation and diminished venous return due to increased capillary permeability and vasodilation, which result in vascular stasis, often with a reduction in local blood flow. Similar non-specific lesions are associated with acute inflammation (Maegraith, 1948). These non-specific lesions can be produced by various vasoactive compounds including kallikrein and kinin which result in a precipitous fall in blood pressure, increased capillary wall permeability and vasodilation (Skirrow et al., 1964).

In addition to their involvement in these protozoan infections, the kinin system has been strongly implicated in the profound hypotension associated with bovine anaphylaxis (Aitken and Sanford, 1969; Eyre and Lewis, 1972; Eyre, Lewis and Wells, 1973), although Eyre et al. (1973) considered that SRS-A was also of major importance. All these workers consider the role of the biogenic amines histamine and 5-HT to be a minor one in the systemic anaphylactic reaction of cattle.

Although Wright (1973, 1975), Wright and Mahoney (1974) and Wright and Kerr (1975) implicated kallikrein and the peptide kinin in the disease process, further work is needed to elucidate their role. Wright (1975) and Mahoney and Wright (1976) have been able to demonstrate plasma kallikrein activation both in vitro and in vivo with parasite extracts, but it is possible that in addition to parasite secretions, other initiating factors such as parasite endotoxin and tissue damage factors may also be involved. The role of endotoxin in this disease merits consideration, since marked similarities exist between the clinical signs of endotoxaemia in calves (Wray and Thomlinson, 1972) and those in acute babesiosis. However, tissue damage does not appear to play a significant role in acute \( B. argentina \) infections, for Wright and Mahoney (1974) injected \( 10^{12} \) haemolysed red cells into calves with no apparent effect on the animal. The factors that initiate systemic hypotension in acute \( B. bovis (= B. argentina) \) infections are still unresolved, but further purification of parasite extracts into enzymatic and other components, and a study of their effects on the vertebrate host, are warranted.
SUMMARY

Systemic blood pressure changes were measured in groups of 4 splenectomized calves on days 3, 6, 7, 8 and 9 after acute infection with Babesia bovis. In addition, changes in five uninfected calves were measured on day 0. Carotid arterial, right atrial, right ventricular pulmonary arterial and ascending vena cava pressures were all lower on day 3 and had fallen dramatically by days 8 to 9. All animals died on days 8 and 9. The PCV began to fall and kallikrein activation started during days 2 to 3. Parasites were first detected on days 2 to 3.

These results are discussed in relation to other protozoan diseases of man and animals and to anaphylaxis in cattle. It is concluded that many of the changes observed in acute B. argentina infections are due to kallikrein and kinins, possibly released by the action of parasite secretions during infection.

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REFERENCES


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