PATHOLOGICAL CHANGES FOLLOWING EXPERIMENTAL MEDIUM VELOCITY PENETRATING HEAD INJURY

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Running Title: Experimental Penetrating Head Injury
INTRODUCTION

Pathological changes following closed head injury in humans have been well documented with descriptions of primary and secondary lesions, both local and diffuse (1). More recently, a primate model has successfully demonstrated some of the important pathogenetic factors in non-penetrating head injury (2, 8). The pathophysiological changes following closed head injury have also been well documented clinically and experimentally (10). By contrast studies of penetrating head injury have been limited and there have been few attempts to relate the outcome of penetrating brain injury to the detailed pathology, either in patients or in experimental studies. Frequently it has been assumed that the pathology of penetrating head injury is similar to that of closed head injury and such pathological studies that do exist have been of low velocity lesions and have been of isolated cases in which death occurred after an interval. Freytag (7), however, analysed the gross abnormality in 254 patients with penetrating injury but microscopy was not included in this study and Clemedson (5) has done a limited experimental study. The physiological consequences of clinical and experimental missile injury have been reported (6) and we have previously reported the results of an experimental high velocity penetrating head injury (3,4). In the present study a side to side medium velocity injury has been produced and physiological and pathological parameters studied.

METHODS AND MATERIALS

Surgical preparation: Fourteen adult baboons (10 experimental, 4 control).
weight range 6 - 10 kilos, of either sex, were tranquillised with phencyclidine and anaesthetised with intravenous barbiturate 60 mg/kg. An endotracheal tube was passed. An intravenous fluid cannula was inserted into the short saphenous vein, the bladder was catheterised and fluid intake and output were monitored throughout the experiment. Blood pressure, heart rate and blood gases were monitored through an arterial cannula and a Swan Ganz catheter inserted through a femoral vein to obtain cardiac output using the thermodilution technique.

An incision was made in the left temporal region and a small craniectomy, 2 cm in diameter, was performed to allow missile penetration through the anterior aspect of the parietal lobes. The animal was positioned and held in a frame so that the missile tract was at 90° to the sagittal plane of the animal's head. Two burr holes were fashioned over the coronal suture, 2 cm lateral to the midline, and pressure transducers were inserted over the convexity of both hemispheres in predetermined constant positions. The animal was allowed to breathe spontaneously and, following surgery, further anaesthetic was required prior to injury. Control observations were made of blood pressure, heart rate, blood gases and cardiac output. The animal's temperature was carefully monitored and maintained on a heating blanket. Control animals had a right frontal burr hole fashioned and a transducer inserted. Two experimental and two control animals had Evans Blue solution (1 ml/kg body weight of a 2% solution of Evans Blue in 0.9% saline mixed with 10 ml freshly drawn venous blood) injected 15 minutes before injury (experimental) or before sacrifice (control).

Method of injury: (Table 1)

A 3/16ths inch (4.8 mm) sphere was propelled from a fixed gun barrel by varying explosive charges to provide a range of impact velocities
which were measured by brake screens and high speed cine photography. The impact energy varied from 7 to 25 joules. Exit velocity, when it occurred, was also calculated using high speed cine photography. High speed x-rays triggered by the brake screens were also used to investigate missile cavitation. The force displacement transducers had a fast response time and were used to calculate the intracranial pressure on impact and also the generation of pressure waves within the cranial cavity.

**Monitoring:**

Following control observations, a missile with predetermined energy was propelled through the exposed brain and the velocity and energy confirmed as already described. All parameters were measured for the first 30 seconds following injury and at intervals of 1, 5, 10, 30 minutes and hourly thereafter. In those animals in which respirations did not return after impact, controlled ventilation was performed for intervals or for the rest of the period of study. At the end of the predetermined period of study the animal was prepared for pathological investigations, as described below.

**Pathological methods:**

The animals were prepared for pathological examination in one of two ways: transthoracic cardiac perfusion with ice cold saline followed by perfusion with mixed buffered aldehydes or with buffered paraformaldehyde was was carried out. Enzyme studies and brain tissues specific gravity measurements were obtained from animals in which samples of brain tissue were removed premortem. Evans Blue was assayed subjectively in animals previously injected with the dye prior to injury.

On removal, the brain was immersed in the perfusion fixative and was left in fixative for several weeks until sectioning and blocking.
Immediately before blocking the brain was washed in running tap water for several hours and the external appearances were recorded photographically and on a standard protocol. The brain was sectioned in the sagittal plane and blocks for embedding in nitrocellulose (L 1 - 6; R 1 - 6) were labelled with Indian ink and processed by standard techniques.

Sections were stained by haematoxylin and eosin (H & E) and by Mallory's phosphotungstic acid, Bielschowsky's axis cylinder stain and Woelcke's myelin stain.

Blocks of tissue for plastic embedding were fixed in either of two solutions.

(a) 4% paraformaldehyde/1% glutaraldehyde in phosphate buffer pH 7.3

(b) 4% paraformaldehyde in phosphate buffer pH 7.3

Sufficient tissue was taken in order to permit routine electron microscopy and specialised sectioning for combined light - electron microscopy. These blocks were processed using standard techniques.

**RESULTS**

Following impact, there was an immediate alteration in respiratory pattern with periods of apnoea, followed by changes in respiratory rate and rhythm. In the animals with the higher impact energy, apnoea was complete and permanent. Bradycardia occurred immediately following impact; and there was an alteration in pulse pressure and blood pressure. The blood pressure always fell and remained lower than control values. Cardiac output was also reduced after impact and in the lower energy group it returned to control values after 30 minutes, but in those in which there was a high energy output, cardiac output remained low for the rest of the period of observation.
When the cardiac output was compared to the strike energy, there was little or no correlation in the first few minutes, but at 30 minutes and at 1 hour there was a linear correlation between the energy imparted and the reduction in cardiac output. When the energy input was greater than 10 joules there was a 30% fall in cardiac output. In those in which there was a higher energy input in the order of 15 - 20 joules, cardiac output was reduced by 30 - 60%. At the time of impact the pressure transducers recorded pressures of 280 lb/sq in, lasting 0.5 msec.

Pathological results:

The method of injury produced a reasonably standard left to right transfrontal wound (Fig. 1a). Occasional variation was found and in some cases the missile had deviated inferiorly to involve basal structures. In most cases, however, the lesion was predominantly transfrontal. In 7 of the animals, a detailed histological study was performed and results of this are summarised in Table 2. In the wound itself there was disorganisation of tissue, haemorrhage and extravasation of fluid (Fig. 1b). Animals surviving more than 4 hours showed cellular necrosis with neuronal encrustations.

Several striking features emerge from the histological study. First of all there was a marked tendency, as we have previously reported in a high velocity experimental model, to widespread dissemination of lesions apparently separated from the primary missile tract. Among common sites of involvement for such lesions was the cerebellum (Fig. 2). Diffuse axonal injury, as characterised by the formation of retraction balls, was not seen. Microglial stars and disseminated cortical ischaemic lesions were also not observed. Various disseminated lesions included increased perivascular staining,
particularly in the region of the wound (Fig. 3a) and a zone of pallor and oedema around the wound (Figs. 3b and 3c). Perivascular astrocytic swelling was apparent adjacent to the wound and in some cases was a more widespread feature. These lesions were apparent in plastic sections (Figs. 4a and 4b). Ultrastructural studies confirmed this finding (Fig. 4c) and also showed extravasation of fluid in the region of the wound. In animals injected with Evans Blue the wound and related contusions were stained. In addition, in some animals there was a faint diffuse staining of white matter; this aspect of the study, related to the specific gravity findings, is not complete and will be reported later.

DISCUSSION

Several important points with possible clinical implications emerge from this correlated physiological pathological study. First of all, the transmitted force in the model had similar effects on blood pressure, respiration and cardiac output as described in other models of closed or penetrating head injury. Pathological findings indicate that even with medium velocity lesions widespread abnormality may be observed and much of this may be based on the dissemination of force through blood vessels and cerebrospinal fluid. Clearly, from the point of view of treatment, little that is observed pathologically in the actual missile tract can be reversible. The more important pathological changes, from the therapeutic aspect, therefore would seem to be those that are disseminated. Of these the diffuse subarachnoid and perivascular ring haemorrhages are lesions which are observed frequently in human penetrating head injury and also, though to a lesser degree, in closed human head injury. It is perhaps somewhat surprising that diffuse axonal injury has not been a feature
of the medium velocity model or of our previous high velocity model. It is possible that the period of observation has been too short for this change to develop or alternatively this particular pathological change may not be associated with this form of head injury.

The changes occurring in relationship to blood vessels and to perivascular astrocytes require further study. Our working hypothesis is that a great deal of the force imparted to the brain by the missile is transmitted through blood vessels with resultant ring haemorrhages or zones of increased staining intensity caused by the leak of protein. Areas of decreased perivascular staining intensity are probably associated with the perivascular astrocytic swelling observed at electron microscopical level. The exact mechanism of the astrocytic swelling is unknown. Similar changes have been observed in experimental trauma in the cat (9) and possible mechanisms include a direct effect of force, chemical triggering by components of extravasated blood cells and serum (e.g. potassium or adenosine) or force-mediated membrane abnormalities, artefactually accentuated during or after perfusion fixation. Clearly, the effect of perivascular astrocytic swelling on blood brain barrier function, on neuronal function and on the subsequent development of oedema could be considerable and our present studies are directed towards elucidating the mechanism of this lesion.

**SUMMARY**

An experimental model of medium velocity missile head injury has been produced in the baboon and the consequent physiological and pathological abnormalities have been studied. Physiological effects include variation in respiratory pattern, hypotension and fall in cardiac output. Pathological studies showed that:-
1. The severity of the pathological lesions is proportional to the force of the missile.

2. Pathological lesions at sites remote from the missile tract are common.

3. The following 'diffuse' lesions are common:
   Subarachnoid haemorrhage.
   Perivascular 'ring' haemorrhages.
   Perivascular 'ring' haemorrhages with a surrounding zone of decreased staining intensity.
   Perivascular increased staining intensity.
   Perivascular decreased staining intensity without haemorrhage.
   Perivascular astrocytic swelling.

4. Diffuse axonal injury is rare.

5. With survival times greater than 4 hours oedema and necrosis are observed.
REFERENCES


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<th>Missile</th>
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Note: specimen not used for histology
SAH = subarachnoid haemorrhage  IVH = intraventricular haemorrhage  RH = ring haemorrhage
TABLE 2. Pathological summary

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<tr>
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<th>SURVIVAL TIME</th>
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<th>SAH</th>
<th>IVH</th>
<th>RH</th>
<th>RH WITH PALE AREAS</th>
<th>PALE AREAS ALONE</th>
<th>INCREASED PERIVASCULAR STAINING</th>
<th>HYPOTHALAMUS</th>
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Figure 1  (a) Left to right transfrontal wound produced by medium velocity missile

(b) Electron micrograph of wound X 1000
Figure 2  (a) Cerebellar haemorrhage associated with transfrontal wound H & E X 100  
(b) Bielschowsky X 250
Figure 3  Pathology of medium velocity missile head injury

(a) Increased perivascular staining H & E X 250

(b) Pallor and oedema adjacent to wound H & E X 25

(c) Oedema at edge of wound H & E X 250
Figure 4  Perivascular astrocytic swelling

(a)  H & E X 100

(b)  1μ Tol Blue X 630

(c)  Electron micrograph X 1200