

## THE EFFECT OF *CLOSTRIDIUM PERFRINGENS* EPSILON TOXIN ON THE BLOOD BRAIN BARRIER OF MICE

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### ABSTRACT

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It was shown that *Clostridium perfringens* epsilon toxin has the effect of allowing the passage of <sup>125</sup>I polyvinyl-pyrrolidone and <sup>125</sup>I human serum albumin into mouse brain. These substances did not enter the brains of normal control mice. The passage of albumin into the brains of mice poisoned with epsilon toxin was extremely rapid. When large doses of toxin ( $\pm 4\ 000$  MLD) were given death ensued within 2-3 min at which stage 1.5% of the injected albumin had already entered the brain. In cases where smaller doses were given and the time interval between injection and death was longer the figure was increased to 2-2½% of the injected plasma albumin.

### INTRODUCTION

*Clostridium perfringens* epsilon toxin is the cause of one of the world's major sheep diseases—enterotoxaemia. Although the disease has been extensively studied and satisfactory vaccines developed for its control the exact mode of action of the toxin is still unknown. An extensive review of work done on the toxins of *Clostridium perfringens*, which are important to animals has been made by Hauschild (1971). Although it is known that epsilon toxin increases the vascular permeability in the brain and causes some kidney damage he concluded that "the cause of death is still open to debate. Future work should be directed to studying the biochemical action of the toxin."

The most important symptoms in enterotoxaemia indicate acute nervous disturbances and it is therefore to be expected that the major pathological changes should be found in the brain. Griner & Carlson (1961) working with lambs showed that the primary encephalic lesion was a perivascular and intercellular oedema. Griner (1961) also showed that epsilon toxin caused an increased transport of <sup>131</sup>I labelled albumin from the bloodstream into the brain. Electron microscopic studies have shown that peroxidase which is injected intravenously remains confined to the blood vessels in control mice (Gardner, 1973). In intoxicated mice the peroxidase escapes from the vessels into the extracellular spaces of the brain tissue. Gardner (1973) also showed that the primary morphological lesion was severe vascular endothelial damage, which gives rise to the development of oedema of brain, heart and lungs. We have shown that epsilon toxin was preferentially adsorbed by brain tissue, while other tissues had little affinity for the toxin (Worthington, Mülders & Van Rensburg, 1973a).

It was the purpose of this study to investigate the effect of epsilon toxin on the blood brain barrier of mice.

### MATERIALS AND METHODS

Epsilon prototoxin which had been purified by DEAE cellulose chromatography (Worthington, Mülders & Van Rensburg, 1973b) was used in all experiments. It was activated with trypsin and toxicity tests performed according to the methods described by Habeeb (1969). Mice were injected intravenously with varying amounts of activated toxin to which was added a fixed dose of a large molecular mass radioactive tracer, which did not cross the blood brain barrier in normal mice. All mice received a constant volume (0.5 ml) of toxin-marker mixture or of marker alone in the case of the controls.

<sup>125</sup>I labelled polyvinylpyrrolidone\* (PVP) with an average mol mass of 30-40,000 or <sup>125</sup>I labelled human serum albumin\* (HSA) was used as marker. Aliquots of all solutions injected were counted in a Phillips NaI crystal gamma counter to determine their activity.

In some experiments mice were sacrificed at varying time intervals and in others allowed to die from the effects of the toxin (see text). In the latter case the time interval between injection and death was recorded. Brains were then removed and the activity of whole brains counted.

### RESULTS

#### A. Toxin dose 2-16 MLD-marker <sup>125</sup>I PVP. Mice allowed to die from epsilon toxin poisoning

Doubling dilutions of toxin (16-2 MLD) were injected together with a fixed dose of <sup>125</sup>I PVP with an activity of  $\pm 500\ 000$  counts per 100 sec (c.p. 100 sec). Five mice were injected with each dose of toxin and an equal number of control mice received <sup>125</sup>I PVP only. The mice were kept under observation and each time a mouse died one of the controls was sacrificed. The time of death varied from 2 h 15 min to 13 h after injection.

The mean activity per brain in mice which had received toxin was 3 142 c.p. 100 sec with a standard deviation (SD) of 1 265. In controls the mean activity per brain was 884 c.p. 100 sec (SD = 165). The difference was significant at the 0.001% level. The difference between the mean activities of the livers of mice from the two groups was not significant. The interval between time of injection and death had no obvious effect on the activity of the brains at the time of death.

#### B. Toxin dose 10 MLD-marker <sup>125</sup>I HSA. Mice sacrificed at varying intervals after injection

Twenty five mice were injected with 10 MLD of toxin together with <sup>125</sup>I HSA ( $\pm 400\ 000$  c.p. 100 sec) and 25 received <sup>125</sup>I HSA only. Mice were sacrificed at varying time intervals after injection of toxin and an equal number of control mice sacrificed at the same time. The results given in Table 1 are expressed as the percentage of the activity injected which was found in the brain, i.e.

$$\frac{\text{c.p. 100 sec/brain}}{\text{total c.p. 100 sec injected}} \times 100. \text{ The amount of}$$

isotope found in the brains increased with time to a maximum at the stage when mice began to die from the effects of the toxin (2h).

\* The Radiochemical Centre, Amersham

TABLE 1 Rate of passage of  $^{125}\text{I}$  HSA into the brains of mice injected with 10 MLD epsilon toxin

Time interval between injection and sacrifice (min)	No. mice per group	Mean percentage injected activity found in brain	Statistical significant difference T & C groups
45.....	5 5	C 0,52 (0,15)* T 0,76 (0,13)	P < 0,05
90.....	5 5	C 0,52 (0,14) T 1,45 (0,66)	P < 0,05
120.....	7 7	C 0,54 (0,15) T 2,30 (0,53)	P < 0,001
160.....	8 8	C 0,45 (0,10) T 1,89 (0,59)	P < 0,001

C = Controls

T = Injected with toxin

\* = Standard deviations given in brackets

TABLE 2 Passage of  $^{125}\text{I}$  HSA into the brains of mice poisoned with large doses of epsilon toxin

Time interval between injection and death (min)	Toxin dose (MLD)	No. mice per group	Mean percentage injected activity found in brain	Statistical significant difference T & C groups
2-3.....	4 000	15 15	C 0,53 (0,11)* T 2,02 (0,67)	P < 0,001
6-10.....	800	15 15	C 0,53 (0,11) T 2,44 (1,10)	P < 0,001
20-30.....	160	15 15	C 0,53 (0,11) T 2,88 (0,99)	P < 0,001
60-120.....	32	15 15	C 0,53 (0,11) T 3,08 (0,55)	P < 0,001
120.....	6,4	15 15	C 0,53 (0,11) T 2,38 (0,53)	P < 0,001

C = Controls. In this experiment all controls were sacrificed at  $\pm 120$  min after injection

T = Injected with toxin

\* = Standard deviations given in brackets

### C. Toxin dose 6,4-4 000 MLD-marker $^{125}\text{I}$ HSA. Mice allowed to die from epsilon toxin poisoning

The results summarized in Table 2 are the average of two separate experiments in which varying doses (five-fold dilutions) of toxin were injected into mice together with  $^{125}\text{I}$  HSA. In three experiments using  $^{125}\text{I}$  HSA the percentage of the total activity injected found in the brains of control mice remained within the range 0,51-0,55%. The activity did not vary at time intervals from 45-160 min after injection. In these experiments only one group of control mice was therefore used in each experiment. They were killed at a time interval of  $\pm 2$  h after injection. The results are given in Table 2 as the percentage of the  $^{125}\text{I}$  activity injected which was found in the brain (see Table 2). In poisoned mice the  $^{125}\text{I}$  HSA passes rapidly into the brain tissue. When 4 000 MLD of toxin was given death ensued within 2-3 min. The brains of these mice contained on average 2,02% of the total activity injected while the brains of controls contained 0,53% of the injected activity. This indicates that 1,5% of the total  $^{125}\text{I}$  HSA injected had passed into the brain tissue. As the toxin dose was decreased the interval from injection to death increased and the  $^{125}\text{I}$  HSA which passed into the brain increased up to an average of 2,5% in mice which received 32 MLD of toxin (time of death 60-120 min).

### DISCUSSION

In our experiments a constant amount of labelled tracer was injected into all mice. We have assumed that the label does not normally pass the blood brain barrier in significant amounts and that the activity counted within the brains of control mice represents the amount of blood within the vessels of the brain. When  $^{125}\text{I}$  HSA was used as marker the percentage of the total activity injected, found in the brain of control mice, remained constant from one experiment to another and also remained constant for extended periods of time thus showing that  $^{125}\text{I}$  HSA does not accumulate in the brain in normal circumstances. It can therefore be assumed that the difference in activity between the brains of mice which received toxin and controls represents that albumin which passed out of the blood vessels into the brain. The percentage of the total counts injected which are found in the brain must closely approximate the percentage of the total plasma albumin which has passed into the brain. These relationships do not necessarily hold for  $^{125}\text{I}$  PVP as it is known that  $^{125}\text{I}$  PVP is excreted fairly rapidly in the urine (Hecht & Scholtan, 1959). The calculation of  $^{125}\text{I}$  PVP as the percentage of total counts found in the brain has not therefore been done.

It was found that from 1.5–2.5% of the total  $^{125}\text{I}$  albumin injected had passed into the brains of different groups of mice at the time of death (see Table 2). In individual mice the figure went as high as 4%. These results confirm the findings of Griner (1961) that epsilon toxin causes an increased permeability of brain vessels. The flow of plasma proteins into the brain must be accompanied by a large amount of water which would cause an increase in intracerebral pressure which may be of significance as a cause of death.

The rapidity with which these changes occurred was surprising. In cases where very large doses of toxin were injected death ensued within 2–3 min. At this stage 1.5% of the albumin marker had entered the brain. The generally accepted view of transport across capillaries in the body is that outflow of fluid through the capillary walls is a result of the hydrostatic pressure, the negative pressure in the interstitial spaces and the osmotic pressure due to proteins in the interstitial fluid. While inflow of fluid is due to the osmotic pressure of the proteins in the plasma in the capillaries (Guyton, 1971). Although the situation in the brain may not be exactly similar to that in other tissues similar principles must pertain. It would appear that epsilon toxin alters brain capillaries very rapidly making them permeable to plasma proteins. The immediate effect would therefore be to abolish osmotic effects due to plasma proteins thus creating a situation where the forces tending to move fluid out of the capillaries would no longer be balanced by any plasma colloidal osmotic pressure tending to move fluid back into the capillaries. The passage of fluid between capillaries and interstitial fluid is extremely rapid, according to Guyton (1971) "the rate at which water molecules diffuse through the capillary membrane is approximately 40 times as great as the rate at which plasma itself flows linearly along the capillary". If brain capillaries are suddenly made permeable to large protein molecules this would result in a very rapid extravasation of fluid into the brain which would cease when the interstitial fluid pressure becomes equal to the hydrostatic pressure in the capillaries.

The rapidity with which changes occur in the permeability of blood vessels in the brain indicates that these changes may indeed be the primary effect of the toxin. Increased intracerebral pressure caused by the flow of plasma proteins and water into the brain might be responsible for the nervous symptoms and acute deaths encountered in enterotoxaemia.

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