THE CELLULAR AND CLINICAL PATHOLOGY OF CLOSTRIDIUM PERFRINGENS TYPE D ENTEROTOXAEMIA

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Abstract.

Enterotoxaemia of sheep is caused by the absorption of the epsilon toxin of Cl. perfringens type D. In preliminary experiments it was found that the sex and nutritional status of mice could influence the outcome of experiments in which this toxin was used. Female mice tended to survive longer than males and food deprivation caused the accumulation of free lipid in the proximal tubules of the kidneys of control, as well as intoxicated, mice.

It appears that there may be species differences in the morphological changes which epsilon toxin induces in the intestinal mucosa. Severe inflammatory necrosis occurred in the mucosa of toxin-containing loops of rabbit intestine but was absent from similar loops of lamb intestine from cases of experimentally induced enterotoxaemia.

While the toxin itself is fairly stable *in vitro* it may deteriorate during storage unless held at low temperatures. The addition of chloroform, as a preservative for the toxin in intestinal contents, does not increase the persistence of the toxin to a significant extent.

The pattern of absorption of epsilon toxin from the intestine into the bloodstream during the course of experimental enterotoxaemia was followed by using a radioactive tracer (I_{125}Polyvinylpyrroldine). It was found that, as with the protein tracers used by other workers, the values of I_{125}PVP in studies of this nature is limited. However, it did reveal that there is a loss of high molecular weight substances from the bloodstream into the extravascular tissues during this intoxication.

Studies of the ultrastructural changes which occur in the tissues of intoxicated animals have revealed that there is a severe generalised vascular endothelial damage, both when epsilon toxin is administered intravenously and when it is absorbed from the intestine. There is no histochemical or histological evidence to suggest that epsilon toxin produces primary morphological changes in tissues, other than endothelium,

although secondary effects are detectable in other organs. These secondary changes include the development of protein-containing effusions in serous cavities, and oedema in a number of tissues.

In the brain, fluid accumulation is primarily intracellular and is confined to the protoplasmic astrocytes. This results in swelling of astrocyte processes in the grey matter so that the increase in fluid content can be detected by electron microscopy as well as quantitatively. The changes in the astrocytes form the basis for early brain lesions which are detectable by light microscopy e.g. vacuolation in the cerebellar granular layer. Associated with the intracellular fluid accumulation there is an extravasation of protein into the extracellular spaces of the brain and this is detectable in mice by using exogenous peroxidase as a tracer.

Fluid loss from the bloodstream is also prominent in the heart and the lungs. In the former tissue there is severe myocardial oedema and fluid accumulation within the cardiac muscle cells, which may explain the electrocardiographic abnormalities that develop during the course of intoxication. Although lung oedema is not a consistent feature of the disease, and may be a reflection of damage caused by high concentrations of epsilon toxin entering the pulmonary circulation, it can be extremely severe with accumulation of fluid in the alveoli and interstitium.

Although it is possible to relate these changes to the vascular damage, no possible mechanism for the action of the toxin on the endothelium has been established. The use of red cell stroma and fluorescent antibody tests has failed to provide any evidence that the toxin is bound to cell membranes either <u>in vitro</u> or <u>in vivo</u>. Warburg respirometry did not reveal any reduction in the metabolic efficiency of tissue slices that were under the influence of epsilon toxin.

The overall loss of fluid into the tissues of intoxicated animals results in a severe haemoconcentration which, because of the severity of the endothelial damage, is not associated with any increase in levels of plasma proteins or inorganic ions such as sodium, potassium or chloride.

One of the most prominent of the biochemical changes that occur in enterotoxaemia is a severe progressive hyperglycaemia which appears to result from the rapid mobilisation of hepatic glycogen. The reduction in the glycogen content of the hepatic tissue can be detected histochemically. A further finding which suggests that hepatic glycogenolysis forms the basis for the blood sugar changes is that the hyperglycaemic response was suppressed in animals in which hepatic glycogen stores had been depleted prior to the administration of epsilon toxin.

Associated with the rise in blood glucose there are also increased amounts of lactate, pyruvate and alphaketoglutarate in the blood. These changes are considered to be a reflection of increased metabolic activity due to the increased amounts of available glucose. The build-up of intermediate substances reflects normal rate-limiting steps in aerobic glycolysis rather than any direct interference with a particular biochemical pathway by the toxin.

The high levels of lactate in the blood cause a severe metabolic acidosis in intoxicated animals which may sometimes be masked by alterations in blood pH associated with deficient respiratory exchange and elevated values of blood pCO_2 caused by the pulmonary oedema.

The importance of the morphological, biochemical, haematological and physiological changes, which have been found in the present investigation, in increasing our understanding of the pathogenesis of enterotoxaemia are discussed. In addition the relevance of these findings to the broader fields of experimental pathology and to the inter-relationships which exist between the cellular and clinical pathology of disease states are described.

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