SOME PATHOGENIC MECHANISMS—THEIR SIGNIFICANCE

Endotoxin shock—distinct entity or universal accompaniment?

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Summary

If an experimental animal is subjected to graded hypotension by bleeding into a reservoir, it will usually recover if the shed blood is returned to the circulation within 2 hr. If hypotension (40 mmHg) is prolonged to 6 hr, most of the animals will die despite the return of all the blood to the circulation at the end of this period. Fine and his co-workers have suggested that this state of irreversible shock is due to endotoxaemia. Fine's evidence in support of this thesis is presented and discussed together with apparently contradictory evidence from other sources.

Introduction

If a dog's blood pressure is reduced to 35 mmHg for 2 hr, by bleeding into a reservoir, and at the end of that time all the blood in the reservoir is allowed to flow back into the animal's circulation, nearly all animals will survive. If the hypotension is maintained for 4 hr nearly all the animals will die, even though all the blood has been re-transfused. After re-transfusion these dogs show a pressor response but this is soon followed by apathy, collapse and bloody diarrhoea, while the blood pressure slowly falls again until death occurs. At necropsy haemorrhagic necrosis of the bowel mucosa will be found (Schweinsberg *et al.*, 1957).

These dogs are said to be in a state of irreversible shock. This is clearly a highly artificial, closely controlled, laboratory situation, but Fine and his co-workers have advanced the hypothesis that these dogs are in irreversible shock because bacterial lipopolysaccharides (LPS) are present in their blood.

Does the blood of these dogs contain a toxin?

If some of this blood is transfused into normal dogs from which an equal amount has been removed, no harm will come to the recipient. If the blood is transfused into dogs prepared by 2 hr of haemorrhagic hypotension the animals will die. The manner of their death and the necropsy findings will be simi-

lar to the findings in dogs subjected to 4 hr hypotension. Similarly the blood will prove lethal if given to reversibly shocked rabbits (Ravin, Schweinsberg & Fine, 1958). In other words the toxin can change reversible to irreversible shock.

Is the toxin endotoxin (LPS)?

The blood is positive for the 10-day-old chick embryo test for endotoxin, i.e. four out of six embryos die with widespread haemorrhages if 0.1 ml is injected into them (Green *et al.*, 1961).

It induces the generalized and the local Schwartzman reaction in suitably treated animals, while normal plasma does not (Rutenburg & Fine, 1956).

It is pyrogenic and normal plasma is not (Green *et al.*, 1961). The toxic substance has also been extracted from shock blood and shown to have the properties of endotoxin (Ravin *et al.*, 1958).

Source of endotoxin

If we accept that these shocked animals have endotoxin in their blood, how does it get there? It might come from the intestine. If endotoxins or bacteria, or both, are absorbed from the intestine of the experimental animal, are they absorbed by the normal animal or only by the shocked animal?

Using the chick-embryo test, Fine and his co-workers (Green *et al.*, 1961) were able to detect LPS in the portal vein blood of 50% of normal rabbits, and in 17% of the same rabbits they found LPS in the inferior vena cava blood.

With a more sophisticated technique, using endotoxin labelled with ^{32}P , they demonstrated endotoxin in the venous blood of shocked rabbits but not in the blood of normal rabbits. The labelled endotoxin was administered by gavage and blood samples taken at intervals thereafter. Endotoxin was found in the livers of both groups but, while the endotoxin from the livers of normal rabbits had been rendered inert, that from the livers of shocked rabbits was still toxic by the usual tests (Ravin *et al.*, 1960).

Similar results were obtained when tissue extracts

were assayed for endotoxin by a haemagglutination-inhibition technique. They, therefore, argued that both normal and shocked animals continuously absorb endotoxin from the gut and that the shocked animal is unable to detoxify it (Ravin *et al.*, 1961).

Sandford & Noyes (1958) carried out a similar set of experiments using endotoxin labelled with ^{51}Cr but they were unable to detect ^{51}Cr in the blood or the tissues following the introduction of labelled endotoxin into the intestinal tract. They admitted that they were suspicious of the stability of their labelled endotoxin and wondered whether the incorporation of ^{51}Cr rendered it inert or prevented absorption from the intestine.

If endotoxin is absorbed from the gut it should be possible to reduce absorption by decreasing the intestinal pool of endotoxin:

- (a) If dogs are pre-treated with oral or parenteral antibiotics and then subjected to haemorrhagic shock, the mortality rate is lowered from 80% or more to 40% or less (Jacob *et al.*, 1954).
- (b) In normal rabbits endotoxin can be detected in portal vein blood of 50% of rabbits tested. If these rabbits are fed endotoxin by gavage the percentage with endotoxin in the portal vein blood rises to 60%, while if specially fed 'coli-form free' rabbits are given neomycin by mouth only 16% have endotoxin in the portal vein blood (Green *et al.*, 1961).

These experiments would seem to indicate that endotoxin is absorbed from the gut in both the normal and the shocked animal and that absorption is related to the amount of endotoxin in the gut.

Detoxification of endotoxin

If endotoxin is being continuously absorbed by the normal animal where is it detoxified?

The spleen has lately been shown to have an important function in inactivating Gram-negative endotoxin. If blood from irreversibly shocked dogs is transfused into 2-hr shock test recipients the dogs will die showing the usual stigmata of irreversibility. If the blood is first perfused through a normal spleen it fails to kill a 2-hr shock test recipient (Palmerio *et al.*, 1964). A healthy spleen perfused with a solution containing a lethal dose of endotoxin can extract it almost completely and inactivate it in a few minutes, whereas a shocked spleen is incapable of destroying or detoxifying endotoxin (Witznitzer *et al.*, 1966).

The liver also plays a part. In 1946 Fine and associates (Frank, Seligman & Fine, 1946) showed that viviperfusion of the liver by a donor protected the shocked dog and allowed recovery from a period of hypotension that would otherwise have been lethal.

Endotoxin can be demonstrated in the portal vein blood of 50% of normal rabbits and in the inferior

vena cava (IVC) blood of 17%, indicating that the liver removes or detoxifies the endotoxin (Greene *et al.*, 1961). If rabbits are primed with thorotrast, 100% of them have endotoxin in their portal vein blood and 89% in their IVC blood. Thorotrast is known to block the reticulo-endothelial system and thorotrast-primed rabbits are extremely susceptible to endotoxin.

These experiments suggest that the reticulo-endothelial system and particularly the spleen, are so damaged by prolonged hypotension that they lose their ability to detoxify the endotoxin that is continuously absorbed from the bowel and, as a result, a fatal toxæmia develops. If the blood supply to the spleen can be maintained, irreversible shock is less likely to develop. Lillehei (1957) found that perfusion of the superior mesenteric artery in dogs shocked to 35 mmHg for $4\frac{1}{2}$ hr changed the survival rate from one out of fifteen to twenty-seven out of thirty.

Recently Fine has shown that denervation of the abdominal viscera and spleen protects the animal from the development of irreversible shock (Palmerio *et al.*, 1963).

Evidence from other sources

Although Fine's thesis that endotoxaemia is the fundamental lesion in irreversible shock appears to be backed by ample evidence, other workers have been unable to confirm it.

Einheber (1961) performed a series of experiments where he transfused 2-hr shock dogs with the blood of dogs dying in irreversible shock. None of the recipient dogs died or showed any evidence of toxicity. He also failed to demonstrate endotoxaemia in rabbits. Blood from a rabbit dying in irreversible shock, produced by graded hypotension or by superior mesenteric artery occlusion, failed on transfusion to kill rabbits primed with thorotrast, nor were rabbits primed with thorotrast unduly susceptible to bowel ischaemia or haemorrhage. He subjected germ-free and conventional chicks to tourniquet shock but the germ-free chicks had significantly higher mortalities than the conventional chicks. Finally, he found no difference in 24- or 48-hr mortalities, or in survival time between germ-free and monocontaminated mice subjected to tourniquet injury.

Zweifach (1959) has described haemorrhagic shock in germ-free rats and has found that they respond qualitatively and quantitatively in much the same way as conventional rats.

Nagler & Zweifach (1961) carried out a series of experiments on rabbits subjecting them to graded haemorrhage. Although passive transfer of 20–25 ml of blood from a donor in irreversible shock to a primed recipient (40 mmHg for 1 hr) was uniformly fatal for the recipient, they were unable to confirm

that the toxin was endotoxin by a number of biological tests. Using the shocked animal as a test subject it was not possible to induce either the generalized or the local Shwartzman reaction with endogenous endotoxin as one of the two provocative factors. They could not produce the accelerated skin test with epinephrine, although it occurs with as little as 1 µg of endotoxin injected intravenously.

The different results obtained by these various workers cannot easily be explained, but it may be that in strictly controlled experiments of this type minor differences in method may explain the apparent contradictions.

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