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The effect of heparin and of acid-citrate-dextrose solution on screen filtration pressure of blood in experimental hypotension

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SUMMARY

Anticoagulation with heparin may not always adequately protect an ischaemic vascular bed. A screen filtration device has been employed to study the in vitro actions of acid-citrate-dextrose (ACD) and heparin on blood from the dog in haemorrhagic shock. With both anticoagulants, blood obtained during shock caused greater filter obstruction than blood taken before shock. However, the increased obstruction was nearly four times greater with heparinized blood than with acid-citrate-dextrose blood. These findings suggest that the anticoagulant action of heparin may be markedly diminished in low blood flow states.

IT is the standard practice of many surgeons to heparinize a regional vascular bed in which circulatory arrest is to occur on the premise that clotting will then be prevented. However, there is both clinical and experimental evidence that the use of heparin is not always successful: the lower limb sometimes does not recover after aortic cross-clamping, and although embolization may be blamed, clot is not always found; in rat kidney (Diethelm and Wilson, 1971) and adrenal (Kovács et al., 1966) and in rabbit brain (Ames et al., 1968) heparin has been ineffective in preventing microcirculatory obstruction.

Thus, it seemed important to establish if heparin effectively inhibited the tendency for fibrin to form under conditions of hypoperfusion. We have studied the changes in screen filtration pressure (SFP) of blood which occur with a standard shock preparation in the dog. The method is an indirect indication of any tendency of circulating blood to cause obstruction in the microcirculation, but is obviously influenced by many factors. For example, blood without anticoagulant and tested immediately causes little or no obstruction to a screen filter of 20 μ pore size (Swank, 1962); however, blood stored in acid-citrate-dextrose (ACD) for 3 weeks produces a marked obstruction, an effect thought to be the result of aggregation of platelets and leucocytes (Swank, 1961). The high SFP of ACD blood takes more than 2 days to develop, by comparison with 16–24 hours in heparinized blood (Swank, 1961). In that platelet aggregation can be induced easily in citrated plasma (Dhall et al., 1969), these observations suggest that the early rise in SFP in heparinized blood is likely to be caused by the development of fibrin. For a variety of reasons heparin is a relatively weak inhibitor of fibrin formation; indeed, heparinized blood cannot be stored for any length of time because of its tendency to produce fibrin clots (Mollison, 1972a). Finally, in relation to normal blood, red cell aggregation is unlikely to be important as a cause of screen filter obstruction (Dhall et al., 1969).

Variable results have been found for SFP in experimental exsanguination. On the one hand, blood without anticoagulant is said to cause marked filter obstruction, possibly because of an increased tendency of platelets and white cells to adhere rather than on account of the presence of preformed aggregates (Swank, 1962); on the other hand, a change in SFP has not been found in some breeds of dog (Swank et al., 1974). In the light of the effect of ACD and of heparin on normal blood, and of preliminary studies which had suggested increased SFP in blood from the dog in shock, a comparison was made between the two anticoagulants.

Materials and methods

The screen filtration technique was essentially that of Swank (1961). The 20 μ filter was mounted in a stainless steel block heated to 37 °C; blood or saline was forced through the system with a mechanical ram attached to a plastic syringe. Pressure was transduced from a side-arm with a strain gauge and traced on a chart recorder. A 2-ml syringe driven at a flow rate of 1 ml/7 sec and a 5-ml syringe at 1 ml/3·8 sec were used for blood and saline respectively. The filter was calibrated with 2 ml of saline; 0·7 ml of blood (5 sec filtration) was then passed through, followed by a further 2 ml of saline. The difference between the two saline pressures gives an indication of the degree of obstruction produced by the blood.

Preparation of heparinized and ACD blood was standardized. A heparin concentration of 3 i.u./ml is required to prevent normal human blood from clotting *in vitro* (Mollison, 1972b), and preliminary studies confirmed that this level would prevent change in SFP for up to 1 hour at 37 °C. Consequently, and because of the expected greater coagulability of the blood of the experimental animal, a level of 5 i.u./ml was decided upon. Heparin Injection (mucous) BP without bacteriostatic (Paines & Byrne Ltd) containing 1000 i.u./ml was diluted 1 : 30 with normal saline; 110 μl were then inserted into a siliconized 2-ml syringe (Silicone Fluid MS 1107, Hopkins & Williams Ltd). A similar quantity of ACD (NIH Solution A) (Mollison, 1972c) was placed in a further 2-ml syringe. Blood obtained by needle puncture of the femoral vein into a siliconized 5-ml syringe was

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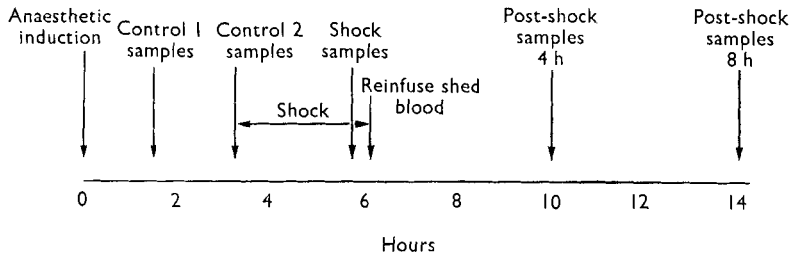

Fig. 1. Plan of the experiment.

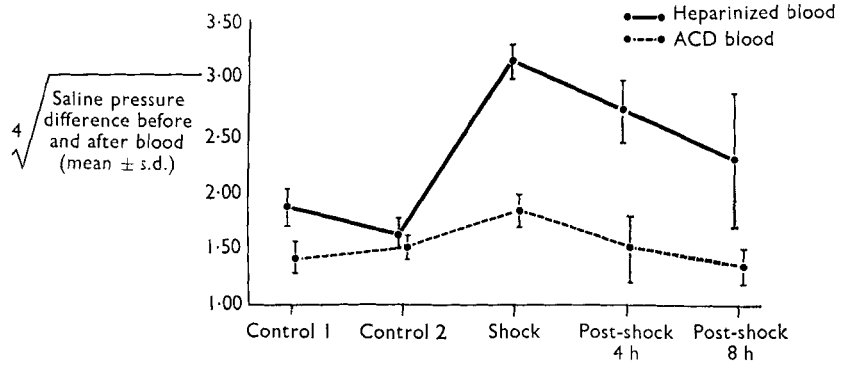

Fig. 2. Effect of haemorrhagic shock on screen filter obstruction by heparinized and ACD blood.

Table I. CHANGES IN ARTERIAL PRESSURE AND MIXED VENOUS Po_2 AND pH

	Control 1	Control 2	Shock	Post-shock 4 h	Post-shock 8 h
Arterial pressure (mm Hg)	201 ±9·7	202 ±11·8	88 ±10·2	184 ±21·9	186 ±20·2
Pvo_2 (mm Hg)	51 ±3·0	48 ±2·1	24 ±4·6	44 ±1·5	43 ±2·1
pHv	7·406 ±0·011	7·402 ±0·020	7·168 ±0·051	7·362 ±0·033	7·390 ±0·016

Results given as mean ±s.d.

aspirated into the prepared syringes in such a quantity as to give a final heparin concentration of 5 i.u./ml in blood at a PCV of 45 per cent. The plasma ACD concentration was approximately that of ACD in whole blood also at a PCV of 45 per cent, that is 1 : 9. The two samples were placed on a mixing table for 1 minute and then in a waterbath at 37 °C. After 1 hour SFP was measured. Coincidentally, a mixed venous blood sample for hydrogen ion activity (pH) and oxygen tension (Pvo_2) was withdrawn from a pulmonary artery catheter and measured with a microelectrode system. Shock was induced in 5 greyhounds of mean weight 27 kg. The procedure is summarized in *Fig.* 1.

Filter resistance was expressed as the fourth root of the measured pressure. Statistical analysis was by the Walsh test for non-parametric paired data (Siegel, 1956).

Results

Changes in mean arterial pressure and mixed venous Po_2 and pH are seen in *Table I.* The comparison between blood treated with ACD and with heparin is shown in *Fig.* 2. At all stages heparinized blood caused greater obstruction than did ACD blood. The pooled values in the control periods differ significantly from each other ($P = 0{\cdot}005$). During shock SFP is increased; 0·38 ± 0·14 (mean ± s.d.) for ACD blood, and 1·49 ± 0·60 for heparinized blood. The incremental ratio is 1 : 3·9, that is, heparin is nearly four times less effective than ACD in the prevention of increased filter obstruction by blood from the dog during shock.

Discussion

These results show that change in SFP during and after haemorrhagic hypotension in the dog is dependent upon the anticoagulant used. Heparin is associated with a marked increase. Although the evidence is, as already discussed, indirect, it is probable that the obstruction is the result of fibrin formation.

There are a number of factors which might cause this ineffectivity of heparin. Increased thromboplastin activity nullifies its anticoagulant action (Jaques, 1963); platelet consumption with thromboplastin release is a known accompaniment of hypovolaemic hypotension. Paracoagulation of soluble fibrin monomer complex by lysosomal protein fraction of polymorphonuclear leucocytes may also be of significance because heparin at the concentration used does not prevent this form of precipitation of fibrin (Hawiger et al., 1969). Further, lysosomal enzymes are released during haemorrhagic shock (Clermont et al., 1972) and these directly antagonize heparin (Stamm, 1966). Mild acidity interferes with the anticoagulant activity of heparin (Jensen et al., 1948; Crowell and Houston, 1961; Hardaway et al., 1964),

but in the current study high SFPs were present at normal pH. Finally, heparin consumption or degradation may interfere with its anticoagulant properties in the animal in shock. Whatever the cause of the increased SFP of heparinized blood in the preparation studied, the evidence is presumptive that a microcirculatory problem might still persist despite the use of heparin. The same may well be true for a regional circulation in which the flow of blood is arrested. A reassessment of heparin for both general and regional use seems called for.

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References

AMES A., WRIGHT R. L., KOWADA M., THURSTON J. M. and MAJNO G. (1968) Cerebral ischaemia II, the no-flow phenomenon. *Am. J. Pathol.* **52**, 437–454.

CLERMONT G. H., ADAMS J. T. and WILLIAMS J. S. (1972) Source of lysosomal enzymes acid phosphatase, in haemorrhagic shock. *Ann. Surg.* **175**, 19–25.

CROWELL J. W. and HOUSTON B. (1961) Effect of acidity on blood coagulation. *Am. J. Physiol.* **201**, 379–382.

DHALL D. P., ENGESET J., MCKENZIE F. N. and MATHESON N. A. (1969) Screen filtration pressure of blood—an evaluation. *Cardiovasc. Res.* **3**, 147–154.

DIETHELM A. G. and WILSON S. J. (1971) Obstruction to the renal microcirculation after temporary ischaemia. *J. Surg. Res.* **11**, 265–276.

HARDAWAY R. M., ELOVITZ M. J., BREWSTER W. R. and HOUCHIN D. N. (1964) Clotting time of heparinised blood. *Arch. Surg.* **89**, 701–705.

HAWIGER J., COLLINS R. D. and HORN R. G. (1969) Precipitation of soluble fibrin monomer complexes by lysosomal protein fraction of polymorphonuclear leukocytes. *Proc. Soc. Exp. Biol. Med.* **131**, 349–353.

JAQUES L. B. (1963) The chemistry, pharmacology and assay of heparin. *Thromb. Diath. Haemorrh.* **11**, (Suppl.) 27–36.

JENSEN R., SNELLMAN O. and SYLVÉN B. (1948) On the inhomogeneity of commercial heparin preparations from the physicochemical point of view. *J. Biol. Chem.* **174**, 265–271.

KOVÁCS K., CARROLL R. and TAPP E. (1966) Temporary ischaemia of the rat adrenal gland. *J. Pathol.* **91**, 235–240.

MOLLISON P. L. (1972a) *Blood Transfusion in Clinical Medicine*, 5th ed. London, Blackwell, p. 57.

MOLLISON P. L. (1972b) *Blood Transfusion in Clinical Medicine*, 5th ed. London, Blackwell, p. 10.

MOLLISON P. L. (1972c) *Blood Transfusion in Clinical Medicine*, 5th ed. London, Blackwell, p. 686.

SIEGEL S. (1956) *Nonparametric Statistics for the Behavioral Sciences.* New York, McGraw-Hill, pp. 83–87.

STAMM H. (1966) Antiheparine. *Thromb. Diath. Haemorrh.* **19** (Suppl.), 181–186.

SWANK R. L. (1961) Alteration of blood on storage: measurement of adhesiveness of 'aging' platelets and leukocytes and their removal by filtration. *New Engl. J. Med.* **265**, 728–733.

SWANK R. L. (1962) Adhesiveness of platelets and leukocytes during acute exsanguination. *Am. J. Physiol.* **202**, 261–264.

SWANK R. L., CONNELL R. S. and WEBB M. C. (1974) Dacron wool filtration and hypotensive shock: an electron microscopical study. *Ann. Surg.* **179**, 427–433.