COMPLEMENT ACTIVATION DURING CARDIOPULMONARY BYPASS BY HEPARIN–PROTAMINE INTERACTION

N. BEST, M. J. SINOSICH, B. TEISNER, J. G. GRUDZINSKAS AND M. McD. FISHER

SUMMARY
Circulating concentrations of split products of the third complement factor (C3c and C3d) were measured in five patients before, during and after cardiopulmonary bypass. In all patients, C3d concentrations increased significantly in samples obtained after the administration of protamine sulphate. Similarly, circulating C3c was seen only in those samples obtained immediately after protamine administration. In vivo experiments demonstrated that activation of the complement system was attributable to the heparin–protamine complex, and was dose-dependent. The activation of complement was not associated with any clinically detectable adverse effects.

Activation of the complement system may cause an increase in vascular permeability (Hugli, 1979) and may be of importance in the pathogenesis of non-cardiogenic pulmonary oedema (Hammerschmidt et al., 1980). The demonstration of complement activation during cardiopulmonary bypass (CPB) (Haslam, Townsend and Branthwaite, 1980; Chenoweth et al., 1981) has led to speculation that the activation of complement may be of significance in the pathogenesis of “post-perfusion lung” (Chenoweth et al., 1981; Hammerschmidt et al., 1981). However, in a more recent study Boralessa and colleagues (1982) were unable to show complement activation during CPB, and suggested that the changes in the concentrations of complement components were a result of dilution, redistribution or unidentified causes. Chenoweth and associates (1981) suggested that the mesh liner of bubble oxygenators caused the activation of complement, and White (1981) suggested that activation could result from the heparin–protamine complex or protamine interaction with reactive protein. We have undertaken the following study to elucidate the role of the heparin–protamine complex in complement activation during CPB using estimation of split products of the third complement factor (C3c and C3d) in vivo and in vitro.

PATIENTS AND METHODS

Patients and cardiopulmonary bypass
Five patients, aged between 38 and 57 yr, underwent cardiac surgery for coronary bypass grafting. All patients were premedicated with morphine and hyoscine. Anaesthesia was induced with fentanyl, and was maintained using nitrous oxide in oxygen, and morphine. Neuromuscular blockade was obtained using pancuronium bromide. The operative procedure involved median sternotomy and anatomosis of reversed segments of autogenous saphenous vein from the aorta to the coronary arteries.

Heparin sulphate 3 mg kg⁻¹ (Commonwealth Serum Laboratories of Australia) was given before cardiopulmonary bypass. A Sarns roller pump and a Cobe Optiflo II bubble oxygenator unit, which included an integral 0.2 µm hydrophobic bacterial gas filter, were used for all patients. The pump oxygenator system was primed with Hartmann’s solution 1000 ml, 4% dextrose with 0.18% saline 1000 ml, and a further 10 000 units of heparin sulphate. Arterial and venous lines were placed in the aorta and right atrium. Initial flow rate on bypass was 2.4 litre/m² body surface area, and the perfusate temperature was 34 °C. During bypass, flow rates were decreased to three-quarters of the initial flow and the temperature of the infusate was decreased to between 22 and 26 °C. All patients received cardio-plegia solution which contained magnesium chloride 16 mmol, potassium chloride 16 mmol, and procaine hydrochloride 1 mmol litre⁻¹. Total bypass time ranged from 45 to 130 min. After normal circulation was re-established, protamine sulphate (Boots


Present addresses:
*Institute of Medical Microbiology, University of Odense, Odense, Denmark, 5000.
†Department of Obstetrics and Gynaecology, The London Hospital (Whitechapel), London E1 1BB.
Correspondence to M. McD. F., Intensive Therapy Unit.

© The Macmillan Press Ltd 1984
Company Limited, U.K.) 6 mg kg \(^{-1}\) was given i.v. over 10–15 min.

**Blood samples**

Blood samples were collected from the radial artery into EDTA tubes (5 mmol litre \(^{-1}\)) at the following times: before the induction of anaesthesia (sample 1); 30 min after the induction of anaesthesia (sample 2); 15 min after heparinization (sample 3); 45–70 min after heparinization (sample 4); off bypass, 15 min after injection of protamine sulphate (sample 5); 3–4 h after surgery (sample 6); 6–8 h after surgery (sample 7); the following morning (sample 8). Plasma was separated within 30 min of collection and stored at \(-70^\circ\)C until assay.

**In vitro experiments**

Blood was collected from healthy volunteers into EDTA 5 mmol litre \(^{-1}\), heparin 143 u./10 ml and anticoagulant-free tubes, respectively. Centrifugation (2000 rev min \(^{-1}\) for 15 min) was performed after 45 min at room temperature (20°C) and serum or plasma separated. Sodium heparin (Commonwealth Serum Laboratories of Australia) was added to a final concentration of 28.6 u. ml \(^{-1}\) to one tube each of serum and EDTA plasma, sodium chloride 0.15 mol litre \(^{-1}\) was added to one tube each of serum, EDTA plasma and heparin-derived plasma. To 200-μlitre aliquots from each of these five tubes was added protamine sulphate 10 mg ml \(^{-1}\) (Boots Company Limited, U.K.) in the following amounts: 0, 10, 20, 50, 100, 200 and 500 μg. Sodium chloride 0.15 mol litre \(^{-1}\) was added to maintain a constant volume throughout. After mixing, the samples were incubated for 1 h at 37°C. Assays for C3 split products (C3c and C3d) were performed immediately after centrifugation at 2000 rev min \(^{-1}\) for 15 min.

**Electroimmunoassay**

Albumin concentrations in the patient samples were measured by rocket immunoelectrophoresis (Brandslund et al., 1981). The concentration of albumin in each patient was expressed as a percentage of the first sample obtained from the patient. C3d measurements were performed by a double-zone rocket immunoelectrophoresis as described previously (Brandslund et al., 1981; Sinosich et al., 1982) and the concentration in the patient samples given in arbitrary units. C3d concentrations in the in vitro experiments were expressed as percentage of the values seen in the control samples, EDTA plasma and heparin, serum, serum and heparin serum, and heparin plasma (all volumetrically adjusted with sodium chloride 0.15 mol litre \(^{-1}\)) C3c was demonstrated by qualitative crossed immunoelectrophoresis only (Brandslund et al., 1981).

The antisera, that is anti-albumin (Code A114), and anti-C3c (Code 10–062) and anti-C3d (Code A063) were purchased from DAKO Immunoglobulins, Copenhagen, Denmark.

**RESULTS**

The circulating concentrations of C3d in the five patients undergoing cardiopulmonary bypass are shown in figure 1. In all patients a decrease in C3d (relative to preoperative values) was seen by the time of heparinization (sample 3) soon after commencement of cardiopulmonary bypass. C3d concentrations were all significantly greater after the administration of protamine sulphate (samples 5 and 6) on completion of cardiopulmonary bypass, returning to normal values by the following morning (sample 8) in all but one patient. The albumin
The presence and significance of complement activation during cardiopulmonary bypass are controversial. Chenoweth and colleagues (1981) showed increases in the plasma concentrations of C3a but not of C5a during CPB, but suggested that C5a-induced granulocyte sequestration occurred. However, Hammerschmidt and co-workers (1981) showed no evidence of C3 conversion or C5a in patient plasma, but also suggested that C5a-induced neutropaenia occurred. Haslam, Townsend and Branthwaite (1980) demonstrated conversion of C3 during both CPB and thoracotomy and, in a more recent study, Boralessa and colleagues (1982) observed a decrease in C3 and C4 during bypass. However, they obtained no evidence of changes in the C3 split product concentration and suggested that the changes were the result of dilution, redistribution, or unidentified factors, and not to activation. Although it was suggested by Chenoweth and colleagues (1981) and Hammerschmidt and co-workers (1981) that the observed changes in complement may contribute to the development of “post-perfusion lung”, this complication did not develop in any of the patients in either their studies or the current study.

The study performed here demonstrated no activation of C3 until protamine was administered, a

**DISCUSSION**

The presence and significance of complement activation during cardiopulmonary bypass are controversial. Chenoweth and colleagues (1981) showed increases in the plasma concentrations of C3a but not of C5a during CPB, but suggested that C5a-induced granulocyte sequestration occurred. However, Hammerschmidt and co-workers (1981) showed no evidence of C3 conversion or C5a in patient plasma, but also suggested that C5a-induced neutropaenia occurred. Haslam, Townsend and Branthwaite (1980) demonstrated conversion of C3 during both CPB and thoracotomy and, in a more recent study, Boralessa and colleagues (1982) observed a decrease in C3 and C4 during bypass. However, they obtained no evidence of changes in the C3 split product concentration and suggested that the changes were the result of dilution, redistribution, or unidentified factors, and not to activation. Although it was suggested by Chenoweth and colleagues (1981) and Hammerschmidt and co-workers (1981) that the observed changes in complement may contribute to the development of “post-perfusion lung”, this complication did not develop in any of the patients in either their studies or the current study.

The study performed here demonstrated no activation of C3 until protamine was administered, a
finding not demonstrated in the previous studies but suggested by White (1981). The protamine–heparin complex has been shown previously to activate complement (Rent et al., 1975), and complement activation has been demonstrated in association with an adverse response to protamine (Best et al., 1983). The in vitro studies confirmed the necessity of both heparin and protamine to activate complement. This activation was not seen in EDTA samples in which chelation of Ca²⁺ and Mg²⁺ had occurred. Further, the effect was dose-dependent, and this may explain the difference between this study and that of Boralessa and associates (1982) in which no activation was seen. However, only one-sixth of the dose of protamine had been given. The degree of complement activation observed was unlikely to be associated with an adverse response and no such response was observed. Although this does not mean that no adverse effect occurs, it may be advisable to use lower doses of protamine to decrease the likelihood of such an effect. While it is noteworthy that in none of the studies cited previously has an adverse effect of the observed activation been documented, activation of greater magnitude has been demonstrated in an anaphylactoid reaction to protamine (Best et al., 1983) and the postulated role of protamine-induced complement activation in post-perfusion lung cannot be discounted.

REFERENCES


**KOMPLEMENT-AKTIVIERUNG WÄHREND KARDIOPULMONALEM BYPASS DURCH HAPARIN—PROTAMIN-INTERAKTION**

**ZUSAMMENFASSUNG**

**ACTIVATION DU COMPLEMENT AU COURS DE LA CEC PAR INTERACTION HEPARINE—PROTAMINE**

**RESUME**
Les concentrations circulantes des produits de dégradation de la troisième fraction du complément (C3c et C3d) ont été mesurées chez cinq patients avant, pendant et après une CEC. Chez tous les patients, les concentrations de C3d augmentaient significativement dans les échantillons prélevés après administration de sulfate de protamine. De même, on ne retrouvait de C3c circulant que dans les échantillons obtenus immédiatement après administration de protamine. Des expériences in vitro ont montré que l'activation du complément pouvait être rapportée au complexe héparine—protamine et était dose-dépendant. L'activation du complément ne s'accompagnait d'aucun effet délétère cliniquement décelable.

**ACTIVACION DEL COMPLEMENTO DURANTE UN PUENTE EXTERNO CARDIO-PULMONAR MEDIANTE LA INTERACCION HEPARINA—PROTAMINA**

**SUMARIO**
En cinco pacientes, se midieron antes, durante y después de un puente externo cardio-pulmonar, las concentraciones circulantes de los productos seccionales del tercer factor de complemento (C3c y C3d). En todos los pacientes, las concentraciones C3d aumentaron de manera significante en las muestras recogidas después de la administración de sulfato de protamina. Igualmente, el C3c circulante se encontraba únicamente en las muestras obtenidas inmediatamente después de la administración de protamina. Las experiencias in vitro demostraron que la activación del sistema de complemento podía atribuirse al complejo heparina—protamina y dependía de la dosis. La activación del complemento no se hallaba asociada con cualesquiera efectos desfavorables clínicamente detectables.