PERIOPERATIVE CHANGES IN COMPLEMENT ASSOCIATED WITH CARDIOPULMONARY BYPASS

H. Boralessa, J. A. Shifferli, F. Zaimi, E. Watts, J. G. Whitwam and A. J. Rees

SUMMARY

The total haemolytic complement (CH50), the complement components C3 and C4, the complement breakdown product C3d, alternative pathway activation and transferrin, were measured before, during and after cardiopulmonary bypass. As expected, CH50 decreased after heparinization, remained low during bypass and decreased further up to 8 h after bypass. C3 and C4 decreased significantly during bypass, continued to decrease for a further 8 h after bypass (by 35% and 40% respectively) and thereafter increased gradually up to 48 h. Although the depletions observed were suggestive of complement activation, there were no demonstrable increases in C3d, and in all patients the concentration of C3d remained within the normal range. Hence it was concluded that complement depletions of this magnitude were unlikely to result from complement activation. Non-specific changes in protein concentrations during bypass, as a result of dilution, redistribution or other unidentified factors, are more probable causes of the observed reductions. The acute phase response to surgery may be a factor in the subsequent increase in C3 and C4 which is seen 24 h after bypass. As transferrin concentrations in the plasma are known to decrease during this response the observed decrease in transferrin concentration would support this view.

The potent peptides C3a and C5a which are released when complement is activated are thought to contribute to the pulmonary dysfunction which sometimes follows cardiopulmonary bypass (CPB) and to the pathogenesis of the post perfusion syndrome (Chenoweth et al., 1981). Both C3a and C5a are known to stimulate release of histamine from mast cells and to cause an increase in vascular permeability (Hugli, 1979) which is a feature of the post-perfusion syndrome (Kirklin, 1980). In addition, plasma C5a concentrations have been shown to be increased in the adult respiratory distress syndrome (ARDS) (Hammerschmidt et al., 1980) where similar organ dysfunction is seen. The demonstration of complement activation by crossed immunoelctrophoresis (Haslam, Townsend and Branthwaite, 1980) and the generation of C3a and C5a during bypass (Chenoweth et al., 1981) suggested that these fragments could contribute to the postoperative complications associated with CPB. However, despite the generation of C3a and C5a the patients studied had no clinical symptoms which could be related to those of active complement fragments either during bypass or in the period after operation.

The present study was undertaken to measure the degree of complement activation during cardiopulmonary bypass by using C3d as an indicator of such activation. Unlike previous studies (Chenoweth et al., 1981) observations were continued up to 48 h after the procedure. In addition, transferrin was used as a protein marker.

METHODS

Patients and surgical procedures

Ten male patients undergoing elective coronary artery bypass surgery were studied. Their mean age was 48 yr (range 32–60 yr, SD 9 yr). Premedication consisting of papaveretum 20 mg, hyoscine 0.4 mg and droperidol 5 mg was administered i.m. 90 min before arrival in the induction area. Anaesthesia was induced with thiopentone 250–300 mg i.v. and papaveretum 6 mg i.v., followed by pancuronium 10 mg i.v. and after intubation was maintained with 67% nitrous oxide in oxygen, incremental doses of papaveretum to a total dose of 1 mg kg$^{-1}$ body weight and a further dose of pancuronium 4 mg during bypass. The mean arterial pressure was maintained in the range 70–80 mm Hg throughout, before bypass with halothane and after bypass with sodium nitroprusside. Surgery consisted of a me-
dian sternotomy and placement of four reversed segments of autogenous saphenous vein grafts from the coronary arteries to the aorta.

Cardiopulmonary bypass equipment was standard and comprised an American Optical Pump, and Bentley BOS10 bubble oxygenator. Cardiotomy filters were used. The pump oxygenator system was primed with Hartmann solution 2 500 ml and sodium bicarbonate 25 mmol. After the administration of bovine lung heparin 300 i.u. kg⁻¹, bypass was instituted with an initial flow rate of 2.4 litre min⁻¹ per m² body surface area. During CPB the patients were cooled to 26–28 °C and flow rates were reduced to 1.4 litre min⁻¹ m⁻². After aortic cross clamping, myocardial preservation was maintained with 1–1.5 litre of cardioplegia (St Thomas’s) solution. After the coronary arterio-venous anastomoses were performed the cross clamp was removed and the aorta-venous anastomoses performed, during which time the patient was gradually rewarmed and resumption of cardiac activity occurred spontaneously.

The lungs were ventilated with 50% oxygen in nitrogen 1.5 litre min⁻¹ throughout bypass. Total bypass time varied between 1 h 10 min and 1 h 30 min.

Potassium was administered to maintain a concentration of approximately 4.5 mmol litre⁻¹ throughout. After removal of the perfusion cannulae protamine was administered in a dose of 1 mg per 300 units of heparin. Coagulation was measured using an automated activated clotting time (Hemochron). At the end of surgery the patients returned to the intensive care unit and were ventilated in the period immediately after operation. Ventilation was terminated and the trachea extubated 4–8 h after surgery. The patients were discharged from hospital 8–10 days later and remained well during this period.

All these patients received 2–3 units of 3–5 day old CPD blood in the period immediately after bypass and after operation a further 1–2 units was administered to restore the haematocrit to normal.

**Collection of samples**

Samples of venous blood were collected at the times shown in table I for complement assays. Two aliquots of blood were taken. One sample was placed in EDTA for C3d estimation and the other allowed to clot to measure C3, C4, CH₅₀, alternative pathway activity and transferrin. The cells were separated by centrifugation, and the plasma or serum was separated and stored at −70 °C. The assays were performed in a single batch.

**Complement measurements**

Total haemolytic activity (CH₅₀) and alternative pathway function were measured as described by Lachmann and Hobart (1978) and the results were expressed as a percentage of the value from a pool of five samples of normal human serum.

Plasma C3 and C4 were measured by single radial immunodiffusion and the results were expressed as a percentage of a pool of five normal human sera.

C3d was measured by the technique of Perrin, Lambert and Miescher (1975).

All concentrations were corrected for dilution relative to the preoperative haemoglobin concentrations during bypass and in the periods after bypass and after operation using the formula:

\[
\text{Corrected concentration} = \frac{\text{Measured concentration} \times \text{initial PCV}}{\text{PCV at time of measurement}}
\]

**Transferrin measurement**

An automated, immunoprecipitation assay (Technicon) was used to measure serum transferrin.

**RESULTS**

No complications were observed in the patients studied during bypass or in the period after operation.

**CH₅₀**

A significant decrease in CH₅₀ (P < 0.05) was seen after heparinization compared with the value before

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time</th>
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<tbody>
<tr>
<td>1</td>
<td>24 h before surgery</td>
</tr>
<tr>
<td>2</td>
<td>After induction of anaesthesia but before surgery</td>
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<td>3</td>
<td>After administration of heparin</td>
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<td>4</td>
<td>10 min after institution of CPB</td>
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<tr>
<td>5</td>
<td>20 min after institution of CPB</td>
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<tr>
<td>6</td>
<td>30 min after institution of CPB</td>
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<tr>
<td>7</td>
<td>60 min after institution of CPB</td>
</tr>
<tr>
<td>8</td>
<td>After administration of protamine</td>
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<tr>
<td>9</td>
<td>1 h after termination of CPB</td>
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<tr>
<td>10</td>
<td>4 h after termination of CPB</td>
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<td>11</td>
<td>8 h after termination of CPB</td>
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<tr>
<td>12</td>
<td>24 h after termination of CPB</td>
</tr>
<tr>
<td>13</td>
<td>48 h after termination of CPB</td>
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operation (fig. 1). Thereafter no further reduction of CH50 values occurred up to 60 min on bypass. CH50 was significantly reduced at 4 h ($P < 0.05$), 8 h ($P < 0.005$) and 24 h ($P < 0.0005$) after bypass (table II).

Haemolytic alternative pathway activity showed changes similar to those in CH50 but these were not significant (table II).

C3d
C3d concentrations did not change during the period of study and for all patients remained in the normal range ($< 2.05 \mu g \text{ ml}^{-1}$).

C3 and C4
No significant difference was seen between the values of C3 and C4 before operation and their values before bypass. Ten minutes after commencing cardiopulmonary bypass, C3 concentration decreased in all 10 patients ($P < 0.005$) and thereafter remained reduced up to 60 min. In contrast, C4 decreased gradually during bypass and at 60 min was seen to be reduced in all 10 patients ($P < 0.0005$).

C3 and C4 continued to decline up to 8 h after the termination of bypass. Thereafter, C3 and C4 concentrations increased gradually (fig. 1); however, 24 h after bypass they were still significantly less ($P < 0.0005$) than the values before operation (table II).

Transferrin
No differences were seen between the transferrin concentrations before operation and those after heparinization, (table II). Ten minutes after commencing CPB the plasma transferrin concentration decreased in all 10 patients. The mean concentration was reduced to 69% of the value before operation ($P < 0.005$) (fig. 1). Unlike C3 and C4, transferrin concentrations returned to normal within 1 h after bypass. Thereafter a slow continuous decrease was observed up to 48 h.

DISCUSSION
The present study demonstrated a reduction in the concentration of C3 and C4 not only during cardiopulmonary bypass (CPB) but also in the period after bypass, an observation which has not been made in previous studies which terminated early in the postoperative period (Hammerschmidt et al., 1981). An important observation made in other studies (Chenoweth et al., 1981) is that, despite complement changes, no substantial complications were seen in the patients studied and this was also
true for the patients in the present study.

The relatively large values of C3 and C4 before operation in these patients could be explained by low values in the standard pool used for the control assays. Alternatively, all the patients were suffering from heart disease and since C3 and C4 are acute phase reactants, the initial values could be expected to be increased in some of these patients.

All the protein concentrations including C3 and C4 were corrected for dilution and since all of them showed a decrease during bypass, it could be assumed that they were all part of a process which may be common to many proteins during bypass. The return of transferrin to pre-bypass concentrations immediately after bypass when C3 and C4 continued to decrease suggests that the reduction in complement concentration after bypass reflects a more specific process. However, as there was no increase in the C3 breakdown product C3d during this period, despite a 40% decrease in complement concentration, it was concluded that complement activation was unlikely to be the cause of this reduction.

Freezing and rewarming blood for complement assays does not cause complement activation in vitro (Garratty, 1970). The failure to demonstrate an increase in C3d in the present study suggests that hypothermia, with temperatures of 28 °C, does not cause complement activation.

Heparin blocks complement activation (Loos, Volkanis and Stroud, 1976; Raepple, Hill and Loos, 1976) and the decrease in CH50 is in keeping with this. Heparin protamine complexes activate the classical complement pathway (Lachman and Hobart, 1978), but the absence of an increase in C3d suggests that these did not cause persistent significant activation in the present study.

C3 and C4 are well preserved in CPD stored blood (Schifferli et al., 1982). Thus in those patients in whom the C3 and C4 concentrations were reduced by 30% and 40%, the 2–3 units of 3–5 day old blood which they received in the period immediately after bypass would tend to increase the concentrations of C3 and C4.

The complement concentrations in blood represent a dynamic equilibrium between synthesis, degradation and distribution into various body compartments, for example intravascular and extravascular. The changes observed could be attributable to any or all of these factors. The reductions in concentration of C3 and C4 observed during bypass were unlikely to be a result of reduced synthesis as they occurred too rapidly. However, reduced synthesis could contribute to the further reduction seen
after operation as the fractional catabolic rates of C3 and C4 are 1.71%/h⁻¹ and 1.4%/h⁻¹ respectively (Ruddy et al., 1976). Clinical and experimental studies show that some proteins are denatured when exposed to cardiopulmonary bypass equipment. For example Pruitt, Stroud and Scott (1971) have shown, in vitro, that gammaglobulins when exposed to the oxygenator pump system undergo denaturation and precipitation with albumin and that the tendency to form such precipitates increases at lower temperatures. Two studies support the view that this denaturation could also occur in vivo. First, significant reductions of immunoglobulins were seen in patients after CPB (Parker et al., 1972), but not after other surgical procedures (Hairston et al., 1969). Second, anti-gammaglobulin antibodies have been demonstrated in the sera of patients who have undergone surgery with CPB (Pretty et al., 1968). Thus it is conceivable that complement proteins could be similarly denatured without the production of C3d. For instance, the altered complement proteins could bind to erythrocytes, similar to C3c binding to red cells seen during storage of blood (Szymanski and Odgren, 1979); or C3 and C4 per se could bind to red cells as a result of physicochemical changes related to bypass, similar to the way in which C3 and C4 bind to red cells when physicochemical alterations are produced in vitro (Heier, Kornstad and Nordhagen, 1981).

Although complement activation during bypass has been demonstrated in several studies (Chenoweth et al., 1981) the patients did not have any clinical complications which could be related to such activation either during or after surgery, which suggests that the degree of complement activation was insufficient to produce complications. Haslam, Townsend and Branthwaite (1980) demonstrated complement activation by two-dimensional immuno-electrophoresis, not only in patients undergoing cardiopulmonary bypass, but also in patients undergoing thoracotomy without bypass and hence did not provide any real evidence that complement activation is a feature peculiar to bypass. Furthermore, changes in complement were not quantified and hence this study also fails to give any indication of the degree of complement depletion. The demonstration by Chenoweth and his associates (1981) of the appearance of complement fragments C3a and C5a during bypass is conclusive evidence of complement activation. However, whilst C3a in plasma was shown to increase progressively during bypass, the concentration subsequently decreased after bypass, which is not consistent with continuous activation. This finding is in complete accordance with the present study which failed to demonstrate increased generation of C3d after CPB and justifies the conclusion that the increasing reduction in C3 and C4 seen after bypass in the present study is not related to activation. Moreover, Chenoweth and his associates inferred C5a production by demonstrating increased leucocyte receptor occupancy by this fragment. However, their patients remained clinically well and they did not show an increase in the plasma concentrations of C5a which is the case in patients with organ dysfunction (Hammerschmidt et al., 1980).

Whereas during cardiopulmonary bypass some complement activation can be detected by sensitive assays (Chenoweth et al., 1981), the continuing reduction in C3 and C4 concentrations seen after bypass in this study were shown to be unrelated to activation; neither did the patients studied have any clinical complications. It was concluded that the observed changes in C3 and C4 were probably related to some other unidentified mechanism which merits further investigation.

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**MODIFICATIONS péri-opératoires du Complément en rapport avec la circulation extracorporelle**

**RESUME**

Le complément hémolytique total (CH50), les fractions C3 et C4 du complément, le produit de dégradation C3d du complément, la voie d’activation alternative et la transferrine, ont été mesurés avant, pendant et après circulation extracorporelle. Comme prévu, CH50 diminuait après héparination, demeurait bas pendant la circulation extracorporelle et continuait à baisser jusqu’à 8 heures après la circulation extracorporelle. C3 et C4 diminuaient significativement pendant la CEC, continuent à diminuer pendant encore 8 heures après la CEC (de 35% et 40% respectivement), puis remontent ensuite progressivement pendant 48 h. Bien que les dépletions observées soient évocatrices d’une activation du complément, il n’y avait pas d’augmentation objective de C3d, et chez tous les patients la concentration de C3d restait dans les limites de la normale. Nous en concluons donc que des dépletions du complément de cette amplitude avaient peu de chance d’être dues à une activation du complément. Des modifications non spécifiques des concentrations protéiques au cours de la CEC, dues à la dilution, à la redistribution ou à d’autres facteurs non identifiés, sont des causes plus probables des diminutions observées. La réponse en phase aiguë à la chirurgie peut être un facteur expliquant l’augmentation secondaire de C3 et C4 constatée 24 heures après la CEC. Comme on sait que les concentrations de transferrine dans le plasma diminuent au cours de cette réponse, les diminutions observées des concentrations de transferrine corroberont cette notion.

**PERIOPERATIVE VERÄNDERUNGEN DES KOMPLEMENTES BEI KARDIOPULMONALEM BYPASS**

**ZUSAMMENFASSUNG**


**CAMBIFS PERIOPERATORIOS DEL COMPLEMENTO ASOCIADOS CON DERIVACIÓN CARDIOPULMONAR**

**RESUMO**

Em curso de derivaciones cardiopulmonares y después de las mismas, se procedió a la medición del complemento hemolítico total (CH50), de las componentes del complemento C3 y C4, del producto de desaminación del complemento C3d, de la activación de la vía alterna así como de la transferrina. Tal como se esperaba, el CH50 disminuyó después de la heparinización, se mantuvo bajo durante la derivación y se redujo aún más hasta 8 h. después de la derivación. Los C3 y C4 disminuyeron de manera significativa durante la derivación, siguieron bajando durante 8 h. más después de la derivación (en un 35% y un 40% respectivamente) y luego aumentaron progresivamente durante las 48 h. siguientes. A pesar de las depéltiones observadas, lo que sugiere un accionamiento del complemento, no hubo aumentos comprobables del C3d y, en todos los pacientes, la concentración del C3d se mantuvo dentro de los límites normales. Por lo tanto, se concluyó que las depéltiones del complemente de tal magnitud, con poca probabilidad, podían resultar de la activación del complemento. Cambios no-específicos de las concentraciones de proteínas durante la derivación resultan principalmente de la dilución, de la redistribución u otros factores no-identificados contribuyen las causas más probables de las reducciones observadas. La respuesta aguda de fase a la cirugía puede ser un factor de los aumentos consecutivos del C3 y del C4 que se verifican 24 h. después de la derivación. Puesto que se sabe que las concentraciones de transferrina en el plasma bajan durante dicha respuesta, la disminución observada en la concentración de transferrina podría corroborar este punto de vista. 