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# Complement Activation Induced by Different Tubings Used for Cardiopulmonary Bypass

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## Abstract

Complement activation induced by latex, polyvinylchloride, and silicone tubings for extracorporeal circulation was studied in 2 *in vitro* situations: Fresh whole human blood mixed with priming solution was circulated through 100 cm tubing lengths for 1 hour, and small tubing pieces were incubated in fresh human serum for 7 hours. Activation of C3 and of the terminal pathway (C5–C9) was assessed in enzyme immuno assays. In both situations complement activation was significantly higher ( $p < 0.05$ ) than in controls. During incubation, C3 activation started later with silicone, but increased to similar levels as with polyvinylchloride after 7 hours. Latex was a significantly more potent C3 activator at 7 hours than the other materials ( $p < 0.05$ ), whereas polyvinylchloride induced most terminal pathway activation ( $p < 0.01$ ). When blood was pumped through intact tubing, complement activation was similar with all materials. The differences found during incubation were apparently overshadowed by other factors in the dynamic situation. The study thus gives no evidence for preferring any of the tested tubing materials to reduce complement activation during *in vivo* extracorporeal circulation, even if the tubing does contribute to overall activation.

## Introduction

It is well known that complement cascade activation during cardiopulmonary bypass (CPB) may have several

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adverse effects (1–4): The activation product C3a acts as a cardiodepressant (5). Another activation product, C5a, may be a pathogenic factor in development of pulmonary capillary leak and non-pressure edema (1,6) and of multi-organ failure (7) after CPB. Deposition of the terminal complement complex (TCC) on erythrocytes may be partly responsible for the hemolysis observed during CPB (8).

Complement is activated via the alternative pathway when blood comes into contact with the artificial polymers used in the CPB circuit (2,9). Investigations of membranes for dialysis have shown that the degree of complement activation in this situation of blood-polymer contact depends on the type of material used (10). The differences in ability to activate complement is related to the binding capacity for the activation product C3b and various regulatory proteins on the surface of the specific material (11).

The aim of the present study was to evaluate complement activation induced by different tubing used for CPB. Tubing segments were first tested in a dynamic situation, i.e. by circulating blood through the tubing using a roller pump, and no significant differences were found. Thereafter, we tested the tubing in a static situation by incubation of small pieces in serum, now observing significant differences between the various materials.

## Materials and Methods

### *Experiments with intact tubing*

Five brands of tubing for extracorporeal circulation were tested, each with 6 parallel experiments: Bypass 65 PVC (a), Latex (b), Rehau Silicone (c), R.X. Silicone (d), and Tygon (e). Each piece of tubing (length: 100 cm,

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a Bentley Laboratories B.V., Uden, Holland

b Kent Latex Products, Kent, Ohio, USA

c Raumedic Rehau, Rehau, West Germany

d Dow Corning, Midland, Michigan, USA

e Norton, Akron, Ohio, USA

inner diameter: 13 mm, wall thickness: 2.4 mm) was formed in a circle by means of a connector including a bleed port. Four pieces of tubing randomly chosen from the 5 brands were delivered in sterile sets (a) where all tubing circles could be filled simultaneously from a common cardiotomy reservoir (f).

Fresh whole human blood from informed volunteer donors was collected in standard CPD-adenin bags (g). For each tubing set, 1 unit of blood with 2,500 IU of Heparin was filled into the reservoir. A baseline sample was drawn before a priming solution containing 125 ml Dextran 60 mg/ml in NaCl, 125 ml Ringer's acetate, 125 ml Glucose 50 mg/ml, 125 ml Mannitol 150 mg/ml, and 50 ml sodium hydrogen carbonate 500 mmol/l was added, and the tubing was filled.

The blood/prime mixture was circulated through the tubing at 4 l/min for 1 hour using 4 different roller pumps (h). Test samples were obtained after 15, 30, 45, and 60 minutes. Controls containing blood mixed with Ringer's acetate (n=8) or priming solution (n=6) were set up in glass tubes. All samples were drawn into glass tubes containing ethylene diamine tetraacetic acid (EDTA) and kept on ice until they were centrifuged shortly thereafter. The plasma was stored at  $-70^{\circ}\text{C}$ .

#### *Incubation experiments*

Fresh human serum was prepared from informed voluntary blood donors. The temperature was maintained at  $4^{\circ}\text{C}$  immediately after collection. "EDTA-serum" (10 mmol/l) for controls was prepared from the same serum.

The following 4 tubing brands were tested in 6 parallel experiments: Bypass 65 PVC, Latex, R.X. Silicone, and Tygon. The tubing was cut into pieces of approximately  $2 \times 5$  mm, and similar amounts of pieces (determined by relative specific weights) of the various types of tubing were incubated in fresh serum at room temperature. Controls consisted of fresh serum without tubing pieces (n=6), tubing pieces in "EDTA-serum" (n=4, i.e. one of each brand) and "EDTA-serum" without tubing pieces (n=2). Samples for examination of complement activation were collected after 4 and 7 hours.

#### *Analyses of samples*

In the dynamic test C3 activation was measured in a double antibody enzyme-linked immunosorbent assay—ELISA (12). Briefly, the plates were coated with a rat monoclonal antibody (Clone 9) specific for a C3 "g" neopeptide expressed after activation at the level of iC3b, but not on native C3. This antibody was a kind gift from

Prof. Peter J. Lachmann. A rabbit anti-human C3d antiserum (l) was used in the second antibody step. Finally a peroxidase conjugated anti-rabbit Ig antiserum (j) was added. Two, 2-azino-di(3-ethyl)-benzthiazoline sulphonic acid (ABTS) was used as substrate. Zymosan activated serum was used as a standard, defined to contain 1,000 arbitrary units (AU)/ml. The results were measured by a spectrophotometer (k) at 405 nm and analyzed with the "Immunosoft" (k) program.

In the incubation experiments C3 activation was measured in a similar ELISA, using a different antibody (13) due to a change of methods at our laboratory. The mouse monoclonal antibody bH6 reacting with a neopeptide expressed in C3b, iC3b and C3c, was used to coat the plates. A rabbit anti-human C3c antiserum (l) was used in the second antibody step. The 2 C3 activation assays have shown close correlation in heterogenous patient populations (13).

The terminal complement complex was quantified in an ELISA as described by Mollnes and coworkers (14). The plates were coated with a mouse monoclonal antibody (MCaE11) specific for a neopeptide expressed only in activated C9. A rabbit anti-human C5 antiserum (i) was applied in the next antibody step. The final steps were similar to those of the C3 activation assays.

In the dynamic experiments the number of white blood cells and hemoglobin (Hgb) were determined in an electronic counter (m). Concentrations of C3 activation products and TCC were corrected for hemodilution by multiplication with the factor: initial Hgb/sample Hgb.

The Friedman test (15, 299–308) was used for statistical analyses of time-dependent parameter changes for each brand of tubing. The Kruskal-Wallis test (15, 229–237) was applied for intergroup comparisons. Results are presented as median with 95% nonparametric confidence interval in brackets.

## **Results**

#### *Experiments with intact tubings*

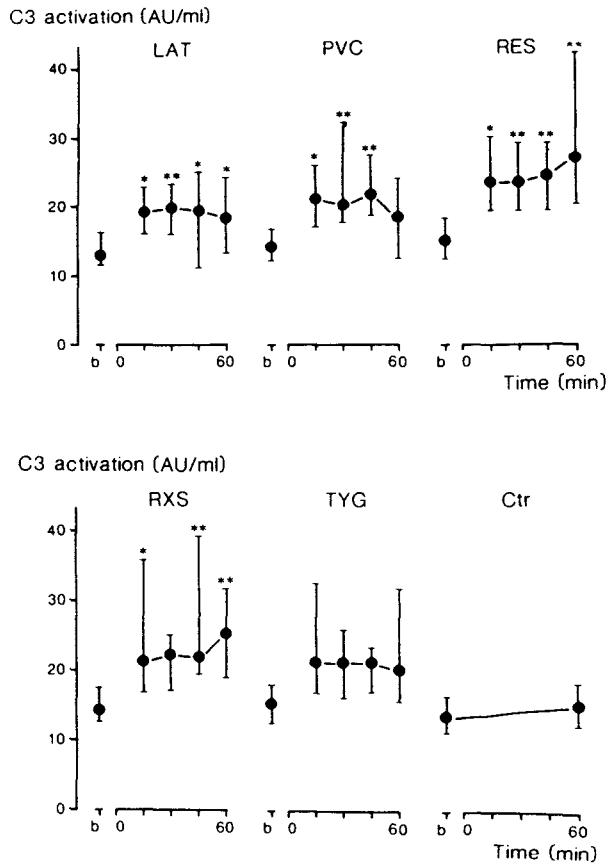
There was a significant rise in C3 activation products compared to baseline after 15 minutes with the Bypass 65 PVC, Latex, Rehau Silicone, and R.X. Silicone tubings ( $p < 0.001$ ), whereas the increase induced by the Tygon tubing did not reach significance ( $p = 0.12$ ) (Figure 1). The maximal increase in C3 activation products from baseline ranged from 37% (20–102) to 75% (22–123) for the various brands of tubing and was 8% (0–22) for con-

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f Polystan, Copenhagen, Denmark  
g Laboratories Travenol, La Chatre, France  
h Gambro, Horten, Norway  
i Dako, Copenhagen, Denmark

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j Amersham, U.K.  
k Dynatech MR 580, Dynatech Laboratories, Alexandria, Virginia, USA  
l Behring, Hamburg, West Germany  
m Coulter Electronics, Harkenden, England



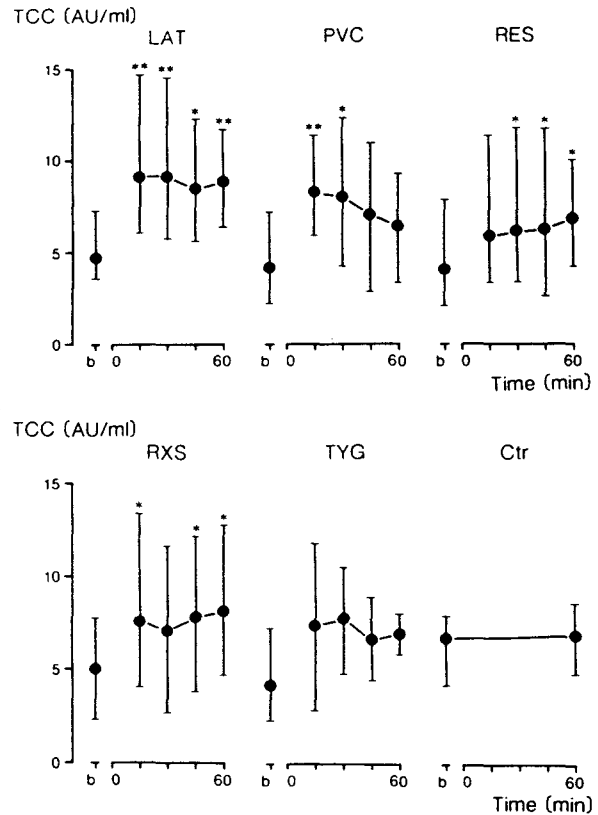
**Figure 1.** C3 activation versus time from start of experiment for 5 different types of tubing for extracorporeal circulation (n = 6 of each). LAT = latex, PVC = polyvinylchloride, RES = silicone (Raumedic Rehau), RXS = silicone (Dow Corning), TYG = tygon. Ctr = blood/primer controls (n = 6), b = baseline values. Vertical lines show 95% nonparametric confidence intervals. Significant changes compared to baseline values (Friedman test); \* p<0.05, \*\* p<0.001.

trols. Thus, all tubing activated C3 significantly more than did the controls (p<0.001). The differences between the various brands of tubing were not significant.

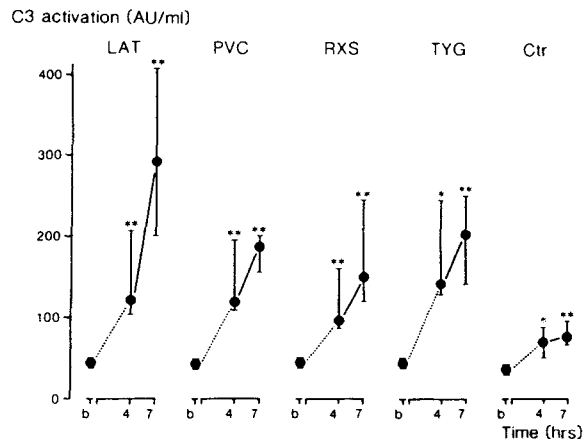
Very similar results were found with respect to formation of TCC (Figure 2): When compared to controls, all types of tubing induced a significant increase in TCC concentration (p<0.05). The maximal increase from baseline ranged from 51% (27–243) to 111% (33–220). The differences between the various types were not significant.

#### Incubation experiments

Compared to the serum controls there was a significantly larger increase in C3 activation products in the serum incubated with all brands of tubing (p<0.01) (Figure 3). However, there were significant differences



**Figure 2.** Activation of the terminal pathway (C5–C9) measured as terminal complement complex (TCC) concentrations versus time from start of experiment for 5 different tubings for extracorporeal circulation (n = 6 of each). Abbreviations and symbols as in Figure 1.



**Figure 3.** C3 activation versus time from start of experiment for pieces of 4 different tubings for extracorporeal circulation incubated in serum (n = 6 of each). Ctr = serum controls (n = 6). Other abbreviations and symbols as in Figure 1.

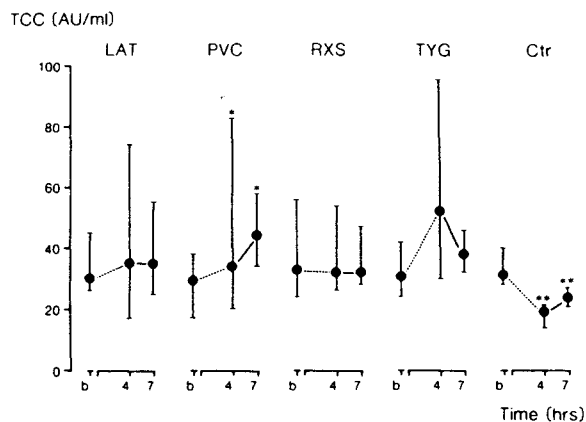
between the various brands: By 4 hours the Bypass 65 PVC and the Tygon tubing pieces had induced a significantly larger increase than the R.X. Silicone tubing ( $p < 0.05$ ); the latter not yet differing significantly from the serum controls. By 7 hours there were no differences between the Bypass 65 PVC, R.X. Silicone, and Tygon tubings, but now the Latex tubings had caused a significantly larger C3 activation than the other brands (R.X. Silicone:  $p < 0.001$ , Bypass 65 PVC:  $p < 0.01$ , Tygon:  $p < 0.05$ ). There was a significant increase in TCC in serum incubated with the Bypass 65 PVC tubing pieces, and stable concentrations in serum incubated with the other 3 tubing brands (Figure 4). In the control sera, on the other hand, the TCC concentrations decreased with time ( $p < 0.001$ ). By 7 hours, the TCC concentrations were significantly elevated compared to serum controls with all tubing ( $p < 0.05$ ), and the Bypass 65 PVC tubings had induced more TCC than the others ( $p < 0.01$ ).

There was no increase in C3 activation products nor in TCC in the controls containing EDTA.

## Discussion

In the present study, complement activation by CPB tubings representing 3 different materials was studied: latex, polyvinylchloride (Bypass 65 PVC and Tygon), and silicone.

No differences between the materials were found in the dynamic part of our study, when blood was pumped through the tubing. Under these conditions all materials led to significant formation of C3 activation products and TCC in comparison with controls.



**Figure 4.** Activation of the terminal pathway (C5–C9) measured as terminal complement complex (TCC) concentrations versus time from start of experiment for pieces of 4 different tubings for extracorporeal circulation incubated in serum ( $n = 6$  of each). Ctr = serum controls ( $n = 6$ ). Other abbreviations and symbols as in Figure 1.

Incubation of tubing pieces in serum showed differences among the types of tubing. Formation of C3 activation products was delayed in the silicone tubing, but after 7 hours was equivalent with polyvinylchloride. At 7 hours, the latex was a significantly more potent C3 activator than the other materials. These findings are consistent with the hypothesis that the ability of a certain material to activate complement is related to its surface properties.

This is not entirely so with respect to TCC formation in the static test. All tubing caused significant TCC formation compared to controls after 7 hours of incubation, but the findings were significantly different for the 2 polyvinylchloride tubing brands. Bypass 65 PVC induced terminal pathway activation more rapidly and led to higher TCC concentrations at 7 hours than did the other tubings, including the Tygons. This may reflect that details in composition/manufacturing of tubing made from the same polymer affect their surface properties (16). Different wetting agents may influence complement activation (17). Dissimilarities in surface smoothness may also be relevant, as trapping of air nuclei in the surface irregularities of synthetic materials increases complement activation (18).

The same factors may explain why our findings are only partly in agreement with earlier incubation studies, where pieces of membranes from dialyzers, oxygenators, plasma separators, and urinary catheters have been tested for complement activation. In these studies silicone induced significant production of C3a and C5a or TCC (1) (P. Garred, unpublished results), whereas PVC did not differ from controls (17, 19). The discrepancies may also have methodological causes. The numerical differences in range of complement activation levels under the 2 experimental conditions are consistent with differences between measurements in plasma (intact tubings) and serum (incubated tubing pieces) observed with similar assays by Mollnes et al. (3).

The conflicting findings in the static versus dynamic experimental situations call for an explanation. However, important differences make a too direct comparison difficult. In the dynamic test blood was anticoagulated with citrate and heparin, substances that both inhibit complement activation in vitro to a certain extent (20). Dextran used in the priming solution may lead to a low level of complement activation (21–22). It cannot be excluded that factors directly related to the pumping of blood, such as turbulent flow or mechanical damage to cells or non-cellular components of serum, may influence complement activation. However, no evidence of activation has been found in intermittent flow centrifugation leukapheresis, another procedure imposing mechanical trauma to blood (23–24). The relation between serum volume and tubing surface area is not the same in the 2

experimental situations. Breillatt and Dorson (17) have shown that complement activation during incubation of dialyzer membranes is a function of this relation. Finally, influence from the change of antibody in the C3 activation assay cannot be totally ruled out even if this seems unlikely on the basis of experience from clinical use.

It is concluded that the tubing per se contributes to the overall complement activation observed during extracorporeal circulation. Differences between various tubing materials found during incubation in serum were overshadowed by other factors when anticoagulated blood mixed with priming solution was circulated through the tubing by a roller pump, a situation more closely resembling the use *in vivo*. Our study thus gives no evidence for preferring any of the tested tubing materials in order to reduce complement activation during CPB.

### Acknowledgements

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