Complement and the damaging effects of cardiopulmonary bypass

Postoperative cardiac, pulmonary, renal and coagulation dysfunction, along with C3a levels, were studied prospectively in 116 consecutive patients undergoing open cardiac operations and 12 patients undergoing closed operations in the same time period The level of C3a 3 hours after open operation was high (median value 882 ng ml^{-1} plasma) and was related to the C3a level before cardiopulmonary bypass (CPB) ($p = 0.03$), the level at the end of CPB ($p < 0.0001$), elapsed time of CPB ($p = 0.07$), and older age at operation ($p < 0.0001$) It was inversely related to the cardiac output as reflected by the strength of the pedal pulses ($p = 0.006$) In contrast, C3a levels did not rise in patients undergoing closed operations The probability of postoperative cardiac dysfunction after open operations (present in 27 of 116 patients) was predicted by C3a levels 3 hours after operation ($p = 0.02$), the CPB time ($p = 0.02$), and younger age ($p < 0.0001$) The same risk factors pertained for postoperative pulmonary dysfunction (present in 41 of the 116 patients), renal dysfunction (present in 24 of the 116 patients) except that CPB time was not a risk factor here, abnormal bleeding (present in 21 of the 116 patients), and important overall morbidity (present in 26 of 116 patients) As regards important overall morbidity, the C3a level effect became evident at about 1,900 ng ml^{-1} (a level reached by 9% of patients), the effect of increasing time of CPB became evident at about 90 minutes of CPB time, and the effect of young age became evident as age decreased from 10 to 4 years This study demonstrates the damaging effects of CPB, relates them in part to complement activation by the foreign surfaces encountered by the blood, and supports the hypothesis that the mechanisms of the damaging effects include a whole-body inflammatory reaction

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Cardiopulmonary bypass (CPB) with maintenance of the circulation by a pump oxygenator has become an established modality for support of the patient during cardiac operations However, whether or not damage to the patient results from its use and, if so, the mechanisms underlying the damage, remain contentious.

Changes in plasma proteins, including those of the coagulation system, have been shown to result from the contact of blood with foreign surfaces, including blood-gas interfaces, during CPB.[1-4] Complement levels fall,[5] and the complement degradation products C3a and C5a are elaborated during CPB.[6] C3a and C5a have physiological effects similar to those observed in many patients after CPB, including vasoconstriction and increased capillary permeability.[7,8] Previous work from this institution has evolved the hypothesis that a whole-body inflammatory reaction of variable magnitude develops as a result of CPB and that a transient damaging effect results [9]

This clinical study was undertaken to investigate further the possibility that CPB has a damaging effect and that certain phenomena, including complement activation on the foreign surfaces of the pump oxygenator, may be related to its magnitude.

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Material and methods

Patients. A prospective study was made of all patients undergoing closed or open cardiac operations on two cardiac surgical services at the University of Alabama Hospital in Birmingham between Feb. 24, 1981, and April 10, 1981 A total of 118 patients were operated upon with the use of CPB and 28 patients by closed methods. Twenty-seven of the closed operations were for congenital heart disease, as were 40 of the open operations. All patients were studied except 18 with congenital heart disease, two treated by the open technique and 16 by the closed technique The 16 patients in the closed series who were excluded did not have an indwelling arterial or venous catheter large enough for sampling. Thus the study group consisted of 116 patients having open (11 deaths) and 12 having closed (no deaths) operations. This experience may not be a representative one, since in the calendar year 1981, among 892 patients undergoing open operation on these services, 59 died (6.6%, CL* 5.8% to 7.6%) and among 148 patients undergoing closed operations of all types, including thoracic aneurysm procedures, 11 died (7%, CL 5% to 10%).

Protocols and methods. The study protocol included collection of blood for measurement of plasma C3a levels, tabulation of intraoperative data about the details of CPB, recording of routine postoperative laboratory and clinical measurements relating to the performance of each subsystem of the patients, and observation and categorization of a number of clinical events indicating abnormal convalescence.

Blood samples for C3a determination, anticoagulated with ethylenediaminetetracetic acid, were obtained from large-bore indwelling venous catheters as near to the cutaneous puncture site as possible to avoid artifactual activation of complement by plastic tubing. In five closed operations, only an arterial line was available for sampling. R. B. Stewart, S. Westaby, and J. K. Kirklin (unpublished data) have shown that no differences exist between arterial and venous C3a levels before, during, or after CPB Samples were obtained before CPB (before the skin incision, in closed operations), just prior to the termination of CPB from the venous port of the oxygenator (in closed operations, just after closure of the chest), 3 hours after termination of bypass (in closed operations, 3 hours after arrival in the intensive care unit), and at 5 AM on the first postoperative morning. All samples were promptly centrifuged and the supernatant plasma stored at −80° C until analyzed.

Levels of human C3a antigen were quantitatively determined by radioimmunoassay.[10] The mean quantity of plasma C3a (ng · ml^{-1}) was defined from duplicate measurements by means of linear standard curves (Bo/B versus concentration C3a standard) whose correlation coefficients were >0.98. These values for plasma levels of C3a were used directly in the analysis, not corrected for hemodilution.

Abnormal convalescence among patients undergoing open operations was assessed by identification of cardiac, pulmonary, and renal dysfunction and abnormal postoperative bleeding. Death was deliberatly avoided as an end-point of the study because of lack of specificity of the event. To assess cardiac performance, routine indices of cardiac function were observed and recorded in each patient for the first 48 hours after operation, including cardiac index measured by indicator-dilution methods, atrial pressures, strength of pedal pulses, pedal skin temperature, and presence or absence of inotropic support. Cardiac performance was considered normal when pedal pulses were Grade 3 or 4 (4 being normal), pedal skin temperature was tepid or warm, cardiac index was 2 L · min^{-1} · m^{-2} of body surface area (BSA) or greater, and no inotropic support more than dopamine 2.5 mg · min^{-1} · kg^{-1} was employed. Cardiac performance which was considered normal or mildly depressed on the basis of all available information was graded 0, moderately depressed cardiac performance graded 1, and severely depressed cardiac performance graded 2.

Pulmonary performance was considered normal (and graded 0) when tracheobronchial secretions were absent or minimal, gas exchange was good, and extubation was accomplished within 24 hours of operation. Postoperative pulmonary dysfunction during the first 48 postoperative hours was graded 1 when secretions were moderate or large in amount, but prolonged (>24 hours) intubation was not required. The dysfunction was graded 2 if prolonged (>24 hours) intubation was required because of pulmonary dysfunction. However, four patients with prolonged intubation were considered to have pulmonary dysfunction Grade 1, because in two of them the intubation was prolonged only because of low cardiac output and in two intubation was required preoperatively because of severe hypoxia, shock, and acidosis; in these latter two, pulmonary dysfunction postoperatively seemed no greater than that preoperatively

Renal performance was considered normal (and graded 0) when the urine was free of gross evidence of blood or hemoglobin, urine flow was equal to or greater than 15 ml · hr^{-1} · m^{-2} BSA, and serum creatinine was less than 2 mg · dl^{-1}. Renal dysfunction during the first 48 hours was judged to be Grade 1 if the urine was red, Grade 2 if oliguria (<15 ml · hr^{-1} · m^{-2} BSA) was

*Throughout the text, CL refers to 70% confidence limits

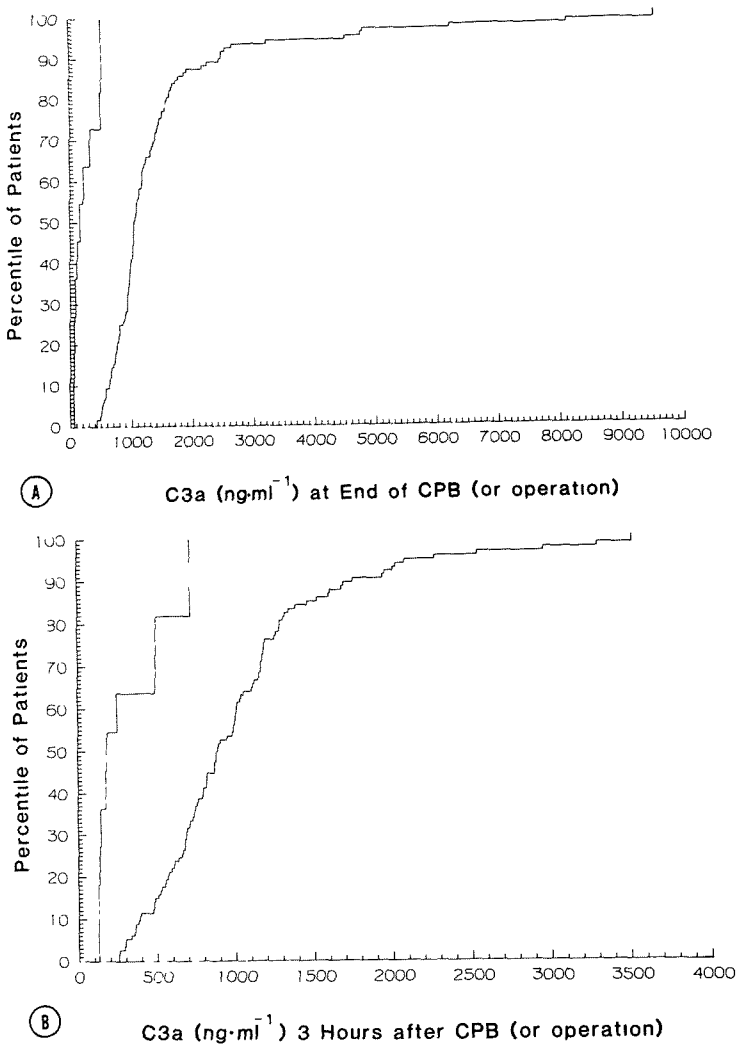

Fig. 1. Percentile distribution of patients according to C3a levels The steep *vertical line* on the *left* represents closed cases and that on the *right,* cases in which cardiopulmonary bypass *(CPB)* was used *A,* End of CPB (or end of operation in closed cases) *B,* Three hours after CPB (or end of operation in closed cases)

present in 2 consecutive hours, Grade 3 when serum creatinine was greater than 2 mg dl^{-1}, and Grade 4 when peritoneal dialysis was used

Abnormal bleeding, or bleeding more than is usually seen after CPB, was considered to be present if a greater than usual bleeding diathesis in the operating room was described by the surgeon (11 patients), if the patient was reentered for bleeding within the first 24 postoperative hours and the bleeding was found to be diffuse (four patients), or if the patient was not reentered but excessive bleeding postoperatively was nonetheless considered to be present by the observing nurse clinician and confirmed by review of his/her notes and of the amount of chest drainage (eight patients) Two patients had abnormal bleeding both intraoperatively and postoperatively, but without reentry Four patients had extreme diffuse bleeding in the operating room, one patient required two reentries (5 and 8 hours after operation), and one patient continued to bleed excessively from all incisions after reentry 25 hours postoperatively These six were judged to have abnormal bleeding Grade 2. All others (15 patients) were considered to have abnormal bleeding Grade 1.

All clinical observations were recorded by one group of nurse clinicians, trained specifically for this task.

An overall appraisal of important postoperative morbidity in individual patients was made Important morbidity was considered to be present if cardiac dysfunc-

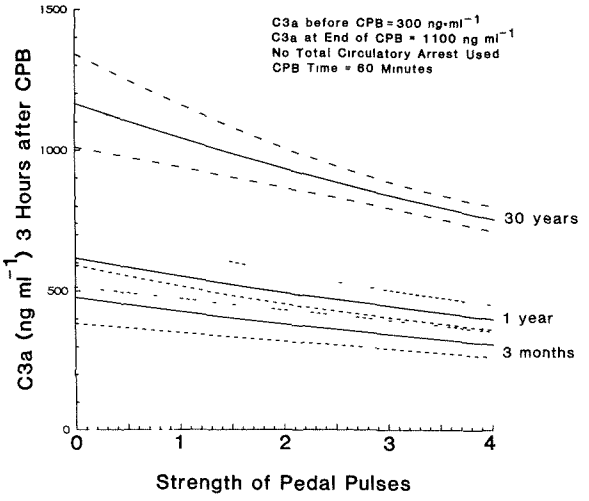

Fig. 2. Nomogram from the multivariate analysis of variables associated with the C3a levels 3 hours after CPB (Table I) It shows the shape of the relation between the pedal pulses at that time and the C3a level, for ages 3 months, 1 year, and 30 years

tion was Grade 2 and/or pulmonary dysfunction Grade 2 and/or renal dysfunction Grade 3 or greater and/or abnormal bleeding Grade 2 were present.

Data analysis. Inspection of the sorted data, simple contingency tables, and comparisons of means were used for initial analysis of the data Multivariate linear regression[11] and logistic regression[12] analyses were then added.

For the multivariate linear regression analysis of C3a levels, the following variables were entered age, weight, BSA of the patient with and without logarithmic transformation and the squaring of these variables to test for quadratic effects, and preoperative serum creatinine levels. Dichotomous variables entered were history of an allergy, congenital heart disease, primary operation, preoperative renal dysfunction by history, and preoperative pulmonary dysfunction by history. The additional factors considered in the analysis of C3a levels at the end of CPB included: surgical service A versus B, pre-CPB methylprednisolone (30 mg · kg^{-1}); Bentley versus Shiley oxygenator; elapsed time of CPB (elapsed time between start of CPB and end of CPB, including low flow and circulatory arrest time); use of total circulatory arrest; surface cooling; total circulatory arrest time; perfusion time (elapsed time of CPB minus total circulatory arrest time); use of low flow ($\leq$0.5 L · min^{-1} · m^{-2} BSA); and prebypass C3a concentration C3a level at the end of bypass and the strength of pedal pulses 3 hours after CPB were also considered for analysis of C3a level 3 hours after bypass. In the analysis of C3a level on postoperative day 1, the following factors

Table I. *Variables associated with higher C3a levels 3 hours after CPB*

Variable	*Coefficient ± SD*	*p Value*
C3a level before bypass (ln)	0 12 ± 0 051	0 03
C3a level at the end of bypass (ln)	0 32 ± 0 079	<0 0001
Total elapsed time of bypass (min)	0 0019 ± 0 00103	0 07
Strength of pedal pulses (0-4) 3 hr after CPB	−0 11 ± 0 039	0 006
Age at operation (ln yr)	0 19 ± 0 034	<0 0001

Intercept 3 4 ± 0 57

Legend CPB, Cardiopulmonary bypass SD, Standard deviation ln, Logarithm
Note If total circulatory arrest was used, add 0 63 ± 0 171 (p = 0 0004) to intercept and remove variable "age at operation"

also were considered: the C3a level 3 hours after bypass; strength of pedal pulses 3 and 5 hours after CPB and at 5 AM on postoperative day 1; cardiac index approximately 5 hours after CPB and on the first postoperative morning, and the presence of abnormal postoperative bleeding In all instances the logarithmic transformation of the various C3a levels was employed in these analyses due to their more normal distribution.

All these variables were also used in the multivariate logistic regression analysis of postoperative subsystem dysfunction Since subsystem dysfunction was graded (polychotomous) rather than considered merely present or absent (dichotomous), the particular generalization of logistic regression for polychotomous data suggested by Walker and Duncan[12] was employed. The assumption of their method is that the same risk factors and logistic regression coefficients apply to all patients, and only the intercept is altered for the various severities; this assumption is considered valid since the same risk factors and not demonstrably different coefficients were estimated for each severity level by analyzing the data according to the more general polychotomous model suggested by Hosmer and co-workers.[13]

In analysis of nomograms of the multivariate logistic analysis, the effect of an incremental risk factor (variable) was considered evident (p value about 0.1) when the upper 70% CL of the lowest probability of event was no longer overlapped by the lower 70% CL of the probability as the strength of the risk factor increased For these analyses we utilized a C3a level of 250 ng · ml^{-1}, 30 minutes of CPB, and age 70 or 10 years as the reference points for determining evident differences.

Results

C3a levels. Among the 116 patients undergoing open operation, the median value (and the fifteenth and

Table II. *C3a levels (ng ml^{-1}) in operations for congenital heart disease*

Time	Closed operations (n = 11)			Open operations (n = 38)			p Value
	n	Mean*	CL	n	Mean*	CL	
End of CPB	10	160	70-370	38	1140	620-2,110	<0 0001
3 hrs after CPB	9	200	90-490	36	710	350-1,440	<0 0001
5 AM on postop day 1	9	220	130-370	36	270	140-510	0 4

Legend CPB Cardiopulmonary bypass (operation, in closed cases) CL, Confidence limits (equivalent to ± one standard deviation of the observations)
*Geometric mean

Table III. *Cardiac dysfunction after open operations (n = 116, 27 patients had events)*

Variable (incremental risk factor)	Logistic coefficient ± SD	p Value
[Higher] C3a levels (ng ml^{-1}) 3 hr after CPB	0 0010 ± 0 00042	0 02
[Longer] Elapsed time of CPB (min)	0 014 ± 0 0058	0 02
[Younger] Age at operation (ln yr)	−0 60 ± 0 138	<0 0001

Intercepts Grade ≥1 = −2 3 ± 0 71, Grade 2 = −4 1 ± 0 82
Legend Standard deviation CPB Cardiopulmonary bypass ln, Logarithm

Table IV. *Pulmonary dysfunction after open operations (n = 116, 41 patients had events)*

Variable (incremental risk factor)	Logistic coefficient ± SD	p Value
[Higher] C3a levels (ng ml^{-1}) 3 hr after CPB	0 0025 ± 0 00094	0 008
[Longer] Elapsed time of CPB (min)	0 025 ± 0 0111	0 02
[Younger] Age at operation (ln yr)	−1 17 ± 0 183	<0 0001
C3a levels × CPB time	−0 015 10^{-3} ± 0 0086 10^{-3}	0 08

Intercepts Grade ≥1 = −0 5 ± 1 01, Grade 2 = −3 7 ± 1 12
Legend SD Standard deviation CPB, Cardiopulmonary bypass ln, Logarithm

eighty-fifth percentile values) for C3a preoperatively, at the end of CPB (Fig. 1, *A*); 3 hours later (Fig 1, *B*), and at 5 AM on postoperative day 1 was, respectively, 304 (150 to 691), 1,052 (684 to 1,726), 882 (486 to 1,390), and 288 (167 to 450) ng · ml^{-1} ($p < 0.0001$)

By multivariate analysis, the C3a level at the end of CPB was correlated only with that before CPB ($p = 0.005$) The C3a level 3 hours after CPB was correlated not only with the previous C3a levels, but also with the elapsed time of CPB, the strength of pedal pulses (Fig 2), and the age at operation (Table I) Total circulatory arrest was associated with increased C3a levels 3 hours after CPB and absence of an age effect By the multivariate analysis, the C3a levels on postoperative day 1 were correlated only with those 3 hours after CPB ($p < 0.0001$) and with the presence of abnormal postoperative bleeding ($p = 0.001$)

Among patients having closed operations, C3a levels before operation, at the end of operation, 3 hours postoperatively, and on postoperative day 1 were not dissimilar (p for difference = 0 5) (Fig 1, *A* and *B*). Thus, among patients with congenital heart disease, C3a levels were higher in those having open operations than in those having closed ones at the end of CPB (or operation) and 3 hours later, but not the next morning (Table II).

Postoperative cardiac performance in patients having open operations. Cardiac dysfunction was present during the first 48 postoperative hours in 27 (23%, CL 19% to 28%) of the 116 patients. Of these, 17 (15%) had moderate (Grade 1) cardiac dysfunction and 10 (9%) had severe (Grade 2) dysfunction. Higher C3a levels 3 hours after CPB, longer elapsed time of CPB, and younger age at operation were incremental risk factors for postoperative cardiac dysfunction, according to the multivariate logistic analysis (Table III).

Postoperative pulmonary performance in patients having open operations. Pulmonary dysfunction was present in the first 48 postoperative hours in 41 (35%, CL 30% to 40%) of the 116 patients. Pulmonary dysfunction was Grade 1 in 30 (26%) of the patients and Grade 2 in 11 (9%). The incremental risk factors for postoperative pulmonary dysfunction were the same as those for cardiac dysfunction (Table IV). In patients 1 year of age, undergoing 60 minutes of CPB, the effect of higher levels of C3a on the probability of important

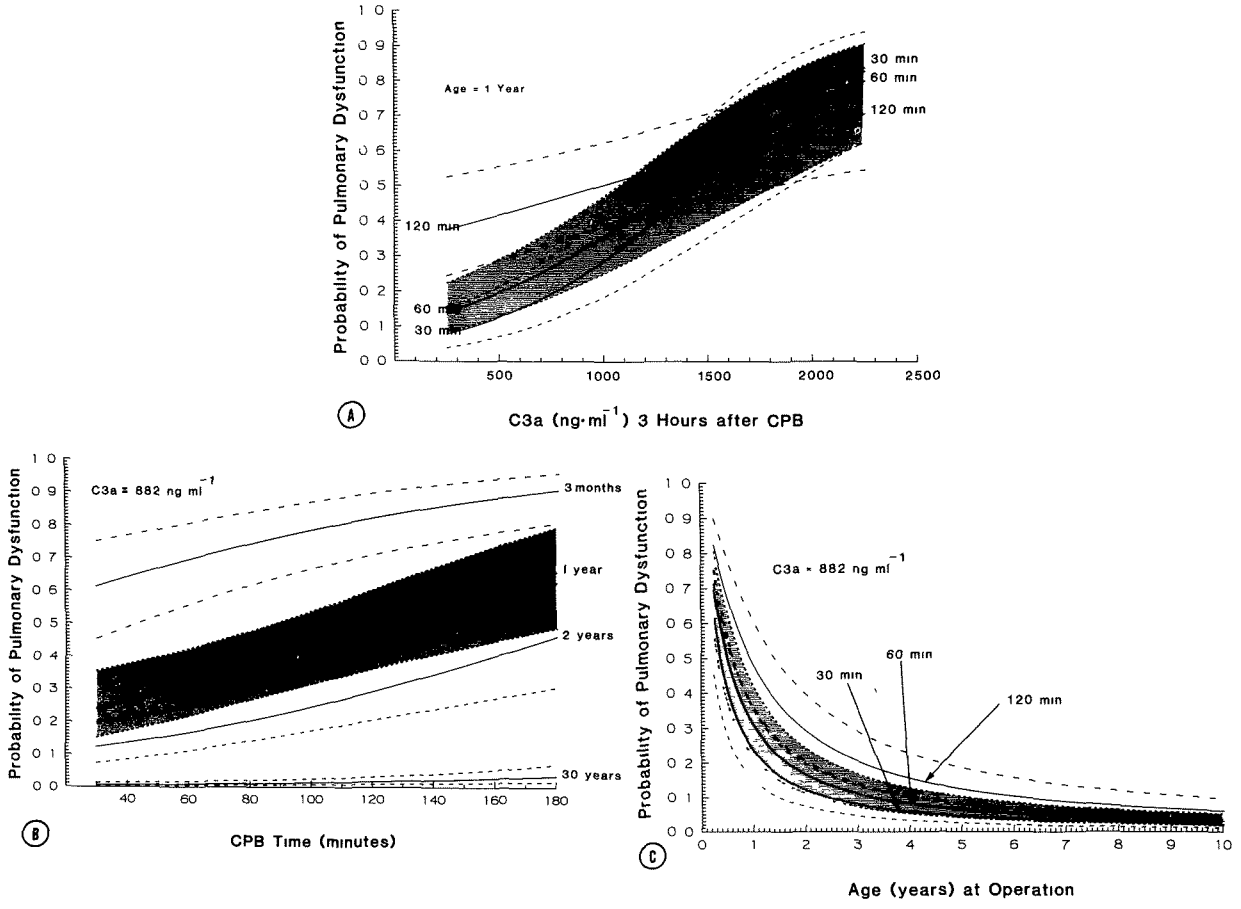

Fig. 3. Nomogram from the multivariate analysis of pulmonary dysfunction (Table IV) for the probability of important (Grade 2) postoperative pulmonary dysfunction, represented by the solid line The *dashed lines* enclose the 70% confidence limits *A,* The relation of C3a levels 3 hours after CPB and CPB time to the probability, when age is 1 year The *shaded area* indicates the 70% confidence limits for 60 minutes of CPB *B,* The relation of CPB time and age to the probability, when the C3a level is 882 ng ml^{-1}. The *shaded area* indicates the 70% confidence limits for age 1 year *C,* The relation of age and CPB time to the probability, when the C3a level is 882 ng ml^{-1}. The *shaded area* indicates the 70% confidence limits for 60 minutes of CPB In *C,* the age at which an evident difference in pulmonary dysfunction occurs, compared to the age at the far *right* of the scale, is indicated for each CPB time by the point of the *arrows*

(Grade 2) postoperative pulmonary dysfunction was evident (see Material and methods) at a level of 916 ng · ml^{-1} (Fig 3, *A*). Forty-seven percent of patients had this or a higher level (Fig. 1, *B*). The effect of longer time of CPB on the probability of pulmonary dysfunction in patients with C3a levels of 882 ng · ml^{-1} (the median value for the 116 patients) was evident at 118 minutes in patients aged 1 year (Fig 3, *B*). The effect of young age was evident when age was reduced from 10 years to 4 years when CPB time was 60 minutes and C3a level was 882 ng · ml^{-1} (Fig. 3, *C*).

The interrelated effects of the three variables (C3a levels, CPB time, and age) on the probability of pulmonary dysfunction are shown in Table V; either high C3a levels or long CPB times increased the probability of pulmonary dysfunction, but the effect of both was greatly reduced in adult patients.

Postoperative renal performance in patients having open operations. Renal dysfunction was present early postoperatively in 24 (21%, CL 17% to 25%) of the 116 patients undergoing open operations. Grade 1 dysfunction was present in five (4%) of the patients, Grade 2 in eight (7%), Grade 3 in six (5%), and Grade 4 dysfunction in five (4%). The incremental risk factors for postoperative pulmonary dysfunction, determined by multivariate logistic regression analysis, were higher levels of C3a 3 hours after CPB and younger age at operation (Table VI).

Table V. *Pulmonary dysfunction Grade 2 after open operations*

Elapsed time of bypass (min)	C3a levels (ng ml^{-1})	Estimated probability of pulmonary dysfunction							
		3 mo*		12 mo*		5 yr*		30 yr*	
		P	CL	P	CL	P	CL	P	CL
30	400	37%	23%-54%	10%	6%-19%	1 8%	0 8%-3 7%	0 2%	0 08%-0 6%
	800	57%	41%-72%	21%	13%-32%	4%	2%-7%	0 5%	0 2%-1 1%
	1,600	87%	74%-94%	58%	39%-74%	17%	9%-30%	2 5%	1 2%-5 3%
60	400	51%	37%-66%	17%	11%-26%	3 1%	1 7%-5 6%	0 4%	0 2%-0 9%
	800	67%	54%-78%	28%	20%-39%	6%	3%-9%	0 7%	0 4%-1 5%
	1,600	88%	77%-94%	59%	43%-73%	18%	11%-28%	2 6%	1 4%-4 9%
120	400	77%	63%-87%	40%	27%-54%	9%	5%-15%	1 2%	0 6%-2 6%
	800	82%	70%-89%	47%	35%-59%	12%	8%-18%	1 6%	0 9%-3 1%
	1,600	89%	79%-94%	60%	47%-72%	19%	13%-27%	2 8%	1 6%-5 0%

Legend P, Probability expressed as percent CL, 70% confidence limits
*Age at operation

Table VI. *Renal dysfunction after open operations (n = 116; 24 patients had events)*

Variable (incremental risk factor)	Logistic coefficient ± SD	p Value
[Higher] C3a levels (ng ml^{-1}) 3 hr after CPB	0 0009 ± 0 00036	0 02
[Younger] Age at operation (ln yr)	−0 70 ± 0 142	<0 0001

Intercepts Grade ≥1 = −0 5 ± 0 47, Grade ≥2 = −0 9 ± 0 47, Grade ≥3 = −1 6 ± 0 50, Grade 4 = −2 6 ± 0 60
Legend SD Standard deviation CPB, Cardiopulmonary bypass ln, Logarithm

Table VII. *Abnormal bleeding after open operations (n = 116; 21 patients had events)*

Variable (Incremental risk factor)	Logistic coefficient ± SD	p Value
[Higher] C3a levels (ng ml^{-1}) 3 hr after CPB	0 0006 ± 0 00037	0 10
[Longer] Elapsed time of CPB (min)	0 017 ± 0 0059	0 005
[Younger] Age at operation (ln yr)	−0 24 ± 0 133	0 07

Intercepts Grade ≥1 = −3 2 ± 0 78, Grade 2 = −4 7 ± 0 89
Legend SD, Standard deviation CPB, Cardiopulmonary bypass ln, Logarithm

Abnormal bleeding in patients having open operations. Fifteen (13%, CL 10% to 17%) of the 116 patients had abnormal bleeding Grade 1, whereas bleeding was Grade 2 in another six (5%, CL 3% to 8%). The risk factors for abnormal bleeding were higher levels of C3a 3 hours after CPB, longer elapsed time of CPB, and younger age at operation (Table VII) The effect of higher C3a levels in patients 1 year of age undergoing 60 minutes of CPB was evident at 2,300 ng · ml^{-1} (Fig. 4, *A*), a level reached by 4% of patients. The effect of longer bypass times in patients 1 year of age with C3a concentration of 882 ng · ml^{-1} was evident at 92 minutes (Fig. 4, *B*) The effect of younger age was evident when age was reduced from 70 years to 1½ years, with C3a concentration of 882 ng · ml^{-1} and with CPB time of 60 minutes (Fig. 4, *C*).

Important postoperative morbidity in patients having open operations. Among the 116 patients, 26 (22%, CL 18% to 27%) had important postoperative morbidity (see Material and methods for definition) within the first 48 hours postoperatively. The incremental risk factors assocated with this were higher levels of C3a 3 hours after CPB, longer elapsed time of CPB, and younger age at operation (Table VIII). In patients 1 year of age undergoing 60 minutes of CPB, the effect of C3a was evident at a level of 1,900 ng · ml^{-1}, a level reached by 9% of patients (Fig. 5, *A*) Even when complement levels were very high, the length of CPB remained associated with morbidity, as did young age (Table IX). The effect of CPB time in patients aged 1 year with a C3a level of 882 ng · ml^{-1} became evident when it exceeded 82 minutes (Fig. 5, *B*). When age at operation decreased from 10 years, the effect of young age became evident at age 4 years (Fig. 5, *C*) when the C3a concentration was 882 ng · ml^{-1} and the CPB time was 60 minutes. The interrelated effects of the three

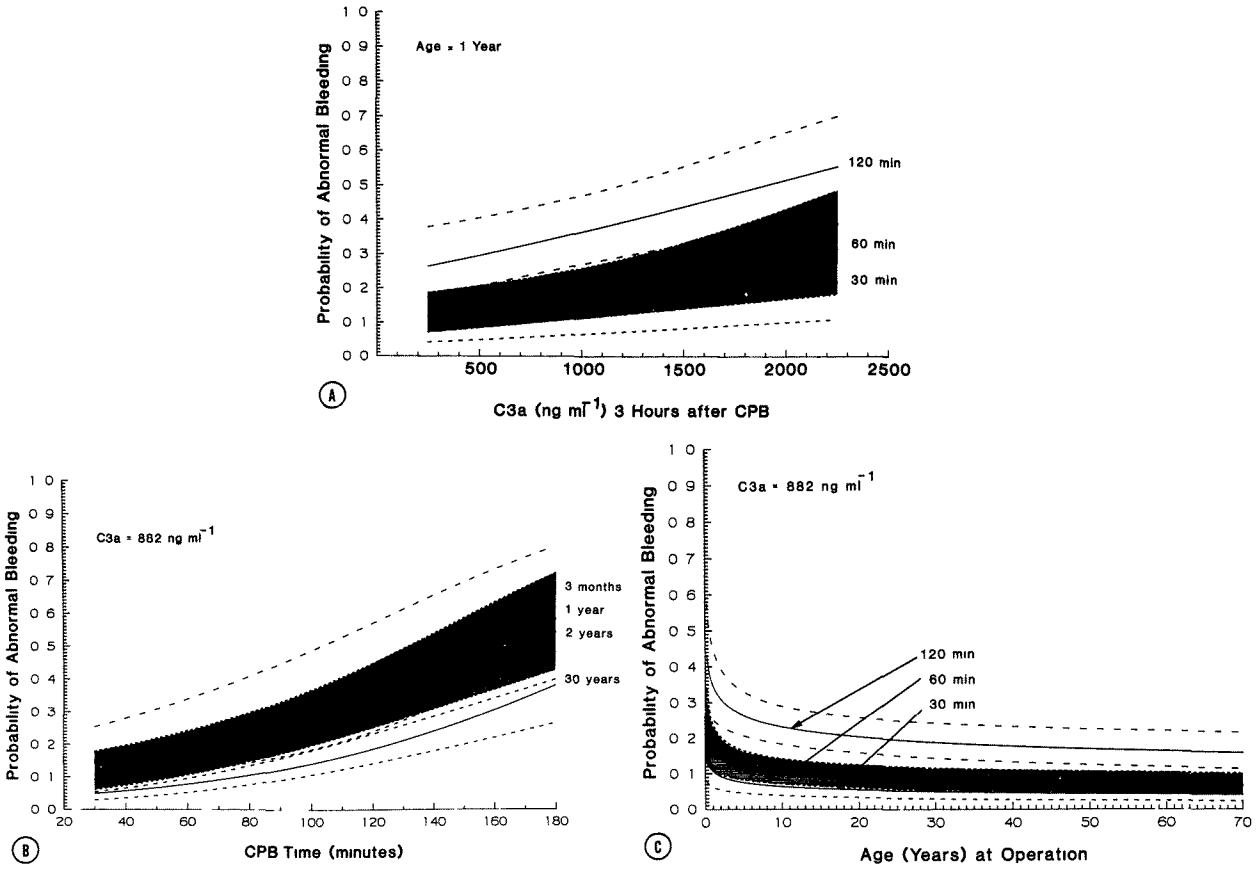

Fig. 4. Nomogram from the multivariate analysis (Table VII) of abnormal bleeding (Grades 1 or 2) The presentation, including *A, B,* and *C,* is as in Fig 2 The *shaded areas* indicate the 70% confidence limits for 60 minutes of CPB *(A),* age 1 year *(B),* and 60 minutes of CPB *(C)*

Table VIII. *Important morbidity after open operations (n = 116; 26 patients had events)*

Variable (incremental risk factor)	*Logistic coefficient ± SD*	*p Value*
[Higher] C3a levels (ng ml−1) 3 hr after CPB	0 0006 ± 0 00033	0 07
[Longer] Elapsed time of CPB (min)	0 017 ± 0 0048	0 0004
[Younger] Age at operation (ln yr)	−0 71 ± 0 131	<0 0001

Intercept −2 0 ± 0 60

Legend SD, Standard deviation CPB, Cardiopulmonary bypass ln, Logarithm

variables (Table IX) are such that when CPB time is increased from 60 to 120 minutes, there is an evident difference in the probability of morbidity at all ages and all C3a levels.

Discussion

Study methods. A study of postoperative morbidity is open to the criticism that many of the observations of subsystem dysfunction are subjective and affected by observer bias. In an attempt to meet this criticism, the observations were made by one group of nurse clinicians rather than by the physicians responsible for the patients, and objective data were recorded for each patient during the real time of his convalescence.

The methodology for measurement of C3a levels appears to be reliable and accurate. Plasma levels were used as measured, and ignoring the hemodilution that occurred at the onset of bypass and to some extent during it may result in as yet unknown errors of interpretation.

In an effort to avoid discarding possibly useful information, we retained variables in the multivariate

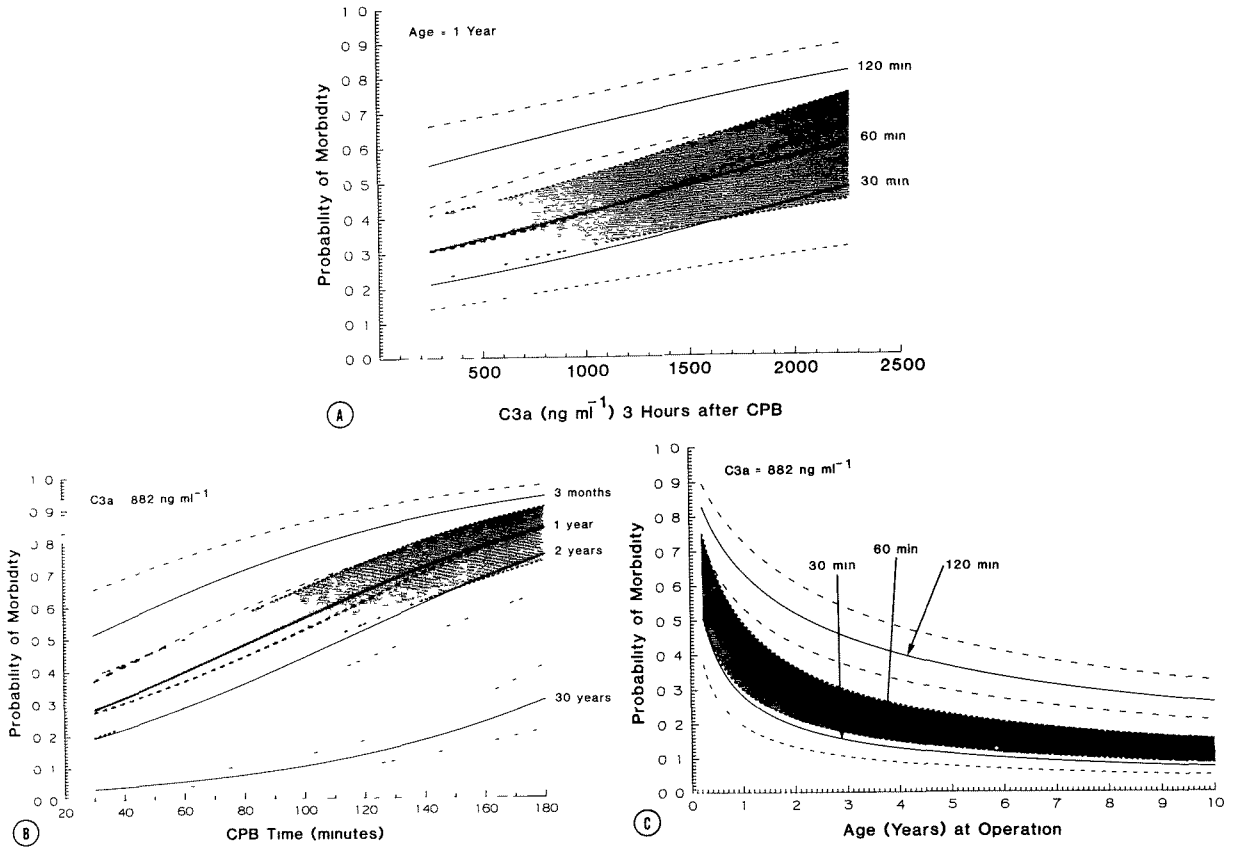

Fig. 5. Nomogram from the multivariate analysis (Table VIII) of important postoperative morbidity The presentation, including *A, B,* and *C,* is as in Fig 2 The *shaded areas* indicate the 70% confidence limits for 60 minutes of CPB *(A),* age 1 year *(B),* and 60 minutes of CPB *(C)* In *C,* the age at which an evident difference in morbidity occurs, compared to the age at the far *right* of the scale, is indicated for each CPB time by the point of the *arrows*

analysis if the p value was less than 0 2, as is the usual practice in this and other institutions [14] It could be argued that elimination of variables with a p value less than 0 05 would be more appropriate, but this would increase the likelihood of type II errors In any event, the data presented must be interpreted in light of the p values.

C3a levels. Complement activation has been shown to occur during hemodialysis for renal failure, and this is believed to be due to contact of blood with the foreign surfaces of the dialysis unit.[15] This activation is postulated to contribute to the pulmonary dysfunction that sometimes occurs after this type of treatment A previous study from this institution demonstrated activation of complement during CPB in man with the production of its degradation products C3a and C5a [6] In the current study, C3a did not increase in patients undergoing closed operations without CPB It did increase in patients undergoing open operations with CPB, and it was still considerably elevated above normal 3 hours after CPB This supports further the idea that complement activation is occurring as a result of contact of blood with the foreign surfaces of the pump-oxygenator system However, in as yet an unknown and unquantified manner, certain pharmacologic interventions such as administration of heparin and/or protamine may play a role,[16-18] as may other immunologic and inflammatory processes within the patient

The previous study[6] and this one suggest a continuing complement activation during CPB, with the levels of C3a 3 hours after CPB being associated, among other things, with the duration of CPB. An alternative hypothesis is that mechanisms for inactivating or metabolizing C3a are impaired by CPB.

The demonstrated association between higher C3a levels and the use of total circulatory arrest is not easily interpreted. It may result from the prolonged exposure of blood only to the pump oxygenator during the circulatory arrest (most of the blood is drained out of the patient into the machine as the circulatory arrest is

Table IX. *Important morbidity after open operations*

Elapsed time of bypass (min)	C3a levels (ng ml^{-1})	Estimated probability of important morbidity											
		3 mo*		6 mo*		12 mo*		24 mo*		5 yr*		30 yr*	
		P	CL	P	CL	P	CL	P	CL	P	CL	P	CL
30	400	44%	31%-58%	33%	22%-45%	23%	15%-32%	15%	10%-22%	9%	6%-13%	2 6%	1 5%-4 3%
	800	50%	36%-64%	38%	27%-51%	27%	19%-38%	19%	13%-26%	11%	7%-16%	3%	2%-5%
	1,600	62%	46%-76%	50%	35%-65%	38%	26%-52%	27%	18%-39%	16%	11%-24%	5%	3%-8%
60	400	57%	43%-69%	44%	33%-56%	33%	24%-43%	23%	17%-31%	13%	10%-19%	4%	3%-6%
	800	63%	49%-74%	51%	39%-62%	38%	29%-49%	28%	21%-36%	17%	12%-22%	5%	4%-8%
	1,600	73%	59%-84%	62%	49%-74%	50%	38%-63%	38%	28%-49%	24%	18%-33%	8%	6%-12%
120	400	78%	67%-86%	69%	57%-78%	57%	46%-68%	45%	35%-55%	30%	23%-38%	11%	7%-15%
	800	82%	72%-89%	74%	63%-82%	63%	53%-72%	51%	42%-60%	35%	28%-43%	13%	10%-18%
	1,600	88%	80%-93%	82%	72%-89%	74%	63%-82%	63%	53%-72%	47%	38%-56%	20%	15%-26%

Legend P, Probability expressed as percent CL, 70% confidence limits
*Age at operation

induced), or from an increased inflammatory reaction from tissue hypoxia during the arrest period, or from a deleterious effect of the circulatory arrest on the mechanisms for inactivating or metabolizing C3a.

The inverse relationship between strength of pedal pulses and C3a levels 3 hours after bypass suggests that C3a may be more rapidly "cleared" in the presence of good circulatory performance. However, it may merely reflect poorer cardiac performance associated with higher levels of C3a.

The explanation for the association between older age and higher levels of C3a is not evident

Subsystem dysfunction in patients undergoing open operations. Cardiac,[19-22] pulmonary,[23-27] renal,[28,29] and coagulation[2-4,30-32] dysfunction after CPB have been described in many studies. Subsystem dysfunction was also noted in the present study and, when analyzed separately or together as morbidity, was probably accentuated by higher C3a levels after CPB, younger age, and longer CPB times. Since the mortality experienced during the study period was higher than that for all other patients during the calendar year for the two services (see Patients), and thus possibly not representative of results in a larger sample, a more realistic estimate of the probability of subsystem dysfunction in a larger sample of similar patients in similar circumstances might be achieved by using the lower 70% CL.

The probable association found in this study between the level of C3a 3 hours after CPB and subsystem dysfunction suggests that complement activation and generation of the anaphylatoxins C3a and C5a, along with other phenomena, may play a role in producing a whole-body inflammatory response. Furthermore, the subsystem dysfunction may be the result of the inflammatory reaction, with vasoconstriction, increased capillary permeability, and other phenomena involving formed and unformed blood elements.[8,9]

In the lungs, for example, increased interstitial water is a nearly uniform accompaniment of CPB.[23] Activation of the alternative pathway of complement produces C5a as well as C3a.[8] Polymorphonuclear leukocytes have specific binding sites for C5a,[33] and transpulmonary sequestration of these leukocytes has been demonstrated during partial bypass.[6] In sheep, white blood cells play an important role in the development of interstitial pulmonary edema following microembolization.[34] Such a mechanism may play a role in the pulmonary dysfunction following CPB.

The incremental risk of young age for dysfunction was noted in all four subsystems studied. Turley, Mavroudis, and Ebert[35] reported no such effect in neonates operated upon in their first week of life as long as the elapsed time of CPB was short, but they observed a high mortality when it was 130 minutes. These observations are not inconsistent with the findings of the current study, nor are the excellent results in young infants reported by Castaneda and colleagues.[36] Good results have been achieved with many types of open operations in infants, particularly in recent years,[36-41] but special efforts and expertise are required, and this present study suggests that this is because of an apparent increased sensitivity of the young to the damaging effects of CPB.

In this study, longer CPB time was an additional risk factor for the presumed damaging effects of CPB. This has been the intuitive opinion of many surgical groups and emphasizes the importance of performing open heart operations in as time-efficient a manner as possible consistent with an accurate and complete repair.

A combination of relatively short CPB times, skillful operations, a patient not already affected by advanced or

long-standing heart failure, and attention to the details of intraoperative and postoperative care can largely neutralize the presumed damaging effects of CPB, as many excellent experiences have shown Even under these circumstances, however, an otherwise unexplainable subsystem dysfunction occasionally occurs Further investigation is required for elucidation of the underlying mechanisms and development of methods for complete neutralization of these damaging effects

The Nurse Clinicians, Ms Marjorie Land, Ms Deborah O'Connell, Mr Joseph Knight, Ms Zenaida Bavez, and Mr Charles McCook, of the Cardiovascular Surgery Intensive Care Unit participated in an invaluable way in making and recording the clinical observations utilized in this study Ms Kathy Peterson, Mr Jack Acton, Mr William Tracy, and Ms Nona Andrews organized the logistics of the study, compiled the data, and handled the blood samples Ms Carol Soderberg assisted in performing the C3a radioimmunoassays Mr Robert Brown and Dr David Naftel, Department of Biostatistics and Biomathematics, assisted in data preparation and analysis Dr Edwin Bradley, Department of Biostatistics and Biomathematics, assisted us in developing the concepts of "evident differences" Ms Sandy O'Brien assisted with the artwork, and Ms Nancy Ferguson typed the manuscript

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Discussion

DR RICHARD M ENGELMAN
Springfield, Mass

I rise to support the results presented by Dr Kirklin and associates and to further define this phenomenon of complement activation by data of our own. In a sequential group of 11 patients undergoing routine coronary revascularization, C5a and C3a inhibitors, enzymes which inactivate chemotactic factor and anaphylatoxin, were assayed quantitatively before, during, and after bypass The C3a inhibitor falls precipitously within 5 minutes of the institution of CPB and does not reach control levels until after 6 hours Sometime between 6 and 24 hours, this value appears to return to normal The inhibitor for the C5a appears to stay within control limits throughout the duration of bypass

These data serve to define as least one mechanism whereby C3a activity, the inverse of the inhibitor activity just described, measureably increases during routine CPB, whereas C5a and C5a inhibitor remain unchanged From previous studies one is aware of that

Dr Kirklin, do you believe it will be possible to conduct CPB without complement activation and, if so, what techniques might be employed?

DR RAY CHU-JENG CHIU
Montreal, Quebec, Canada

In our own study, which will appear in *The Annals of Thoracic Surgery,* we also found rapid consumption of both C3 and C4 right after the start of CPB This phenomenon could not be accounted for by hemodilution alone and thus indicates acute complement activation However, we did not detect further consumption in serial sampling of C3 and C4 after protamine administration, even though protamine, and especially protamine-heparin complex, are known to be strong activators of complements in vitro, and had been thought to

play a role in protamine hypotension encountered not infrequently in cardiac operations

Dr Kirklin, did you see futher complement activation after protamine injection in your series, and, if you did, could you correlate it both in timing and in magnitude with the hemodynamic changes associated with protamine administration? I recall you made some comments on this subject at the previous meeting of the Association

MR JOHN R KEATES
London England

We also see the postperfusion syndrome, which includes hemorrhagic diathesis, systemic inflammatory reaction, and diffuse organ dysfunction A retrospective analysis of the last 1,500 bypasses at King's College Hospital revealed 10 florid examples of this syndrome which could not be explained by low cardiac output or infection Five of the 10 patients subsequently died We also feel that complement activation could be implicated in this syndrome

Our efforts at studying complement have included analysis of C3d, release of which is accepted by immunologists as being unequivocal evidence of complement activation We studied 10 patients undergoing routine coronary artery bypass We noted that there was a progressive rise in C3d throughout the perfusion, that there was no extra rise after the administration of protamine, and that the levels fell rapidly to near normal but remained slightly elevated up to the fifth day

Although these patients made an uneventful recovery, we would suggest that the degree of complement activation found, which is in excess of that found in severe disseminated lupus, is disturbing, particularly in view of the correlation found by Dr Kirklin between C3a release and organ dysfunction Moreover, we suggest that these types of investigations represent a potentially highly sensitive yardstick for the evaluation of all components of the extracorporeal circuit in respect to their potential production of diffuse organ damage

Finally, has Dr Kirklin any data incriminating particular parts of the bypass circuit in complement activation?

DR J K KIRKLIN *(Closing)*

I would like to thank the discussers for providing very important and interesting additional information to this material

Dr Engelman's data are extremely interesting In response to his specific question regarding whether CPB can be conducted without complement activation, there are some preliminary studies which suggest that it may be possible However, although certain components of the oxygenator system, for example, the nylon components, are known to be powerful activators of complement, the mere presence of complement activation is perhaps not the most important thing We view this as a mere window through which we can get some glimpse into a very much more complicated process of an overall inflammatory effect of CPB The other components of what is called the humeral amplification system, which includes the coagulation system, fibrinolytic system, kallikrein system, and the complement system, are probably all very important and closely interrelated I think the problem is much more complicated than whether or not we can prevent complement activation

Regarding protamine, in this particular study we did not serially follow measurements of plasma C3a levels after the administration of protamine We do have other unpublished data, however, which suggest very strongly that protamine or the protamine-heparin complex is a powerful activator of complement, not via the alternative pathway, as is true with CPB, but via the classical pathway involving C4a. We do have some studies regarding patients receiving protamine in a prospective manner However, as you know, protamine reactions are extremely rare. These studies were carried out in patients having routine coronary artery bypass grafting, and no patients manifested an adverse reaction to protamine In the preliminary data, we have seen marked changes in hemodynamics or pulmonary or systemic vascular resistance with protamine. Of course, that says nothing about the situations in which there is a profound protamine reaction

Regarding the components of the bypass circuits, we know that multiple components of the bypass circuit activate complement in vitro Nylon has been shown in a previous study to markedly activate complement, but, again I think the solution is much more complicated than merely removing nylon from the circuit Thus many aspects of the CPB circuit need to be thoroughly investigated both in vitro and in vivo

In answer to Dr. Spencer's question about any other operations studied These are the first data that we have accumulated regarding closed operations We have not yet studied noncardiac procedures, but certainly other situations, such as trauma, in which multiple blood products are given, merit investigation

Regarding the possible role of cardiotomy suction on complement activation, we currently have no data to answer this question

Regarding the membrane oxygenator, I believe there is no secure evidence at present relating to complement activation in the membrane versus the bubble oxygenator system, but certainly this is an area which needs to be actively investigated