

# Studies of contact activation of blood in haemodialysis

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**SYNOPSIS** A study of patients on Kiil and Travenol coil dialysis has shown that kallikrein esterase, the kinin precursor, is increased in the venous blood during dialysis and that this is accompanied by an increase in the venous effluent of the dialysers. In addition Hageman factor depletion and an increase of platelet factor 4 has been found in the venous effluent. Such studies indicate surface activation of coagulation in the dialysers and will be of use in future studies of the suitability of different dialysers.

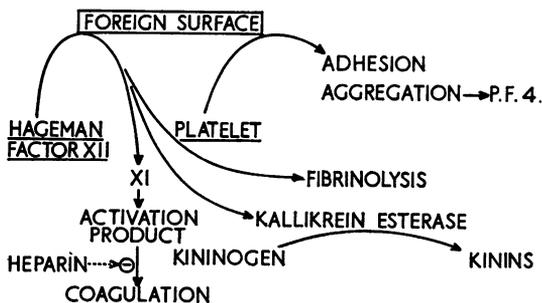
In the early days of extracorporeal circulations many problems came to light relating to activation of the coagulation and fibrinolytic systems (Kendall and Lowenstein, 1962; Porter and Silver, 1968; Barkhan, 1968). Essentially it has been found that there is a relationship between the chemical structure and the surface properties of synthetic polymers to platelet interaction with the surface and to the adsorption of blood proteins (Lyman, Muir, and Lee, 1965; Lyman, Brash, Chaikin, Klein, and Carini, 1968). As shown in the Figure there is activation of Hageman factor XII by adsorption to foreign surfaces, as there is by contact with glass, and in turn this can lead to production of kallikrein esterase generating the production of kinins (Mar-

golis, 1963; Buluk, Czokalo, and Malofiejew, 1970) and activation of fibrinolysis by plasmin (Iatradis and Ferguson, 1961; Erdös, 1966; Back, 1966). In comparing dialysers used in the artificial kidney not only should attention be drawn to the data on transport rates at varying blood flow of urea, creatinine, and ions but also haemoglobin losses and damage to blood coagulation in the circuit (Muir and Martin, 1971). Inevitably blood is slightly haemolysed, not only by the shearing effects of pumps, but also in relation to the surface of the dialyser (Hyde and Sadler, 1969). In turn it is possible that other coagulation changes may have some deleterious effects on the patient, quite apart from the usual haemodynamic upsets during dialysis that are attributable to blood volume changes (Kim, Neff, Cohen, Somerstein, Chinitz, Onesti, and Swartz, 1970). Thus it could be that some episodes of hypotension might be due to the release of plasmin or kinins (Back, Guth, and Munson, 1963).

We have therefore studied arterial and venous blood samples adjacent to the artificial kidney haemodialyser, and also systemic venous blood taken from the patient before and just after dialysis, to examine the possibility of kinin activation. This has been done as a preliminary exploration as to whether such studies might help in the evaluation of different dialysers.

## Methods

Siliconized plastic syringes and siliconized glassware were used throughout. Systemic venous blood was taken into sodium citrate anticoagulant (1.0 ml 3.8 %



**Fig.** A foreign surface leads to platelet aggregation and simultaneous activation of coagulation, fibrinolysis, and kinin generation. The substrate kininogen is referred to as kallikreinogen in the text.

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sodium citrate to 9.0 ml blood), and immediately kept in iced water, from chronic renal failure patients before and after haemodialysis. Initially 12 patients undergoing Kiil dialysis and 12 undergoing Travenol coil dialysis were studied. Specimens were also taken from the arterial inflow and venous outlets, both shortly after the start of dialysis and again after six hours. The standard technique was to estimate spontaneous kallikrein esterase activity by its arginine esterase hydrolysis of tosyl-arginine methyl ester (TAME) and the levels of kallikrein inhibitors by the method of Colman, Mason, and Sherry (1969a).

Additionally on the arterial-venous samples taken from the machine assay of Hageman factor activity was made by the two-stage method of Müller-Berghaus and Lasch (1969). The test plasma activated with Celite over a five-minute incubation period to reveal its Hageman factor content, is added to a buffered substrate, in which Hageman factor is inhibited by lysozyme, but to which platelet factor 3 has been added. After the addition of calcium chloride the clotting time is a measure of the original Hageman factor content. As a further check an indirect assessment of Hageman factor content of arteriovenous samples from the dialyser was made by estimating the effect of Celite in the activation of the remaining factor XII. For this latter purpose 0.1 ml plasma, 0.1 ml saline, and 0.1 ml Celite (20 milligrams per ml) were incubated at 37°C for 10 minutes in a siliconized tube, and then the clotting times were determined after the addition of 0.1 ml Cephalin (Sigma) and 0.1 ml 0.025 M calcium chloride in quick succession.

Further tests which were applied to arteriovenous samples from the dialysers were estimation of plasma fibrinogen by the method of Ratnoff and Menzie

(1951), the coagulability of the fibrinogen by extraneous addition of isotopically labelled fibrinogen according to Regoeczi (1967), estimation of the euglobulin lysis time by the method of Menon, Martin, and Weightman (1969), of fibrin monomer by the method of Lipiński and Worowski (1968), and of platelet factor 4 using bovine fibrinogen lysate as substrate by the method of Farbiszewski, Niewiarowski, Worowski, and Lipiński (1968).

## Results and Comment

The results for the assay of spontaneous kallikrein esterase activity, plasma kallikreinogen substrate, and the kallikrein inhibitors are given in Table I expressed as  $\mu$ mole TAME hydrolysed per millilitre plasma per hour. Inhibitors are also given as these absolute units and not as the arbitrary units which were used by Colman, *et al* (1969a). Chronic uraemic patients had slightly higher values than normal control patients for kallikrein esterase activity and for the kallikreinogen substrate, and had lower inhibitor values, but the differences had no statistical significance. Higher mean values for esterase activity were obtained from the venous effluent side of both Kiil and coil dialysers and these values were higher by the sixth hour of dialysis than shortly after the onset. On the whole the statistical significance was not great, nor was any difference found in arteriovenous samples for the kallikreinogen substrate nor for the inhibitors. However, there was a marked increase in the spontaneous kallikrein esterase activity between systemic venous samples taken before dialysis and just before cessation of dialysis, and these results were highly significant. Moreover a significant fall of serum kallikreinogen

	Spontaneous Esterase Activity		Kallikreinogen		Kallikrein Inhibitors	
	Artery	Vein	Artery	Vein	Artery	Vein
Normals (10)		3.46 $\pm$ 5.3		50.4 $\pm$ 34.0		29.04 $\pm$ 12.8
Uraemic (10)		7.07 $\pm$ 7.0		66.2 $\pm$ 42.0		20.27 $\pm$ 10.65
Coil Before dialysis (12)		2.25 $\pm$ 2.18		71.07 $\pm$ 8.2		18.88 $\pm$ 10.2
After dialysis (12)		10.12 $\pm$ 6.10		60.95 $\pm$ 8.12		19.21 $\pm$ 10.6
		P < 0.001		P < 0.005		
Kiil Before dialysis (12)		1.34 $\pm$ 1.69		75.6 $\pm$ 17.5		20.0 $\pm$ 10.6
After dialysis (12)		10.0 $\pm$ 7.12		68.2 $\pm$ 16.4		15.2 $\pm$ 7.6
		P < 0.001				
Coil $\frac{1}{2}$ hour (12)	1.5 $\pm$ 2.73	10.0 $\pm$ 4.8	62.6	62.5	18.8	20.4
		P < 0.001				
6 hour (12)	8.2 $\pm$ 13.2	16.6 $\pm$ 14.5	62.9	59.2	19.3	14.6
Kiil $\frac{1}{2}$ hour (12)	2.9 $\pm$ 5.1	6.6 $\pm$ 5.9	69.8	69.9	16.5	22.5
		P < 0.2				
6 hour (12)	2.0 $\pm$ 2.9	9.2 $\pm$ 9.0	62.9	70.1	16.0	16.5
		P < 0.05				

Table I Kallikrein enzyme activation<sup>1</sup>

<sup>1</sup>Mean values are given and where relevant the standard deviations. Significance values by Student's t test

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substrate was found during the course of dialysis, for those patients dialysed by Travenol coil. No inhibitor variations were apparent.

Table II summarizes the results of the veno-arterial differences of the Hageman factor assay, and assays of plasma fibrinogen, fibrinogen coagulability, fibrin monomer, platelet factor 4, and euglobulin lysis time. The significant points are that the Hageman factor assay showed by both techniques a consistent negative veno-arterial difference across the dialyser, which means that Hageman factor was depleted in the venous effluent. The fact that the differences were greater with the second indirect technique probably means that other factors were also detected in this test. There was, however, no change in the clottability of fibrinogen itself and, because of the haemoconcentration due to ultrafiltration, plasma fibrinogen levels were increased by 5.5% on the venous side.

On the other hand, fibrin monomer complexes detected by protamine sulphate precipitation showed a slight mean negative value of -1.6% which must mean that some smaller fibrin products are being filtered by the membrane. In contrast to this there was a mean increase of platelet factor 4 of 105% on the venous side of the system and this must reflect platelet destruction with platelet factor 4 release in the dialysers.

It was found that the euglobulin lysis time was affected by the speed of the blood pump, and this is the subject of further investigation, but nevertheless mean lysis times were more prolonged on the venous side of the dialyser by 7.0%. This poorer fibrinolysis could be due to a haemoconcentration of inhibitors.

**Discussion**

The Figure summarizes the interrelationships of the various factors and enzymes referred to in the study. In fact the kallikrein esterase enzymes are formed of a trio of enzymes consisting of a slow gamma globulin MW 99800, a fast gamma globulin MW

163000, and an alpha globulin (Colman, Mattler, and Sherry, 1969b). This knowledge is relevant to the interpretation of the results since only molecules of molecular weight less than 5000 show any appreciable loss through the membranes of the artificial kidney (Alexander and Galletti, 1965). Also it should be noted that thrombin, plasmin, activated clotting factors X and XI, and C' esterase, as well as kallikrein, are capable of hydrolysis of the artificial substrate, TAME. However thrombin would be immediately consumed *in vivo* and in view of the poorer fibrinolysis on the venous side of the circuit, it appears that plasmin would be held in check, hence it is probable that the assay is in fact detecting kallikrein esterase.

Our normal values for spontaneous kallikrein esterase activity and for the substrate kallikreinogen differ from those given by the originators of the method. Thus our normal kallikreinogen values were 50.4 ± 34.0 whereas Colman *et al* (1969b) quote 97 ± 27.4. The reason for the discrepancy is unknown but might be related to the fact that initially we had difficulty in finding a kaolin preparation that caused maximum activation of plasma kallikrein within one minute. In our results inhibitor units are expressed as absolute values.

The important points to emerge from this study are that with both Kiil and coil dialysers there is evidence of activity of kinin-producing enzymes in the systemic venous blood of the patients over the course of six to 12-hour dialysis. The significant increase of kallikrein esterase activity coupled with a fall in kallikreinogen but no change in inhibitors is evidence of this. In addition increase of esterase activity has been shown on the venous effluent side of the dialyser with both the Kiil and Travenol coil, at both half an hour and at the sixth hour of dialysis. Taken in conjunction with the fact that depletion of Hageman factor has also been shown, this must mean that the foreign surface of the dialysers has produced contact activation of the blood passing over it. Further support for this comes from the

	Absolute Units <sup>1</sup>		Percentage Differences
	Artery	Vein	
Hageman factor			
Direct assay	—	—	- 30 ± 10.2
Indirect assay	—	—	- 46 ± 18.4
Fibrin monomer (OD units)	0.848 ± 0.28	0.839 ± 0.29	- 1.66
Plasma fibrinogen (mg %)	520 ± 105	540 ± 120	+ 5.46
Fibrinogen clottability (%)	94.9 ± 0.04	95.0 ± 0.05	Nil
Platelet factor 4 (OD units)	0.222 ± 0.016	0.045 ± 0.100 (p < 0.001)	+ 105
Euglobulin lysis time (units)	—	—	- 6.96

Table II Mean veno-arterial differences of coagulation factors in 20 assays

<sup>1</sup>Absolute units have not been given where the values were found to be affected by pump speeds

evidence that there is an increased amount of platelet factor 4 in the venous effluent. This finding adds credence to previous reports of platelet destruction and serotonin release during haemodialysis (Lawson, Crawford, Dawson-Edwards, and Blainey, 1965).

Normally activation of factor XII by a foreign surface will lead to activation of fibrinolysis as well as to kinin activation (Erdos, 1966; Back, 1966). However in general in this study it was found that fibrinolysis as measured by the euglobulin lysis time was poorer in the venous effluent. This could be the result of haemoconcentration of plasma inhibitors in the dialyser rather than a true reflection of the state of plasminogen activator. Thus it was also found that beta-lipoprotein, which is a fibrinolytic inhibitor, was increased on the venous side by the order of 5%, a figure comparable to the 5% increase of fibrinogen. Indeed the effects of ultrafiltration and turbulence in the dialyser makes full interpretation of such studies difficult. Hence no deductions have been made from small changes of any of the parameters studied.

It is important to clarify why it is that complete heparinization does not protect the blood from initial activation of coagulation. Not only does a foreign surface cause adhesion to and damage of platelets, a process which can only be prevented by a platelet inhibitor such as aspirin or dextran, but also activation of Hageman factor XII which then stimulates factor XI, the so-called 'activation product', which is at the beginning of the coagulation cascade. Heparin cannot inhibit the initial steps, since it is mainly an antithrombin, but also has its earliest inhibitory action at the point of activation of factor IX by the 'activation product' (Gurewich and Thomas, 1965; Ratnoff and Davie, 1962). It should also be known that heparin is known to accelerate plasma kinin formation (Armstrong and Stewart, 1962). In fact no evidence was found in this limited study that kinin activation had any systemic effect upon the patients, but an opportunity to study this in detail has not so far arisen. Indeed kinins are normally limited to a local action because of highly effective inhibitors and it is only if fibrinolytic activation becomes generalized that there can be gross activation of kininogenesis producing hypotension and collapse (Back, 1966). Nevertheless, it is quite possible that further research may show that occasionally kinins are responsible for such events during dialysis as unexplained headache or hypotension, for kallikrein is a potent proteolytic enzyme producing characteristics of the inflammatory process (Kellermeyer and Graham, 1968).

As already mentioned activated factor XII forms a complex with factor XI and the product of their

interaction leads to stimulation of factor IX. In fact factor XI also has proteolytic activity. Whereas, under normal circumstances of clotting only a trace of XII or XI is consumed, if the activating surface is extensive (as with dialyser membranes), then sufficient factor XII may be completely removed to lead to the total incorporation of factor XI in a surface bound complex with its subsequent absence from serum (Haanen, Morselt, and Schoenmakers, 1967). This possibility requires further study.

One of the purposes of this study was to examine any differences between two popular dialysers in their tendency to initiate thrombosis. No significant differences were detected but the results do indicate which parameters deserve attention in future comparisons, namely, platelet factor 4, Hageman factor, and kallikrein esterase activity. Studies of this kind could profitably be introduced into the routine of dialyser testing, which is currently confined to the measurement of performance without any attention to blood cell damage or to thrombogenicity.

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