EFFECT OF DIALYZER REUSE ON LEUKOPENIA, HYPOXEMIA AND TOTAL HEMOLYTIC COMPLEMENT SYSTEM

R. M. Hakim and E. G. Lowrie

The reuse of artificial kidneys has become common practice in many dialysis centers. Approximately 30% of large centers in the United States (with patient population exceeding 100) reuse dialyzers and the proportion of home patients reusing their dialyzers is estimated to be 15%[1]. These figures are higher in Europe, where approximately 37% of patients dialyzed in hospitals reuse their dialyzers and more than 75% of home patients practice reuse[2].

Recent evidence suggests that dialyzer reuse may lead to significant medical benefits in addition to economic benefits. A survey by Wing et al[2] involving a large number of patients in the United Kingdom showed a significant decrease of mortality in patients reusing dialyzers. This was evident in patients dialyzed in hospital centers, where the mortality at one year of patients reusing dialyzers (6.5%) was less than half that of patients not reusing (15.6%). In addition, in home dialysis patients there was no mortality in patients reusing dialyzers, compared to a 12.5% one year mortality for patients not reusing dialyzers.

Recently a study by Kant and Pollack[3] showed that adverse symptoms such as chest pain, respiratory distress and cramps were significantly less frequent with reuse than with first use, and that the length of hospitalization was also significantly less for patients dialyzed in centers practicing reuse than for the same patients dialyzed in centers using dialyzers only once.

At The Kidney Center in Boston, a large outpatient dialysis facility with over 340 patients, hollow fiber dialyzers are reused on a regular basis. A number of patients had complained of increased adverse symptoms such as nausea, vomiting and itching and showed increased incidence of hypotension during the first use of their dialyzers. In an attempt to explain these observations, and as a followup to ongoing studies of the biocompatibility of different dialyzer membrane materials[4], the effects of reuse on a number of biochemical parameters were investigated. Specifically, the effect of reuse on the leukopenia and hypoxemia that occurs during dialysis was investigated as a function of first use and third use for different dialyzers. In addition, the extent of complement activation which is thought to initiate the leukopenia[5] was investigated for these dialyzers as a function of reuse.

MATERIALS AND METHODS

Patients. Eight long-term chronic hemodialysis patients undergoing thrice weekly, 5 hr maintenance hemodialysis were chosen for the study. Informed consent was obtained prior to the study. None of the patients were known to have had recent or current symptoms of fluid overload or cardiorespiratory diseases or infections. Patients were dialyzed according to their regular schedules and fluid was removed to achieve their "dry weight", 2.5 ± 1.0 kg (Mean ± 1 SD) on the average.

Dialysis System. Each patient was dialyzed with 3 different dialyzers, namely the Cordis-Dow (C-D 1.3) cellophane hollow fibers; the Travenol CF-1200, using cuprophane hollow fibers, and Toray Industry's B-2-M manufactured with polymethylmethacrylate (PMMA) hollow fibers, all dialyzers having the same surface area[6]. Each patient was dialyzed 3 times with the same dialyzer and studies were performed during the first and third use. Prior to reuse, all dialyzers were tested to be free of formalin (the sterilizing solution) by the modified Schiff's reagent, which is sensitive to 30 parts per million (ppm) of formalin. The dialysate used was Eri-lyte 8107, which when diluted appropriately contains acetate of 40 mEq/L, and Na of 138 mEq/L. A single pass counterflow system of dialysate circulation was used in all cases.

Studies. Arterialized blood samples were drawn anerobically from the arterial line prior to the start of dialysis and at intervals of 7.5, 15, 30, 60 and 120 mins after the start of dialysis. Samples for pH, PO_2 and PCO_2 were drawn in a heparinized syringe. All samples were kept on ice until just prior to measurement, which was less than 30 mins from the time of sampling. Blood for the white blood cell and differential counts was collected in EDTA tubes.

Blood gases were measured using the Corning Medical (Medfield, MA) Automatic pH/Blood Gas System, Model 161. Calibration with 2 known gaseous standards was performed before each measurement. In addition, calibration with tonometered liquids for PO_2, PCO_2, pH and total CO_2 (General Diagnostics, NJ) was done at the beginning of each set of experiments. Accuracy of PO_2 measurements at PO_2 of 100 mm Hg was 2.1% and repeatibility was ± 1 mm Hg. Accuracy of PCO_2 measurements at PCO_2 of 40 mm Hg was 0.8%, and repeatibility was ± 1 mm Hg.

White blood cell counts were done with a Coulter Counter, Model S (Miami, FL). Differential counts were done on 100 leukocytes. Total hemolytic complement assays were measured by the Kent and Fife[7] technique (courtesy of Dr. P. Schur at the Robert Breck Brigham Hospital) prior to the initiation of dialysis and at 15 and 30 mins after the start of dialysis.

From the Affiliated Hospitals Center, Inc. (Peter Bent Brigham Division), Harvard Medical School, and The Kidney Center, Boston, Massachusetts.

Reuse Procedure. Procedures for reuse of hollow fiber artificial kidneys are carried out under carefully controlled conditions by trained technical personnel. Briefly, the procedure involves the gentle rinsing of the dialyzer immediately after use with heparinized saline, then a determination of the blood compartment volume by the standard saline rinse technique[8]. Dialyzers are reused until their post-use volume is 75-80% of the original volume, since their clearance is not affected significantly[9,10] at these volumes. The ultrafiltration coefficient is adjusted for the fractional remaining volume. Further rinsing and reverse ultrafiltration cleansing (i.e. from the dialysate into the blood compartment) is done with water. Endcaps are removed and entrapped blood products are mechanically removed. The blood compartment is then filled with a 1.75% solution of formaldehyde and the dialyzer is stored in an iodoform solution to sterilize the outside of the kidney.

Data Analysis. Data from all patients were grouped and analyzed by the type of dialyzer (i.e. membrane) and number of reuse. Analysis of variance was used to test statistical significance[11]. The analytical model assumes a 2-factor design (dialysis membrane and number of uses).

All parameters were converted to percent predialysis value of each experiment in order to reduce the effects of different predialysis values for different patients and for the same patient on different days. Percent fractional changes $\frac{P_{initial} - P_{minimum}}{P_{initial}} \times 100$ were also calculated. All values are reported as mean ± SEM. The average predialysis value of PO_2 was 95.4 ± 3.2 mm Hg, WBC = 6.7 ± 0.5 x 10^3 cells/mm^3, and neutrophils = 4.0 ± 0.6 x $10^3/mm^3$.

RESULTS

The relative change in the fraction of neutrophils during dialysis, calculated from the total white blood cell and differential counts is shown in Figure 1 for the 3 types of dialyzers during first and third use. It is seen that during first use the fall in neutrophil count is greatest for cellulosic membrane dialyzers, decreasing to a minimum of 5.2 ± 1.0% and 11.3 ± 3.4% of the predialysis value for the cellophane (C-D 1.3) and cuprophane (CF-1200) material, respectively. For the PMMA membrane (Toray B-2-M), the minimum neutrophil count is only 69.2 ± 12.4% of the predialysis value. During third use, the relative decrease in neutrophil count is considerably less for both cellulosic materials, being 53.6 ± 14.1% of predialysis value for cellophane and 62.4 ± 10.7% for the cuprophane material, while it remained practically unchanged for the PMMA membrane. The difference in the relative neutropenia between first and third use is statistically significant ($p < 0.001$) for both cellophane and cuprophane material but shows no statistical significance for the PMMA membrane. This is shown more clearly in Figure 2, which shows the percent fractional change for the 3 types of dialyzers during first and third use.

This early leukopenia is followed by a relative leukocytosis. The degree of leukocytosis is again greatest for the cellolosic membrane dialyzers, being 123.0 ± 8.5% for the C-D 1.3, 144.8 ± 10.4% for the CF-1200, but only 110.3 ± 11.8% for the Toray B-2-M during their first use. During third use, the extent of leukocytosis is considerably attenuated for all dialyzers.

The relative change in the total hemolytic complement levels (THC) is shown in Figure 3. During the first use of the cellophane membrane dialyzer, THC level decreases to 68.9 ± 7.3% of predialysis value at 15 mins and rises to 79.4 ± 3.0% of predialysis value at 30 mins. The corresponding values for the cuprophane material (CF-1200) are 72.5 ± 6.6% at 15 mins and 87.5 ± 4.7% at 30 mins. For the PMMA membrane of the Toray B-2-M, the change in THC levels during first use is less than for cellulosic materials; it decreases to only 80.7 ± 3.1% at 15 mins and 88.6 ± 6.5% at 30 mins of predialysis value.

The change in THC levels during third use is significantly less than during first use in cellulosic membrane dialyzers - similar to the change in neutrophils described above. The THC levels of the C-D 1.3 is 90.8 ± 4.2% and 92.4 ± 5.1% at 15 and 30 mins, respectively, while it is 86.2 ± 5.0% and 90.4 ± 4.2% for the CF-1200 at the same times. The THC levels of the PMMA membrane dialyzer is not significantly different between first and third use at 15 or at 30 mins. The percent fractional fall in THC levels for all 3 dialyzers during first use is 31.4 ± 7.3% for cellophane, 28.9 ± 6.5% for cuprophane and 20.4 ± 2.8% for PMMA. During third use, the fractional fall of THC is significantly less for cellulosic membranes - 14.1 ± 4.2% for cellophane, 16.9 ± 3.5% for cuprophane - and is unchanged for the PMMA - 21.9 ± 3.7%.

The relative change of PO_2 as a function of time for different dialyzers is shown in Figure 4. Similar to the neutrophils and THC, the maximum relative change of PO_2 occurs for the cellulosic membrane materials during their first use. For the cellophane membrane of the C-D 1.3, the maximum relative change of PO_2 occurs for the cellulosic membrane materials during their first use. For the cellophane membrane of the C-D 1.3, the maximum relative change of PO_2 occurs at 30 mins and is 79.6 ± 4.1% of predialysis value, while it is 80.9 ± 2.0% for the cuprophane membrane at 30 mins. For the PMMA membrane of Toray B-2-M, the maximum relative change occurs at 60 mins and is only 87.5 ± 2.7% of predialysis value. The difference in the fractional fall of PO_2 between the cellulosic and noncellulosic dialyzer is statistically significant ($p < 0.04$). During third use, the relative change in PO_2 is somewhat less than during first use and at 30 mins is 84.6 ± 3.5% of predialysis value for cellophane, 83.8 ± 3.3% for cuprophane, and is unchanged for the PMMA membrane (87.3 ± 4.3%) (although it

occurs at 30 mins, rather than at 60 mins as during first use). Although the relative change of PO_2 was less during third use than during first use with both cellulosic membrane dialyzers, the differences were not statistically significant.

There was no significant difference between different dialyzers and between first and third use for pH or PCO_2.

DISCUSSION

The results outlined above clearly show a relationship between the extent of the leukopenia and complement activation (as measured by the total hemolytic complement level) and the type of membrane used, as well as its state of prior use, i.e. whether it is a new or reused dialyzer. Thus, the degree of leukopenia and hypoxemia, as well as the extent of complement activation during first use, is significantly more for the 2 cellulosic membrane materials than for the PMMA membrane dialyzer. During third use, the degree of leukopenia, hypoxemia and complement activation is less than during first use for both cellulosic materials and remains unchanged with prior use for the PMMA membrane.

Although the cellular deposits found in hollow fiber kidneys after use are mostly leukocytes[12], and to a lesser degree platelets, it is well known from inlet-outlet sampling across the dialyzers that the dialysis-associated leukopenia is not due to sequestration of the WBC in the dialyzer[13]. Animal studies have clearly shown that the sequestration of the leukocytes occurs primarily in the pulmonary vasculature[14].

The fact that the leukopenia, as determined from dialyzer inlet WBC, occurs maximally at 15 mins, at a time when approximately 20% of the vascular volume has been in contact with the surface of the dialyzers, suggests that a systemic factor is responsible for this leukopenia. Craddock et al[15] have shown that the leukopenia is due to the activation of the complement system via the alternative pathway. This phylogenetically older system can be activated by several polysaccharides such as inulin, zymosan and endotoxin (lipopolysaccharides)[16] and therefore it is not surprising that the polysaccharide structure of cellulose (as cellophane or cuprophane) may initiate complement activity. This is in agreement with our results, which showed a more pronounced activation of the complement system as measured by the total hemolytic assay in cellulosic membrane dialyzers than in PMMA membranes.

Several activated components of the complement system (C_{3a}, C_{5a}) have strong chemotactic abilities[17] and these activated components are likely to be the systemic factor responsible for the dialysis-associated leukopenia. Indeed, Craddock and his colleagues[15] have shown that plasma from a genetically C_5 deficient donor was incapable of producing granulocyte aggregation in vitro. Recently, a component of C_3 activation, namely C_{3e} which is a fragment of the α-chain of C_3 (MW 10,000) has been shown to induce in animals an initial leukopenia 15-30 mins after infusion, followed by leukocytosis which is maximum at 2 hrs - similar to the dialysis-associated sequence of WBC changes[18,19]. Although the presence of C_{3e} fragments has not been demonstrated during dialysis, it is known that other components of C_3 activation are present[15].

Concomitant with the contact of blood with membranes, there is a rapid deposition of various protein components present in blood, predominantly fibrinogen, even in well heparinized blood[20]. This rapid deposition of autologous protein serves to decrease the direct blood-material interaction. This, in turn, leads to a decrease in complement activation after a certain time during the dialysis procedure, as shown by our results of THC levels at 15 and 30 mins.

In addition, despite the rinsing procedures used, there is still a certain amount of autologous proteinaceous material that remains adherent to the inner surface of the hollow fibers. This also would decrease the direct blood-material interaction and thus the extent of complement activation and leukopenia would be less during subsequent uses, again as shown by our results.

It is important to note that the dialysis-induced leukopenia is also associated with significant functional and structural changes of the neutrophils[21]. Preliminary studies of random migration and chemotaxis of neutrophils during the period of leukopenia showed severe impairment of these functions[22]. Although there has been no direct correlation between these laboratory studies of neutrophil functions and adverse clinical conditions, it is well known that neutrophil adherence, chemotactic and phagocytic activities are essential mechanisms in the complex process for combating infection[23,24]. Dialysis patients seem prone to bacteremic infections and infection is the principal cause of hospitalization of patients with chronic renal failure[25]. The cyclical variation of the number and function of neutrophils with each dialysis and the decreased chemotactic responsiveness of neutrophils may well contribute to this high rate of infection.

Additional evidence is gradually accumulating that systemic complement activation may not only be the hallmark of many disease processes such as SLE and rheumatoid arthritis, but may indeed cause pathological conditions such as leukoembolization with occlusion of vessels up to 60 μm diameter[26]. In fact, there is considerable evidence that the dialysis-associated hypoxemia may be related to the sequestration of leukocytes in the pulmonary vasculature[15], and that the high incidence of pulmonary fibrosis and calcinosis found in post-mortem lung specimens of dialysis patients[27] may well be due to the chronic complement-induced activation of the

neutrophils, with resultant release of their lysosomal enzymes, during dialysis. Finally, it is becoming evident that the activation of complement is but one aspect of blood-materials interaction and that other aspects such as vasoactive kinin generation due to Hageman factor activation[28] is also occurring and may well play an important clinical role in dialysis-associated symptoms.

CONCLUSION

Reuse of cellulosic membrane dialyzers such as C-D 1.3 and CF-1200 leads to a significant decrease in the extent of complement activation, leukopenia and, to a lesser degree, hypoxemia. The correlation between these studies and the reduced morbidity and mortality associated with dialyzer reuse is not firmly established, but the evidence is highly suggestive. Finally, noncellulosic materials, such as PMMA, appear to be inherently more biocompatible than cellulosic materials.

ACKNOWLEDGMENTS

The cooperation of the patients participating in the study and of the staff of The Kidney Center in Boston is acknowledged and appreciated. The technical assistance of J. McCarthy is gratefully acknowledged. Miss R. Canty gave invaluable secretarial assistance.

REFERENCES

1. Deane N, Blagg C, Bower J, DePalma J, Gutch C, Kanter A, Ogden D, Sadler J, Siemsen A, Teehan B, Sosin A. A survey of dialyzer reuse practice in the United States. Dial Transplant 7:1128, 1978.
2. Wing AJ, Brunner FP, Brynger HOA, Chantler C, Donckerwolcke RA, Gurland HJ, Jacobs C, Selwood NH. Mortality and morbidity of reusing dialysers. Br Med J 2:853, 1978.
3. Kant KS, Pollak VE. Dialyzer reuse: Safety and efficacy. (Abstract) Chronic Renal Disease Conference, NIAMDD, Jan. 9-11, 1980, p 33.
4. Hakim J, Lowrie EG. Hemodialysis associated neutropenia and hypoxemia. The effect of dialyzer membrane materials. (Submitted for publication)
5. Craddock PR, Fehr J, Brigham KL, Kronenberg R, Jacob HS. Complement and leukocyte-mediated pulmonary dysfunction in hemodialysis. N Engl J Med 296:769, 1977.
6. Kunitomo T, Kirkwood RG, Kumazawa S, Lazarus JM, Gottlieb MN, Lowrie EG. Clinical evaluation of post-dilution dialysis with a combined ultrafiltration-hemodialysis system. Trans Am Soc Artif Intern Organs 24:169, 1978.
7. Kent JF, Fife EH. Precise standardization of reagents for complement fixation. Am J Trop Med 12:103, 1963.
8. Gotch FA, Sargent J, Keen M, Holmes G, Telsinger C. Development and long-term clinical evaluation of a thromboresistant hollow fiber kidney. Trans Am Soc Artif Intern Organs 18:135, 1972.
9. Juncos LI, Marbury TC, Cade R, Mahoney JJ, Bucci CM, Ayers JA. Reusing the hollow-fiber dialyzer. Dial Transplant 6:32, 1977.
10. Farrell PC, Eschbach JW, Vizzo JE, Babb AL. Hemodialyzer reuse: Estimation of area loss from clearance data. Kidney Int 5:446, 1974.
11. Snedecor G, Cochran W. Statistical Methods. Iowa State Univ. Press, Ames, Iowa, 1967, p 299.
12. Mason RG, Zucker WH, Bilinsky RT, Shindoa BA, Sharp DE, Mohammad SF. Blood components deposited on used and reused dialysis membranes. Biomat Med Dev Artif Organs 4:333, 1976.
13. Kaplow LS, Goffinet JA. Profound neutropenia during the early phase of hemodialysis. JAMA 203: 1135, 1968.
14. Toren M, Goffinet JA, Kaplow LS. Pulmonary bed sequestration of neutrophils during hemodialysis. Blood 36:337, 1970.
15. Craddock PR, Fehr J, Dalmasso AP, Brigham KL, Jacob HS. Hemodialysis leukopenia: Pulmonary vascular leukostasis resulting from complement activation by dialyzer cellophane membranes. J Clin Invest 59:879, 1977.
16. Fearon DT, Austen KF. Immunochemistry of the classical and alternative pathways of complement. In: Glynn LE, Steward M, eds. Immunochemistry. New York: John Wiley & Sons, 1978, pp 365-397.
17. Schur P. Complement testing in the diagnosis of immune and auto-immune diseases. Am J Clin Path 68:647, 1977.
18. Ghebrehiwet B, Muller-Eberhard HJ. C3e: An acidic fragment of human C3 with leukocytosis-inducing activity. J Immunol. 123:616, 1979.
19. McCall CE, DeChatelet LR. New biological activity following intravascular activation of the complement cascade. Nature 249:842, 1974.
20. Gunnarsson B, Asaba H, Kiibus A, Soderborg B, Wiklund S, Bergström J. Fibrin deposition in disposable dialyzers before and after re-use. Clin Nephrol 12:117, 1979.
21. Bjorksten B, Mauer SM, Mills EL, Quie PG. The effect of neutrophil chemotactic responsiveness. Acta Med Scand 203:67, 1978.
22. Geotzl E. Private communication.

23. Gellin J, Wolf S. Leukocyte chemotaxis: Physiological considerations and abnormalities. Clin Hemat 4:567, 1975.
24. Miller ME. Pathology of chemotaxis and random migration. Hematology 12:59, 1975.
25. Nsouli K, Lazarus JM, Schoenbaum S, Gottlieb M, Lowrie EG, Shocair M. Bacteremic infections in hemodialysis. Arch Intern Med 139:1255, 1979.
26. Hammerschmidt DE, Harris P, Waylant JH, Jacob HW. Intravascular granulocyte (PMN) aggregation in live animals: A complement (C)-mediated mechanism of ischemia. Blood 52,Suppl 1:125, 1978.
27. Conger JD, Hammond WS, Alfrey AC, Contiguglio SR, Stanford RE, Huffer WE. Pulmonary calcification in chronic dialysis patients: Clinial and pathological studies. Ann Intern Med 83:330, 1975.
28. Spragg J, Austen KF. Plasma factors: The Hageman-factor dependent pathways and the complement sequence. In: Hadden JW, Coffey RG, Spreafico F, eds. Immunopharmacology. New York: Plenum Publishing Corp., 1977, pp 125-143.

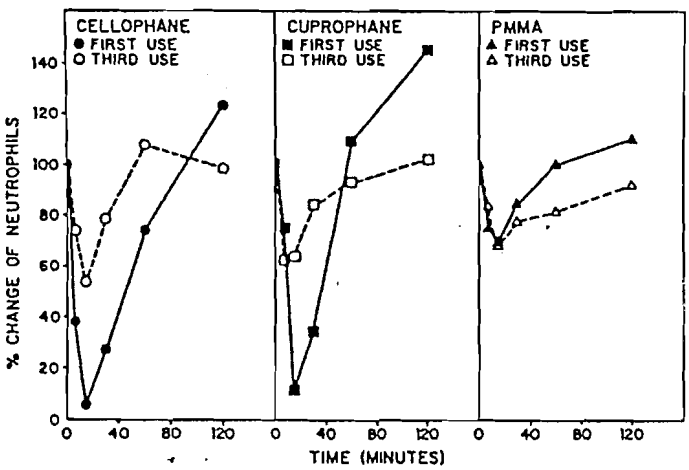


Figure 1. The percent change of neutrophils during dialysis for cellophane, cuprophane and PMMA membrane hollow fiber dialyzers during first and third use.

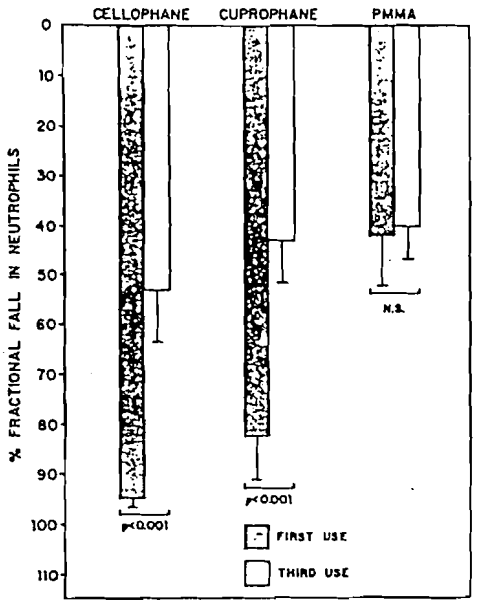


Figure 2. The percent fractional fall of neutrophils for cellophane, cuprophane and PMMA membrane dialyzers during first and third use.

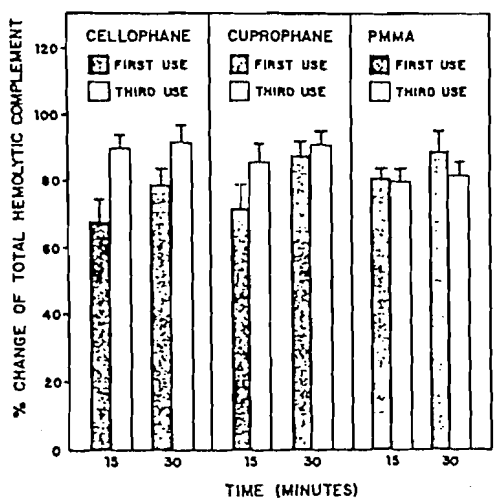


Figure 3. The percent change of the total hemolytic complement level at 15 and 30 mins after start of dialysis for cellophane, cuprophane and PMMA membranes during first and third use.

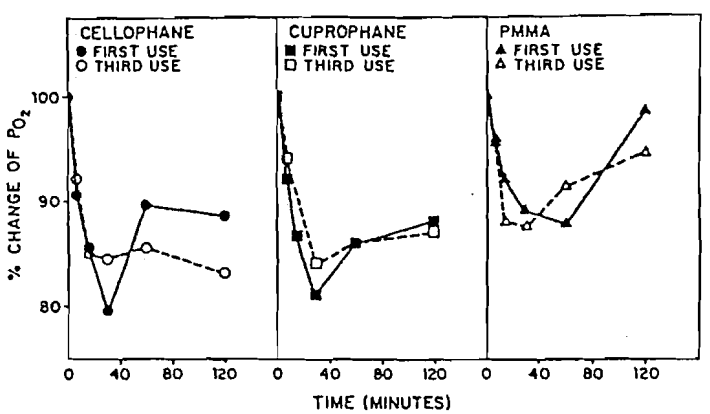


Figure 4. The percent change of the PO_2 during dialysis for cellophane, cuprophane and PMMA membrane dialyzers during first and third use.

DISCUSSION OF MANUSCRIPT #32

DR. NOSÉ: I am extremely happy to see your paper stressing importance of blood compatibility of dialysis membranes, and also shows a clear advantage for reused dialyzers. I recall in 1965 we had the same results with cardiac prostheses. We reported to the New York Academy of Science that the silastic cardiac prosthesis implanted for the first time and then the second time, with formaldehyde solution storage between the first and the second use, we had less occlusion; the third time we had much less occlusion. Based upon this, in 1971 we proposed the so-called "crosslinked" hypothesis. I believe you're treating in-between formaldehyde, so I am sure that the surface is crosslinked and the effect is the same as ours, and I would be extremely happy to hear your result.

DR. HAKIM: I think there may well be an effect of formaldehyde on the surface, but even before our first use, to make our dialyzers as much the same as possible, we had treated our dialyzers with formalin. Of course, after that, we had some proteinaceous material that formaldehyde may act on differently.

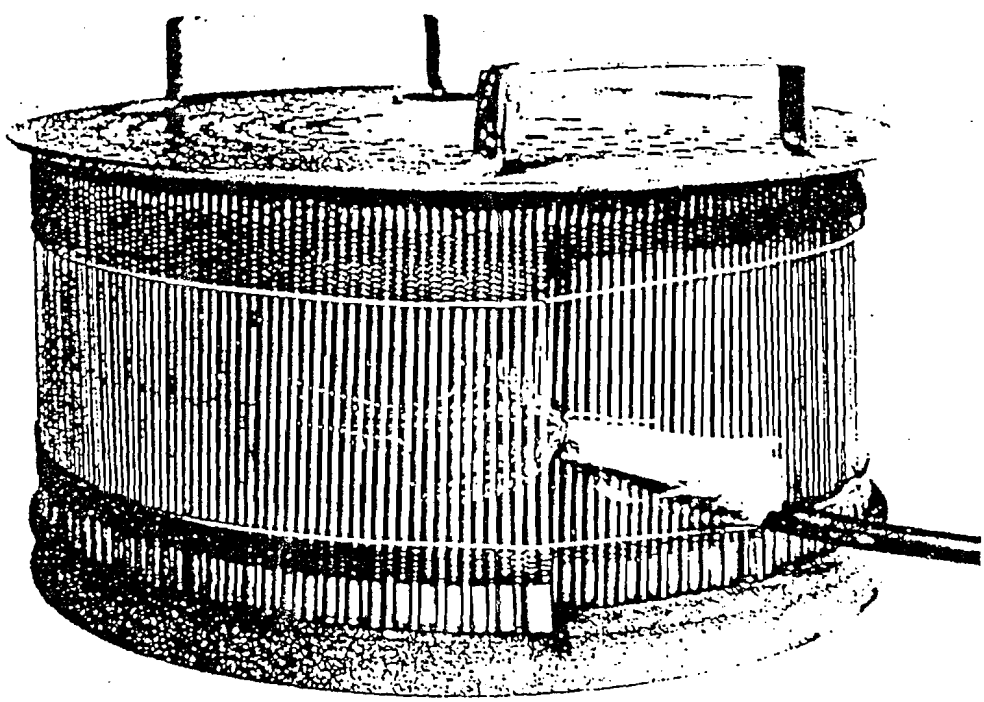
DR. PATERAS: As you alluded to the fact that recently there has been some evidence that the membrane activates the complement alternate pathway, I was wondering if you had any evidence to support the work from Minneapolis recently, that states that the fifth component is in fact responsible for this phenomenon. Did you study the other components, as well?

DR. HAKIM: We are in the process of studying all the other components selectively. But, as you well know, it's very expensive and very time consuming because you test using functional, not just quantitative, assays. To answer your question, specifically, no, we have not yet tested the C5 component but we have tested other components of the alternative pathway. These show a much more dramatic fall with cellulosic membranes than what we see here.

DR. DEANE: I think this is a significant paper in that it begins to fill in an important gap in our overall quantitative information about multiple usage. Wing presented mortality data and Kant morbidity data, and now Dr. Hakim is beginning to present biochemical data. I think the avenues, and I'd like to hear if you are beginning to do anything about this, that you've opened up are those which would look at the effluent from first use dialyzers and ways that might not have been done before; and also what are the natures of the changes on the lining of the membrane that almost seem to make it more like self with repeated usage?

DR. HAKIM: We haven't done that type of work yet, but it's in the planning stages.

* * * * * * * * * * *



The first concentrically wrapped kidney by Von Garrelts of Stockholm, Sweden, 1948; weight of the kidney was 130 lbs.