Introduction: Symposium Feature Specialty—Dialysis

Appearing for the first time we present this new impact area. Dr. Charley F. Gutch has consented to be our feature dialysis author for four issues. Realizing The Journal is primarily for technologist manuscripts, we feel that further guideance can be made into many facets of the technology by our special section physician writers. We are gratefully appreciative of this our inaugural step.

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The Middle Molecule Muddle – A Pragmatic Approach (or Diogenes without a Lantern)

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The concept of dialysis as a means of removing solute from a solution was developed in 1861 by T. Graham, a Scotch chemist.[1] Graham used a parchment membrane and among other things demonstrated removal of urea from urine. Dialysis became, and continues to be, an important and sophisticated tool which is widely used in chemistry and industry.

Abel, Rowntree and Turner are credited with the original concept, development and experimental use of an "artificial kidney" in 1913. Collodion tubes were used as the dialysis membrane, and hirudin (from the heads of leeches) was their anticoagulant. With this vividiffusion apparatus they were able to demonstrate that substances such as salicylate and urea could be successfully removed from the blood of living animals.

In the 1930's cellophane was developed and soon noted by chemists to have excellent properties for dialysis. Heparin of sufficient purity for human use had also become available. Thus, in 1943, Kolff, who had been working for five years

on prototypes, developed with Berk the rotating drum artificial kidney. The first patient was treated March 17, 1943.[2] That patient had malignant hypertension and end stage renal disease, and received twelve treatments. The first human hemodialysis was for a chronic patient.

However, in the late 1940's and throughout the fifties, hemodialysis was primarily used for acute renal failure. It was a drastic, dramatic procedure used to tide the patient over until his own kidney function began to recover. Then B. H. Schriner in 1960 began long term dialysis of chronic patients. He proved that maintenance dialysis was feasible, and could keep patients in a reasonable state of health for prolonged periods of time. Currently there are more than 10,000 patients receiving such dialysis treatment in the United States, many for more than five years.

Kolff, while developing his ideas for a clinical artificial kidney in 1938, was primarily interested in removing the 20 or so grams of urea that are produced each day. In the first clinical uses of the rotating drum kidney, it was demonstrated that not only urea but creatinine, uric acid, and phosphate were removed also. Indoxyl and substances giving a xanthoprotein reaction were recovered in dialysate. Kolff at that time expressed his conviction that uremia is not the result of a single toxic substance but the sum of deleterious effects of all retained substances.

In the 1960's, as the numbers of patients receiving long term dialysis began to increase, it became apparent that some patients developed a peripheral neuropathy after awhile.[3] Usually the onset was gradual; often with parasthesias and a burning sensation in the feet, followed by progressive weakness of the lowers. Occasionally the neuropathy was fulminant—rendering the patient unable to walk in a few days. Other problems—anemia, clotting abnormalities and bone disease—became recognized as complications of hemodialysis. It was also noted that patients maintained for long periods by peritoneal dialysis seemed to have much less neuropathy and less of other complications even though their BUN and creatinine values stayed somewhat higher than those of hemodialysis patients.[4]

Various hypotheses were offered. Perhaps essential substances were being removed by hemodialysis. Or metabolic breakdown products of large molecular weight, which would cross the artificial membrane poorly, might tend to accumulate in the body. Such molecules might pass the living peritoneal membrane more efficiently than the artificial cellulosic membranes.

The Seattle group tried increasing the number of hours of dialysis each week, and found patients were less likely to develop neuropathy. In some cases it was possible to halt or even reverse progression of neuropathy.

Plasma concentration of urea falls rapidly early in the dialysis procedure, while at the end it is slow. Extending dialysis time actually produces little additional urea removal. Larger molecules, at a lower concentration in the blood stream, and with a low net removal rate per hour, maintain a relatively high diffusion gradient throughout a prolonged dialysis. The net removal of such molecules per week would then correlate well with the increased total hours of dialysis, whereas urea and creatinine removal would be less influenced.

From this premise Babb and the Seattle group derived the square meter-hour hypothesis in 1971.[5] It was felt to be clinically established that 30 hours dialysis per week on a standard Kiil (effective surface area 0.7 M^2) with Cuprophan 150 membrane was adequate to prevent neuropathy. 30 times 0.7 yields 21 M^2 hours. If the membrane area were reduced by one-half, 60 hours dialysis time would be necessary to achieve the 21 M^2 hours. If the effective membrane area could be doubled, only 16 hours dialysis time would be needed. Blood flow rate and dialysis flow rate became much less important in this concept, since the large molecule removal is more related to time and surface area than to flow.

Various approaches have been tried to test the hypothesis. One group used a small surface area dialyzer (0.44 M^2) which had better clearance for urea and creatinine than the standard 1 M^2 Kiil.[6] Patients were dialyzed 20 hours per week on this unit and most maintained predialysis creatinine values not significantly different than with the larger surface area device. With passage of time all patients had a decrease in motor nerve conduction time and some developed clinical neuropathy. This seemed to confirm that something other than efficient removal of urea and creatinine is necessary.

In vitro studies and clinical work by the Seattle group suggests that since most current dialyzers and membranes in clinical use have negligible clearance for solutes of greater than 2000 molecular weight, molecules larger than that probably are not involved in the development of neuropathy. Therefore, attention has centered on molecules in the range of 300 to 2000 M. W. as the offenders, in so far as development of neuropathy is concerned.

Unfortunately the only clinical test currently available for the validity of the square meter-hour hypothesis—or as most people now prefer, the middle molecule hypothesis—is the presence or absence of peripheral neuropathy. Such neuropathy may appear early or relatively late; it may be gradual in onset, or fulminate and incapacitating in a few weeks. Once developed, it is slow to improve. Nerve conduction velocity measurement is widely used as an index of subclinical neuropathy. The reliability of this test is not great; there is a wide range of normal values, and the day-to-day variation in uremic patients is considerable. Comparison of groups of patients or progress of a given patient based upon nerve conduction is subject to considerable error.[7]

In vitro testing of membranes and dialyzers has been extensively done in the middle molecule range. Some of the test solutes commonly used include:

Sucrose	— M. W.	342
Raffinose	—	512
Bromsulfophthalim	—	838
Vitamin B_{12}	—	1355
Inulin	—	5200

Thus it is possible to demonstrate in vitro that the mathematics of the middle molecule (square meter-hour) hypothesis are correct. Neither blood flow rate or dialysis fluid flow rate are critical in the middle range of 300 to 1500 molecular weight. Babb et al derived certain predicted pre-dialysis serum concentration levels of molecules in this range and was able to show mathematically that patients

without neuropathy conformed to the square meter-hour theory in terms of time and surface area exposure using the standard Kiil with Cuprophan 150 membrane.

However, no middle molecules have thus far been identified individually, or demonstrated individually or collectively to induce a neuropathy syndrome experimentally. There is indirect evidence. Man, et al,[8] tested dialysate (using a highly permeable polyacrylonitrile membrane) from two patients with advanced neuropathy for tissue cell culture toxicity and for gupy's fry toxicity. The toxicity was 4 to 8 times that of non-neuropathic patients (the latter toxicity was equal to that of the ultrafiltrate of normal urine). It was also shown that after a number of such dialyses, the ultrafiltrate toxicity dropped to that of the non-neuropathic patient. By fractionation on Sephodex columns, the peak activity corresponded to molecular weight 500 to 3000. Hattler collected urine of patients following successful kidney allografts, on the premise that poorly dialyzed molecules would be rapidly excreted in the early urine.[9] Concentrates of such urine had a marked inhibitory effect on mixed lymphocyte cultures. It was also toxic for mice. It was no longer demonstrable after the third day post-transplantation.

Lonergan, et al,[10] explored the possibility of transketolase (an enzyme, which in conjunction with thiamine may be involved in neural energy production). By concentrating dialysate of uremic patients, a material was obtained which inhibited TKA activity of normal red cells. This material was fractionated by selective filtration, and the inhibitory fraction found to be only in that portion having molecular weight under 500. Kopple[11] however, found that TKA activity in red cells of patients receiving regular dialysis was normal, casting doubt on the previous work.

Millora[12] applied the technic of electroencephalographic frequency analysis as a measure of uremic encephalopathy to assess the square meter-hour hypotheses. Patients adequately dialyzed by this criteria had significant improvement toward normal by these EEG criteria, while those who had less dialysis did not, even though serum urea and creatinine levels in both groups were similar.

Use of small volume dialyzers, such as the HFAK in multiple arrangements to provide high surface area has been tried by some workers and seems to confirm that fewer hours of dialysis are needed to maintain patients in relative well being.

Other work[13] has been done with membranes of increased permeability. The cellulose acetate used was approximately three times as permeable for bromsulfophthalin and inulin as Cuprophan 150. Applying the square meter-hour hypothesis to this more permeable membrane, patients were dialyzed with low fluid flow rates. The patients appeared to do and feel well, but neurological manifestations were variable and not totally in keeping with the theoretical premises.

The similarity to problems in the dialysis of relatively large molecular weight exogenous poisons was pointed out by Schreiner. Here flow rate becomes much less important, while dialysis time and dialyzer membrane surface area become critical.

More recently, attention has been focused on the fact that in dialyzers, clearance of inulin (M.W.-5200) is only 2.5% of creatinine clearance, whereas

the normal human kidney clears both inulin and creatinine at essentially the same rate. Since many maintenance dialysis patients have some residual function of their own kidneys—this could account for a significant degree of middle molecule removal and the absence of neuropathy in some patients underdialyzed according to the square meter-hour hypothesis.[14] For several years the early institution of dialysis has been advocated to avoid onset of neuropathy. It may be that some such patients have been dialyzed primarily for fluid control and for removal of solutes other than those contributing to nerve damage, while their residual renal function takes care of potentially neurotoxic agents. It has been suggested that when endogenous creatinine clearance falls under 2 ml/min. on the average (residual function, plus dialyzer clearance) neuropathy is likely to occur. In such case, anephric patients should be particularly prone to neuropathy. However, one study of a group of anephric individuals[15] did not demonstrate significant change in nerve conduction time over a mean 28 month period of observation, even though the patients were not adequately dialyzed by the middle molecule hypothesis. They did have decreased hematocrits, problems with nutrition and generally did not fare well—but they did not show accelerated neuropathy.

Some of the arguments concerning the middle molecule hypothesis are reminiscent of those arguments about parallel-flow vs. coil dialyzers of ten years ago—embellished with higher mathematics. This is unfortunate.

The middle molecule hypothesis provides a new look at clinical and experimental hemodialysis. However, uremia is a complex syndrome. Teschan[16] pointed out that the characteristic metabolic defects of uremia affect the whole organism and involve the integration of organ systems. The biochemical and hormonal derangements are multiple. A defect of the middle molecule hypothesis is its concentration on one clinical manifestation, neuropathy, as the index criterion. Neuropathy is a vicious complication which appears to be causally related to inadequate dialysis. But absence of neuropathy in the presence of other problems—severe anemia, bone disease, malnutrition, pericarditis or ascites—does not represent adequate dialysis, or adequate treatment. Two hundred or so nitrogenous compounds have been shown to accumulate in the tissues and fluid of uremic individuals. Indoles and guanidines compounds (guanidinosuccinic acid, methyl guanidine, guanidinoacetic acid) have been shown in experimental animals to produce anemia, alteration in coagulation factors, and tissues wasting at serum levels comparable to those in uremia. Methylguanidine in some instances induced prolongation of nerve conduction time. Its size is well under molecular weight 300. Trace metals have been implicated in certain bizarre neuro-encephalopathic conditions encountered in some hemodialysis patients. The multiplicity of cellular enzymatic disturbances—present even in the well dialyzed uremic—seems to mitigate against a single offending agent in uremia. Even though currently not in favor, urea has an effect on cellular membrane transport.

Application of the middle molecule hypothesis can contribute to development and investigation of improved dialysis membranes. It has significant implication for dialyzer design in terms of optional effective surface area, and possible incorporation of sorbant devices into the overall system. This should ultimately

result in better dialysis equipment, and can lead to better clinical dialysis of patients. It may open new avenues of approach to the total treatment of uremia.

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