Acceleration of Peritoneal Dialysis by Surface-Active Agents

By S. C. PENZOTTI and A. M. MATTOCKS

Peritoneal dialysis is an effective method of removing endogenous poisons, but it is a slow process. In a search for agents to accelerate dialysis across the peritoneum, a number of wetting agents were tested and used in the peritoneal fluid. Dialysis tests were run on rabbits with radioactive urea and rate constants were calculated from dialysate and blood curves. The anionic and cationic wetting agents had significant effects, while the nonionic wetting agents were much weaker. On the basis of effectiveness and gross toxicity dioctyl sodium sulfosuccinate 0.01 percent was chosen as the most promising accelerator. It was evaluated in more detail and found to accelerate the dialysis of urea to about four times the rate of controls. When tested with radioactive creatinine the acceleration was two to threefold. It was concluded that dioctyl sodium sulfosuccinate is worthy of the further work required to bring it to the stage of clinical trial.

PERITONEAL DIALYSIS is an effective means for removal of endogenous poisons (1). It has the advantage over the artificial kidney of requiring no special equipment or facilities and being a relatively simple technique which might be performed in any physician's office. It has the serious disadvantage of being slow to clear the body of toxic agents, requiring up to 72 hr. in treatment of kidney failure and being so slow in removal of drugs as to have questionable value in suicidal or accidental drug poisonings (2, 3). If the process could be accelerated this would be of utmost importance, making possible the treatment of a larger number of uremic patients in a given institution and offering an effective treatment for drug poisoning in any locality.

In a search for substances which might accelerate diffusion across the peritoneal membrane, wetting agents were among the first type of compound to be considered. It is conceivable that incomplete wetting of the membrane by the dialysis fluid might occur with the common solutions, possibly due to fatty material on the membrane surface or on other tissues where penetration of the fluid to the membrane might be blocked. Also, diffusion acceleration of unknown nature has been observed in laboratory experiments with cellophane membranes when certain wetting agents were added (4). In this laboratory it was found that tris-hydroxymethyl aminomethane (THAM), a substance with weak surface-active properties, accelerated peritoneal dialysis by an unknown mechanism (5).

A group of common wetting agents was selected for test in peritoneal dialysis, including some anionic, cationic, and nonionic compounds. Experiments were planned to observe dialysis rates of urea and gross toxic effects with one to three animals as a screening test, then to select optimum concentrations of any found to be effective, finally to run a series of tests with the selected concentration of the best agents found. Since creatinine as well as urea is used as an indicator of the progress of peritoneal dialysis, it was planned to test the most promising of the agents for its effect on the dialysis of creatinine also.

As was pointed out in a previous paper (5), the effectiveness of peritoneal dialysis is best evaluated by means of a rate constant, since the amount dialyzed in a given time is dependent on the blood-dialysate gradient at each instant of time throughout the period, while the blood level is affected by urinary excretion and metabolism as well as diffusion into dialysate. Thus, experiments were set up to measure both blood level and dialysate level over the period of experiment, allowing the rate constant to be calculated from suitable equations.

MATERIALS AND METHODS

Healthy, mature male albino rabbits were selected as the experimental animal. No general anesthesia was used, but 50 mg. of chlorpromazine hydrochloride was administered intramuscularly 20 min. prior to the beginning of the experiment. A urinary catheter was inserted and 1 Gm. of ^{14}C-tagged urea (200 mg./ml.) was injected intravenously. Twenty to 30 min. was allowed for distribution of the drug throughout the body fluids before beginning dialysis, then 150 ml. of dialysis fluid was introduced into the peritoneum by means of a pediatric-size peritoneal catheter. Blood samples were taken from the marginal ear vein. Dialysate samples were withdrawn through the peritoneal catheter, taking care to return contents of the outlet tubing and catheter after removal of the sample. Samples of blood and dialysate were removed at 10–15 min. intervals over periods up to 200 min. Urine

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samples were taken but are not used in this analysis and are not presented here. With creatinine dialysis the same procedure was used except that 0.5 Gm. of ^{14}C-tagged creatinine (50 mg./ml.) was injected and the beginning of dialysis was delayed until about 1 hr. after injection in most cases.

The urea injection was prepared by dissolving an amount of radioactive urea equivalent to about 70 microcuries and sufficient stable urea to make 10 Gm. total urea in bacteriostatic water for injection (0.9% benzyl alcohol) to make 50 ml. This was sterilized by filtration and stored in the refrigerator.

Creatinine injection was prepared by dissolving an amount of creatinine-1-^{14}C equivalent to about 70 microcuries and sufficient stable creatinine to make a total of 5 Gm. in water for injection, then adding 0.2 ml. of 0.2 *N* hydrochloric acid and sufficient water for injection to make 100 ml. The solution was filtered, sealed in multidose injection vials, and autoclaved. The acid and heat were used to insure that any traces of creatine would be converted to creatinine.

The dialysate solution used for control was a commercial formula, Inpersol No. 46244—Abbott containing dextrose 1.5%, sodium lactate 0.5%, sodium chloride 0.56%, calcium chloride USP 0.026%, and magnesium chloride hexahydrate 0.015%. The surface-active agents were merely dissolved in this fluid to prepare the test solutions, except where concentrations of wetting agents were high enough to alter significantly the osmolality. In such cases the same formula was used except that sufficient dextrose or dextrose and sodum chloride was deleted from the formula to balance the number of moles of added substance. The only fluids of this type of the data tables of this paper are those containing THAM.

The wetting agents used, their grade and source were: sodium desoxychlorate, purified, (Fisher); polysorbate 60 and polysorbate 85,[1] pharmaceutical grade; trishydroxymethyl aminomethane (THAM), primary standard (Fisher); sodium lauryl sulfate USP (Amend); benzalkonium chloride[2] 17% solution; dioctyl sodium sulfosuccinate (DSS), solid, (Fisher); cetyl trimethyl ammonium bromide (CTAB), technical, (Matheson); and cetyl trimethyl ammonium chloride (CTAC), practical, (Fisher).

Measurements of concentrations in blood and dialysate were made by standard radioactive counting methods. For blood, a 0.2-ml. sample of whole blood or serum was added to 1 ml. of 6% trichloroacetic acid in a centrifuge tube and immediately shaken vigorously, then centrifuged. A 0.5-ml. aliquot of the clear supernatant was added to 10 ml. of dioxane phosphor solution and counted. For dialysate, 0.5 ml. of the solution was transferred to the counting vial containing 10 ml. of phosphor solution for counting. An accurate dilution of the injection used served as a standard which was counted with each set of samples. The dioxane phosphor solution contained 0.7% PPO, 0.005% POPOP, and 5% reagent naphthalene in spectro quality dioxane. All samples were counted with 1% error or better in a Packard 314-X Tri-Carb liquid scintillation counter and corrected for quenching by channels ratio.

[1] Marketed as Tweens 60 and 85 by Atlas Chemical Corp., Wilmington, Del.
[2] Marketed as Zephiran by Winthrop, New York, N. Y.

RESULTS AND TREATMENT OF THE DATA

The dialysis rate constant describing the diffusion from blood to dialysis fluid is depicted as k_1 in the following equation:

$$\begin{array}{c} \overset{k_1}{\rightleftarrows} \\ \mathrm{B} \quad \mathrm{D} \\ k_3 \downarrow \quad k_2 \\ \mathrm{U} + (\mathrm{M}) \end{array} \qquad \text{(Eq. 1)}$$

where B represents the compound, urea or creatinine, in blood, D, in dialysate, and U and M represent urinary excretion and metabolism, respectively. Values of k_1 were calculated from the equations of Rescigno and Segre (6) as previously described (5) using an analog computer. These rate constants are tabulated in Table I. Figures 1 and 2 illustrate curves obtained with controls and effective accelerating agents, respectively.

DISCUSSION AND CONCLUSIONS

In the design of these experiments, it was planned to run each test solution on a minimum number of animals as a screening test, then to run repeated tests with more animals where comparisons of rate constants with controls were desired. Thus, only in the cases where the agents showed promise as accelerators were more than two tests run. Substances were judged to be promising if no gross toxicity was evident and the rate constants were two to three times those of controls.

From the data it is seen that polysorbate 60, a nonionic wetting agent, appeared to have a weak effect at relatively high concentration. The low concentration of polysorbate, 0.01%, was run to determine whether this solution, with surface tension almost as low as the 1.8% solution, might give an equal effect. Since it did not, it appears, with this limited experience, that the polysorbate effect was not due simply to lowered surface tension. Polysorbate plus THAM was run to obtain an indication as to whether the two compounds affect dialysis by the same mechanism. Since the results showed a higher rate than either had alone, the indication, based on limited data, is that the two mechanisms are not the same.

In general, results show that both anionic and cationic wetting agents increase the rate of dialysis of urea across the peritoneal membrane and that the effect reaches a maximum, with higher concentrations yielding no appreciable increase in dialysis rate. The cationic agents, CTAB, CTAC, and benzalkonium chloride were all effective with the maximum in each case being at about 0.02%. With these agents, however, toxic effects and deaths were detected at concentrations 2–3 times as high as the most effective level. With benzalkonium chloride, deaths resulted with 0.02%. All deaths observed were similar, respiratory in nature, accompanied by fall in blood pressure, convulsions, appearance of a deep orange to red color in urine and dialysis fluid, and occasionally appreciable hemolysis as seen in the hematocrit. Because of the toxic manifestations observed at levels close to the effective concentrations, attention was focused on the less toxic anionic wetting agents.

TABLE I—DIALYSIS RATE CONSTANTS, k_1, FOR UREA AND CREATININE DIALYSIS WITH VARIOUS PERITONEAL FLUIDS

Fluid	Animal No.	Weight, Kg.	k_1, min.$^{-1}$ × 10^2
Urea Dialysis			
Polysorbate 60 1.8%	8	3.51	1.3
	9	3.08	1.7
Polysorbate 60 1.8% plus THAM 1.8%	10	3.32	3.9
Polysorbate 60 0.01%	11	2.55	0.5
Polysorbate 85 1.8%	29	2.79	1.1
Dimethylsulfoxide 1.2%	12	3.60	1.9
Benzalkonium chloride 0.02%	20	4.27	5.0[a]
	21	3.19	4.9
	22	3.13	3.9[a]
	23	3.51	4.4[a]
	24	3.61	4.8
Average		3.54	4.6
Benzalkonium chloride 0.1%	19	3.56	5.9[a]
CTAB 0.02%	33	2.79	5.3
	34	4.15	4.2
	35	4.43	4.9
	36	2.84	3.3
	37	2.77	4.9
Average		3.40	4.5
CTAC 0.02%	42	2.49	3.7
	45	3.07	2.8
CTAC 0.01%	43	3.05	2.6
	46	3.33	3.4
CTAC 0.05%	47	2.90	4.9
	48	3.58	4.6
Sodium lauryl sulfate 0.2%	27	2.87	2.8
	28	3.27	7.7
	30	3.33	3.1
	31	2.45	2.7
	32	4.02	5.3
Average		3.19	4.3
DSS 0.01%	16	2.63	4.4
	17	3.19	4.2
	18	3.61	3.4
	25	3.99	4.6
	26	4.08	5.3
	50	3.13	3.5
	51	2.98	4.5
	53	3.34	4.9
	55	4.26	3.0
	57	3.49	2.9
Average		3.47	4.1
DSS 0.18%	13	3.42	3.7
DSS 0.033%	14	3.80	5.0
DSS 0.02%	15	2.87	5.0
DSS 0.04%	38	2.79	4.3
	44	3.04	4.6
DSS 0.001%	39	4.02	0.8
DSS 0.005%	40	2.86	2.5
	41	3.96	3.1
Control	1	3.61	0.5[b]
	2	3.87	0.6[b]
	3	4.46	0.9[b]
	4	3.96	1.2[b]
	5	4.26	1.0[b]
	6	2.09	1.3[b]
	7	1.90	1.3[b]
	49	2.51	1.3
	52	2.71	0.8
	54	3.44	1.1
	56	3.78	1.0
	58	3.55	1.2
Average		3.34	1.0
Creatinine Dialysis			
DSS 0.01%	61	3.22	2.0
	63	2.79	1.5
	66	3.53	1.6
	67	3.97	1.3
Average		3.38	1.6
Control	59	2.45	0.48
	60	3.21	0.63
	62	3.27	0.84
	64	3.00	0.77
	65	3.10	0.53
Average		3.01	0.62

[a] Animal died shortly after last sample taken. [b] Controls taken from earlier data (5).

The difference in effect of CTAB and CTAC is unexpected, but since both are mixtures rather than pure compounds, it is presumed that certain effective components are present in greater proportions in one mixture than the other. The bromide ion would be undesirable in dialysis fluid since bromism might result. It was for this reason that the chloride was tested.

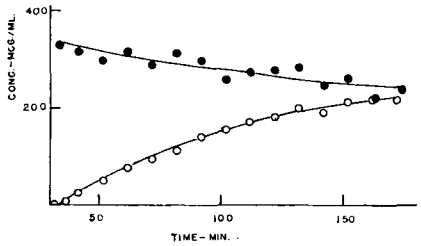

Fig. 1—Dialysis with control fluid, animal no. 54. Key: ●, blood; ○, dialysate.

Among the anionic agents tested, sodium lauryl sulfate, DSS, and sodium desoxycholate, DSS was the most effective. No gross toxicity was observed

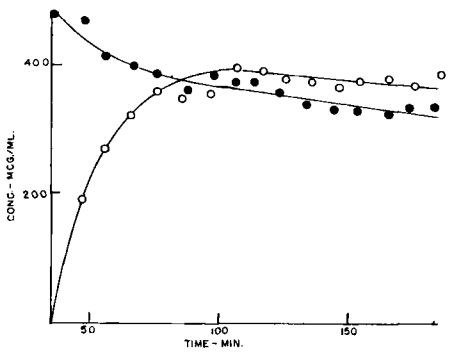

Fig. 2—Dialysis with an effective wetting agent, 0.01% DSS, animal no. 16. Key: ●, blood; ○, dialysate.

at levels five times the concentration of maximum activity, 0.01%. Sodium lauryl sulfate required a concentration of 0.2% to yield dialysis rate roughly equivalent to those with 0.01% DDS. Higher concentrations of sodium lauryl sulfate were not tested, since the 0.2% caused irritation in the abdomen evidenced by squirming and discomfort of the animal when the solution was introduced. It did not show any evidence of permanent damage, however. The rates obtained with sodium lauryl sulfate varied more than with any other agents used.

From these experiments it was concluded that DSS was the most promising of the wetting agents, accelerating the dialysis rate for urea to about four times that of the controls. This would constitute a significant improvement in the dialysis process.

Since DSS appeared to be the most promising agent it was also tested for its effects on the dialysis of creatinine. As seen in Table I, it also accelerates the removal of the compound, rates being about three times the controls.

The experiments with sodium desoxycholate were quite surprising. Death resulted with concentrations as low as 0.01%, and when lower concentrations were tested the effect on dialysis rate decreased as toxicity appeared to decrease. At nontoxic levels no acceleration of dialysis was observed.

The results of this work indicate that DSS may be a useful accelerator of peritoneal dialysis and is deserving of further work. The compound accelerates the dialysis of urea and creatinine, but whether it also accelerates the removal of the multitude of other substances, some known, some unknown, which accumulate in uremia must be determined by experiments which assess the effect of general uremic symptoms and long-term treatment of the diseased condition. Before DSS can be evaluated in man, of course, a measure of its absorption and accumulation in the body must be made and correlated with possible toxic effects which might result. It seems possible that once a given level of DSS is established in the system that the concentration in the dialysis fluid might be reduced without loss of effect. These are problems which must be faced in further work leading toward clinical trials.

This work also suggests the possibility of using DSS or other wetting agents in the removal of drugs such as salicylates and barbiturates which are commonly encountered in poisonings. This study is being conducted in this laboratory.

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Keyphrases

Peritoneal dialysis
Surfactants in dialysis solutions
Dialysis acceleration—surfactants
^{14}C-labeled urea—test compound
^{14}C-labeled creatinine—test compound
Liquid scintillation counting—analysis

Studies with Ion-Exchange Resins on Cinchona Alkaloids II

Effects of the Ionic Form and of the Solvent Medium

By C. V. BHAT, B. R. KAMATH, R. S. SHAH, S. S. KANHERE, and S. L. BAFNA

The effect of the ionic form of the sulfonic acid cation-exchange resins of different degree of crosslinking and of the added sulfuric acid on the equilibrium exchange of four cinchona alkaloid sulfates as well as the equilibrium uptake of four cinchona alkaloid bases by the hydrogen form of the same resins from six aliphatic alcohols has been studied and the results are discussed.

EARLIER (1) the equilibrium exchange of four cinchona alkaloid (quinine, quinidine, cinchonine, and cinchonidine) sulfates on styrene divinylbenzene copolymer based sulfonic acid cation-exchange resins of different degree of crosslinking and particle size in hydrogen form had been studied. This includes the study on the effect of the ionic form of such resins and of added sulfuric acid on the equilibrium exchange of the four cinchona alkaloid sulfates as well as the

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