caperatic acids are 3.0×10^{-3} and $2.4 \times 10^{-3}M$, respectively. Norcaperatic acid seemed to have a greater affinity to the enzyme than either of the substrates or agaricic acid. The order of decreasing affinities to aconitase was: norcaperatic acid, *cis*-aconitic acid, agaricic acid, and *dl* + allo, trisodium isocitric acid.

A chemical-steric relationship exists between the test acids and fluorocitric acid. Peters (13) states that fluorocitric acid is a competitive inhibitor of aconitase. *In vivo* it produces effects similar to those seen with agaricic and norcaperatic acids.

The basic concepts of enzyme action and steric requirements have been discussed by Koshland (14) and Ogston (15, 16) and a three-point attachment of citric acid to aconitase has been postulated. The citric acid is the correct size and shape to bind to the enzyme surface at three points as the conformation of the enzyme changes to accommodate it. In this natural situation, the proper alignment of bonds needed for catalytic activity is formed. It may be that when the bulky molecule of norcaperatic acid (citric acid with a $C_{14}H_{29}$ substituted for a H atom) approaches and attaches to the enzyme surface, it is bound; but because of its size, the proper formation of the catalytic bonds is prevented (steric hindrance). Agaricic acid and fluorocitric acid could act in the same manner since a $C_{16}H_{33}$ group or a F atom is also sterically much larger than a H atom. Moreover norcaperatic, agaricic, and fluorocitric acids all contain two asymmetrical carbon atoms, while citric acid does not. This makes four stereoisomeric forms possible for each structure. These forms would each have varying abilities to form enzyme and/or receptor attachments (affinity). In conclusion, the two naturally occurring acids studied here may be useful biochemical tools—especially for seeking antidotes, since the gross symptoms of certain toxic mushrooms have been shown here to be due, at least in part, to subcellular activity.

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Effect of Tris(hydroxymethyl)aminomethane on Removal of Urea by Peritoneal Dialysis

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From earlier *in vitro* studies it was thought that tris(hydroxymethyl)aminomethane (tromethamine) might accelerate diffusion of substances across the peritoneal membrane by a mechanism other than causing ionization of a weak acid in the dialysis fluid. This concept was tested by measuring the rate of dialysis of urea in rabbits, urea being essentially nonionized at the pH of the tromethamine fluid. Blood and dialysate concentrations were measured following intravenous injection of tagged urea and following incorporation of tagged urea in the dialysis fluid (reverse dialysis). Dialysis rates were calculated. It was found that tromethamine increased the dialysis rate two to threefold. It is concluded that tromethamine accelerates dialysis by an unknown mechanism.

Several workers have reported on the use of tris(hydroxymethyl)aminomethane (tromethamine)[1] in peritoneal dialysis (1–6). These have dealt with the use of tromethamine to remove salicylates, barbiturates, and urate, the theory being that the alkalinity of the dialysis fluid would cause ionization of the drug in the peritoneum and thus maintain a higher concentration gradient of unionized drug across the dialyzing membrane. It is important that the mechanism by which tromethamine exerts its effect on dialysis be understood in order that a search for other more effective substances be facilitated.

In a study conducted in these laboratories, it was found that both ionized and unionized pento-

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[1] Tham.

barbital and salicylate diffused rapidly and at almost equal rates across isolated rabbit mesentery (7). This agreed with other work (8–10) which demonstrated the diffusion of rubidium and phosphate ions across the isolated membrane. These findings suggest that the effect of tromethamine in accelerating dialysis might be something other than simple change of pH of the peritoneal fluid. On the other hand, it remains to be shown that the isolated membranes truly represent the diffusion characteristics of the live organ; thus *in vivo* tests are required.

One approach to determine whether tromethamine has an effect other than that of causing ionization of the compound in the peritoneum is to measure its effect on the dialysis of a substance which is not ionized at the pH of the tromethamine dialysis fluid or that of the blood. Urea is such a substance, having a pKa of 0.18, and it was selected for this study.

At the outset it was recognized that the rate of dialysis of a substance at any time would be a function of the blood (and extracellular fluid) concentration at that instant. The blood level, in turn, is affected not only by dialysis of the drug into the peritoneal fluid, but also by excretion and metabolism. Thus evaluation of dialysis rate simply by the amounts in dialysis fluid at intervals of time would be subject to errors resulting from different rates of metabolism and excretion by different animals. To avoid these variations experiments were designed to measure the blood and dialysate levels throughout the procedure. Results could then be calculated in terms of rate constants which are independent of the other variables.

MATERIALS AND METHODS

Healthy, mature male albino rabbits were selected as the experimental animal. No general anesthesia was used. A urinary catheter was inserted and an intravenous injection of 1 Gm. of ^{14}C-tagged urea (200 mg./ml.) was administered. Twenty to thirty minutes were allowed for the 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 peritoneal fluid were removed at 10–15-min. intervals over periods up to 200 min. Urine samples were taken also but are not used in this analysis and are not presented here.

Several tests of "reverse dialysis" were performed, wherein the tagged urea was placed in the dialysis fluid prior to its injection into the peritoneum. In these cases 2 ml. of the urea-^{14}C injection was mixed with 150 ml. of dialysis fluid just prior to injection, and samples of dialysate and blood were taken at 5–8-min. intervals over periods up to 120 min.

The urea injection was prepared by dissolving aseptically 1.2 mg. of urea-^{14}C (specific activity 3.67 mc./mmole) in 50 ml. of a sterile solution containing 200 mg. urea/ml. and 0.9% benzyl alcohol as a preservative. The injection was stored in the refrigerator.

The control dialysis fluid was a standard hospital formula containing: dextrose, 1.5%; sodium chloride, 0.62%; sodium lactate, 0.39%; calcium chloride monohydrate, 0.026%; and magnesium chloride hexahydrate, 0.015%. Tromethamine-I fluid was the same solution to which was added 18.17 Gm. (150 mmoles) tromethamine/liter. This fluid, although equivalent to control in content of dextrose and salts, was more hypertonic, and it is possible that an effect observed might be partially due to withdrawal of fluid by osmosis. Thus, a second fluid, tromethamine-II, was prepared containing 18.17 Gm. tromethamine/L. and sodium chloride 0.40%, no dextrose, and the same quantities of calcium chloride, magnesium chloride, and sodium lactate as the control solution. This solution was measured on an osmometer and found to have the same osmolality as the control (0.351). The dextrose and lactic acid used were USP; all other chemicals were analytical or reagent grade. The reagent grade of tromethamine is of such purity as to be commonly used as a primary standard in acidimetry.

Measurements of concentration in blood and dialysate were made by standard radioactive counting methods. Blood was prepared for counting in two ways, which were shown to give equal values: (*a*) 0.5 ml. of serum was added to 1 ml. of a solubilizer[2] and the mixture was allowed to stand 24 hr. Then 0.1 ml. concentrated hydrochloric acid and 10 ml. of dioxane phosphor counting solution were added. (*b*) Approximately 0.5 ml. of blood was drawn directly into a graduated centrifuge tube containing 1 ml. of 6% trichloroacetic acid; the volume was recorded and the contents shaken thoroughly. The tube was then centrifuged, and an aliquot of the clear colorless supernatant was added to 10 ml. of dioxane counting solution. The latter method was preferred, since no quenching was observed with this procedure. A 0.5-ml. portion was added with dialysate samples directly to the dioxane counting solution. An accurate dilution of the injection was used as a standard, which was counted with each set of samples. This was also used as the internal standard whenever quenching corrections were required. The dioxane counting solution consisted of 7 Gm. PPO (2,5-diphenyloxazole), 0.05 Gm. POPOP [(1,4-bis-2,5-phenyloxazolyl) benzene], and 50 Gm. naphthalene in sufficient spectro quality dioxane to make 1 L. All samples were counted at least 3 times in a Packard 314-X Tri-Carb liquid scintillation counter.

RESULTS AND TREATMENT OF THE DATA

Data on dialysis with control and the 2 tromethamine fluids are presented in Table I. Typical curves for blood and dialysate concentrations are shown in Figs. 1 and 2. As one might anticipate,

[2] Hyamine 10X, Rohm and Haas Co., Philadelphia, Pa.

TABLE I—CONCENTRATIONS OF EXOGENOUS UREA IN BLOOD AND DIALYSATE FOLLOWING INTRAVENOUS INJECTION AND DURING PERITONEAL DIALYSIS

Time, min.	Blood, mcg./ml.	Dialysate, mcg./ml.
Rabbit No. *1*, Control Dialysis Started, 20 min.		
10	581	0
29	507	13
40	385	30
50	433	53
62	...	78
72	320	107
83	246	140
94	299	157
104	233	167
114	236	181
125	201	192
134	...	189
145	...	180
Rabbit No. *2*, Control Dialysis Started, 20 min.		
12	498	0
27	449	27
37	439	74
53	464	92
62	436	126
72	...	160
83	415	172
92	443	177
102	461	191
114	462	205
124	435	218
134	...	244
144	425	243
154	442	264
164	409	270
174	444	298
185	406	293
194	433	301
Rabbit No. *3*, Tromethamine-I Dialysis Started, 30 min.		
11	485	0
22	425	0
32	332	34
42	402	87
53	343	142
62	366	228
72	364	253
82	322	270
90	330	269
102	310	290
113	310	273
122	351	282
132	310	259
144	337	268
154	314	270
164	284	302
174	270	267
185	271	272
194	271	251
204	262	260
215	258	263
Rabbit No. *4*, Control Dialysis Started, 30 min.		
15	374	0
30	329	0
40	304	9
50	303	25
60	277	46
70	274	70
80	265	84
90	265	96
100	243	115
110	243	136
120	237	134
130	261	137
140	213	161
150	204	168
160	208	164
170	216	160
Rabbit No. *5*, Tromethamine-I Dialysis Started, 30 min.		
11	441	0
30	392	0
40	324	23
51	348	104
61	294	120
71	293	142
80	287	156
91	236	206
100	309	224
110	316	237
121	276	262
132	227	266
142	303	268
150	277	278
161	276	291
171	299	287
180	293	283
193	261	293
204	278	290
Rabbit No. *6*, Tromethamine-I Dialysis Started, 20 min.		
10	578	0
24	238	0
35	441	77
45	394	153
55	405	311
65	366	...
75	362	342
85	402	309
97	380	353
106	358	337
117	...	363
125	354	375
135	344	366
145	...	358
155	347	330
166	343	299
177	338	292
187	324	288
197	341	...
207	310	...
Rabbit No. *7*, Control Dialysis Started, 23 min.		
10	535	0
25	426	0
35	406	97
45	397	151
55	377	164
67	398	191
75	367	196
85	321	217
95	349	223
105	330	218
115	329	231
125	369	252
135	352	238
145	327	252
155	299	255
165	249	260
175	...	259
185	253	270
195	294	265
205	276	264
Rabbit No. *8*, Control Dialysis Started, 30 min.		
20	318	0
29	310	0
40	310	14
51	287	46
60	296	75
70	262	89
80	261	106
91	270	118

(Continued on next page.)

TABLE I—(*Continued.*)

Time, min.	Blood, mcg./ml.	Dialysate, mcg./ml.
100	. . .	137
110	240	137
121	229	141
132	247	148
140	224	154
150	246	158
160	. . . .	168
170	. . .	176
180	231	177
190	227	180
200	213	178
210	227	181
Rabbit No. *9*, Tromethamine-II Dialysis Started, 35 min.		
30	302	0
46	272	21
56	263	54
65	239	99
76	255	133
86	225	181
96	250	198
106	251	199
115	220	203
125	193	211
135	235	223
146	236	228
155	. . .	224
165	231	234
175	229	230
185	253	244
195	216	236
205	. . .	237
Rabbit No. *10*, Tromethamine-II Dialysis Started, 30 min.		
46	558	133
56	494	199
68	548	333
78	544	355
85	492	356
98	507	392
105	451	403
117	455	415
125	460	420
137	443	417
147	416	408
155	440	412
166	407	420
176	. . .	414
186	455	412
196	. . .	412
206	396	405
216	404	408
Rabbit No. *11*, Tromethamine-II Dialysis Started, 30 min.		
43	657	91
53	755	227
64	470	273
73	410	329
83	394	368
94	450	377
104	515	397
114	397	384
123	405	388
133	378	388
143	. . .	400
153	378	402
164	353	386
173	328	378
183	319	386
193	324	382
203	. . .	375
213	325	307
Rabbit No. *12*, Control Dialysis Started, 30 min.		
32	664	0
42	536	85
53	518	128
62	489	171
73	474	188
82	392	203
92	365	219
102	391	230
112	345	239
122	321	254
132	339	253
142	318	264
153	285	266
162	279	271
172	273	264
182	279	264
192	278	260
202	286	260
212	285	263
Rabbit No. *13*, Control Dialysis Started, 30 min.		
33	422	0
43	. . .	89
54	431	119
64	391	139
73	371	159
83	357	174
94	365	199
103	. . .	206
113	325	215
123	358	227
133	368	233
143	314	248
153	286	248
163	277	261
173	280	276
183	319	279
193	272	284
205	280	270
213	. . .	280
Rabbit No. *19*, Tromethamine-II Dialysis Started, 30 min.		
30	376	16
40	313	40
50	292	65
60	290	133
70	272	172
80	250	195
90	277	214
100	270	224
110	262	239
120	277	246
130	253	245
140	279	260
150	294	245
160	304	269
170	302	265
Rabbit No. *20*, Tromethamine-II Dialysis Started, 30 min.		
33	481	28
40	415	59
50	462	110
60	445	187
70	412	227
80	439	266
90	407	303
100	431	329
110	420	341
122	378	347
131	402	355
140	405	361
150	413	372
160	412	381
170	337	387
Rabbit No. *21*, Tromethamine-II Dialysis Started, 58 min.		
60	331	0

(*Continued on next page.*)

TABLE I—(*Continued.*)

Time, min.	Blood, mcg./ml.	Dialysate, mcg./ml.	Time, min.	Blood, mcg./ml.	Dialysate, mcg./ml.
68	318	50	139	283	257
79	294	93	149	247	266
89	279	135	159	275	269
98	290	180	169	220	265
108	287	216	179	260	263
118	292	240	188	272	270
129	285	241	199	243	290

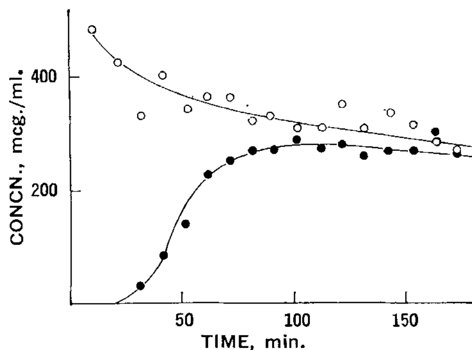


Fig. 1—Blood and dialysate concentrations of exogenous urea, rabbit No. 3, tromethamine-I dialysis fluid. Key: O, *blood;* ●, *dialysate.*

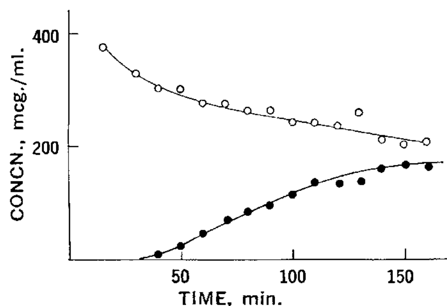


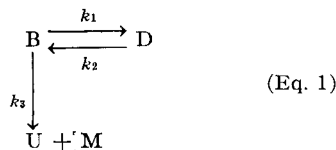
Fig. 2—Blood and dialysate concentrations of exogenous urea, rabbit No. 4, control dialysis fluid. Key: O, *blood;* ●, *dialysate.*

the blood levels and dialysate levels varied from one animal to another due to differences in metabolism and excretion rates. On the other hand, the more rapid rate at which the dialysate curves approach the blood curves in the case of tromethamine is obvious in most instances. As has been mentioned, the impression formed from such an observation might be quite misleading, since the reason for blood and dialysate curves approaching one another rapidly may be due to rapid rate of excretion or metabolism rather than rapid dialysis. For this reason it is necessary to compute dialysis rates to make a meaningful comparison.

The data on reverse dialysis are presented in Table II, and curves of dialysate concentrations are shown in Figs. 3 and 4. In Fig. 3 it is seen that the 2 tromethamine fluids lost urea at a consistently faster rate than the control. In Fig. 4 the tromethamine curve is quite close to control, the difference being mainly in curvature. As is shown later, the analysis yields higher rate constants in this case, since the rate constant reflects the differences in curvature between initial and final portions of the curve. The rate is slower in this instance, however, and may not be significant.

Several methods were utilized to analyze the data, all giving approximately the same results. The one judged to be most generally acceptable is presented in this paper. Rather than use equations relating total amounts in compartments which requires estimates of distribution volumes (11, 12), equations relating concentrations in the compartments were used.

The distribution of exogenous urea after an initial period for mixing throughout the body fluids may be depicted as follows:



(Eq. 1)

where B is blood or equivalent body fluid, D is dialysate in the peritoneum, and U and M represent urinary excretion and metabolites. Equations for treating this type of system may be found in Rescigno and Segre (13) and in Rodiguin and Rodiguina (14). The equation for dialysate concentration may be stated as follows:

$$X_2 = \frac{X_0 k_1}{m_2 - m_1}(e^{-m_1 t} - e^{-m_2 t}) = A(e^{-m_1 t} - e^{-m_2 t}) \quad \text{(Eq. 2)}$$

where X_2 is concentration in dialysate, X_0 is concentration in blood at time dialysis is begun, m_1 and m_2 are the roots of the auxiliary equation (13, 15). The equation for the concentration in blood is:

$$X_1 = \frac{X_0}{m_2 - m_1}[(k_2 - m_1)e^{-m_1 t} + (m_2 - k_2)e^{-m_2 t}] = B_0 e^{-m_1 t} + C_0 e^{-m_2 t} \quad \text{(Eq. 3)}$$

where X_1 is the concentration in blood, and other constants are the same as in Eq. 2. If t is zero at the time of starting dialysis, the concentration in blood at that time becomes B_0 plus C_0, and this sum may be taken as X_0 in subsequent calculations.

The dialysate curve is far more sensitive to the magnitude of k_1, since the blood concentration is

TABLE II—CONCENTRATIONS OF EXOGENOUS UREA IN BLOOD AND DIALYSATE DURING REVERSE DIALYSIS

Time, min.	Blood, mcg./ml.	Dialysate, mcg./ml.
Rabbit No. *14*, Control		
0	0	2666
6	11	2446
12	26	2290
17	35	2081
23	45	1915
28	47	1850
35	58	1710
43	56	1613
50	67	1464
58	69	1371
67	74	1253
77	78	1157
85	83	1088
94	81	1046
102	83	957
110	84	889
118	81	821
126	86	788
Rabbit No. *15*, Control		
0	0	2666
7	15	2237
13	33	1975
19	49	1815
24	62	1777
29	70	1587
37	66	1448
46	64	1429
54	114	1293
62	77	1209
70	105	1153
79	93	1063
88	93	981
95	86	924
103	103	851
111	93	786
119	97	739
Rabbit No. *16*, Tromethamine-II		
0	0	2666
5	28	2507
11	63	2299
16	81	2024
21	93	1849
26	95	1611
31	101	1469
36	118	1315
44	110	1147
52	120	1076
60	120	1006
68	122	920
76	133	842
84	133	776
91	127	689
100	118	627
109	136	560
Rabbit No. *17*, Tromethamine-II		
0	0	2666
5	18	2430
10	28	2321
15	42	1990
20	49	1781
25	50	1687
30	61	1496
36	51	1454
41	60	1455
46	64	1443
53	62	1333
61	64	1269
69	70	1147
77	80	1098
85	81	1080
93	80	1022
102	79	1007
Rabbit No. *18*, Tromethamine-II		
0	0	2666
6	40	1898
11	66	1975
16	79	1786
22	74	1576
31	94	1227
36	80	1132
41	95	1081
47	96	1026
52	83	961
58	98	834
67	96	804
75	100	669
85	96	635
94	92	585
103	91	531
112	98	471

affected less than 10% by the amount diffused into dialysate, hence the dialysate curve and Eq. 2 were used to calculate the values of m_1 and m_2, while the blood curve and Eq. 3 were used to obtain values of X_0. Equation 2 was solved on an analog computer, and the values of m_1 and m_2 were put into Eq. 3 on the computer to be certain that they fit the blood curve as well. This is easily and rapidly done with the analog computer and a modern x-y plotter. The rate constant was then calculated from an expression derived from Eq. 2:

$$k_1 = \frac{A}{X_0}(m_2 - m_1) \qquad \text{(Eq. 4)}$$

Values obtained for k_1 are presented in Table III.

With reverse dialysis the dialysis curve was again used for evaluation of m_1 and m_2 and for the values X_{20} and D of the following equation, which is the one applicable for the condition where the drug is administered in the dialysis fluid (13):

$$X_2 = \frac{X_{20}}{m_2 - m_1}[(\mathrm{D} - m_1)e^{-m_1t} + (m_2 - \mathrm{D})e^{-m_2t}] \qquad \text{(Eq. 5)}$$

where X_2 is concentration in dialysate, X_{20} is its initial value, D is k_1 plus k_3, m_1 plus m_2 equals the sum of k_1, k_2, and k_3, the k's having the same meaning as in Eq. 1. The value of k_2, representing transport from the dialysate to the blood, was calculated as $m_1 m_2 - \mathrm{D}$. The values obtained for k_2 are shown in Table III.

It is seen from Table III that an increased rate of dialysis was observed with tromethamine rather consistently. One control was as high as 2 of the tromethamine solutions, but it was high for a control solution. The average rate with tromethamine was more than twice that of controls. Tromethamine-I and tromethamine-II fluids showed no difference, thus all tromethamine values were averaged together.

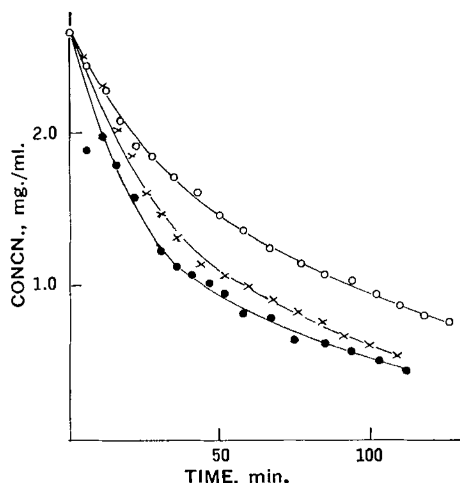


Fig. 3—Dialysate concentrations of exogenous urea during reverse dialysis. Key: O, rabbit No. 14 with control solution; ●, rabbit No. 18 with tromethamine-II solution; ×, rabbit No. 16 with tromethamine-II solution.

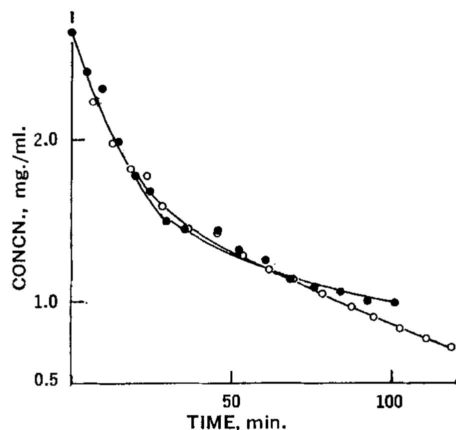


Fig. 4—Dialysate concentrations of exogenous urea during reverse dialysis. Key: O, rabbit No. 15 with control solution; ●, rabbit No. 17 with tromethamine-II solution.

TABLE III—RATE CONSTANTS, k_1 AND k_2, FOR DIALYSIS OF EXOGENOUS UREA

Dialysis Fluid	Rabbit No.	Wt., Kg.	k_1 min.$^{-1}$ × 10^2
Control	*1*	3.61	0.8
	2	3.86	0.9
	4	4.45	1.0
	7	3.96	1.9
	8	4.26	1.1
	12	2.09	1.4
	13	1.90	0.9
Av.		3.45	1.2
Tromethamine-I	*3*	4.26	4.3
	5	3.85	1.7
	6	4.00	2.4
Tromethamine-II	*9*	3.96	2.4
	10	2.32	3.4
	11	2.42	3.6
	19	3.98	2.2
	20	2.84	1.9
	21	3.41	2.3
Av.		3.45	2.7
	Reverse Dialysis		k_2 min.$^{-1}$ × 10^2
Control	*14*	3.73	1.6
	15	3.37	1.9
Tromethamine-II	*16*	2.94	4.1
	17	3.69	2.9
	18	3.96	4.1

DISCUSSION AND CONCLUSIONS

The blood curves for urea showed greater deviations than one might generally expect for a typical drug or injected substance. These variations were the subject of much laboratory study, and a number of variations in assay were attempted in an effort to obtain better uniformity. It did not appear that the variation was due to assay procedure, or that urea was entrapped in protein precipitates since results were nearly identical when several methods were used, including those not requiring protein precipitation on the same set of samples. It is believed that these variations result from traces of radioactive carbon dioxide present in the blood and tissues following metabolism of urea, removal of this metabolite being a slow and nonuniform process.

The fitting of the dialysate curve to the data points is reasonably precise. A change in values of curve parameters of 5% or more will not yield a satisfactory fit. Furthermore, a variation in one constant tends to be corrected for in another; for example, if one uses too large a value for m_1 a correspondingly higher value for m_2 is required, making the difference $m_2 - m_1$ about the same. Thus, the values for A, m_1, and m_2 appear to be within 5%. The greatest error in calculating k_1 results from the estimation of X_0, initial blood level. This value is restricted, however, by the requirement that m_1 and m_2 of the dialysis curve must also apply to the blood curve.

Reverse dialysis was run with the aim of determining whether tromethamine might have a uni-directional effect on dialysis. One possible mechanism for its action might be the formation of a tromethamine–urea complex which is nondiffusible or slowly diffusible, thus increasing the effective blood to dialysate concentration gradient. If this were the mechanism, then in reverse dialysis the presence of tromethamine in the peritoneum would cause a much slower transport from fluid to blood than would be observed in controls. Since the tromethamine actually accelerated transport in 2 of 3 cases, this hypothesis for tromethamine action was rejected. The reason for a less significant effect in one case of reverse dialysis (Fig. 4) is not clear, but since the failure to retard dialysis answered the purpose of the experiment, this was not investigated further.

The values of dialysis rate constants, which are independent of individual variations in blood levels, excretion, and metabolism rates, clearly demonstrate that the diffusion of exogenous urea across the peritoneal membrane is accelerated by the presence of tromethamine. In these experiments the pH of the

tromethamine dialysate was found to change to a variable degree. The original fluid was pH 9.7, and it dropped rapidly after injection to 8.0–8.2, then more slowly as it approached the pH of body fluids. This drop occurs as a result of neutralization in the abdomen and due to absorption of tromethamine (5). One might expect the dialysis rate constant to vary with these changes, but a single constant was found to give acceptable fit to the data throughout the dialysis period.

The rate constants obtained in this work are based only on exogenous urea which may be considered to diffuse from one compartment to another quite independently of all other molecules. The constants themselves should be applicable to any urea present in the animal, but the total amounts of urea in the various compartments in the animals studied cannot be deduced without having measurements of specific activity. Since this work was designed to evaluate the rates for the added urea only, specific activities in the various fluids were not measured. Thus, by following only the exogenous urea the complexities of analysis and errors in measurement of total urea were avoided. If one treats the kinetics of endogenous urea, the biosynthesis of urea must be taken into account, and an equation of form similar to that of Teorell (11) for amount of drug in tissues with 3 exponential terms would be required. Obviously a direct comparison of blood and dialysate concentrations would not reflect the dialysis rates. This greater complexity of the system, coupled with the relatively low urea levels found in normal animals, probably accounts for the failure to detect the effect of tromethamine on urea dialysis by other workers, such as Knochel and Mason (6) where urea diffusion was followed.

The acceleration of urea dialysis by tromethamine cannot be attributed to a pH effect on ionization since urea is essentially nonionized under all conditions of the experiments. The mechanism of the tromethamine effect in this case is unknown, but it is certainly worthy of further investigation, particularly with the aim of finding other more effective substances. Some possible mechanisms are: (*a*) a wetting effect to improve contact between the membrane and dialysis fluid, especially where fatty deposits are present on the membrane, (*b*) a direct chemical effect on the peritoneal membrane to increase membrane permeability, (*c*) improved circulation of the extracellular fluid to the membrane. Some preliminary tests of these possibilities have been made. Measurements of protein content of dialysates showed the tromethamine fluids to have extracted no more protein than the control solutions. Examination of the membranes after dialysis revealed no gross visible changes, except a possibility that the membranes have less fatty film. Knochel *et al.* (1) reported no gross changes in the membrane with tromethamine fluids. Three tests with isolated membrane in the manner used by Shenouda and Mattocks (7) indicated only small increases in permeability with tromethamine. Further studies are in progress to define the nature of the tromethamine effect.

Although this work suggests the possibility of the use of tromethamine in dialysis for uremia, it should be noted that tromethamine is rapidly absorbed from the dialysis fluid (5) and is primarily eliminated *via* the kidneys. In the uremic patient elimination may be extremely slow, resulting in rapid accumulation in the body. Thus, the toxicity in the uremic patient may be much greater than in the normal. Additional studies with nephrectomized animals are needed to define the safe and effective ranges of the procedure before application in the clinic. Such experiments are being undertaken in this laboratory.

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