PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 151, 126-131 (1976)

Influence of Volume Expansion on Sodium Excretion During Parabiotic Dialysis[1] (39158)

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Despite the varied techniques employed and the abundance of available data, the question as to whether or not a natriuretic hormone exists still remains open (1). Cross-circulation has been one of the approaches used in investigating the possibility that a hormone is involved in the natriuresis following infusion of large volumes of saline (2–5). However, while in theory appropriate, these experiments present considerable technical difficulties. In parabiotic dialysis the blood of two animals is circulated past a permeable membrane so that while remaining physically separate the two blood supplies can equilibrate diffusable material. Any intervention that results in an increase in concentration of a hormone in one animal of the pair will bring about a gradient for diffusion to the second animal providing the membrane is permeable to the hormone. It has been suggested that the greater part of the increase in Na excretion observed when small volumes of saline are infused is related to an increase in filtered Na, whereas with large volumes of infused saline Na excretion increases more than filtered Na (6). Although there is no agreement as to the mechanism involved, this increase in Na excretion has been attributed by some to a natriuretic hormone. This area has been reviewed recently by Buckalew and Nelson (1). If the natriuretic response to intravascular volume expansion is the result of the release of a hormone, it would be expected that if one animal of a parabiotic pair is given a large infusion of saline, it should respond by increasing its circulating level of a natriuretic hormone. This would result in the diffusion of the hormone to the second animal of the pair which should also show a natriuresis. Although such a result could be explained by the contribution of an antinatriuretic hormone, parabiotic dialysis can be used to distinguish between a natriuretic and an antinatriuretic hormone. If the intravascular volume of one dog of a parabiotic pair is expanded with an isotonic iso-oncotic solution, the concentration of natriuretic or antinatriuretic substance should decrease. As a result, natriuretic or antinatriuretic substances should diffuse from the second animal into the infused animal. If the former responds with antinatriuresis it is preesumably due to the loss of a natriuretic hormone.

Methods. An artificial kidney was adapted to establish parabiotic dialysis as described previously (7). The unit employed consisted of plates and a frame with a surface area of 1/2 sqm, and a dead space including tubing of 120 ml. It has a low flow resistance so that the animals' own arterial pressure circulates the blood through the dialyzer at blood flows generally between 100 and 200 ml/min. The unit was filled with heparinized saline after the unit had been assembled and sterilized. The membranes and tubing were replaced after each experiment.

The side wall pressures of the arterial lines entering the dialysis were monitored using mercury manometers. A screw clamp on the arterial inlet lines was used to keep the two arterial inlet pressures equal so that hydrostatic flow across the membranes of the dialyzer and, thus, fluid shifts between the animals, was prevented. The parabiotic dialysis system is illustrated in Fig. 1.

Bemberg Cuprophane PT-150 mem-

[1] Supported by Grant HE 11387-02 from the National Institutes of Health, U.S. Public Health Service, and NASA Institutional Subgrant 68-426-L-A.

[2] This study carried out while a recipient of Research Career Development Award 1-K3-HE 36,005 from the National Institutes of Health, U.S. Public Health Service.

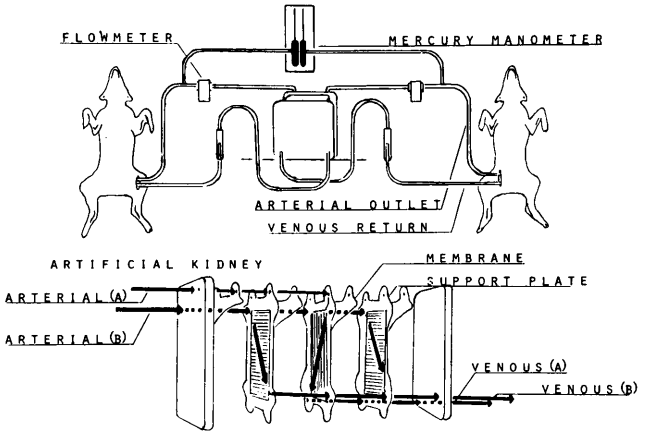

FIG. 1. Parabiotic dialysis system.

branes were used. Isotope studies have shown that molecules such as low molecular weight dextran, insulin, and inulin will readily pass across it. We have observed its permeability to ADH. Employing the method of Lane and Riggle (8), we determined the effective pore size to be 37 Å in diameter. This method does not confirm the existence of such pores but merely indicates that the observed permeability of the membrane is compatible with the existence of pores of 37 Å diameter.

Two series of experiments were carried out with the conditions common to both as follows. Dogs weighing between 13 and 24 kg, anesthetized with pentobarbital sodium (20–40 mg/kg) were employed. At least 1 h before the experiment began, 10 mg of DOCA and 2.5 units of vasopressin tannate in oil were injected im into each of the parabiotic pair. The trachea was intubated, and the arterial and venous connections to the artificial kidney were made by catheterizing a femoral artery and vein. Both animals were given anticoagulant to prevent clotting in the extracorporeal tubing and artificial kidney. Parabiotic dialysis was then begun. A solution containing 10 g creatinine/liter, 5 g PAH/liter, 20 μg ADH/liter and 16 mg fluorohydrocortisone/liter was infused into both animals at a rate of 0.33 cc/min for the duration of the experiment. After beginning dialysis, urine was collected through a catheter inserted into the bladder. The collection periods varied from 20 to 30 min with an air and distilled water flush done at the end of each period. When both animals reached a relatively constant urine flow, blood and urine samples were taken for creatinine and sodium determinations. A stable urine flow was reached generally after about 80 min of dialysis.

Series I. ($n = 13$). Following the control period, one animal of the pair (Animal A) received 1 liter of isotonic saline in 20 min followed by 1 liter of isotonic saline per hr for 2 hr for a total of 3 liters of saline. The second animal of the pair (Animal B) received no infusion. Urine samples were collected at 30-min intervals during infusion and blood samples taken at regular intervals.

Serial II. ($n = 7$). After the control period, one animal of the pair (Animal A) was given 4% of body weight of 6% dextran (av mol wt = 70,000) in isotonic saline in 20 min or less. Blood and urine samples were taken at 30-min intervals during the postinfusion period.

Results. Series I. The data for this series are shown in Table I. Animal A showed a substantial natriuresis and diuresis following infusion. Animal B usually had a period of peak urine flow in the postinfusion period. Subsequent urinary Na analysis showed clearly that Animal B had either a peak period of Na excretion or values which were still increasing when the experiment was terminated. The postinfusion values listed in Table I for Animals A and B are the values determined during the peak period of Na excretion for each animal. In

TABLE I. INFLUENCE OF SALINE INFUSION ON RENAL FUNCTION IN THE RECIPIENT (ANIMAL A) AND ITS PARABIOTIC PARTNER (ANIMAL B).[a]

	V (ml/min)		Ccr (ml/min)		Filtered Na (μEq/min)		UNaV (μEq/min)		PNa (mEq/liter)		Hematocrit (%)	
Expt	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
						Animal A						
1	0.45	3.10	45	86	7,200	11,970	4.5	409.0	160	138		
2	0.30	3.60	52	95	7,860	13,900	5.0	511.0	151	146	44	27
3	0.48	7.00	67	95	10,150	13,950	57.0	1,146.0	149	147	38	28
4	0.84	7.70	14	33	2,720	4,950	66.0	218.0	159	153	44	31
5	0.26	2.76	57	26	8,100	4,000	11.0	455.0	143	154	28	22
6	0.66	2.23	78	27	10,240	3,320	25.0	55.3	132	126	32	22
7	0.57	3.69	17	35	2,013	3,410	5.3	740.0	124	120	23	16
8	0.36	1.50	61	30	8,360	4,050	2.0	120.0	138	136	46	31
9	0.16	4.74	22	26	4,230	3,870	2.0	218.0	142	141	23	15
10	0.43	3.80	69	124	9,810	18,800	8.0	422.0	143	152	32	21
11	0.32	2.65	66	28	8,200	4,260	46.0	2,048.0	127	155	46	30
12	0.27	2.92	38	10	5,220	1,230	11.0	261.0	133	118	41	29
13	0.28	4.23	66	59	8,560	7,440	10.0	600.0	131	126	40	28
Mean	0.41	3.84	50	52	7,128	7,319	19.5	554.1	140	138	36	25
± SE	0.05	0.49	6	10	758	1,512	6.2	147.8	3	3	2	2
						Animal B						
1	0.45	0.93	79	82	11,800	10,660	14.4	152.0	150	130		
2	0.33	0.93	50	57	7,050	8,480	39.6	188.0	141	148	46	45
3	0.31	0.26	43	52	6,260	7,750	9.0	11.0	148	151	30	30
4	0.22	0.20	28	29	4,270	4,370	8.7	15.6	152	151	41	40
5	0.54	0.71	34	28	4,960	4,300	23.2	166.0	133	151	26	24
6	0.18	0.34	29	18	3,620	1,950	9.0	20.3	125	110	41	34
7	0.22	0.23	43	12	6,500	1,800	5.5	36.1	152	151	23	22
8	0.20	0.44	74	84	10,500	11,900	5.0	60.4	142	142	43	42
9	0.36	0.59	19	11	2,420	1,458	1.8	25.0	130	131	20	21
10	0.23	0.31	70	111	9,750	15,250	10.3	18.7	140	137	37	34
11	0.29	0.50	40	40	5,410	5,280	4.9	48.2	134	131	46	30
12	0.39	0.84	70	19	8,950	2,520	13.4	44.7	128	132	49	48
13	0.38	0.22	44	54	6,610	7,170	6.4	7.3	150	132	28	35
Mean	0.32	0.50	48	46	6,777	6,376	11.6	61.0	140	138	36	34
± SE	0.03	0.08	5	9	773	1,196	2.8	17.6	3	3	3	3

[a] V = urine flow rate; Ccr = creatinine clearance; UNaV = rate of excretion of Na; PNa = plasma sodium.

this and in Series II, peak sodium excretion correlated well with cumulative sodium excretion.

The nonnormal distribution of the data indicated that the Wilcoxon signed rank test for paired data was the appropriate method for determining the significance of the differences between pre- and postinfusion values. There was a significant increase in sodium excretion for both Animal A ($P < 0.01$) and Animal B ($P < 0.01$) without a significant change in filtered Na or creatinine clearance. Urine flow increased significantly in both Animals A ($P < 0.01$) and Animals B ($P < 0.015$). Although hematocrit decreased significantly following infusion in Animal A ($P < 0.01$), there was no significant change ($P > 0.1$) in Animal B.

The maximum natriuresis in Animal B always occurred after the peak natriuresis in Animal A. Although the peak natriuresis for Animal B occurred an average of 80 min after peak natriuresis in Animal A, there was a variation of ±40 min. A comparison of the time course for excreted Na for Animals A and B in Series I is shown for one experiment in Fig. 2.

Series II. The changes in urine flow and Na excretion were not as pronounced as in Series I, since volume expansion was done with a smaller volume. Therefore, cumulative urine flow and Na excretion were plotted against time and the pre- and postinfu-

sion values determined from the slope of the resulting line. An additional advantage of this analysis is that a deviant value is readily observable. This may be of importance at low urine flow rates where sampling by means of a catheter in the bladder might be most subject to error. The data for the individual experiments from this series are shown in Table II.

The Wilcoxon signed rank test for paired data was used to determine the significance of change. There was a significant increase in Na excretion ($P < 0.02$), urine flow ($P < 0.01$), creatinine clearance ($P < 0.02$), and

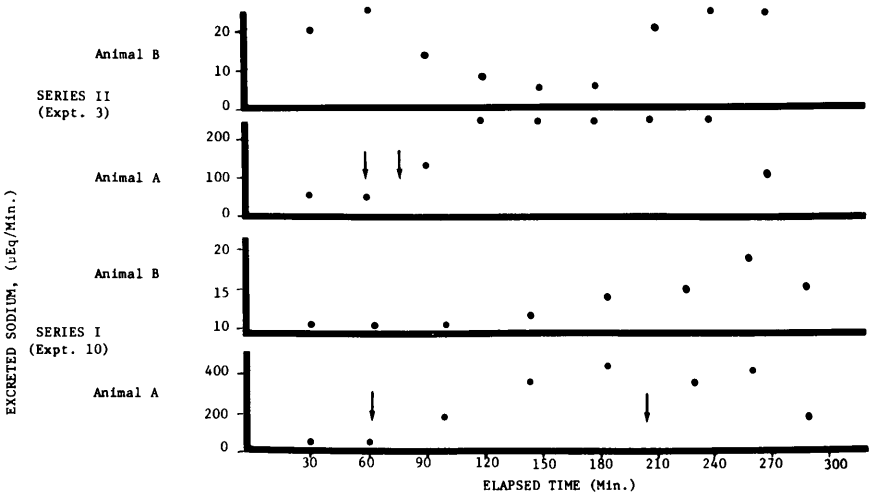

FIG. 2. Representative time course for excreted sodium in Series I and Series II experiments (→, duration of intervention).

TABLE II. INFLUENCE OF DEXTRAN INFUSION ON RENAL FUNCTION IN THE RECIPIENT (ANIMAL A) AND ITS PARABIOTIC PARTNER (ANIMAL B).[a]

	V (ml/min)		Ccr (ml/min)		Filtered Na (μEq/min)		UNaV (μEq/min)		PNa (mEq/liter)	
Expt	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
					Animal A					
1	0.10	2.57	19.0	78.0	2,870	11,870	5.0	103.0	151	151
2	0.10	0.57	15.0	37.0	2,260	5,350	1.8	1.8	150	144
3	0.66	3.00	59.0	40.7	9,030	6,500	58.3	237.0	153	159
4	0.15	0.25	30.0	59.4	4,820	9,500	2.3	18.7	160	159
5	0.10	2.50	9.7	51.0	1,318	7,540	1.2	4.2	136	147
6	0.28	1.32	56.7	83.3	8,950	12,800	3.3	73.5	158	153
7	0.10	1.17	41.0	83.2	6,540	13,050	1.5	39.0	159	162
Mean	0.21	1.63	32.9	61.8	5,113	9,516	10.5	68.2	152	154
± SE	0.08	0.40	7.5	7.5	1,191	1,185	8.0	31.4	3	3
					Animal B					
1	0.93	0.83	99.0	46.0	11,600	7,600	53.7	25.8	146	165
2	0.13	0.18	30.0	39.5	4,280	5,650	4.0	1.3	142	143
3	0.31	0.31	81.5	55.0	12,700	8,600	41.3	6.6	156	156
4	0.22	0.25	20.0	32.3	3,140	5,100	2.2	1.0	157	158
5	0.37	0.37	81.6	88.8	13,200	14,200	10.0	1.5	161	160
6	0.24	0.32	63.6	73.5	10,000	11,740	3.6	2.4	157	160
7	0.21	0.21	54.0	34.3	8,680	5,250	0.45	0.18	160	153
Mean	0.34	0.35	61.4	52.8	9,086	8,306	16.46	5.54	154	156
± SE	0.10	0.08	10.9	8.0	1,508	1,323	8.19	3.46	3	3

[a] V = urine flow rate; Ccr = creatinine clearance; UNAV = rate of excretion of Na; PNa = plasma sodium.

filtered Na ($P < 0.02$) in Animal A (Table 2). In Animal B there was a significant decrease in excreted Na ($P < 0.02$) without a significant change in filtered Na ($P > 0.1$), urine flow ($P > 0.1$), or creatinine clearance ($P > 0.1$). The reduction in sodium excretion occurred as early as 10 min and as late as 35 min and occurred on the average in 20 min following infusion of Dog A. The time course of the changes in sodium excretion from one experiment is shown in Fig. 2.

Discussion. The results of Series I clearly show that a significant natriuresis occurs in one animal of a parabiotic pair when its partner receives a saline load. In both animals, natriuresis occurs without consistent or significant changes in creatinine clearance, filtered or plasma sodium. However, in Series I there were some large variable changes in GFR following saline loading. The reasons for this are not clear. It may be argued that because of the manner in which urine was collected, i.e., directly from the bladder, complete collection of the bladder urine was not made. However, the data showed no consistent relationship between the level of urine flow and the variability of the GFR changes. Also, in other experiments not employing parabiotic dialysis, we have collected urine in a similar manner and did not find the same variable changes in GFR following volume expansion. Thus, tentatively we would suppose that the apparent variability in the response of creatinine clearance in the present study was real. It should also be noted that the greatest variability occurred in Group A animals of Series I, that is, the animals that had received large infusions of sodium chloride. The validity of the position we are proposing does not rest upon the consistency of the response of GFR in the infused animal, but rather upon reasonable demonstration than in the noninfused animal of the pair (Animal B) there were no consistent increases in GFR. In Group B of Series I, Animals 7 and 12 showed large increases, and Animal 10 a large decrease in creatinine clearance following infusion of its parabiotic pair. However, even if these experiments are eliminated from the series, the data still show no significant change in creatinine clearance following infusion of its parabiotic pair.

Any effects on sodium excretion due to changes in ADH or aldosterone levels were precluded by infusing large amounts of these hormones into each of the parabiotic pair. There is no reason to suspect that a shift of fluid occurred between the parabiotic pair due to hydrostatic flow, since the inflow pressures to the artificial kidney and, thus, transmembrane hydrostatic pressure, were essentially equal. However, infusion of saline into the one animal would presumably decrease plasma protein concentration. As a result, a small diffusion gradient would exist for movement of water from the infused animal to the noninfused animal, i.e., from Animal A to Animal B. Such a water movement might be expected to be reflected by significant changes in hematocrit. Although there were variable changes in hematocrit, we found no statistically significant decrease in Animal B. It could be argued that monitoring the weight of the animals would have provided information concerning net fluid movement. Although we realize the advantages of doing so, the experimental design precluded measurements of body water distribution. Even if changes were seen in the weight of the noninfused animal, we would have no indication as to where this fluid was distributed. It could have been distributed throughout the total body water, intravascular space, interstitial space, gastrointestinal tract, etc. In any event this problem would be less of a factor in Series II since the solution infused was iso-osmotic, so that the transfer of water between the animals of the pair due to a different osmotic pressure would not be anticipated. Although the primary purpose of the study was to demonstrate a new technique, namely parabiotic dialysis, it nevertheless provides data from Series I which are best interpreted in terms of a hormonal agent. The time delay between Animals A and B in terms of the increase in sodium excretion may reflect the time required for an effective level of a "natriuretic" hormone to be established in the noninfused member of the parabiotic pair. If one does assume the mechanism to be humoral, then the question arises as to whether the increase in sodium output of the noninfused animal in Series I was brought about by movement of a natriuretic

hormone to Animal B or movement of an antinatriuretic hormone from Animal B. To distinguish between the two possibilities the experiments described in Series II were carried out.

A humoral agent as part of a physiologic control system usually exerts its effect by changes in concentration around some normal value. Thus, the rapid infusion of some appropriate solution should result in an acute decrease in the concentration of any circulating substance. It has also been suggested that for the natriuretic hormone, a large volume of solution must be infused before any action of this hormone is seen. Hence, in Series II, a relatively small infusion (4% of body weight) was given so that the level of any humoral agent would be altered simply by dilution. Six percent dextran in saline was used since most of it remains intravascular for at least several hours. This dilution would create a concentration gradient for the movement of natriuretic or antinatriuretic hormones from B to A. The data for Series II show that Animal B had a significant decrease in excreted Na during the postinfusion period without a change in filtered Na or creatinine clearance. Presumably, dextran infusion acutely reduced the level of circulating natriuretic hormone in Animal A which should lead to antinatriuresis. However, the increase in creatinine clearance and filtered Na in Animal A would mask the above effect on sodium excretion.

Although dilution of a natriuretic hormone presumably occurred in the infused member in Series I (saline infusion) which in itself would, on the basis of the results of Series II, be expected to cause an antidiuresis in the noninfused animal, this effect was presumably offset by the production of a natriuretic factor secondary to the large expansion of extracellular fluid volume.

Although parabiotic dialysis and cross-circulation (2, 4) differ, the results are consistent in that both imply the action of a natriuretic hormone. Indeed, with both techniques the recipient animals show comparable natriuresis when the donor animals are given similar infusions.

Summary. Dialysis of a saline-infused against a noninfused dog was associated with a significant increase in Na excretion in both animals without a significant change in creatinine clearance or filtered Na. This indicates that there was movement by diffusion of a humoral agent between the two animals. The observed increase in Na excretion in the noninfused animal resulted from gain of a natriuretic hormone, since in a second series of experiments a natriuretic rather than antinatriuretic hormone appeared to be involved.

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Received September 2, 1975. P.S.E.B.M. 1975, Vol. 151.