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Comparative Effects of Saline and Isoncotic Albumin in Saline on Sodium Excretion.* (32141)

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(Introduced by T. S. Danowski)

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The infusion of isoncotic albumin in saline has been reported to produce the same increase in sodium excretion as saline alone (1,2,3). Although infusion rates were kept constant in relation to animal size by Mills *et al*(1), comparisons were based on different groups of animals. Levinsky and Lalone(2) based their observations not only on different infusion rates relative to the size of the animal, but apparently upon different groups of animals. Isoncotic albumin in saline produced a greater expansion of the vascular volume than saline(1,3), and since Mills and his coworkers(1) could not correlate the increase in blood volume or interstitial volume with the rise in sodium excretion, it seems odd that two solutions with different patterns of extracellular volume expansion should produce the same effect on sodium excretion. The present experiments were undertaken to determine whether isoncotic albumin in saline actually produced the same effect as saline alone. It was reasoned that if the effects were the same, then paired experiments in the same animal performed under comparable conditions should confirm the observations mentioned above(1, 2). The data indicate that under the experi-

mental conditions employed saline infusion produces a greater increase in sodium excretion than does the infusion of isoncotic albumin in saline.

Methods. Twenty-one experiments were performed in 12 female dogs ranging in weight from 7 to 27 kg (mean 14.7 kg). Experiments in 6 dogs were paired and performed at intervals of at least one month with identical infusions of 0.9% NaCl and 6% bovine albumin in 0.9% NaCl infused at a rate of 0.7 or 1.0 ml/kg/min. In half these experiments (dogs 26, 23, 29) saline was infused first and in the remainder (dogs 31, 27, 25) the order of the infusions was reversed. In dog 33 with a similar interval between experiments the saline was infused at 0.6 ml/kg/min, and isoncotic albumin in saline was infused at a rate of 1.0 ml/kg/min. Two single experiments were performed (dogs 28 and 23). Four additional experiments were performed infusing isoncotic albumin in saline at a constant rate until urine flow stabilized for at least 4 periods. The infusion was then suddenly changed to saline and continued at the same rate. In a fifth, the order of infusion was reversed.

The dogs were hydropenic for 24 or 48 hours, and all animals were fasted for 24 hours. Sixteen hours before starting the ex-

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periment the animals received intramuscularly, 5 units of vasopressin in oil and 15 mg of DOCA in oil. Three hours before the start of control collections all animals received, intramuscularly, another 15 mg of DOCA in oil. The animals were anesthetized with pentobarbital, 25 mg/kg, intravenously, and additional pentobarbital was administered periodically to maintain light anesthesia. An endotracheal tube was kept in place throughout each experiment. After a priming injection of inulin, 300 mg, sodium p-aminohippurate, 400 mg, and aqueous vasopressin, 500 milliunits, these drugs were infused at a constant rate in relation to animal weight delivering inulin at 13 to 16 mg/min and sodium PAH at 5.0 to 6.3 mg/min, and vasopressin at 50 to 80 milliunits per hour. Plasma concentrations of inulin and PAH ranged from 17 to 45 and 0.8 to 3.5 mg percent, respectively.

Following at least 2 stable control periods of 30 minutes each the experimental solutions were infused at a constant rate until urine flow stabilized for 4 to 6 10-minute periods before the experiment was terminated or the infusion was changed abruptly in the 5 experiments noted earlier. The duration of infusion varied up to 3 hours, and the total volume infused ranged from 700 to 2300 ml. Urine was collected in a volumetric cylinder through an indwelling catheter and each experimental period was terminated by suprapubic compression. Seven milliliters of blood were drawn during each experimental period. The total volume of blood removed in each experiment varied from 63 to 154 ml. Inulin was measured by the method of Walser *et al*(4), PAH by the method of Smith *et al*(5), total protein by the biuret reaction(6), solute concentrations in the Fiske osmometer and sodium in a Beckman flame photometer. Clearances were calculated in the conventional manner, and the changes in plasma volume were estimated from the changes in hemoglobin and hematocrit(3). Femoral venous pressure was measured manometrically using saline through an indwelling Cournand needle maintained at a constant level with respect to the estimated position of the right atrium.

Results. The comparative effects given in Table I represent the responses obtained in separate experiments on the same animal under similar conditions of dehydration and infusion rate based upon the animal's weight. The minimum infusion volume of 700 ml was chosen arbitrarily as a reference point for these experiments, and because of differences in overall expansion due to variations in animal size the values given do not necessarily represent maximum responses.

Control glomerular filtration rates for paired experiments (Table I) ranged from 34 to 79 ml/min (mean, 51.6 ml/min) in saline experiments and 35-81 ml/min (mean, 51.4 ml/min) in isoncotic albumin in saline experiments. GFR increased in 5 of 8 experiments with saline and in 3 of 7 isoncotic albumin in saline experiments (Table I). The mean increases were 9.3 and 2.3%, respectively, and were not significantly different from zero. Control PAH clearances ranged from 85 to 218 ml/min (mean, 140 ml/min) in saline studies and 82 to 189 ml/min (mean, 139 ml/min) in isoncotic albumin in saline studies. PAH clearance increased in 5 of 7 saline and 4 of 6 isoncotic albumin in saline experiments (Table I). The mean increases for each group were 19.7 and 36.0%, respectively, but comparison of the changes showed no significant difference ($P > .4$).

Isoncotic albumin in saline produced a significantly greater increase in femoral venous pressure ($P < .02$) (Table I).

In all 6 paired experiments urine flow and sodium excretion increased more when the animals were infused with 0.9% saline. Calculation of sodium excretion in these animals based upon the infusion of 30, 50 and 70 ml/kg of both experimental solutions showed that the mean differences (saline minus albumin in saline) were 560, 830, and 720 μ Eq/min, respectively. Of interest is the experiment in dog 33 who exhibited approximately the same changes with a slower saline infusion as she did when receiving the more rapid albumin in saline infusion (Table I). At the infusion of 70 ml/kg, or a total of about 1600 ml, of each experimental solution, however, the increase in sodium excretion was 1160 μ Eq/min for saline and 680

TABLE I. Experimental Data on Effects of Saline and Isoncotic Albumin Saline Infusion. All data pairs represent separate experiments in each animal. Pre-infusion control and experimental values are given, the experimental values being taken at a time when the smallest volume 700 ml, was infused in any single experiment.

Dog #	Wt Kg	Exp. Sol.	Plasma Volume* %	Tot. Prot. G %	V ml/min.	UNaV μ Eq/min.	Filt. Na μ Eq/min.	Cinulin ml/min.	CPAH ml/min.	Fem. Ven. Pressure Cm. Sal.
26	16.8	C		8.3	.68	197	10085	69	203	
		S	10	7.1	14.2	2420	12710	82	229	
	16.4	C		7.1	.44	116				
23	15.9	S+A	75	6.8	3.3	766				
		C		6.2	.75	200	7450	51	147	3.5
	16.8	S	21	5.0	12.2	2140	9680	69	174	4.1
29	12.3	C		6.4	.76	199	5735	81	189	2.0
		S+A	60	6.9	7.0	371	5090	77	272	9.4
	13.2	C	13	5.4	.54	74	7780	48	124	
31	7.0	S		6.0	10.0	1620	7420	47	101	
		C		6.4	.14	21	6900	42	82	6.0
	7.3	S+A	20	5.5	.45	71	8050	49	149	8.8
27	9.1	C		7.4	.24	54	6020	39	85	
		S	76	5.0	3.8	678	6740	44	140	
	10.0	C		6.6	.45	37	7320	43	109	
25	9.1	S+A	140	7.3	1.0	205	6350	38	98	
		C		6.7	.22	47	6200	41		
	10.0	S	56	5.0	5.1	1016	6300	42		5.1
33	9.1	S+A	100	6.1	3.4	325	4500	28		10.6
		C		6.3	.22	32	5450	34	93	7.0
	10.0	S	91	4.4	4.1	624	4530	29	74	9.1
28	14.1	C		5.0	.42	36	5550	37	101	6.2
		S+A	190	5.2	4.0	438	6850	44	100	11.5
	23	C		8.1	.20	13	12635	79	218	2.0
23	15.0	S	48	5.3	.55	173	16300	102	254	2.0
		C		5.9	.26	24	9380	62	181	4.5
	22.7	S+A	56	5.9	.60	112	10000	66	296	7.0
28	14.1	C		6.3	.30	88	9430	63	172	3.1
		S+A	152	7.0	2.3	565	8950	63	198	11.5
	15.0	C		7.0	.46	108	8525	52	112	2.0
		S		6.4	9.9	1980	7260	44	157	3.0

* The volumes of blood removed for sampling were the same for each pair of experiments in an animal and were not accounted for in calculating the change in plasma volume. Control values are taken as 100%, and the data given are increases from control.

μ Eq/min for albumin in saline infusion.

In Fig. 1 it is apparent that during the early course of infusion sodium excretion rose to a greater extent with a smaller increase in venous pressure during saline infusion, and rose to a lesser extent with a larger increase in venous pressure during isoncotic albumin in saline infusion. Since oncotic pressure is not reduced by the albumin infusion it was considered possible that the diminished rise with this solution may be related at least in part to the maintained oncotic pressure. The effects of changing the infusion from isoncotic albumin in saline abruptly are given in Table II. No consistent change in sodium excretion can be detected, although plasma concentration of protein declined following the abrupt change of the

infusing solution. Analysis of the failure of the rate of sodium excretion to increase is complicated by a further rise in venous pressure.

Upon analyzing the changes produced by the infusion of isoncotic albumin in saline it appears that following the initial increase in sodium excretion (Table I) a short period of stabilization follows. As the infusion is continued, the response to total volume infused varying among animals, there occurs a decline in the rate of sodium excretion, while venous pressure continues to rise and the plasma concentration of protein remains relatively stable (Table III). Plotting the regression of the percent change in sodium excretion on the rise in venous pressure indicates an inverse correlation ($r = .548$,

TABLE II. Effects of an Abrupt Change in the Type of Infusion on Sodium Excretion, Plasma Proteins, Venous Pressure and Glomerular Filtration Rate. Each value represents the mean of 3 periods taken when rate of sodium excretion was stable.

Dog #	Wt kg	Exp. Solution	UNaV μ Eq/min.	Plasma proteins g %	Venous pressure cm saline	Cinulin ml/min.
35	14.3	Cont.	123	5.5	2.0	19
		Alb.+Sal.	978	6.3	6.3	46
		Sal.	804	5.8	8.0	60
38	19.5	Cont.	183	6.3	10.0	27
		Alb.+Sal.	667	6.3	12.1	83
		Sal.	678	5.7	15.5	71
32	26.8	Cont.	189	6.7	2.0	40
		Alb.+Sal.	779	6.9	6.0	34
		Sal.	957	5.8	6.3	31
11	22.7	Cont.	74	5.5	6.0	40
		Alb.+Sal.	1328	6.3	15.7	55
		Sal.	1585	5.5	20.0	61
31	7.3	Cont.	80	6.7	4.0	28
		Sal.	544	4.2	8.0	24
		Alb.+Sal.	233	5.5	24.7	24

$P < .05$). Comparison of saline and isoncotic albumin in saline experiments in dogs 23, 25 and 33 (Table III) shows that for similar volumes of infusion the percent fall from maximum in the rate of sodium excretion was greater when isoncotic albumin in saline was infused (64 vs 10, 29 vs 2 and 6 vs 0%, respectively). Saline infusion produced virtually no further increase in venous pressure (Table III).

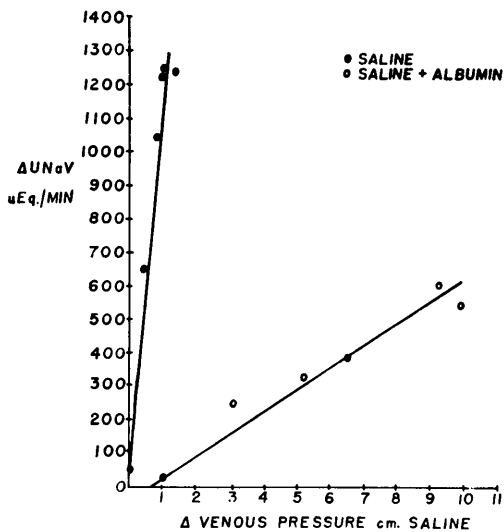


FIG. 1. Relation of the change in rate of sodium excretion (Δ UNaV) to the change in venous pressure. Values for saline experiments represent means for 4 experiments and for isoncotic albumin in saline the means for 6 experiments, and include consecutive 20 minute periods from start of infusion to stabilization of sodium excretion.

Discussion. The data indicate clearly in paired experiments that saline infusion produces a greater increase in sodium excretion than is the case when the same infusion is given with isoncotic albumin incorporated. Support for this finding is found in the observations of Earley, Martino and Friedler (7) that saline reduces proximal tubular reabsorption of sodium whereas isoncotic albumin in saline does not decrease proximal sodium reabsorption. The failure to demonstrate this phenomenon by Mills *et al* (1) and Levinsky and Lalone (2) may well have been related to their experimental designs, in that different groups were compared, the infusion rates were not controlled relative to animal size or a combination thereof.

The differences in sodium excretion observed in the present experiments cannot be explained by alterations in the glomerular filtration rate or effective renal plasma flow. Since the increase in sodium excretion following saline loading has been attributed in part to an increase in renal medullary blood flow (8), it remains possible that the solutions employed had different effects on medullary blood flow, a parameter not measured in the present experiments. If this were the case, then the observed differences would probably be quantitative rather than qualitative, since both solutions increased sodium excretion.

Other explanations for the differences in sodium excretion appear possible from the

TABLE III. Relations of Venous Pressure, Plasma Proteins and the Increase in Sodium Excretion When Sodium Excretion Achieves a Maximum and at the End of Continuous Infusion of Saline and Isoncotic Albumin in Saline.

Dog	Vol. infused ml/kg		Venous pressure cm saline		Plasma proteins g %		Δ UNaV μ Eq/min.	
	Max.	End inf.	Max.	End inf.	Max.	End inf.	Max.	End inf.
Saline								
23	42	109	4.1	4.1	5.0	5.1	1940	1740
25	45	98	8.6	9.0	4.9	4.1	654	641
33	76	122	2.0	2.0	5.2	5.2	1414	1414
23	29	52	2.0	3.0	6.4	6.4	1930	1930
Isoncotic albumin in saline								
28	76	150	15.2	26.8	5.9	6.7	780	212
27	29	135	10.6	11.0	6.3	6.4	247	227
25	42	86	9.5	15.5	4.8	5.1	547	387
23	21	103	6.0	18.0	6.6	7.5	367	133
33	62	101	11.4	18.5	6.2	6.2	681	639
29	68	79	17.6	18.5	5.3	5.3	199	117
26	73	127			7.2	7.7	1803	1320
38	41	68	9.5	13.0	6.3	6.3	597	447
35	55	71	6.0	6.5	6.1	6.5	850	850
32	38	55	6.0	6.0	7.3	7.0	790	499
11	37	75	10.0	18.0	6.1	5.1	1516	1198
31	96	113	14.5	25.8	5.2	5.5	594	71
Mean difference			5.5 \pm 4.8		.18 \pm .507		237 \pm 192	
P			<.01		>.2		<.01	

observed data. Since albumin in saline produced no significant fall in the plasma protein concentration (Table I) it must be considered that the maintenance of plasma oncotic pressure may have been responsible for the diminished response to this solution. Bresler(9) has suggested that oncotic forces in the peritubular capillaries could result in appreciable reabsorption by the tubules, and infusion of hyperoncotic albumin solutions reduces the rate of sodium excretion (10,11) during saline infusions. In addition it is recognized that an increase in venous pressure impairs the rate of sodium excretion(12,13). The rise in venous pressure was a significant finding during isoncotic albumin in saline infusion in our experiments. Thus, it must be considered that maintaining oncotic pressure, increasing venous pressure or both could have played a role in causing the diminished response to albumin in saline infusion.

During the early phase of infusion it is difficult to separate the maintenance of oncotic pressure from the rise in venous pressure as retarders of the increase in sodium excretion. Indeed it must be considered possible that the diminished response is a function of both maintained oncotic pressure

and rising venous pressure. On the other hand as the infusion of isoncotic albumin in saline is continued it becomes apparent that the rising venous pressure outweighs any effect that oncotic pressure might have, and the subsequent fall in sodium excretion appears then to correlate with the increasing venous pressure.

Summary. Paired experiments were performed to determine whether saline infusion produced an effect different from the effect of isoncotic albumin in saline. The results obtained indicate that for equal volumes of infusion delivered at identical rates, saline infusion provides a greater stimulus to the excretion of sodium than isoncotic albumin in saline. During the earlier phase of infusion the diminished response to isoncotic albumin in saline may be related to both the maintained oncotic pressure and a rising venous pressure. As the infusion of isoncotic albumin in saline is continued, after a period of stabilization, the rate of sodium excretion declines and then appears to correlate with a continuing increase in venous pressure.

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Influence of Cell Density on Growth Inhibition of Human Fibroblasts *in vitro*.* (32142)

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The division cycle of phase II human fibroblasts during logarithmic and postlogarithmic growth *in vitro* was studied previously(1,2). It was shown that the percentage of cells synthesizing deoxyribonucleic acid (DNA) between subcultivation and confluency was maximal during the second day after subcultivation. This fraction declined later, eventually reaching zero when the population became crowded(3,2). An increasing proportion of cells was delayed in the G2 period of the cell cycle(2) as the cultures became confluent. The previous work was done always with the same initial inoculum under conditions where high mitotic indices were obtained together with a relatively long logarithmic growth.

The present work extends the previous findings by studying the influence of cell density on the division cycle and analysing in more detail the periods of the cell cycle affected by cell growth inhibition.

Materials and methods. Cell culture. A cell strain (HEB) derived from human embryonic fibroblasts(2) was used, maintained in Eagle's minimal essential medium(4) supplemented with 10% calf serum, streptomycin (50 μ g/ml), penicillin (100 U/ml) and

aureomycin (50 μ g/ml). Methods used for subcultivation are the same as described by Hayflick and Moorhead(5). Cell counts were done electronically in a Celloscope (AB Lars Ljungberg & Co., Sweden) as described by Santen(6). The terminology used to designate how the cells were subcultivated is the following: a 2:1 split means that cells contained in 2 dishes were plated into one new dish, a 1:1 split means that the cells from 1 dish were plated into 1 new dish, a 1:2 split means that cells from 1 dish were plated into 2 new dishes, a 1:4 split means that cells from 1 dish were plated into 4 new dishes.

Autoradiography. Tritium-labeled thymidine (H^3 -TdR) with a specific activity of 1.9 C/mM was used at a concentration of 0.01 μ C/ml, previously shown to be non-toxic(2). For the experiments cells were pooled and subcultivated into new 60 mm plastic Petri dishes containing coverslips. The techniques used in the autoradiographic procedure have been described(1). To determine the amount of labeled interphases, 1000 cells were analysed. Mitotic indices and the percentage of labeled mitosis were obtained from the analysis of 3000 cells. Mitotic indices are expressed as percentage mitoses of the total number of cells.

Results. Influence of inoculum on amount

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