that the energy yield from glucose to lactate, not affected by arsenite, may have some bearing on this difference.

The table shows that oxamate fails to alter the FA of uterine horns isolated from estrogen-dominated rats and maintained in a lactate-containing medium. This situation is in keeping with findings of previous experiments[5].

Although we do not have direct evidences that, under estrogen dominance and blockade of LDH system, lactate may be metabolized by the rat uterus via a pathway by passing pyruvate formation[5]; there exist observations indicating that, in several tissues exposed to low oxygen tension, lactate is directly converted into fumarate[10].

It has been shown in the rat uterus suspended in a medium with lactate that the presence of oxamate plus arsenite can block the formation of pyruvate from lactate as well as the entrance of pyruvate into the Krebs cycle[5,7].

However, the FA of estrogen dominated uterine horns isolated in lactate media and exposed to the combination of both agents, is significantly less reduced ($p < 0.02$) than when arsenite was the sole enzyme inhibitor, i.e. the presence of oxamate ameliorates the impairment of uterine motility following arsenite. This puzzling finding is also compatible with the possibility for the estrogenized rat uterus to obtain, at least under certain conditions, some contractile energy from lactate metabolized by alternative metabolic reactions, other than the formation of pyruvate, as has been documented to be the case in vascular smooth muscle preparations[11].

1 This work has been supported by grants 6638 and 6294 from the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.
2 Senior Investigator of the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.
3 Junior Investigator of the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.
4 A. Goldraij, A.L. Gimeno and M.F. Gimeno, Acta physiol. latinoam. *23*, 113 (1973).
5 A.L. Gimeno, A. Goldraij, M.F. Gimeno and R. Santillán de Torres, Reproducción *3*, 5 (1976).
6 A. Goldraij, A.L. Gimeno and M.F. Gimeno, Acta physiol. latinoam. *25*, 229 (1975).
7 J.L. Webb, in: Enzyme and Metabolic Inhibitors, vol. III. Academic Press, New York 1966.
8 A. Goldraij, A.L. Gimeno, J.F. Vastik and M.F. Gimeno, Reproducción *1*, 31 (1975).
9 E.A. Newsholme, P.J. Randle and K.L. Manchester, Nature. Lond. *193*, 270 (1962).
10 E. Mensen De Silva and A. Cazorla, Am. J. Physiol. *224*, 669 (1973).
11 B.M. Altura and B.T. Altura, Am. J. Physiol. *219*, 1698 (1970).

Presence of sodium transport inhibiting factor in dog plasma during volume expansion[1]

R.H. Sterns, I. Singer and P.U. Feig

Department of Medicine, University of Pennsylvania, School of Medicine and Veterans Administration Hospital, Philadelphia (Pennsylvania 19104, USA), 10 July 1978

Summary. Plasma dialysates from volume-expanded dogs (E) were compared directly to dialysates from the same dogs when hydropenic. In a double-blind study, E caused relative inhibition of short-circuit current in toad urinary bladder. We therefore confirm the presence of a sodium transport inhibiting factor in plasma of volume-expanded dogs.

The search for a natriuretic hormone associated with volume expansion has been recently reviewed[2,3]. Due to the multiple variables involved in natriuresis in vivo[4], an effort has been made to develop in vitro bioassays for the hormone. After earlier experiments showing inhibition of sodium transport when frog skin was incorporated into the circulation of volume-expanded dogs[5], isolation of an antinatriferic factor was attempted using the anuran membrane for assay[6–8]. Buckalew et al. showed that dialysates of plasma from volume-expanded dogs inhibit sodium transport, as measured by short-circuit current (SCC)[9] in toad urinary bladder (TUB) and frog skin[6,10], but these studies could be criticized on several grounds: a) volume-expanded and control dialysate were from *different animals;* b) control animals were treated with furosemide, which was *assumed* to have negligible effects on the assay; c) assays were not performed on paired quarter-bladders, a model which offers better control against biological variability in the assay system; d) while the antinatriferic effect may be directly related to membrane resistance[11], low resistance membranes were not necessarily discarded; and e) assays were not 'double-blind'.

Other authors[2,12,13] were unable to confirm Buckalew's results, possibly because of one or more of the above factors. Moreover, preliminary data by Buckalew suggested that antinatriferic effect could be inhibited by diffusion of potassium from the electrical bridges into the bathing media[14]. The present study attempts to eliminate the problems listed above. Under 'double-blind' conditions, plasma dialysates obtained from volume-expanded dogs (E) were compared directly to plasma dialysates obtained from the same dogs when hydropenic (H). The comparisons were rigorously controlled in paired quarter-bladders, rejecting membranes of low resistance in advance, and specifically examining the effects of changes in medium potassium concentration.

Methods. 4 female mongrel dogs were studied twice each in alternating sequence with 2 weeks interval between studies. For one study, each dog, previously on ad libitum food and water, was anesthetized with pentobarbital sodium (30 mg/kg) and given an i.v. infusion of 1200 to 1500 ml of 0.9% saline solution over 90 to 120 min; at the end of the infusion, 75–100 ml of blood were obtained from the jugular vein. In the other study, each dog was deprived of sodium and water for 24 h, anesthesia was induced in the same manner and after 90 to 120 min without infusion (as a time control) blood was obtained, as above. Plasma ultrafiltrates were prepared with Diaflo PM-10 membranes (American Corp. Lexington, Mass.) with a mol. wt cutoff of 10,000[15].

For the bioassay, urinary bladders were removed from doubly-pithed female toads from the Dominican Republic (National Reagents, Inc., Bridgeport, Conn.). Half-bladders were placed in double lucite chambers with a 2.7 cm^2 cross-sectional area for each channel. Bladders with a SCC less than 20 μA, with a resistance less than 1000 Ω/cm^2 or less than 20% match between 2 contiguous quarter-bladders were discarded prior to the bioassay. (Mean resistance of bladders used was 2000 Ω/cm^2.) The bathing media contained (in mM): Na^+ 115, K^+ 3.5, Cl^- 95, HCO_3^- 25, Ca^{++} 1.0, $PO_4^{=}$ 0.5. For the studies on the effect of increasing K^+-concentrations, K^+ was 7.0 mM and Cl^-

98.5 mM. Agar electrical bridges contained only NaCl, in the same concentration as the bathing media. Constant stirring was maintained with an air bubble lift containing 3% CO_2 and 97% O_2. The pH of the solutions was 7.7. Plasma dialysates were diluted with distilled water to isosmolality with the bathing media (220±5 mOsm/l). Dialysates from each dog yielded 5–6 bioassays. SCC was continuously monitored by automatic voltage clamp and recorded on double-channel recorder (Kipp & Zonen Model BD-9).

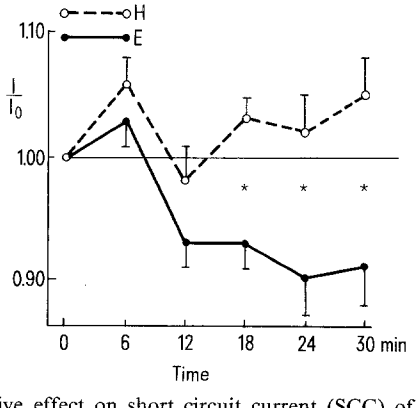
After a stabilization period of 1 h, the serosal media of 2 contiguous quarter-bladders were simultaneously replaced: one by sample H and one by sample E from the same dog. To study the effect of K^+, 2 half-bladders from the same toad were bathed by media containing either 3.5 mM K^+ or 7 mM K^+; samples H and E were placed in contiguous quarter-bladders as above, and the K^+-concentration in samples added to the half-bladder with the high medium potassium was increased by 3.5 mM.

Results. The table shows that initial ('baseline') SCC in the contiguous quarter-bladders was almost identical. At 30 min after addition of samples from each of the 4 dogs, SCC in the quarter-bladder treated with E was significantly lower than in the quarter-bladder treated with H, by a mean of 13% ($p < 0.001$).

Relative effect of samples E as compared to H, from each of 4 dogs on short-circuit current (SCC)

	Dogs I	II	III	IV	Pooled
n	6	5	5	5	21
'Baseline'	0.98 ±0.05	0.99 ±0.06	0.99 ±0.06	1.04 ±0.08	1.00 ±0.03
'Effect'	0.92 ±0.05	0.82 ±0.07	0.85 ±0.06	0.89 ±0.09	0.87 ±0.03
'Effect/baseline'	0.94 ±0.02	0.83 ±0.04	0.86 ±0.02	0.85 ±0.04	0.87 ±0.03
p	<0.05	<0.02	<0.005	<0.02	<0.001

All values are expressed as mean±SEM. 'Baseline' values measured immediately prior to adding samples and 'effect' values measured 30 min after adding the samples are expressed as the SCC in the quarter-bladder exposed to E divided by the SCC in the one exposed to H. 'Effect/baseline' is the ratio of these 2 ratios. n: number of bioassays; p: 2-sided Student's t-test for paired variables.



The relative effect on short circuit current (SCC) of hydropenic samples (H) compared to volume expanded samples (E) for all 21 bioassays. I/I_0 represents SCC after adding sample divided by SCC immediately prior to adding sample. *$p < 0.001$, Student's t-test for paired variables.

The pooled data from all 21 bioassays are represented in the figure. The relative inhibition of SCC produced by E (compared to H) became highly statistically significant 18–30 min after adding the samples.

5 additional experiments showed that the inhibition of SCC by E was identical at K^+-concentrations of 3.5 and 7.0 meq/l (17% vs 18%) and the relative inhibition by E remained statistically significant ($p < 0.05$) at the higher K^+-concentration.

Discussion. Preliminary data suggesting that increasing medium K^+-concentration inhibits antinatriferic activity were intriguing because this could explain the inability of other authors to reproduce Buckalew's findings. Up to a concentration of 7.0 meq/l, however, K^+ did not affect the results. The inability of other investigators to demonstrate the existence of a sodium transport inhibiting factor during volume expansion[2,12,13] is thus unexplained, but may be due to some of the experimental variables that were avoided in the present study. Contrary to earlier experiments by Buckalew, paired samples were obtained from the same dogs and furosemide was not used in hydropenic dogs. To minimize possible systematic errors, hydropenia and volume-expansion were alternated in the dogs, and to minimize variability in the bioassay system, matched, paired, high-resistance quarter-bladders from the same animal were used. NaCl electrical bridges were used and the effects of K^+ studied directly. Investigator bias was eliminated by the use of coded samples. In this fashion, the effects of E and H on SCC in TUB could be differentiated and E and H were correctly identified in each of the coded paired samples. It should be noted that this study cannot definitively distinguish between inhibition of SCC by E and stimulation of SCC by H.

The present study demonstrates a 13% relative inhibition of SCC by E, which, while highly significant, is lower than the 30% inhibition of SCC by E, observed by Buckalew et al.[6,10] and by Kramer et al.[8]. Since the toad bladder is a model of the distal nephron, decrements in Na-transport even smaller than 13%, could result in significant natriuresis.

1 Acknowledgments. This study was supported by the Philadelphia Veterans Administration Hospital (103.6M). We would like to thank the laboratory of Dr V. Buckalew at Bowman Gray School of Medicine, Winston-Salem, North-Carolina, for preparation of plasma dialysates; G. Huber and E. Fink for technical assistance and K. Conrad for secretarial assistance.

2 S. Klahr and H.J. Rodriguez, Nephron *15*, 387 (1975).
3 N.G. Levinsky, Adv. metab. Disorders *7*, 37 (1974).
4 R.W. Schrier and H.E. De Wardener, New Engl. J. Med. *285*, 1231 (1971).
5 D.M. Nutbourne, J.D. Howse, R.W. Schrier, L.B. Talner, M.G. Ventom, P.J. Verroust and H.E. De Wardener, Clin. Sci. *38*, 629 (1970).
6 V.M. Buckalew, J. Martinez and W.E. Green, J. clin. Invest. *49*, 926 (1970).
7 H. Favre, F.H. Hwang, R.W. Schmidt, N.S. Bricker and J. Bourgoignie, J. clin. Invest. *56*, 1302 (1975).
8 H.J. Kramer and F. Krück, Abstr. 5th Int. Cong. Nephrol., Mexico City, p. 144 (1972).
9 A. Leaf, J. Anderson and L.B. Page, J. gen. Physiol. *41*, 657 (1958).
10 V.M. Buckalew, Jr, and D.B. Nelson, Kid. int. *5*, 12 (1974).
11 V.M. Buckalew, Proc. int. Workshop Natriuretic Horm., Bonn (FRG), July 1976 (in press).
12 D.C. Brown, M. DuBois, C.A. Knock, E.M. Clarkson and H.E. DeWardener, Kid. int. *6*, 388 (1974).
13 N.G. Levinsky, Proc. int. Cong. Nephrol., 5th abstr. Symp., p. 65 (1972).
14 V.M. Buckalew, Jr, Presented at int. Workshop Natriuretic Horm., Bonn (FRG), 1976.
15 V.M. Buckalew, Jr, Nephron *9*, 66 (1972).