media respectively). An incubation time too short to reach a steady-state could explain these results[19].

Discussion. In accordance with previous findings[19], the present study shows Ehrlich ascites cells to be well-suited for studies on potassium transport processes. The present findings support the notion that bilirubin inhibits cellular potassium influx[19]. Potassium influx levelled off under the present experimental conditions at a concentration of about 6 mM in medium lacking inhibitor. Evidently, that concentration was sufficient to saturate nondiffusive components of influx. The fraction of influx due to simple passive diffusion under the present experimental conditions is about 10% of total influx[22]. Bilirubin and ouabain lowered the concentration of potassium at which influx became saturated. It is noteworthy that the maximal degree of inhibition of potassium influx induced by bilirubin and ouabain differed even in the presence of saturating concentrations of potassium in the medium. In that neither bilirubin (present paper) nor ouabain[22] induced impairment of potassium efflux, the decrease induced by bilirubin or ouabain in cellular potassium concentration implies that transport processes which contribute to net influx were inhibited. This finding is consistent with the fact that ouabain is a highly effective inhibitor of primary active transport of potassium[22,24] and suggests that bilirubin also inhibits this process. In addition, the fact that a greater degreee of inhibition of potassium influx is induced by bilirubin than by ouabain suggests that bilirubin inhibits another fraction of potassium net influx, in addition to primary transport processes. Perhaps bilirubin influences the Na/K/Cl cotransport system[15,25].

Bilirubin and ouabain were also found to differ in preincubation studies in that only the inhibitory action of ouabain was found to be reversible. This difference between effects of bilirubin and ouabain suggests that they differ also in the strength with which they bind to membrane sites.

Further studies on effects of bilirubin and other organic compounds on potassium transport processes may provide further information on the partition of potassium flux in cells, a topic of recent interest[22].

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3 M. Lubin, Nature *213*, 451 (1967).
4 G. Moroff and E.E. Gordin, Biochim. biophys. Acta *325*, 406 (1973).
5 M. Lubin, in: The cellular functions of membrane transport, p. 193. Ed. J.F. Hoffman. Prentice-Hall, Englewood Cliffs, N.J., 1964.
6 M.R. Quastel, D.S. Dow and J.G. Kaplan, in: Proc. of the 5th Leucocyte Culture Conf., p. 97. Ed. J.E. Harris. Academic Press, New York-London 1970.
7 S.G. Schultz and P.F. Curran, Physiol. Rev. *50*, 637 (1970).
8 J.T. Tupper, Biochim. biophys. Acta *394*, 586 (1975).
9 V.L. Lew and L. Beaugé in: Membrane transport in biology, vol. 2, p. 81. Eds G. Giebisch, D.C. Tosteson and H.H. Ussing. Springer, Berlin 1979.
10 I.M. Glynn, J. Physiol. *136*, 148 (1957).
11 J.R. Sachs and M.E. Conrad, Am. J. Physiol. *215*, 795 (1968).
12 D.L. Kropp and J.R. Sachs, J. Physiol. *264*, 471 (1977).
13 M.J. Hunter, J. Physiol. *268*, 35 (1977).
14 L. Beaugé, Biochim. biophys. Acta *401*, 95 (1975).
15 P. Geck, C. Pietrzyk, B.C. Burckhardt, B. Pfeiffer and E. Heinz, Biochim. biophys. Acta *600*, 432 (1980).
16 M.J. Dunn and R. Grant, Biochim. biophys. Acta *352*, 117 (1974).
17 J.R. Sachs, J. gen. Physiol. *57*, 259 (1971).
18 S. Kashiwamata, S. Goto, R.K. Semba and F.N. Suzuki, J. biol. Chem. *254*, 4577 (1979).
19 J.L. Corchs, R.E. Serrani and M. Palchick, Biochim. biophys. Acta *555*, 512 (1979).
20 K.H. Norwich, J. theor. Biol. *32*, 47 (1971).
21 J.R. Sachs, J. gen. Physiol. *63*, 123 (1974).
22 B. Mills and J.T. Tupper, J. Membrane Biol. *20*, 75 (1975).
23 H.G. Hempling, J. gen. Physiol. *41*, 565 (1958).
24 B. Sarkadi and D.C. Tosteson in: Membrane transport in biology, vol. 2, p. 117. Eds G. Giebisch, D.C. Tosteson and H.H. Ussing. Springer, Berlin 1979.
25 F. Aull, Biochim. biophys. Acta *643*, 339 (1981).

Relationship of specific granules to the natriuretic and diuretic activity of rat atria[1]

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Summary. The isolation of several fractions from rat atrial homogenates, by the use of differential and sucrose gradient centrifugation, indicates that the diuretic and natriuretic activity is restricted to the fractions rich in specific granules. Our preliminary results suggest that the active substance is a small peptide which is probably different from the natriuretic substance(s) already known.

A high, and a low molecular weight natriuretic substance have been found in plasma and urine of volume-expanded experimental animals and human subjects. It has been suggested that the low molecular weight natriuretic factor may be a small peptide[3-6].

The origin of this natriuretic factor remains to be elucidated. Recently, it has been reported that crude rat atrial homogenates produce a substantial, rapid, and short increase in diuresis and natriuresis when injected into a rat[7,8]. Similar results were obtained by Trippodo et al.[9]. It seems that this natriuretic factor is primarily active in the medullary collecting duct[10].

Mammalian atrial cardiocytes contain specific granules with morphological characteristics very similar to secretory granules found in peptide-secreting endocrine cells[11,12]. Based on this morphological appearance and on the fact that these granules are rich in protein in a variety of species[13], we wanted to know whether the natriuretic activity of rat atrial homogenates is localized in these granules.

Materials and methods. Female Sprague-Dawley rats (200–350 g) were anesthetized with ether; atrial and ventricular tissues were separated, washed, and homogenized in 0.25 M sucrose containing EDTA (ethylene-diaminetetraacetic acid) 1×10^{-3} M and PMSF (phenylmethylsulfo-

Specific activity of atrial and ventricular homogenates and of atrial specific granules

	Δμl urine/20 min/mg-protein	Δμ Eq Na/20 min/mg-protein
Ventricular homogenates (n = 5)	−2.27± 3.48	−0.73± 0.31
Atrial homogenates (n = 5)	126.68± 36.10*	28.85± 7.27*
Subfraction III (n = 4)	4288.25± 1702.66**	518.28± 268.49**
Subfraction IV (n = 4)	5702.00± 1650.00**	644.47± 325.00**

Mean ± SEM. * $p < 0.01$ vs ventricular homogenates; ** $p < 0.01$ vs atrial homogenates.

nyl fluoride) 1×10^{-3} M. The homogenates were centrifuged at 3000 × g and the supernatants kept at −20 °C until assayed. Prior to the bioassay, protein concentration in the supernatants was measured[14].

Female Sprague-Dawley rats (180–200 g) were used as bioassay animals. They were anesthetized with pentobarbital (Nembutal) (60 mg/kg i.p.) and a bladder and an intrajugular vein catheter were installed. The animals received an infusion of 5% dextrose, 3ml/h, 45 min before the assay and during the collection period. Three different doses of atrial and ventricular extracts 0.93, 1.87 and 3.75 mg-protein, were injected. Urine was collected in preweighed vials for 20-min periods, and after a 20-min basal collection the extracts were injected. Sodium concentration was measured in a flame photometer and expressed as μEq excreted in 20 min.

The atrial specific granules were isolated by using differential and density gradient centrifugations[15]. At the end of the procedure, 5 subfractions were obtained. Subfractions I and V did not yield a pellet. By electron microscopy, subfraction II was seen to be essentially composed of mitochondria with a few smooth and rough vesicles. Subfraction III contained a mixture of mitochondria, smooth and rough vesicles and specific granules. Subfraction IV had the highest concentration of specific granules, a few lysosome-like structures and a little mitochondrial debris. The specific granules in subfraction IV were morphologically identical to those observed in situ in the atria. Each subfraction was injected into the bioassay animal as described above. Four experiments were carried out, each of them based on the material extracted from atrial homogenates from 80 rats.

Samples of subfractions III and IV were incubated with 100 μg trypsin and 1 sample of subfraction IV was incubated with 100 μg prolidase, all for 3 h at room temperature. Three samples of subfraction III were incubated alternately at 100 °C for 5 min, at 37 °C for 3 h and at 37 °C for 3 h in the presence of EDTA and PMSF.

The response to the active material was fast, and was completed within 20 min; the results represent the difference between the basal and the 1st collection period after the injection. The results are expressed as means ± SE. Comparisons were made by Student's unpaired t-test (fig.) and by the 1-way analysis of variance and the Newman-Keuls a posteriori test (table).

Results. As seen in the figure, there is a significant and dose-related increase in both diuresis and sodium excretion in the animals receiving atrial extracts. The values shown represent a 8-fold increase in diuresis and a 40-fold increase in natriuresis when the equivalent of 3.75 mg-protein was injected. No changes were observed in either diuresis or natriuresis in the animals receiving ventricular extracts.

Subfractions I, II and V did not elicit any diuretic or natriuretic response in the assayed animals.

Subfraction III produced a 25-fold increase in diuresis and an almost 80-fold increase in natriuresis. This effect was completely eliminated by previous incubation with trypsin, but was not hindered by boiling for 5 min. The incubation for 3 h at 37 °C also destroyed its activity; however, this inhibition was prevented by the addition of EDTA and PMSF.

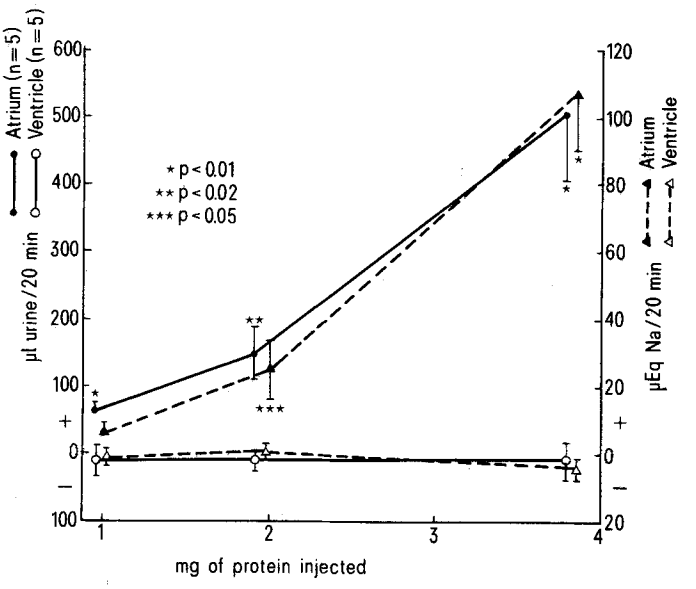
The results obtained with subfraction IV were similar to those observed with subfraction III. Its activity is also destroyed by trypsin, but not by prolidase.

As can be seen in the table, the specific activities (increment in urinary volume and sodium excretion in 20 min/mg-protein) of subfractions III and IV increased substantially when compared with that of crude atrial homogenates. The specific activity for the latter and for the ventricular homogenate was calculated using the response observed with the higher dose (3.75 mg-protein).

Discussion. Much evidence has been accumulated favoring the existence of a natriuretic and diuretic factor secreted in response to volume expansion; however, the origin of this factor remains elusive.

Stimulation of atrial baroreceptors by distending a balloon results in an increase in urinary flow and sodium excretion[16,17]. Linden[18] suggested that the increase in urinary volume after distension of the left atrium is probably due to a diuretic factor of unknown origin.

The similarity of atrial specific granules to secretory granules found in peptide-secreting endocrine cells, their content of proteins, and the changes in atrial granularity observed in experiments which modify salt and water balance[19,20] suggest that atrial specific granules may play a role in the mechanism by which atrial distension modifies diuresis and natriuresis in response to volume changes.



Effects of rat atrial and ventricular homogenates on diuresis and natriuresis. Asterisks: atrium vs ventricles homogenates.

Our data confirm the evidence of the presence of a potent diuretic and natriuretic factor in rat atria. Moreover, they strongly suggest that the specific granules may be the place of storage for this factor. The fact that its activity is destroyed by trypsin but not by boiling suggests that it is a small peptide. It is also possible that the cardiac tissue contains proteases, which may break down this peptide and which are inhibited by EDTA and PMSF.

The absence of inactivation of the atrial factor by prolidase, a protease known to destroy the activity of the natriuretic factor described by de Wardener[5], suggests that this natriuretic factor and the 'peptide' we have observed are not related.

It is tempting to speculate that mammalian atria, through the release of a polypeptide material produced in the specific granules, may play a role in the regulation of salt and water balance.

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3 H.E. de Wardener, I.H. Mills, W.F. Clapham and C.J. Hayter, Clin. Sci. *21*, 249 (1961).
4 H.E. de Wardener, Clin. Sci. molec. Med. *53*, 1 (1977).
5 E.M. Clarkson, S.M. Raw and H.E. de Wardener, Kidney int. *16*, 710 (1979).
6 K.A. Gruber and V.M. Buckalew, Jr, Proc. Soc. exp. Biol. Med. *159*, 463 (1978).
7 H. Sonnenberg, A.T. Veress, H.B. Borenstein and H.J. de Bold, Physiologist *23*, 13a (1980).
8 A.J. de Bold, H.B. Borenstein, A.T. Veress and H. Sonnenberg, Life Sci. *28*, 89 (1981).
9 N.C. Trippodo, L.P. Ziegler and A.A. MacPhee, Fedn Proc. *40*, 534a (1981).
10 W.A. Cupples, A.T. Veress, A.J. de Bold and H. Sonnenberg, Fedn Proc. *40*, 554a (1981).
11 M. Cantin, S. Benchimol, J.C. Berlinguet, Y. Castonguay and M. Huet, J. Ultrastruct. Res. *52*, 179 (1975).
12 M. Huet and M. Cantin, Lab. Invest. *30*, 514 (1974).
13 L. Yunge, M. Ballak, J. Bergeron, J. Lacasse and M. Cantin, Can. J. Physiol. Pharmac. *58*, 1463 (1980).
14 O.H. Lowry, M.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
15 A.J. de Bold and S.A. Bencosme, Cardiovasc. Res. *7*, 351 (1973).
16 J.P. Henry, O.H. Ganer and J.L. Reeves, Circulation Res. *4*, 85 (1956).
17 H. Lydtin and W.F. Hamilton, Am. J. Physiol. *207*, 530 (1964).
18 R.J. Linden, Am. J. Cardiol. *44*, 879 (1979).
19 J.P. Marie, H. Guillemot and P.Y. Hatt, Path. Biol. *24*, 549 (1976).
20 A.J. de Bold, Proc. Soc. exp. Biol. Med. *161*, 508 (1979).

Differential effects of cadmium and mercury on amino acid and sugar transport in the bullfrog small intestine[1]

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Summary. Amino acid transport in the bullfrog small intestine was specifically inhibited by application of Cd^{2+} to the mucosal fluid. This inhibition was dose-dependent, reversible and competitive in nature. In contrast, Hg^{2+} inhibited both amino acid transport and sugar transport: the inhibition by Hg^{2+} was non-graded, irreversible and non-competitive in nature.

Heavy metals can serve as tools for exploring the chemical nature of sites of membrane function[3,4], because these agents interact with several ligands (particularly sulfhydryl, carboxyl and imidazole groups) of biologically important molecules. The effects of several heavy metals on the transport of amino acids and sugars were examined in the bullfrog small intestine, in order to obtain structural information about these transporting sites in the intestinal brush border membranes.

Bullfrogs of either sex were used. The experimental procedures were similar to those described by Hoshi and Komatsu[5]. After decapitation, the initial short (about 4 cm) segment of the upper intestine below the opening of the common bile duct was isolated. The transepithelial potential difference was measured in the everted sac of the intestine incubated in an aerated saline solution via calomel cells and salt bridges with respect to the reference electrode in the mucosal fluid. The basic saline solution employed in the present study had the following composition (in mM): Na_2SO_4 11.5, $KHCO_3$ 2.5, $CaSO_4$ 1.0, mannitol 187.7, and Tris-H_2SO_4 5.0 (pH 7.4±0.1). To change the Na^+ concentration, mannitol was replaced with equiosmolar amounts of Na_2SO_4, or vice versa. All experiments were done at room temperature (24–29 °C), unless otherwise noted.

The magnitude of the transepithelial potential difference (PD) was dependent on the Na^+ concentration in the bathing solution ($[Na^+]_o$), and the serosal positivity decreased (or reversed) as the $[Na^+]_o$ was decreased. The mean PD value measured in 26 preparations was -6.2 ± 0.6 (SE) mV in the basic solution ($[Na^+]_o = 23$ mM). The addition of sugars or amino acids to the mucosal fluid resulted in an immediate decrease in the serosal negativity or in reversed polarity. It has been well established[6–9] that such evoked potentials correspond quantitatively with the Na^+-dependent, 'secondary' active transport of these organic solutes, although there is still room for debate about the origin of the evoked potentials[10]. The PD changes evoked by 5 mM D-glucose and 10 mM D-galactose were 6.5 ± 1.0 mV (n = 11) and 6.2 ± 1.0 mV (n = 8), respectively. Amino acid-evoked potentials were 13.8 ± 1.6 mV (n = 8) for 10 mM L-α-alanine and 9.2 ± 1.0 mV (n = 10) for 20 mM glycine. These sugar- or amino acid-evoked potentials exhibited the Michaelis-Menten-type kinetics versus the $[Na^+]_o$ values as well as versus the concentrations of those organic solutes, as observed in rat[11] and toad[5] small intestine. These evoked potentials were markedly suppressed by cooling the tissue to 2 °C, or by adding 0.3 mM ouabain to the serosal solution.

The addition of $CdCl_2$ (up to 3 mM) to the mucosal fluid neither affected the transepithelial potential difference in the absence of sugar and amino acid, nor the sugar-evoked potential (fig. 1, b). However, the alanine- or glycine-evoked potential was rapidly suppressed by the mucosal application of Cd^{2+}. This inhibition was dose-dependent (fig. 1, a) and reversible. A similar rapid, graded and reversible inhibition of amino acid- (but not of sugar-) evoked