non-cardiocyte nuclei is only about 5% of that of cardiocyte nuclei and sustained aortic coarctation greatly increases enzymatic activity. It is apparent that the increase in enzymatic activity parallels the in vivo augmentation of thymidine incorporation into DNA (fig., D), which phenomenon is an index of cellular hyperplasia; therefore poly ADP-ribosylation in cellular proliferation behaves as is it would directly correlate with DNA synthesis. In vivo thymidine incorporation into DNA (fig., C and D) clearly shows that cardiocytes respond to circulatory stress with hypertrophy[5,6] and hyperplasia is confined to cells other than cardiocytes. The inverse correlation between poly ADP-R synthetase activity and rates of DNA synthesis in various cell types has been noted earlier[20] as it was found that nondividing cardiocytes exhibit a much larger poly ADP-R synthetase activity than liver cells, which are known to undergo mitosis. Characteristically non-cardiocyte cell types which possess the potential of proliferation also exhibit a much lower poly ADP-R synthetase activity.

The present results emphasize the differentiation dependence of regulation of both poly ADP-R and DNA synthesis potential of the 2 cell types within the same organ, and are consistent with a regulatory (inhibitory) effect of poly ADP-ribosylation on RNA synthesis[14]. De-repression of RNA synthesis is induced by inhibition of poly ADP-ribosylation in cardiocytes, resulting in hypertrophy[9,14,17]. Poly ADP-ribosylation is non-cardiocyte nuclei parallels DNA synthesis and it is assumed that induction of poly ADP-ribose synthetase constitutes a terminating signal for DNA synthesis in cell types capable of proliferation because poly ADP-ribosylation is known to inhibit also DNA synthesis[21-24]. Developmental hormones are probable mediators of stress on the poly ADP-ribose regulated systems[14,18].

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Inhibition of potassium (^{86}Rb) influx in Ehrlich ascites cells by bilirubin and ouabain[1]

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Summary. Bilirubin inhibited influx of potassium into Ehrlich ascites cells without altering efflux. The data showed that compared with ouabain, net potassium influx components were impaired in a higher degree by bilirubin. The reversal of this effect was shown, in our experimental conditions, only for ouabain.

Potassium is an essential ion for the function of all animal cells. Cellular membrane transport processes regulate the intracellular concentration of potassium within certain limits[3-6]. Potassium influx is influenced by a) active pump (primary) and cotransport (secondary) mechanisms, b) exchange processes, and c) diffusion through selective channels[7-9]. Organic compounds have been used extensively to study cellular ionic transport mechanisms[10-17]. Recently, unconjugated bilirubin was founds to inhibit the Na-K ATPase in the microsomal fraction of the brain[18] and the potassium influx into Ehrlich ascites cells[19]. The present study was carried out to obtain further information on the effects of bilirubin on potassium fluxes. A comparison was made between the effects of bilirubin and of ouabain, a well-established inhibitor of potassium transport processes[8,10].

Materials and methods. The general methodology used to study potassium influx and intracellular ion content has been described in detail previously[19]. Briefly, radioactive rubidium (^{86}Rb) was used as a marker for potassium. An aliquot (10-15 µl) of a solution of ^{86}RbCl of high specific activity (10-15 Ci/g) was added to cellular suspensions in the range of 5×10^6-1×10^7 cells/cm^3. 1-ml samples of the suspensions were taken at intervals and centrifuged for 30 sec at 2500 × g to obtain a cell pellet. The cells were washed thrice by resuspension in 0.3 osM $MgCl_2$ and then centrifuged again for 30 sec at 2500 × g. The radioactivity of packed cells and aliquots of incubation medium was deter-

mined by liquid scintillation spectrometry (Beckman D233 Sci. Spectrometer). Cellular uptake of ^{86}Rb was linear with time up to about 15 min of incubation, and the shortest time interval (generally less than 5 min) of incubation which gave counts differing from background was selected to avoid convective passage of ^{86}Rb due to non-steady-state intracellular potassium concentration[20]. In preincubation experiments, cells were suspended at 27 °C for 60 min in a potassium-free medium[21] containing either bilirubin or ouabain. Then, the cells were centrifuged at 2500 × g and washed in medium without bilirubin or ouabain. Potassium influx was measured subsequently as described above. Potassium influx was calculated from the incorporation of ^{86}Rb by cells and the specific activity of the incubation medium. Fluxes were expressed in pmoles/sec per 10^5 cells[19]. The unidirectional efflux rate coefficient was determined from the kinetics of ^{86}Rb washout[22]. Cells were preloaded with a tracer amount of ^{86}Rb during 60 min without or with the addition of bilirubin (10^{-4}M). The suspension was centrifuged and the supernatant discarded. The cells were then washed twice in buffered saline solution and resuspended to the desired cell concentration in a medium of identical composition to the preloading solution without isotope. At intervals samples of the suspension were removed and centrifuged, and the cell pellet treated as described above for the study of radioactivity.

Cellular potassium and sodium contents were determined by flame-photometry[19]. Nigrosin exclusion at the end of the experiments amounted to 93-97% cells. The composition of the incubation medium was as follows (M): NaCl 0.11; KCl variable; $MgSO_4$ 0.001; $CaCl_2$ 0.0001; Tris-HCl (pH 7.4) 0.05. When inhibitors (ouabain and bilirubin) and albumin were used, a molar ratio of 20:1 was employed. All determinations were run in duplicate. Bilirubin, ouabain and bovine serum albumin were purchased from Sigma (St. Louis, MO, USA). Radioactive Rb was obtained through the Comisión Nacional de Energía Atómica Argentina, as $^{86}RbCl$ of high specific activity (0.5-10 Ci/g) in sterile solution. All the other reagents were also of analytical grade.

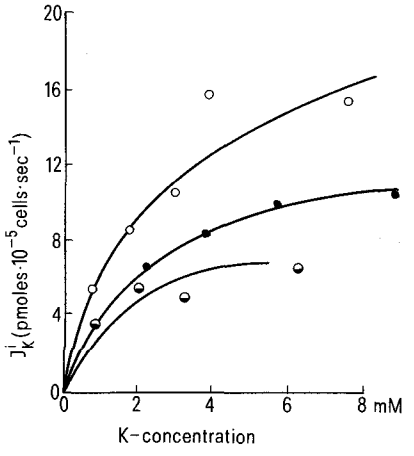
Results. The figure presents the effects of ouabain and bilirubin on potassium influx. The value of influx levels off as the potassium concentration is increased.

Table 1 shows the values for potassium influx obtained in Ehrlich ascites cells exposed to ouabain or bilirubin either before (preincubation) or during incubation with ^{86}Rb. As expected, the presence of ouabain or bilirubin in the medium during incubation inhibited potassium influx significantly, and potassium influx was inhibited significantly more by bilirubin (10^{-4}M) than by ouabain (10^{-3}-10^{-4}M). In contrast to previous findings[8], the effects of ouabain at concentrations (M) of 10^{-4} and 10^{-3} failed to differ significantly.

Preincubation with bilirubin significantly inhibited potassium influx, but preincubation with ouabain did not affect potassium influx significantly (table 1).

The rate coefficient for potassium efflux (h^{-1}) under control conditions was 2.5 ± 0.38 (mean ± SEM, N = 7) and it did not differ significantly from the value obtained in the presence of bilirubin (10^{-4}M) (2.91 ± 0.42, N = 5). As previously reported[22] ouabain failed to affect the efflux rate constant in these cells.

Table 2 shows the amount of potassium and sodium in Ehrlich ascites cells after 60 min incubation in control medium and in medium containing either ouabain (10^{-4} M) or bilirubin (10^{-4} M). The content observed in control cells was in accordance with a previous report[23]. Potassium content decreased significantly and sodium content increased significantly in cells incubated with either ouabain or bilirubin. The effects of bilirubin and ouabain on intracellular content of potassium differ significantly. Loss of electrolytes from the cells was observed with both inhibitors. It was greater with bilirubin (sodium + potassium contents at the end of incubation period amounted to about 90 and 70% of control value in ouabain and bilirubin



Influx of potassium (^{86}Rb) into Ehrlich ascites cells as a function of external potassium concentration in the presence of either no inhibitor (○), ouabain 10^{-4}M (●) or bilirubin 10^{-4}M (◒). The indicated values represent average estimates of duplicate determinations for 3 representative experiments.

Table 1. Effects of bilirubin and ouabain on the influx of potassium (^{86}Rb) into Ehrlich ascites cells

Inhibitor (M) Preincubation medium	Incubation medium	Potassium influx (pmoles 10^{-5} cells sec^{-1})
-	-	15.81 ± 1.42 (8)[a]
-	Bilirubin (10^{-4})	5.86 ± 1.0 (9)[b]
-	Ouabain (10^{-4})	10.72 ± 1.17 (7)[c]
-	Ouabain (10^{-3})	10.77 ± 1.98 (7)
Bilirubin (10^{-4})	-	6.29 ± 2.0 (4)
Ouabain (10^{-3})	-	15.87 20.58*

Potassium concentration in the medium for influx determinations was 4-6 mM in all the experimental conditions. Values shown are mean ± SEM for duplicate determinations in the number of experiments shown in parentheses. * Individual determinations. Student's t-tests: b, statistically different from a, $p < 0.001$ and from c, $p < 0.01$; c, statistically different from a, $p < 0.05$.

Table 2. Effects of bilirubin and ouabain on intracellular potassium and sodium content in Ehrlich ascites cells after 60 min incubation

Inhibitor (M)	mmoles l^{-1} cells Potassium	Sodium
-		
-	132.76 ± 12.01 (10)[a]	24.34 ± 6.3 (6)[d]
Bilirubin (10^{-4})	63.15 ± 6.2 (9)[b]	44.66 ± 3.26 (9)[e]
Ouabain (10^{-4})	86.15 ± 7.0 (9)[c]	52.84 ± 3.86 (6)[f]

Potassium concentration in the medium was 4-6 mM in all experimental conditions. Values shown are means ± SEM for duplicate determinations in the number of experiments shown in parentheses. Student's t-tests: b, statistically different from a, $p < 0.001$ and from c, $p < 0.05$; c, statistically different from a, $p < 0.01$; e, statistically different from d, $p < 0.01$; f, statistically different from d, $p < 0.01$

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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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-