Actions of Aldosterone on Polyadenylated Ribonucleic Acid and Na^+ Transport in the Toad Bladder†

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ABSTRACT: Polyadenylated ribonucleic acid [poly(A)(+)-RNA] has been isolated from the cytoplasm of the epithelium of the urinary bladder of the toad (*Bufo marinus*) by oligo(deoxythymidylate)cellulose chromatography. Aldosterone increased the incorporation of [^{3}H]uridine, and of [^{3}H]uridine and [^{3}H]adenosine (given in combination) into 7S, 12S, and 18S poly(A)(+)-RNA during the first 30 min of the action of the hormone, as defined by either a pulse or a pulse-chase sequence. The quantity of cytoplasmic poly(A)(+)-RNA that hybridized to [^{3}H]poly(uridine) was also increased by aldosterone. These results are consistent with the inference of induction of messenger RNA synthesis. This effect was most marked during the first 30 min of the action of the hormone.

In a previous report, we noted that aldosterone increased the incorporation of [^{3}H]uridine into a nonmethylated, 9–12S rapidly labeled RNA[1] (Rossier et al., 1974). This effect was not elicited by either cortisol or the inactive isomer, 17α-isoaldosterone, at equimolar concentrations and was inhibited by the competitive antagonist, spirolactone. These findings are consistent with the possibility that modulation of mRNA content mediates the effect on Na^+ transport. Further characterization of the effects of aldosterone on various classes of RNA is needed to evaluate their respective roles in the mechanism of action on Na^+ transport. The poly(A) sequences in eucaryotic RNA provide a means of further analysis of the action of the hormone. Jelinek et al. (1973) concluded that mature, cytoplasmic mRNA in HeLa cells is derived from polyadenylated HnRNA. In the mouse L-cell, 80% or more of the cytoplasmic mRNA contained poly(A) sequences (Perry et al., 1973). Although the functional role played by poly(A) is not clear, the efficiency of translation and stability of mRNA appear to be enhanced by this moiety (Jelinek et al., 1973; Huez et al., 1974). Moreover, the presence of poly(A) segments allows isolation of this class of RNA (putatively mRNA) by affinity chromatography either on oligo(dT)-cellulose (Aviv and Leder, 1972; Swan et al., 1972) or poly(U)-Sepharose (Wagner et al., 1971). This strategem has been used to partially purify ovalbumin-mRNA after hormonal stimulation (Chan et al., 1973).

In this paper, we describe the isolation of poly(A)(+)-RNA from toad bladder epithelium by oligo(dT)-cellulose chromatography, and its characterization by hybridization to poly(U) and sucrose density centrifugation. The effects of aldosterone on incorporation of [^{3}H]uridine into poly(A)(+)-RNA and on poly(A)(+)-RNA hybridized to poly(U) are also described. A preliminary report of these results was presented at the IVth International Congress on Hormonal Steroids (Wilce et al., 1974).

Experimental Section

Materials

The incubation medium (frog, Ringer's solution) contained (all in mM) 90 NaCl, 3 KCl, 25 $NaHCO_3$, 3.9 $MgSO_4$, 0.5 KH_2PO_4, 1 $CaCl_2$ and 6 glucose (pH 7.6, osmolality = 230 mOsm/l.). Gentamycin was added to a final concentration of 10 μg/ml and the medium was filtered through 0.45 μm Millipore filters. All glassware and other solutions were heat sterilized. *d*-Aldosterone was obtained from Calbiochem Corp. and oligo(dT)-cellulose (T_2) from Collaborative Research, Inc. All of the conventional reagents were either reagent grade or spectroquality. [5-^{3}H]Uridine (26–28 Ci/mmol), L-[*methyl*-^{14}C]methionine (10 mCi/mmol), and [2,8-^{3}H]-adenosine (30–50 Ci/mmol) were purchased from New England Nuclear Corp., and [5-^{3}H]poly(U) (>300 mCi/mmol of P_i), [8-^{3}H]poly(A) (10–30 mCi/mmol of P_i) from Miles Laboratories, Inc. Aquasol was obtained from New England Nuclear Corp.

Methods

Colombian, female toads (*Bufo marinus*) obtained from Tarpon Zoo, Florida, were partially immersed in 75 mM NaCl for 48 h, at room temperature before use. The urinary bladders were removed and mounted as sacs (mucosal side outside) on plastic cannulas, filled with 5 ml and immersed in 90 ml of the frog Ringer's solution, and maintained at a constant temperature of 25 °C, as described previously (Rossier et al., 1974). Both sides of the sacs were oxygenated with 97% O_2–3% CO_2. The potential differences (pd) and short-circuit currents (scc) were measured at 30-min intervals by the method of Walser et al. (1969). The scc is a convenient and accurate index of the

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[1] Abbreviations used: oligo(dT), oligo(deoxythymidylate); poly(U), poly(uridine); poly(A)(+)-RNA, polyadenylated ribonucleic acid isolated by oligo(dT)-cellulose chromatography; pd, spontaneous electrical potential difference across the wall of the toad bladder; scc, the current required to clamp the pd to zero (i.e., the short-circuit current) measured in μA/hemibladder; Tris, tris(hydroxymethyl)aminomethane; SEM, standard error of the mean; mRNA, messenger ribonucleic acid; tRNA, transfer RNA; HnRNA, heterogeneous nuclear RNA; rRNA, ribosomal RNA; P_i, inorganic phosphate; EDTA, ethylenediaminetetraacetic acid; A and C, aldosterone and control populations, respectively.

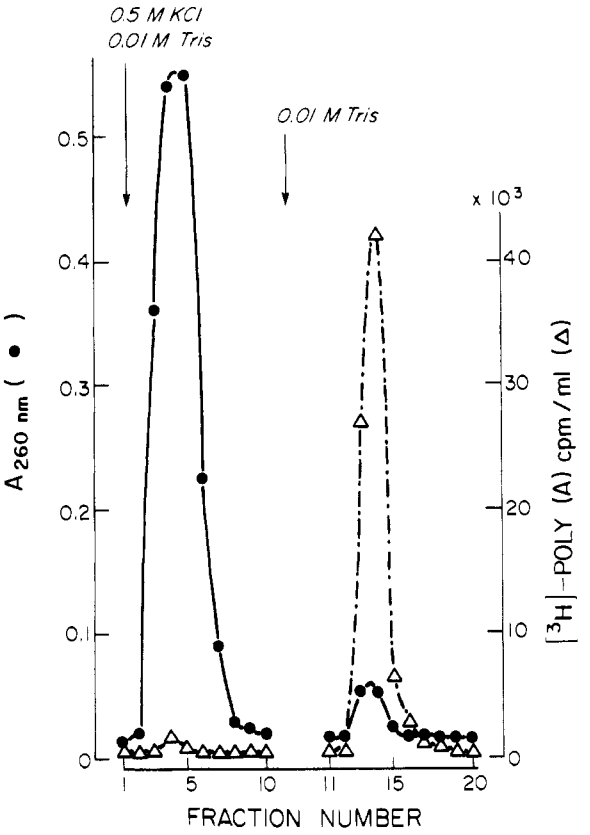

FIGURE 1: Calibration of the oligo(dT)-cellulose column with [^{3}H]-poly(A) and unlabeled cytoplasmic RNA extracted from toad bladder epithelium. Two A_{260nm} units (~64 mg) of cytoplasmic RNA and 10^5 cpm of [^{3}H]poly(A) were layered on the column and washed with high salt buffer (0.5 M KCl) and then eluted in low salt buffer (0.01 M Tris). One-milliliter fractions were collected and analyzed for RNA content (A_{260}, ●), and ^{3}H activity (△).

rate of transepithelial active Na^+ transport both in the presence and absence of aldosterone (Porter and Edelman, 1964). Aldosterone (final concentration, 7×10^{-8} M) was added to the mucosal and serosal solutions of the test hemibladders and an equal volume of the diluent (frog Ringer's solution) to the control, 4 h after mounting. The labeled precursors, [5-^{3}H]uridine and [2,8-^{3}H]adenosine, were added to the serosal sides of the hemibladders at the concentrations and for the intervals of time (pulse or pulse-chase) as stated under Results.

Isolation of Nuclear and Cytoplasmic RNA. The hemibladders were washed twice with ice-cold incubation medium and all subsequent steps were carried out at 0–4 °C. The epithelial cells were removed by scraping with a glass slide and the cells harvested from five to ten paired hemibladders were pooled into "test" and "control" groups. Nuclear and cytoplasmic RNA were isolated by a modification of the method of Penman (1966), as described previously (Rossier et al., 1974). Pooled epithelial scrapings (from five hemibladders) were suspended in 2 ml of hypotonic medium (0.001 M NaCl–0.0015 M $MgCl_2$–0.001 M Tris-HCl, pH 7.4) and homogenized in an Elvehjem-Potter Teflon–glass homogenizer, with 15 strokes at top speed. The homogenates were centrifuged at 600*g* for 2 min. The pellets were washed once with 2 ml of the hypotonic medium and once with 2 ml of this medium supplemented with Triton X-100 (1.5% v/v) and sodium deoxycholate (1.5% w/v). The washed nuclear pellets were then incubated in 2 ml of high-salt medium (0.5 M NaCl–0.05 M $MgCl_2$–0.01 M Tris-HCl, pH 7.4) containing 2 mg/ml of DNase (electrophoretically pure) at 37 °C for 30 s. The reaction was stopped by the addition of EDTA (final concentration, 0.03 M) and sodium dodecyl sulfate (final concentration, 0.5% w/v). Nuclear RNA was then extracted with 2 ml of phenol at 55 °C for 1 min. Two milliliters of chloroform was then added and the extraction mixture was reincubated at 55 °C for 1 min. The residual nuclear fraction was collected by centrifugation and reextracted with phenol and chloroform–phenol as described above. The extracted nucleic acids were precipitated with 2 volumes of ice-cold ethanol. Cytoplasmic RNA was obtained by extracting the 600*g* supernatants (twice) with phenol–sodium dodecyl sulfate (0.5% w/v) at 25 °C for 5 min with constant shaking. The RNA was precipitated by addition of 2 volumes of ice-cold ethanol. The cytoplasmic and nuclear RNA precipitates were dissolved in 0.01 M NaCl–0.05 M sodium acetate, pH 5.2, and stored at −60 °C.

Oligo(dT)-Cellulose Chromatography. Poly(A)(+)-RNA of the nuclear and cytoplasmic extracts was collected by a modification of the method of Aviv and Leder (1972). The RNA extracts in 0.5 M KCl–0.01 M Tris-HCl (pH 7.6) (i.e., high-salt buffer) were applied to the oligo(dT) columns and the bound RNA was eluted in one step with 0.01 M Tris-HCl (pH 7.6) (i.e., low-salt buffer) without the use of an intermediate buffer. Chromatography was executed at room temperature with sterile glassware and buffers.

Binding of Cytoplasmic RNA to [^{3}H]Poly(U). Hybridization of the cytoplasmic RNA to [^{3}H]poly(U) and characterization of the product were accomplished by the technique of Fraser and Loening (1973). The RNA extracted from the cytoplasmic fraction was dissolved in 0.1 M NaCl and 0.05 M sodium acetate (pH 5.2) and mixed at 20 °C (1:4) with [^{3}H]poly(U) in 0.05 M Tris-HCl–0.15 M NaCl–0.001 M EDTA–0.5% sodium dodecyl sulfate (pH 7.6). The entire sample of the hybrid, equivalent to 30 μg of cytoplasmic RNA, was immediately layered on a linear (12.5 ml) 7–25% sucrose gradient in 0.05 M Tris-HCl–0.15 M NaCl–0.001 M EDTA–0.5% sodium dodecyl sulfate (pH 7.6). The tubes were centrifuged at 20 °C, at $2 \times 10^5 g$ for 4 h in a swinging bucket rotor (SB 283) in a B-60 International Centrifuge. Thirty fractions (total volume, 12.5 ml) were collected with the ISCO Model 222 gradient fractionator monitored at 254 nm.

Radioassays. The fractions (chromatographic and density gradients) were added to 10 ml of Aquasol and the ^{3}H and ^{14}C activities were assayed in a Mark II Nuclear Chicago liquid scintillation spectrometer. The recovery of radioactivity from the columns and the gradients varied from 90 to 95%.

Results

Oligo(dT)-Cellulose Chromatography. Two techniques were used to validate the recovery of poly(A)(+)-RNA in the low-salt extracts of the oligo(dT)-cellulose columns. (1) Unlabeled RNA obtained by extraction of the cytoplasmic fraction of toad epithelium was suspended in high salt buffer at a concentration of 1.0 A_{260} unit/ml. [^{3}H]Poly(A) (7–11S) was added to this buffer to a final concentration of 5×10^4 cpm/ml. Two milliliters of the mixture was layered on the oligo(dT) column and eluted with the low-salt buffer. As shown in Figure 1, 97% of the bulk, unlabeled RNA was recovered in the high-salt eluates (fractions 1–10); in contrast 95% of the [^{3}H]poly(A) was eluted with the low-salt buffer in fractions 11–20. Most, if not all, of the 3% of the unlabeled RNA that was collected in the low-salt eluates probably represents poly(A)(+)-RNA extracted from the epithelial cytoplasm. Aviv and Leder (1972), for example, found that ~2% of the

TABLE I: Oligo(dT)-Cellulose Chromatography of Cytoplasmic RNA of Toad Bladder Epithelium.[a]

Expt. No.	Pairs of Hemi-bladders	[^{3}H]-Uridine (μCi/ml)	[^{3}H]-Adenosine (μCi/ml)	0.01 M Tris–0.5 M KCl (cpm/μg RNA) A	C	A/C	0.01 M Tris (cpm/μg RNA) A	C	A/C	scc$_t$/scc$_0$ A	C	A/C
				I. 30-Minute Pulse								
1	5	10	0	620	540	1.14	3350	2980	1.12	0.98 ± 0.02	1.05 ± 0.02	0.93
2	5	15	0	1390	1930	0.72	3020	2050	1.47	1.23 ± 0.12	1.11 ± 0.12	1.10
Mean:						0.93			1.29			1.01
				II. 30-Minute Pulse–150-Minute Chase								
1	5	5	0	370	300	1.24	2010	660	3.04	2.50 ± 0.30	1.04 ± 0.09	2.40
2	5	5	0	90	130	0.68	980	320	3.04	1.40 ± 0.13	0.97 ± 0.09	1.45
3	5	5	0	170	110	1.60	930	325	2.83	1.81 ± 0.20	0.92 ± 0.19	1.97
4	10	15	15	1330	1400	0.95	12400	6690	1.85	1.32 ± 0.10	0.57 ± 0.08	2.31
5	10	15	15	1360	1460	0.93	2580	3080	0.83	1.58 ± 0.16	0.52 ± 0.03	3.03
		Mean ± SEM				1.08 ± 0.16			2.31 ± 0.43[b]			2.23 ± 0.25[b]

[a] Paired hemibladders were incubated in aldosterone (7×10^{-8} M) (denoted "A") or the diluent (denoted "C") and labeled with [^{3}H]uridine or both [^{3}H]uridine + [^{3}H]adenosine for either 30 min (group I) or for a 30-min pulse followed by 150-min chase (group II). The cytoplasmic fractions were prepared and chromatographed through oligo(dT)-cellulose as described in the text. The specific activity was based on A_{260} (1 unit of A_{260} = 32 μg of RNA/ml; light path = 10 mm); 0.01 M Tris-0.5 M KCl denotes the high salt eluates and 0.01 M Tris, the low salt eluates. The ratio scc$_t$/scc$_0$ denotes the short-circuit current at time t (30 min after aldosterone in group 1 and 180 min after aldosterone in group II) divided by that of the same hemibladder at time zero—the time of addition of the hormone or the diluent. [b] Statistically significant (i.e., $p < 0.01$).

bulk cytoplasmic RNA of the reticulocyte was polyadenylated. These results indicate that the poly(A) polymer was separated, with a high degree of precision, from a large excess of RNA by this method. (2) Toad bladder epithelium was labeled simultaneously with [^{3}H]uridine and L-[*methyl*-^{14}C]methionine for 30 min, followed by a 150-min chase, and a cytoplasmic extract was prepared as described previously (Rossier et al., 1974). The cytoplasmic extract was diluted 1:5 with the high-salt buffer and 2.0 ml of the labeled RNA containing 1.5 A_{260} units of bulk RNA was layered on the oligo(dT) column. Once again, 97% of the bulk RNA (OD units) was eluted with high salt in fractions 1–10 (Figure 2). In accord with the much higher degree of methylation of rRNA and tRNA than in mRNA, all of the detectable [^{14}C]methyl activity was recovered in these fractions (Bard et al., 1974; Perry and Kelley, 1974). In contrast the [^{3}H]uridine activity was distributed between both the high- and low-salt fractions; about 30% appeared in the latter. The pulse-chase format took advantage of the rapid labeling of mRNA. Thus, the high specific activity (i.e., ^{3}H/A_{260}) and the minimal ^{14}C activity in the low-salt fractions are in accord with the recovery of poly(A)(+)-RNA, i.e., putative mRNA, in these fractions (Perry et al., 1973; Perry and Kelley, 1974).

To assess the effects of aldosterone on both labeling of poly(A)(+)-RNA and on active Na^+ transport, pulse and pulse-chase protocols were used. Aldosterone has a 60–90-min latent period with respect to Na^+ transport activity and is sensitive to inhibitors of RNA synthesis during this interval (Lahav et al., 1973). As summarized in Table I, in the first 30 min, aldosterone elicited a 29% increase (mean of two experiments of five paired hemibladders each) in incorporation of [^{3}H]uridine into poly(A)(+)-RNA (0.01 M Tris fraction) and no change in labeling of the high-salt fraction or in scc. The 30-min pulse–150-min chase experiments yielded no change in labeling of the high-salt fraction, and more than twofold increases in incorporation of [^{3}H]uridine into poly(A)(+)-RNA and in scc. Enhanced labeling of the poly(A) fraction was recorded in four of the five experiments. The one experiment in which aldosterone failed to stimulate incorporation (i.e., A/C = 0.83) was probably a result of significant degradation of the RNA during incubation of the tissue or isolation

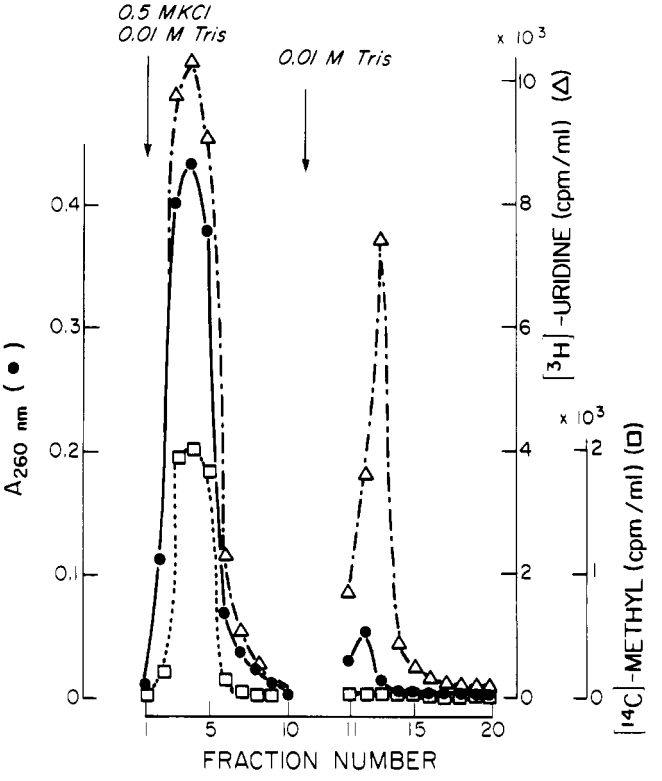

FIGURE 2: Calibration of oligo(dT)-cellulose column with labeled cytoplasmic RNA extracted from toad bladder epithelium. Toad bladders were labeled with [^{3}H]uridine and L-[methyl-^{14}C]methionine for 30 min, followed by a 150-min chase as described previously (Rossier et al., 1974). One and a half A_{260} units (≃48 μg) of the cytoplasmic extract was layered on the column, washed with high salt buffer (0.5 M KCl), and then eluted in low salt buffer (0.01 M Tris). One-milliliter fractions were collected and analyzed for RNA content (A_{260}, ●), ^{14}C activity (□), and ^{3}H activity (Δ).

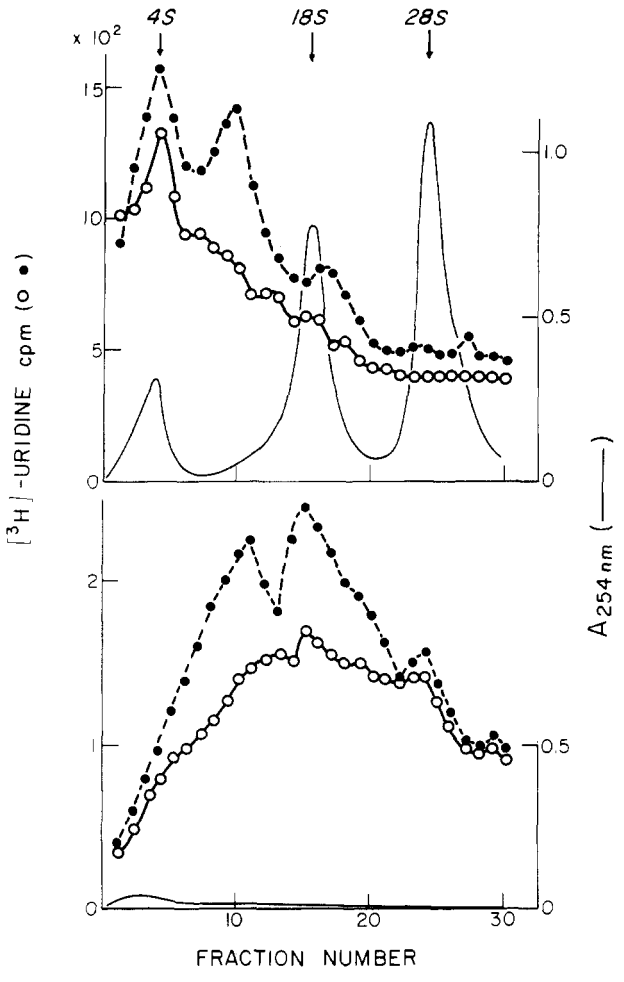

FIGURE 3: Effects of aldosterone on the incorporation of [^{3}H]uridine into cytoplasmic RNA and poly(A)(+)-RNA of toad bladder epithelium analyzed by sucrose density gradients. Paired hemibladders were incubated with aldosterone (7×10^{-8} M) on the diluent and labeled for 30 min with [^{3}H]uridine. The ^{3}H activity of the control (○) and of the aldosterone (●) treated tissue is indicated by the ordinates on the left. The absorbance at 254 nm (solid line) is indicated by the ordinates on the right. Sedimentation is from left to right. (Upper panel) Sucrose density gradient analysis of total cytoplasmic RNA before oligo(dT)-cellulose chromatography. The ^{3}H activity is normalized to the total RNA (i.e., the area under the absorbance curve). (Lower panel) Sucrose density gradient analysis of cytoplasmic poly(A)(+)-RNA obtained by oligo(dT)-cellulose chromatography. Equal amounts of RNA (0.05 A_{260} unit) were layered on the gradients. The effect of aldosterone on Na^+ transport is given in Table I (group I, experiment 2).

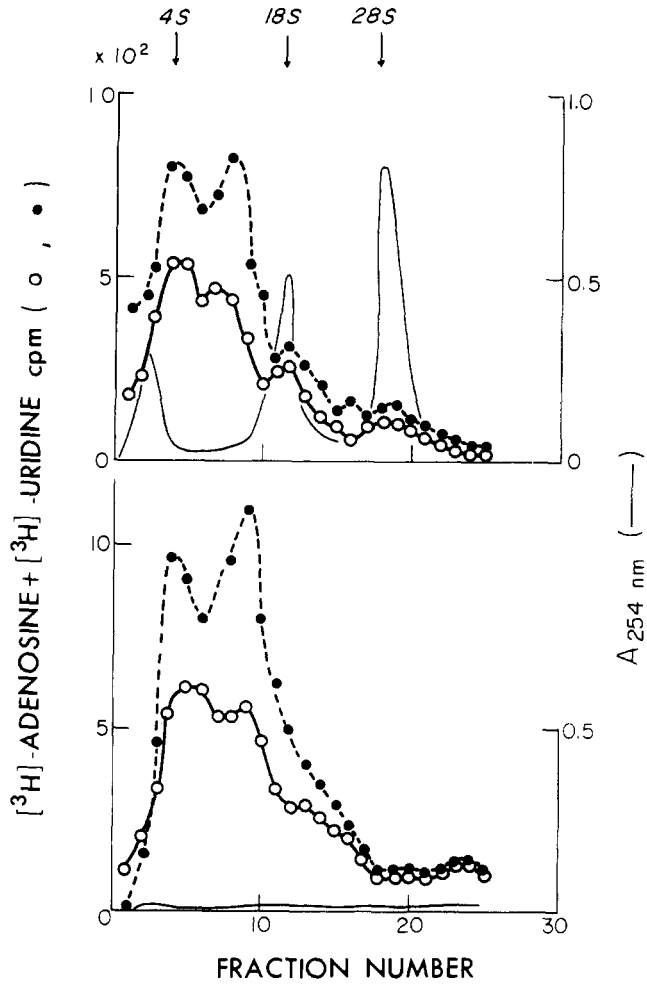

FIGURE 4: Effects of aldosterone on the incorporation of [^{3}H]uridine and [^{3}H]adenosine into cytoplasmic RNA and poly(A)(+)-RNA of toad bladder epithelium analyzed by sucrose density gradients incubated with aldosterone (7×10^{-8} M) or the diluent and labeled by a 30-min pulse followed by a 150-min chase. The ^{3}H activity of the control (○) and of the aldosterone (●) treated tissue is indicated by the ordinates on the left. The absorbance at 254 nm (solid line) is indicated by the ordinates on the right. Sedimentation is from left to right. These results represent a single pool of ten paired hemibladders. (Upper panel) Sucrose density gradient analysis of total cytoplasmic RNA before oligo(dT)-cellulose chromatography. The ^{3}H activity is normalized to the total RNA (i.e., the area under the absorbance curve). (Lower panel) Sucrose density gradient analysis of cytoplasmic poly(A)(+)-RNA obtained by oligo(dT)-cellulose chromatography. Equal amounts of RNA (0.02 A_{260} unit) were layered on the gradients. The effect of aldosterone on Na^+ transport is given in Table I (group II, experiment 5).

of the RNA, in that sucrose density gradient analysis of this preparation revealed a preponderance of low-molecular-weight RNA. These results imply differential stimulation by aldosterone of poly(A)(+)-RNA synthesis.

To explore this possibility further, we analyzed the labeled RNA products before and after fractionation by oligo(dT)-cellulose chromatography. Unfractionated cytoplasmic RNA labeled for 30 min gave a typical bulk RNA absorbance pattern consisting of a 4S tRNA and 18S and 28S rRNAs (Rossier et al., 1974). Exposure to aldosterone resulted in augmented labeling of the cytoplasmic RNA that was particularly pronounced in the 11–14S fractions (upper panel, Figure 3) as reported previously (Rossier et al., 1974). The labeled poly(A)(+)-RNA gave a heterogeneous distribution in the sucrose density gradient and almost no detectable bulk RNA (lower panel, Figure 3). The aldosterone-treated tissues yielded peaks in the 12S and 18S regions. Neither peak was evident in the control preparation.

To obtain higher specific activities and an increased yield of labeled poly(A)(+)-RNA, [^{3}H]uridine and [^{3}H]adenosine were both used to label the RNA and the 30-min pulse was followed by a 150-min chase. The results (on a pool of ten paired hemibladders) in Figure 4 (upper panel) revealed an equivalent absorbance pattern for the bulk, unfractionated cytoplasmic RNA as in the 30-min chase experiment (cf. Figure 3). The RNA in the 7S and 12S regions was more intensely labeled in the aldosterone-treated extracts as compared with the controls. The aldosterone-induced peaks at 7S and 12S in the density gradients were preserved on passage through the oligo(dT)-cellulose column (lower panel, Figure 4). The 12S peak was also apparent in earlier studies (Rossier et al., 1974) and in the 30-min pulse experiments (Figure 3). The 7S peak

may be indicative of an effect of aldosterone that is more readily detected with [^{3}H]adenosine as the precursor.

The early increase in labeling of the poly(A)(+)-RNA induced by aldosterone may result in a net accumulation of these species during the action of the hormone. Hybridization of poly(A)(+)-RNA to [^{3}H]poly(U) provides a method for evaluating this possibility (Fraser and Loening, 1973). Aldosterone (final concentration, 7×10^{-8} M) was added to "test" hemibladders and the diluent to the controls. Four hours later, the epithelial layer was collected by scraping and cytoplasmic extracts were prepared as described previously (Rossier et al., 1974). At the time of collection of the epithelium, the scc_t/scc_0 ratio in the presence of aldosterone was 2.29 ± 0.59 and in the controls, 1.13 ± 0.09 ($p < 0.05$). The cytoplasmic RNA was hybridized to the [^{3}H]poly(U) in the following ratios: (a) 30 μg:0.1 μg; (b) 30 μg:0.15 μg; and (c) 30 μg:0.2 μg. At the lowest concentration of [^{3}H]poly(U) (0.1 μg) only a heterogeneous pattern was obtained on sucrose density gradient centrifugation, without any differences in the aldosterone-treated and control populations (pool of ten paired hemibladders) (upper panel, Figure 5). At an intermediate concentration of [^{3}H]poly(U) (0.15 μg), the hybrids from the aldosterone-treated group gave a differential sedimentation peak at 9 S (middle panel, Figure 5). The 4S peaks, of the same magnitude in both groups, represent free [^{3}H]poly(U), as defined by the calibration curve shown in the lowest panel. At the highest concentration of [^{3}H]poly(U) (0.20 μg), the free peak (4 S) is increased in both but to a greater extent in the control than in the aldosterone-treated populations. In addition, the differential peak at 9 S remains invariant but another differential peak, of even greater magnitude, appears in the 14S region. The results shown in Figure 5 are representative of two separate experiments, each made up of a pool of ten paired hemibladders. These findings imply either that aldosterone augmented the pool of poly(A)(+)-RNA or the affinity of the cytoplasmic RNA for binding to poly(U). The evidence summarized above is consistent with the inference that aldosterone stimulates poly(A)(+)-RNA synthesis during the latent period, i.e., the first 30 min of exposure to the steroid.

As the enhanced Na^+ transport is maintained indefinitely as long as the aldosterone is present in the medium, the effect on labeling of the poly(A)(+)-RNA might also be sustained. To evaluate this possibility, labeling experiments were done in paired hemibladders: during the first 30 min of exposure to aldosterone in one, and after preincubation in aldosterone (7×10^{-8} M) for 150 min in the other.[2] At the time of addition of the [^{3}H]uridine to the medium, the scc_t/scc_0 ratio was 2.56 ± 0.47 in the experimental hemibladders and 1.44 ± 0.13 in the controls ($p < 0.05$). The results in Table II indicate that labeling of the poly(A)(+)-RNA in both the nuclear and cytoplasmic fractions was greater during the first 30 min of exposure to aldosterone than after 150 min of exposure to the steroid. Similar differences are apparent in the high-salt eluates. It is unlikely that the differences in active Na^+ transport account for the differences in RNA metabolism since the scc was lower in the control group (i.e., exposed to aldosterone for only 30 min). These findings raise the possibility that aldosterone evokes an initial burst of synthesis of HnRNA and mRNA some of which is not sustained.

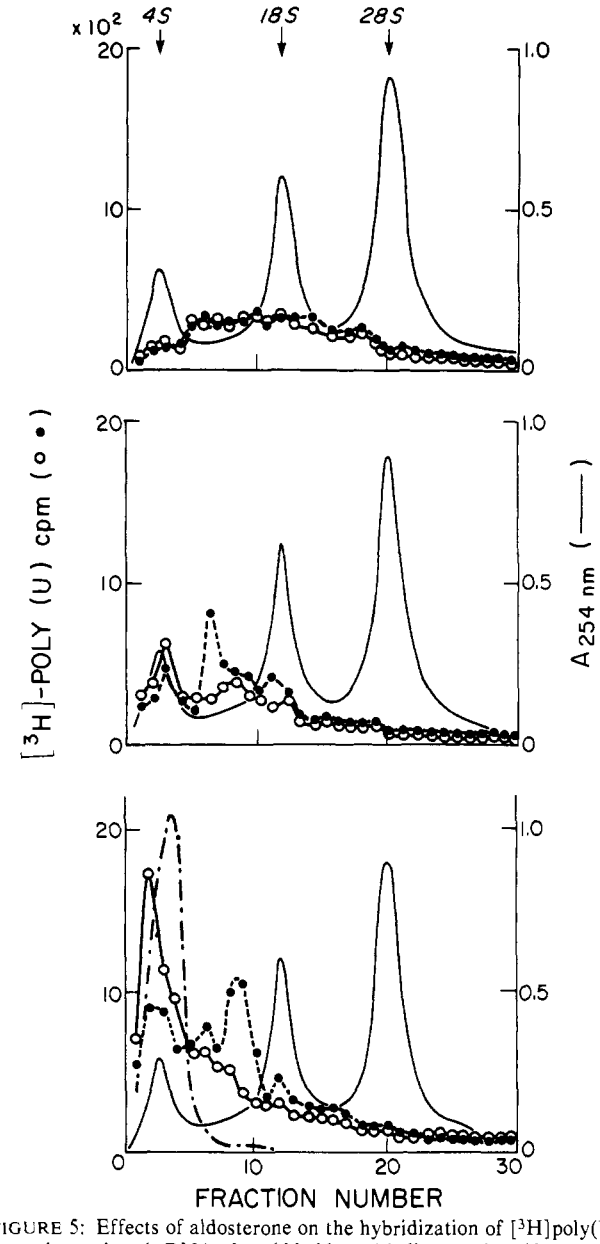

FIGURE 5: Effects of aldosterone on the hybridization of [^{3}H]poly(U) to total cytoplasmic RNA of toad bladder epithelium analyzed by sucrose density gradient. Pairs of hemibladders were incubated with aldosterone (7×10^{-8} M) or the diluent for 240 min and cytoplasmic extracts of the epithelia were prepared and hybridized with [^{3}H]poly(U), as described previously in the text. The ^{3}H activity of the control (O) and of the aldosterone treated tissue (●) is indicated by the ordinates on the left. The absorbance at 254 nm (solid line) is indicated by the ordinates on the right. Sedimentation is from left to right. (Upper panel) Sucrose density gradient analysis of the products of hybridization of 30 μg of total cytoplasmic RNA with 0.1 μg of [^{3}H]poly(U). (Middle panel) Sucrose density gradient analysis of the products of hybridization of 30 μg of total cytoplasmic RNA with 0.15 μg of [^{3}H]poly(U). (Lower panel) Sucrose density gradient analysis of the products of hybridization of 30 μg of total cytoplasmic RNA with 0.2 μg of [^{3}H]poly(U). The dashed line indicates the sedimentation profile of 0.2 μg of free [^{3}H]poly(U) run on a parallel gradient. scc_t/scc_0 ratio of the aldosterone treated group was 2.29 ± 0.59 and of the control group 1.13 ± 0.09 SEM ($n = 9, p = <0.05$). scc_t/scc_0 is the short-circuit current in μA per hemibladder at time t (t = 240 min) divided by the short-circuit current at t_0 (scc_0) i.e., time of addition of aldosterone (7×10^{-8} M) or the diluent.

Discussion

The calibration experiment shown in Figure 2 indicates that

[2] Eight pairs of hemibladders were preincubated for 5 h in steroid-free, glucose (5 mM)-frog Ringer's solution. At time zero aldosterone (7×10^{-8} M) was added to the media (both sides) of the experimental hemibladders and the diluent to the controls. At 150 min, aldosterone (7×10^{-8} M) was added to the media of the controls and all hemibladders were labeled with [^{3}H]uridine for 30 min. Thus, at the time of sampling, the epithelia were exposed to aldosterone for 180 min in the experimental and for 30 min in the control groups.

TABLE II: [^{3}H]Uridine Incorporation into Poly(A)(+)-RNA Either 0–30 min or 0–180 min after Aldosterone.[a]

Cell Fraction	Time after Aldosterone (min)	0.5 M KCl (cpm/A_{260})	0.01 M Tris (cpm/A_{260})
Nucleus	0–30	8730	72 000
	150–180	2980	39 360
Ratio of incorporation [(0–30)/(150–180)]:		2.92	1.83
Cytoplasm	0–30	3550	48 800
	150–180	2500	28 300
Ratio of incorporation [(0–30)/(150–180)]:		1.42	1.72

[a] Eight paired hemibladders were exposed in sequence to aldosterone (7×10^{-8} M). One of each pair was treated at zero time and the other 150 min later. Both pairs were then incubated in [^{3}H]uridine for 30 min. The nuclear and cytoplasmic fractions were prepared and analyzed by oligo(dT)-cellulose chromatography as described in the text. The nuclear fractions were not cleared of DNA prior to chromatography. Thus the specific activities of the high salt eluates (0.5 M KCl) of the nuclear fractions had to be expressed per A_{260} units. To preserve uniform dimensions, all other fractions were also referred to the A_{260} values.

little rRNA or tRNA is present in the poly(A)(+)-RNA fraction, as no [^{14}C]methyl was detected in the low-salt eluates. Owing to the low specific activity of the [^{14}C]methyl label (10 mCi/mmol), however, minor degrees of contamination with rRNA cannot be excluded. Perry and Kelley (1974) recently showed that mammalian mRNA is also methylated but only at about 14% of that in rRNA. Although the exclusion of [^{14}C]methyl from the low-salt eluates may indicate that anuran mRNA is not methylated, it is entirely possible that the specific activity of the methyl donor was too low to detect the methyl moiety in the poly(A)(+)-RNA.

Augmentation by aldosterone of the labeling of cytoplasmic poly(A)(+)-RNA with [^{3}H]uridine during the latent period, i.e., the first 30 min, implies either: an effect on the precursor pool, the synthesis of the gene product (e.g., HnRNA), processing of the gene product to poly(A)(+)-RNA, transport of the poly(A)(+)-RNA into the cytoplasm, or a diminished rate of degradation. A precursor pool effect seems improbable for two reasons: (1) in earlier studies (Rossier et al., 1974) aldosterone did not alter the recovery of [^{3}H]uridine in the acid-soluble fraction; (2) the augmented incorporation of [^{3}H]uridine was not uniformly distributed across the sucrose density gradient profiles of the poly(A)(+)-RNA (Figures 3 and 4). The results presented here do not, however, distinguish among the remaining possibilities alluded to above. In other systems, steroid hormones promote a net accumulation of specific mRNAs, e.g., ovalbumin-mRNA, tryptophan oxygenase-mRNA (Rhoads et al., 1971; Comstock et al., 1972; Palmiter, 1974; Schutz et al., 1973). With [^{3}H]uridine, aldosterone enhanced the labeling of 12S and 18S poly(A)(+)-RNA (Figure 3) and with [^{3}H]uridine + [^{3}H]adenosine, the peaks were at 7 S and 12 S (Figure 4). These results are in accord with selective labeling of particular mRNAs, rather than a general effect, e.g., processing by polyadenylation or transport into the cytoplasm. Augmented labeling of 12S RNA by aldosterone was noted previously (Rossier et al., 1974). The 18S peak (Figure 3) may represent a distinct class of higher molecular weight mRNAs. Alternatively, it may result simply from aggregation of the labeled poly(A)(+)-RNA to other species in that we did not use denaturing conditions in the sucrose density gradients. The 7S peak obtained on labeling with [^{3}H]adenosine may represent either low-molecular-weight mRNAs, with relatively long poly(A) tails, or degraded species in which the poly(A) tail is preserved owing to its resistance to ribonucleases (Perry et al., 1973).

Both in the present studies and that reported previously (Rossier et al., 1974), aldosterone enhanced the labeling of a 12S species, largely nonmethylated. This effect may be indicative of an hormonally induced increase in the quantity of poly(A)(+)-RNA in that the sucrose density gradient analysis of the low-salt eluates from the oligo(dT) column revealed peaks at 7, 12, and 18 S. To assess this possibility further, hybridization to [^{3}H]poly(U) was used. The results shown in Figure 5 are very similar to those of Fraser and Loening (1973). At suboptimal concentration ratios of [^{3}H]poly(U) to cytoplasmic RNA, only a broad, nondistinctive pattern was obtained (uppermost panel). Under optimal conditions, free [^{3}H]poly)u) was present in the gradient and aldosterone-dependent peaks of labeled hybrids were obtained at 9 and 14 S (middle and lowest panel). These results are consistent with the accumulation of increased amounts of 7S and 12S poly(A)(+)-RNA under the influence of aldosterone. Fraser and Loening (1973), for example, found that hybridization to [^{3}H]poly(U) resulted in a 1.5- to 2-unit shift in the sedimentation coefficient of globin mRNA. The alternative possibility of an aldosterone-dependent increase in the affinity of the poly(A)(+)-RNA for the [^{3}H]poly(U) must also be considered. For example, Slater and Slater (1974) noted extranuclear polyadenylation of RNA after fertilization of sea urchin embryos. Thus, if aldosterone increased the poly(A) chain length, enhanced binding to poly(U) might be observed. This explanation, however, is less likely to apply in the presence of a large excess of poly(U) (lowest panel). It appears, therefore, that aldosterone promoted a net gain in the epithelial content of poly(A)(+)-RNA.

The possibility that aldosterone produces a transient effect on labeling of poly(A)(+)-RNA was evaluated by comparing incorporation of [^{3}H]uridine during the first 30 min of exposure to the hormone to incorporation in the interval from 150 to 180 min. The incorporation of [^{3}H]uridine into the poly(A)(+)-RNA fraction was significantly greater during the first 30 min of the action of the hormone. This result agrees closely with the findings of Yu and Feigelson (1969): Cortisone augmented the synthesis of hepatic (A+U)-rich RNA during the first hour of treatment and the effect waned during the 2d to the 6th hours. If these findings signal an initial burst of synthesis of mRNA that is only partially sustained thereafter, measurements of the time course of the rate of degradation of the newly synthesized species may be of particular importance.

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References

Aviv, H., and Leder, P. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 1408.

Bard, D., Efron, E., Marcus, A., and Perry, R. P. (1974), *Cell 1,* 101.

Chan, L., Means, A. R., and O'Malley, B. W. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 1870.

Comstock, J. P., Rosenfeld, G. C., O'Malley, B. W., and Means, A. R. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 2377.

Fraser, R. F., and Loening, U. E. (1973), *Eur. J. Biochem. 34,* 153.

Huez, G., Marbaix, G., Hubert, E., Leclercq, M., Nudel, U., Soreq, H., Salomon, R., Lebleu, B., Revel, M., and Littauer, U. Z. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 3143.

Jelinek, W., Adesnik, M., Salditt, M., Sheiness, D., Wall, R., Molloy, G., Philipson, L., and Darnell, J. E. (1973), *J. Mol. Biol. 75,* 515.

Lahav, M., Dietz, T., and Edelman, I. S. (1973), *Endocrinology 92,* 1685.

Palmiter, R. D., and Carey, N. H. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 2357.

Penman, S. (1966), *J. Mol. Biol. 17,* 117.

Perry, R. P., Greenberg, J. R., Kelley, D. E., LaTorre, J., and Schochetman, G. (1973), in Gene Expression and Its Regulation, Kenney, F. T., Hamkalo, B. A., Favelukes, G., and August, J. T., Ed., New York, N.Y., Plenum Publishing Co., p 149.

Perry, R. P., and Kelley, D. E. (1974), *Cell 1,* 37.

Porter, G. A., and Edelman, I. S. (1964), *J. Clin. Invest. 43,* 611.

Rhoads, R. E., McKnight, G. S., and Schimke, R. T. (1971), *J. Biol. Chem. 246,* 7407.

Rossier, B. C., Wilce, P. A., and Edelman, I. S. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 3101.

Schutz, E., Beato, M., and Feigelson, P. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 1218.

Slater, I., and Slater, D. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 1103.

Swan, D., Aviv, H., and Leder, P. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 1967.

Wagner, A. F., Bugianesi, R. L., and Shen, T. Y. (1971), *Biochem. Biophys. Res. Commun. 45,* 184.

Walser, M., Butler, S. E., and Hammond, V. (1969), *J. Clin. Invest. 48,* 1714.

Wilce, P. A., Rossier, B. C., and Edelman, I. S. (1974), *J. Steroid Biochem. 5,* 351.

Yu, F. L., and Feigelson, P. (1969), *Biochem. Biophys. Res. Commun. 35,* 499.