Treatment with strophanthidin increases the sensitivity of the resting membrane potential to external K^+. (v) This increase is related to the internal sodium concentration, and is (vi) prevented by previous exposure to cyanide.

This demonstration that the sodium pump of squid axon, too, is electrogenic reinforces the hypothesis (*1*) that a truly neutral Na-K pump may not, in fact, exist.

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7. In 20 axons the drug-induced depolarization was stable, as in Fig. 1; in another seven axons the membrane potential decreased and then returned within 1 to 3 minutes to a value somewhere between the original resting potential and the peak depolarization. We could not correlate this unusual behavior with obvious parameters such as age, resting potential, or sodium content.
8. These experiments were performed at the Marine Biological Laboratory, Woods Hole, Massachusetts. Supported by PHS grants NS09380 and NS08977. P.D.W. was holder of a research career development award (GM 46373) of the National Institute of Neurological Diseases and Stroke.
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Polyadenylic Acid in Visna Virus RNA

Abstract. *Visna virus 70S RNA contains long stretches of polyadenylic acid [poly(A)]. The homogeneity in length of poly(A) regions is observed in 70S RNA from visna virus and all RNA tumor viruses tested, and not with other types of RNA. By this criterion visna virus resembles RNA tumor viruses.*

Data have been presented showing that the genomic RNA of all RNA tumor viruses (oncornaviruses) that have been studied contain polyadenylic acid [poly(A)] residues of large and rather homogeneous size (*1*). In that study, poly(A) was detected by molecular hybridization to [^{3}H]polyuridylic acid [poly(U)]; although more conventional assay techniques have obtained the same result (*2*). The genome of the RNA-containing tumor virus appears to be unique in having 70*S* RNA with long, relatively homogeneous stretches of poly(A). Poly(A) regions from most mature RNA preparations are heterogeneous in size. This appears to be the case for pure RNA preparations such as poliovirus RNA (*3*) and hemoglobin messenger RNA (mRNA) (*4*), as well as for the bulk poly(A)-containing RNA in the cytoplasm of cells (*5*). Similar results are presented in this report. The one case where poly(A) in RNA appears to be of uniform size is that of newly synthesized cellular RNA (*6*); however, unlike the RNA tumor virus genome, *this RNA* is of heterogeneous size.

The list of RNA tumor viruses having a genomic 70*S* RNA with long, relatively homogeneous stretches of poly(A) now includes Rous sarcoma virus, avian myeloblastosis virus, feline leukemia virus (Rickard), feline sarcoma virus (Gardner), Rauscher leukemia virus, murine sarcoma virus (Kirsten-Gross), murine mammary tumor virus (C_3H), and a primate virus, the Mason Pfizer agent. Since this configuration has not been observed in RNA from any other source, it is reasonable to assume the situation is diagnostic and to search for it in agents where oncogenic potential is controversial.

Visna virus constitutes such an agent. This RNA-containing particle causes a chronic neurological disorder in sheep (*7*). Although similar in several characteristics to the tumorigenic RNA-containing viruses (*8, 9*) it was not classified with this group. The reports that visna virus preparations contain RNA-dependent DNA polymerase activity (*10–12*) and 70*S* RNA (*9, 13*) led to the suspicion that this virus may also be tumorigenic. These biochemical observations encouraged further biological evaluation and led to the finding that visna virus can induce transformation in vitro (*14*).

We report here that the 70*S* RNA of visna, like that of the RNA tumor viruses, also contains large, homogeneous residues of poly(A).

On rate zonal centrifugation, RNA isolated from visna virus shows (Fig. 1) a peak of poly(A)-containing material in fractions 7 and 8. This is the same position where the poly(A)-containing RNA of avian myeloblastosis virus was found in a separate gradient (not shown). Hybridization of the 70*S* material to [^{3}H]poly(U) was completely abolished by preliminary treatment with 0.4*M* NaOH for 18 hours at 30°C, a finding consistent with its identification as an RNA molecule. Of two preparations of visna virus RNA examined, both showed this 70*S* poly(A)-containing material; however, the gradient which is not presented also showed a poly(A)-containing peak in fractions 3 and 4. We have observed similar rapidly sedimenting RNA, larger than the 70*S* RNA species, in preparations of RNA from tumor

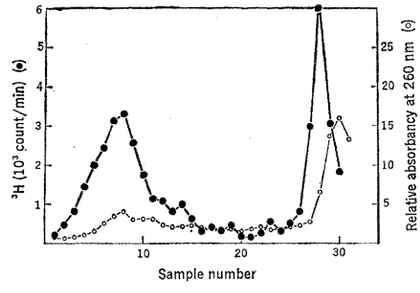

Fig. 1. 70*S* poly(A)-containing RNA from visna virus. The procedure for the growth and purification of visna virus was essentially the same as described previously (*10, 11*). Virus was precipitated in 6 percent polyethylene glycol (6000 daltons). The resuspended virus was clarified by centrifugation at 5000*g* for 10 minutes, and then sedimented at 100,000*g* for 60 minutes. The virus was resuspended in TNE buffer (0.01*M* tris, *p*H 7.5, 0.1*M* NaCl, 0.001*M* EDTA) and centrifuged on preformed gradients of 20 to 60 percent sucrose in TNE. The virus band was removed, diluted with TNE buffer, centrifuged for 1 hour at 100,000*g*, and resuspended in TNE buffer. The virus suspension was extracted twice with TNE buffer saturated with a mixture of phenol and cresol (1 : 0.15) and once with chloroform. The resulting aqueous phase was treated with potassium acetate to a concentration of 2 percent, and nucleic acids were then precipitated with 2.2 volumes of ethanol. The precipitate was centrifuged, dissolved in 0.5 ml of TNE buffer, and applied to a linear glycerol gradient (10 to 30 percent) in TNE buffer. The RNA was sedimented for 4 hours at 40,000 rev/min (4°C in a SW41 Beckman rotor). Fractions of 0.4 ml were collected. The poly(A) content of 0.025 ml of each fraction obtained from glycerol gradient centrifugation was determined by hybridization to [^{3}H]poly(U) (2×10^7 count/min per microgram) as previously described (*1*).

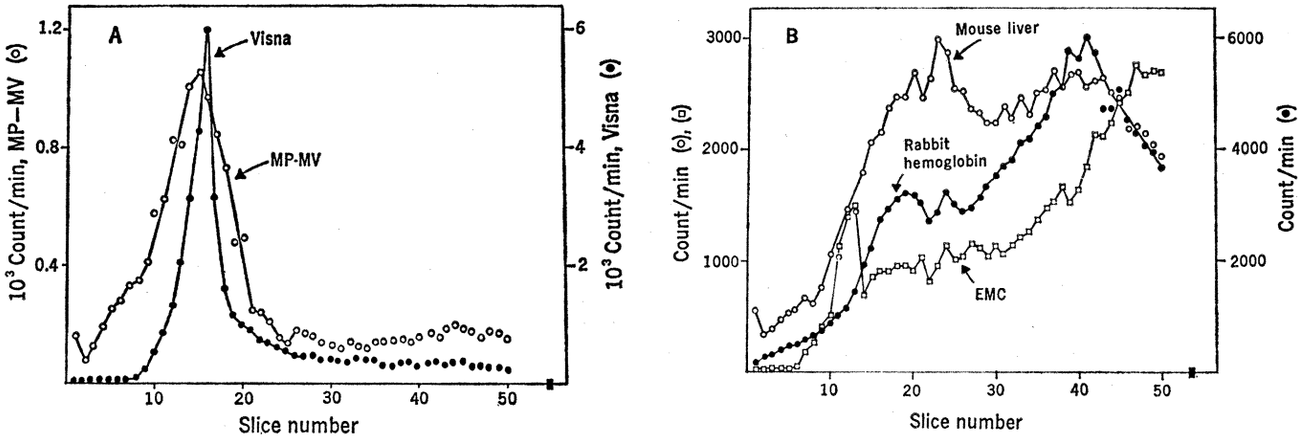

Fig. 2. Size of the poly(A) regions in visna virus 70*S* RNA. [^{3}H]Poly(U) was hybridized to 0.2 ml of glycerol gradient fraction number 7 (Fig. 1) in a hybridization mixture (0.6 ml) of standard composition (*1*) at 36°C for 24 hours. After hybridization, nuclease digestion was carried out at 30°C for 2 hours by the addition of 2 ml of a solution containing 5 μg (per milliliter) of boiled pancreatic ribonuclease and 5 μg of deoxyribonuclease in 0.01*M* tris, *p*H 7.4; 0.01*M* $MgCl_2$; and 0.5*M* NaCl. The products of nuclease digestion were extracted once with chloroform and isoamyl alcohol, precipitated with ethanol, and analyzed in 7.5 percent polyacrylamide gels (*1*). The size of the poly(A) regions in (A) RNA from the Mason Pfizer agent and visna virus and in (B) mouse liver RNA, encephalomyocarditis virus RNA, and rabbit reticulocyte total hemoglobin messenger RNA were determined as above from appropriate amounts of RNA.

viruses and from human leukemic lymphocyte cytoplasm (*15*). Its origin has not been established.

Poly(A) material in the sucrose gradient (Fig. 1) is not restricted to the high-molecular-weight RNA. Material having a sedimentation of 4*S*, or slightly larger, also contains poly(A). It is not known whether the low-molecular-weight material originates in the virion, but similar material has also been routinely observed in gradients of RNA prepared from several batches of Rauscher leukemia virus and avian myeloblastosis virus, while it is not found in RNA isolated from their host cells.

The size of the poly(A) region in visna virus 70*S* RNA was measured by polyacrylamide gel electrophoresis (Fig. 2A). The size distribution of the poly(A) regions is extremely homogeneous and is approximately the same as the size of poly(A) regions recovered from the Mason Pfizer agent (Fig. 2A). This agent was isolated from a monkey mammary tumor and is capable of causing lymphoadenopathy when introduced into normal monkeys (*16*). The poly(A) in the RNA of this agent is the same size as poly(A) regions in RNA of other tumor viruses (*1*). As reference standards, the size of poly(A) regions from mouse liver RNA, rabbit reticulocyte hemoglobin mRNA, and encephalomyocarditis (EMC) virus RNA are also presented (Fig. 2B). To facilitate direct comparison, these analyses were carried out in parallel with the tumor virus RNA analyses, with the same poly(U) preparation. In the reference standards, the poly(A) regions display a more heterogeneous size distribution and an average higher electrophoretic mobility than the Mason Pfizer virus and the visna virus. This heterogeneity does not arise from the formation of complexes between poly(U) and RNA regions that do not contain poly(A) because (i) poly(U) saturates cellular RNA when the ratio by weight of poly(U) to poly(A) is 2:1 (*17*) and (ii) the inclusion of a 1000-fold excess of RNA that does not contain poly(A) (for example, polyguanylic acid) does not affect the rate of formation of hybrids between poly(U) and poly(A) RNA (*18*). The poly(A) regions of visna virus RNA resemble those in the RNA of RNA tumor viruses more than the other RNA types examined.

Interestingly, RNA from EMC virus does exhibit some long poly(A) sequences in our assay. This virus belongs to the picornavirus group, as does poliovirus, although in the case of poliovirus, the genomic poly(A) tracts are reported to be 50 (*1*, *19*) or 100 (*3*) nucleotides in length. Possibly the length of the poly(A) regions from these virus particles will depend on factors of physiology, such as the host cell line, state of maturity, or other undetermined factors.

Our results indicate that the average size of poly(A) tracts in RNA from proliferating cells active in protein synthesis (for example, the mouse liver RNA presented in Fig. 2B) is smaller than the size of poly(A) tracts from the RNA tumor viruses. The latter regions appear to be equivalent in size to poly(A) tracts only of newly synthesized RNA in cells—that is, about 200 nucleotides (*6*, *20*). These data support the idea that the length of poly(A) regions in mRNA may decrease as a function of time in the cytoplasm, possibly as the mRNA molecule is used as a template for protein synthesis (*4*). The size distribution of the poly(A) tracts in rabbit reticulocyte mRNA (Fig. 2B) is also consistent with this model.

We have stressed the potential use of the [^{3}H]poly(U) hybridization technique for detecting poly(A)-containing RNA in situations where only small amounts of RNA can be isolated and where labeling with radioisotopes is not feasible (*1*). We have now demonstrated that the method can be used where only a minute quantity of material was obtained. Moreover, experiments with human milk preparations (*21*) have indicated that the assay is effective for detecting the presence of viral-like RNA when virus particles either cannot be detected with certainty or are observed only with difficulty. Also, the technique has been used as an indirect measure of the titer of mRNA in developing cells (*17*).

Finally, our findings add further information on the close physical, biochemical, and biological similarities between visna virus and oncornaviruses. These now include virion morphology and size (*7*, *8*, *13*), buoyant density (*11*, *13*), 70*S* RNA (*9*, *13*), reverse

transcriptase activity (*10–12*), transforming ability in vitro (*14*), and poly(A) content and size.

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22. We thank S. Gillespie and C. Davis for assistance in preparing and analyzing the visna RNA; to A. Shatkin, D. Housman, J. Schlom, and S. Marshall for preparations of EMC virus RNA, rabbit reticulocyte hemoglobin mRNA, MP-MV RNA, and mouse liver total RNA, respectively. M.R. holds a fellowship from the Leukemia Society of America, Inc.

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Isolated Adrenal Cortex Cells: Hypersensitivity to Adrenocorticotropic Hormone after Hypophysectomy

Abstract. *Cells from the adrenals of hypophysectomized rats (up to 28 days after operation) require less adrenocorticotropic hormone to induce one-half maximal rate of production of 3′,5′-adenosine monophosphate than do cells from the adrenals of intact rats. A corresponding increase in sensitivity is reflected in the steroidogenic response to adrenocorticotropic hormone up to 2 days after hypophysectomy.*

In the absence of pituitary tropic hormones, target endocrine glands develop a depression of secretory capacity. For example, in vivo studies have demonstrated that hypophysectomy is followed by a reduction in the rate of secretion of corticosteroids in response to a given dose of adrenocorticotropic hormone (ACTH) (*1*). On the other hand, rate of accumulation of the "second messenger," 3′,5′-adenosine monophosphate (cyclic AMP) is not diminished in response to the administration of ACTH (*2*). This interesting difference prompted us to examine the two responses in vitro; both ACTH-induced accumulation of cyclic AMP and steroidogenesis have been determined in suspensions of isolated cells prepared from adrenals removed at various times after hypophysectomy.

Suspensions of cells were prepared from the fasciculata-reticularis region of 32 to 40 rat adrenals according to the method of Sayers *et al.* (*3*). Thirty microcuries of [8-^{14}C]adenine were added to the dispersion medium for the labeling of adenosine triphosphate, the precursor of cyclic AMP. After dispersion with trypsin, the cell suspensions were centrifuged, and the pellet was resuspended in a volume of buffer medium so that each 0.9-ml portion taken for incubation contained approximately 350,000 cells. Cortrosyn ($ACTH_{1-24}$) (Organon Inc.) was added in a volume of 0.1 ml of buffer medium. This medium only, in a volume of 0.1 ml, was added to blanks. After 60 minutes of incubation, cells plus medium were extracted with methylene chloride. In samples of the aqueous layer cyclic AMP labeled with ^{14}C was determined by the method of Kuo and De Renzo (*4*), as modified for use in isolated adrenal cortex cells by Beall and Sayers (*5*). Corticosterone in the methylene chloride extracts was determined by the method of Silber *et al.* (*6*).

Logarithmic dose response curves for production of cyclic AMP and of corticosterone by isolated adrenal cortex cells (350,000 cells per milliliter of incubate) in response to various doses of $ACTH_{1-24}$ are displayed in Figs. 1 and 2, respectively. The isolated cells are characterized by the following parameters: (i) $cAMP_{max}$ and B_{max}, the maximum rate of production of cyclic AMP and the maximum rate of production of corticosterone, respectively, and (ii) A_{50C} and A_{50B}, the doses of $ACTH_{1-24}$ that induce one-half $cAMP_{max}$ and B_{max}, respectively. Computer estimates of these parameters with standard errors represent least square fits by a nonlinear method (*7*). The data presented in Fig. 1 show that $cAMP_{max}$ for cells prepared from the adrenals of hypophysectomized rats was greater than $cAMP_{max}$ for cells from intact rats. The increases for rats hypophysectomized for 2, 14, and 28 days were 33 ± 2.6, 15 ± 2.5, and 25 ± 3.4 percent, respectively, over values for intact rats. The A_{50C} was estimated to be 760 ± 150 pg for cells from intact rats and 160 ± 20, 240 ± 30, and 160 ± 30 pg for cells from rats hypophysectomized for 2, 14, and 28 days, respectively. The data indicate that the cells from hypophysectomized rats are about five times more sensitive to ACTH than cells from intact rats.

As expected, B_{max} progressively decreased with time after hypophysec-

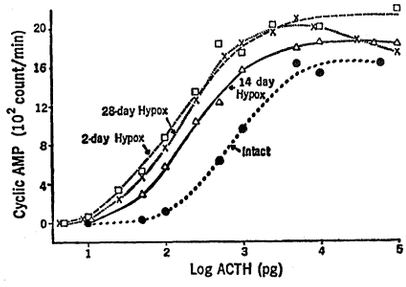

Fig. 1. Logarithmic dose response curves for cyclic AMP production by samples of adrenal cortex cells in suspension prepared from the fasciculata-reticularis region of adrenals of intact rats and of rats killed 2, 14, and 28 days after hypophysectomy. Net cyclic AMP production, as counts per minute of cyclic [8-^{14}C]AMP per 350,000 cells per 60 minutes of incubation, is plotted on the ordinate; dose of $ACTH_{1-24}$ in picograms on the abscissa. The points are the means of analyses on three samples of the cell suspension; *Hypox*, hypophysectomized.