tration was varied. The percent of bound methadone increased as the concentration of albumin increased, and this relationship was linear on a semilogarithmic plot of percent of bound methadone versus the log of the albumin concentration.

The percentage of methadone bound to albumin was, however, relatively independent of the methadone concentration when examined at two albumin concentrations, 0.400 g per 100 ml of solution ($6.13 \times 10^{-5}M$) and 5.00 g per 100 ml of solution ($7.67 \times 10^{-4}M$), except that slightly less methadone was bound at the higher concentrations of methadone (Table 2). Because the molar concentrations of drug do not exceed the molar concentrations of albumin studied, saturation of protein binding sites is not likely a factor in the results obtained.

Borgå *et al.* (*2*), using dilutions of human plasma, found that, for desmethylimipramine and nortriptyline, the plot of the ratio of unbound to bound drug against the reciprocal of the plasma concentration was a straight line through the origin. A similar plot of the methadone data from Table 1 is a straight line, but it does not go through the origin. The regression equation for this line is $R = (6.79 \times 10^{-4})A + 0.213$ (*7*), where R is the ratio of unbound to bound L-[1-^{3}H]methadone, and A is the reciprocal of the molar concentration of albumin.

A Scatchard plot r/D versus r, where r is the number of moles of drug bound per mole of albumin, and D is the concentration of unbound drug, results in a curved line (with the binding data at an albumin concentration of 0.400 g/100 ml from Table 2), an indication that there may be several binding sites on the albumin molecule with different affinities for methadone.

To my knowledge, this is the first report on the binding of methadone to human plasma albumin, and the first quantitative study on the protein binding of any of the narcotic analgesics. The results presented here and the two studies by Borgå *et al.*, and Franksson and Änggård (*2*) on other basic drugs, reveal that the binding to protein of some basic drugs differs from that of some acidic drugs as warfarin (*8*), insofar as the percent of the basic drug that is bound seems to be relatively independent of drug concentration.

GEORGE D. OLSEN
University of Oregon Medical School, Portland 97201

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4. A report of postmortem studies of concentrations of methadone in human blood indicated a range of 0.22 to 3.04 μg/ml. Several of the subjects had taken overdoses of methadone, so the upper value may be atypical for therapeutic situations [A. E. Robinson and F. M. Williams, *J. Pharm. Pharmacol.* **23**, 353 (1971)].
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Polyadenylic Acid Sequences in the Virion RNA of Poliovirus and Eastern Equine Encephalitis Virus

Abstract. *Poliovirus virion RNA contains a single covalently bound sequence of polyadenylic acid which is approximately 49 nucleotides long. A single, slightly longer polyadenylic acid sequence is contained in Eastern Equine Encephalitis virus RNA. Since these viruses are otherwise dissimilar these sequences may play a common role in viral replication, possibly in translation of the viral RNA.*

The virion RNA of two small, unrelated animal viruses, poliovirus, a picornavirus, and Eastern Equine Encephalitis virus (EEE), a group A arbovirus, has been found by us to contain polyadenylic acid [poly(A)]sequences. We now report the quantity, size, and base composition of poly(A) sequences released from each viral RNA by ribonucleases. The data is discussed in light of the known infectious nature of purified RNA from these two viruses and the discovery of poly(A) sequences in the messenger RNA's (mRNA) from mammalian cells (*1–4*) and from several animal viruses (*5–7*).

The EEE virus (New Jersey strain) was prepared in chick embryo cell cultures infected at a multiplicity of 0.1 plaque-forming unit per cell. After an attachment period of 1 hour at 37°C, 240 μc of carrier-free $^{32}PO_4$ was added to each 5-cm dish in phosphate-free Hanks salt solution containing 3 percent calf serum, freed of γ-globulin. Virus was harvested 18 hours later from the supernatant. The virus was concentrated and purified by elution from aluminum phosphate gel (*8*) and zone sedimentation on sucrose gradients (5 to 20 percent), and subsequent sedimentation to a pellet at 100,000*g*.

Poliovirus (type 1, Mahoney) was grown in suspension culture HeLa cells with an input multiplicity of 100 plaque-forming units per cell. The cells were at a concentration of 4×10^6 per milliliter in phosphate-free Eagle's minimal essential medium supplemented with 4 mM glutamine and actinomycin D (4 μg/ml). After 30 minutes, dialyzed calf serum (5 percent) and 18 mc of carrier-free $^{32}PO_4$ was added. The cells were harvested 6.5 hours later by centrifugation and were then subjected to four cycles of freezing and thawing to liberate the virus. The virus was purified by banding at sedimentation equilibrium in a cesium chloride solution (*9*).

RNA was purified from both viral preparations by three successive extractions with hot phenol of virus suspended in buffered sodium dodecylsulfate solution, as described (*10*). The size of the poliovirus RNA was analyzed by sucrose gradient sedimentation with HeLa cell ribosomal RNA's as sedimentation markers. A portion of poliovirus RNA was also sedimented on a sucrose gradient after being dissolved in a dimethyl sulfoxide (DMSO) solution together with an excess of small ($< 10S$), unlabeled, synthetic poly(A). In this latter analysis the DMSO would have denatured any double-stranded RNA present (*11*) while the added unlabeled poly(A) would prevent any contaminating small radioactive poly(A) molecules from readhering to the viral RNA before sedimentation. These sedimentation analyses both indicated that

the poliovirus RNA preparation was predominantly composed of intact 35*S* molecules. A portion of RNA taken from the sedimentation peak of the poliovirus RNA dissolved in DMSO solution with unlabeled poly(A) was collected to provide a sample of viral RNA that could be assumed to be free of any adventitiously bound radioactive poly(A).

The purified viral RNA's were quantitatively analyzed for poly(A) by a method (*2*) which includes digestion with pancreatic and T1 ribonucleases followed by binding of any poly(A) present to polythymidylate cellulose. Bound poly(A) was subsequently eluted by a change of temperature and salt concentrations, and a portion of the eluate was used for a determination of the percentage of initial ^{32}P in poly(A) sequences. An internal standard of synthetic [^{3}H]poly(A) added to the viral RNA before nuclease treatment permitted correction for losses during the procedure.

Two different preparations of poliovirus RNA each contained 0.75 percent poly(A), while a similar proportion of poly(A), 0.69 percent, was found in EEE RNA. We conclude that the poly(A) detected in poliovirus RNA is covalently part of that RNA sequence, since the proportion, 0.76 percent, of poly(A) was unchanged when the RNA was further purified by zone sedimentation following solution in the presence of DMSO and unlabeled poly(A) molecules.

The reported molecular weight of the poliovirus virion RNA is 2.4 to 2.5 $\times$ 10^6 (*12*). From this value and the reported base composition of the RNA (*13*), it can be calculated that the poly(A) sequences in poliovirus RNA constitute about 53 to 55 nucleotides. Electrophoretic and compositional data indicate that poliovirus poly(A) has a size of this order, so there must be one such sequence per virion RNA molecule. Exact physical measurements of the size of EEE virion RNA are not available, but from its sedimentation rate in sucrose gradients, the molecular weight has been estimated to be 3.5 $\times$ 10^6 (*14, 15*). We have determined that the base composition of ^{32}P-labeled EEE virus virion RNA by conventional methods (*2*) is: adenosine monophosphate (AMP), 26.4 percent; uridine monophosphate (UMP), 27.2 percent; guanosine monophosphate (GMP), 24.8 percent; cytidine monophosphate (CMP), 21.4 percent. From these data we calculate that the poly(A) of EEE RNA includes approximately 70 nucleotides. Our electrophoretic analysis of EEE poly(A) suggests that, as with poliovirus RNA, this poly(A) is present as one sequence in each EEE virion RNA molecule.

Table 1. Nucleotide analysis of poly(A) sequences. The poly(A) content of ^{32}P-labeled virion RNA and the nucleotide composition of the poly(A) were determined as in (*2*).

Nucleotide	Poliovirus		EEE	
	Count/min	%	Count/min	%
CMP	900	2.0	12	1.2
AMP	45,000	97.4	970	95.8
GMP	194	0.31	10	0.9
UMP	213	0.33	22	2.1

The poly(A) from each virus migrates in acrylamide gel electrophoresis as one peak (Fig. 1), with a mobility higher than that of the poly(A) from the corresponding host cell RNA but lower than the mobility of transfer RNA (tRNA). The relative mobility of poly(A) from EEE RNA appears to be slightly less than that from poliovirus RNA, indicating a small difference in length between the two. The fact that the viral poly(A)'s move more slowly in gels than tRNA is not incompatible with their being shorter in chain length (about 50 as opposed to 75 to 80 nucleotides) since molecules of poly(A) have little secondary structure at neutrality, unlike the compactly folded tRNA's. We find that poly(A) fractions sedimenting at 8*S* to 10*S* migrate more slowly than 18*S* ribosomal RNA in polyacrylamide gel electrophoresis.

Analysis of a heavily radiolabeled sample of poliovirus poly(A) gave the nucleotide composition shown in Table 1. The CMP was detected as a distinct peak that followed the AMP peak. The CMP was present as one nucleotide per poly(A) sequence of about 49 nucleotides, whereas GMP and UMP were found only as traces. The poly(A) available from EEE RNA contained insufficient radioactivity for such an accurate analysis, but the results (Table 1), indicate that this poly(A) also was essentially free of nucleotides other than AMP.

The ratio of one CMP residue to 49 AMP residues in isolated poliovirus poly(A) probably results from cleavage by ribonuclease A at a CMP residue adjacent to the 3′ end of a sequence $(Ap)_{48}ApCp$ within the viral RNA.

Poly(A) sequences of approximately 150 to 200 nucleotides have been found in heterogeneous nuclear RNA (Hn-

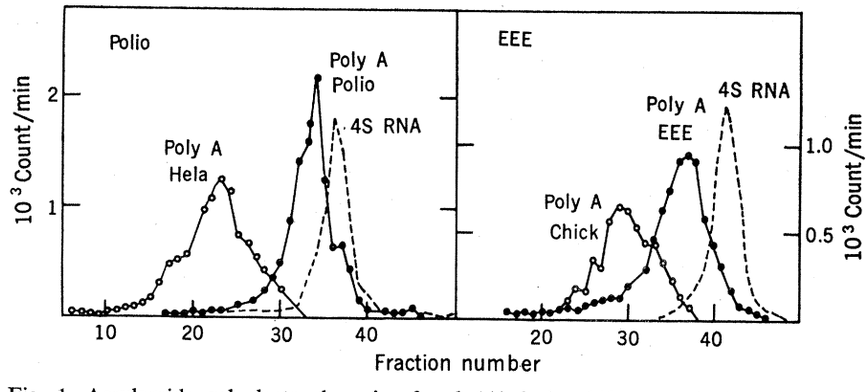

Fig. 1. Acrylamide gel electrophoresis of poly(A) isolated from poliovirus and EEE virus RNA's and from HeLa and chick cell RNA. Poly(A) was isolated from ^{32}P-labeled poliovirus and EEE virus RNA as described (*2*); the ribonuclease treatment was followed by hybridization to polythymidylate-cellulose, elution, and ethanol precipitation. The ^{32}P-labeled poly(A) from the total nuclear RNA of HeLa cells labeled for 1 hour with $^{32}PO_4$ was prepared as described (*2*). ^{14}C-labeled poly(A) from chicks was isolated from the polysome RNA of chick muscle cell cultures labeled for 90 minutes with [^{14}C]adenosine. The growth of the chick cells (*19*), isolation of polysomes (*20*), RNA extraction (*10*), and isolation of poly(A) (*2*) were as described. The poly(A) samples were dissolved in a 10 percent sucrose solution in 0.01*M* NaCl, 0.02*M* EDTA, 0.1 percent sodium dodecyl sulfate (SDS), 0.01*M* tris, *p*H 7.0, with a trace of bromophenol blue dye. They were applied to 3.5 percent acrylamide gels (8.5 cm by 6 mm diameter) cross-linked with 0.175 percent bis-acrylamide in an SDS-phosphate buffer system (*p*H 7) as described (*2*). The gels were subjected to electrophoresis for 45 minutes at 10 volt/cm before the sample was applied; after the samples were applied to the gels, the 10 volt/cm gradient was resumed for 1 hour and 15 minutes. The gels were processed with a Maizel gel fractionator before the radioactivity was determined by scintillation counting.

RNA), in cytoplasmic mRNA of HeLa cells (*2, 3*), and in mouse ascites cells (*4*). We now show that similar poly(A) sequences are found in rapidly labeled RNA of chick polysomes, presumed to be mRNA. The function of these sequences is unknown, but it seems likely that they function in the synthesis of mRNA or its transport to the cytoplasm or in its translation or a combination of these processes. A direct function of poly(A) in the translation process is suggested by the finding that adenine-rich sequences are present in mRNA synthesized by viral RNA polymerases in vaccinia (*5*) and vesicular stomatitis (*6, 16*) virions, both of which reproduce in the host cell cytoplasm. Since the vaccinia mRNA is transcribed from a double-stranded DNA genome and the vesicular stomatitis mRNA from a single-stranded RNA genome, the common occurrence of poly(A) in these mRNA's seems more likely to be related to their role in translation than to their synthesis. Poly(A) has also been observed in the mRNA of adenovirus (*7*), which is synthesized in the host cell nucleus but transported to the cytoplasm for translation (*17*).

Our demonstration of poly(A) sequences in virion RNA from poliovirus and EEE virus provides additional evidence consistent with a functional role of poly(A) in the process of translation of mRNA in animal cells. Both of these viral RNA's are known (*18*) to be infectious when purified from their respective virions, so that they are capable of functioning as mRNA's with their host cell translation mechanism. The common presence of poly(A) sequences is especially significant because of the dissimilarity of these viruses. For example, EEE, like other group A arboviruses, has a lipoprotein envelope about its capsid, while poliovirus, of the picornaviruses, has none. The base compositions of the virion RNA's of these viruses are also quite different [data above and (*13*)]. The significant chemical feature which EEE virus and poliovirus do have in common is a poly(A) sequence in their virion RNA; hence it seems reasonable to hypothesize that these sequences play some common role in the functioning of the RNA. While several possibilities might be raised, the one process in which poliovirus RNA, EEE virus RNA, vaccinia virus mRNA, vesicular stomatitis virus mRNA, and the host cell mRNA's all function in common is clearly translation. We have no direct evidence at present as to what function poly(A) might play in the translation process.

The presence of small poly(A) sequences that can be accurately analyzed in viral RNA molecules is also significant from another standpoint, the physical-chemical characterization of that RNA. As an example, the analytical data regarding poliovirus RNA poly-(A) in Table 1, together with the known base composition of poliovirus RNA (*13*) permit a direct calculation of the molecular weight of the polio RNA: 2.77×10^6. This is in excellent agreement with the figure previously determined by physical methods: 2.4 to 2.5×10^6 (*12*). This calculation is possible only when the size of the poly-(A) sequence is accurately known, and therefore we are unable at present to apply it to EEE virion RNA.

JOHN A. ARMSTRONG
Department of Epidemiology and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania 15213
MARY EDMONDS
HIROSHI NAKAZATO
Department of Biochemistry, Faculty of Arts and Sciences
BRUCE A. PHILLIPS
Department of Microbiology, School of Medicine
MAURICE H. VAUGHAN
Department of Biochemistry, Faculty of Arts and Sciences

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Receptor-Estrogen Complex in the Nuclear Fraction of Rat Uterine Cells during the Estrous Cycle

Abstract. *A quantitative method was used to determine the concentration of receptor-estrogen complex in the nuclear fraction of rat uterine cells throughout the estrous cycle. The concentrations of nuclear receptor-estrogen complex were: metestrus, 0.22; diestrus, 0.75; proestrus, 1.29; and estrus, 0.31 picomoles per milligram of DNA. This cyclic fluctuation in the nuclear complex closely parallels the secretion of ovarian estrogen during the estrous cycle, an indication that the accumulation of receptor-estrogen complex by the nuclear fraction of uterine cells may be of physiological significance, and under the control of endogenous estrogen.*

Estrogenic hormones are selectively retained by the uterus and other tissues sensitive to estrogen (*1*). It is generally accepted that the interaction of estrogen (E) with the uterus is characterized by the binding of E to a cytoplasmic protein (R) with the subsequent movement of the receptor-estrogen complex (RE) from the cytoplasm to the nucleus of uterine cells. The appearance of the RE complex in the nuclear fraction of the uterine cells is a specific process initiated by estrogenic hormones, but not by progesterone or testosterone (*1–3*). It has been suggested that the nuclear RE complex