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The 3′-Terminal Nucleosides of the High Molecular Weight RNA of Avian Myeloblastosis Virus

(sucrose density centrifugation/[^{3}H]borohydride reduction/60–70S RNA)

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ABSTRACT **The RNA isolated from avian myeloblastosis virus was fractionated by sucrose density gradient centrifugation. The 3′-OH terminal nucleosides of various fractions were determined by periodate oxidation followed by tritiated borohydride reduction. The 60–70S fraction and the 35S RNA derived from it by heating both have adenosine as the major terminal nucleoside, with cytidine as the next most frequent terminal. Control samples of $tRNA^{met}_{f,coli}$ and 28S ribosomal RNA from mouse ascites tumor cells gave the expected terminal residues and molecular weights.**

The RNAs of many plant and bacterial viruses show a degree of similarity at the 3′-OH terminus of the molecule (1–9), and it is believed that the 3′-termini are important in binding the viral RNA synthetase (10). All of these RNAs studied have been found to terminate in -CCA (11). In addition, Yot *et al.* (12) have suggested that the terminal piece of turnip yellow mosaic virus RNA appears to be a complete tRNA molecule capable of accepting valine and ending in -CCA. The function of such a tRNA ending for a large viral RNA molecule is not yet known.

In contrast to bacterial or plant RNA viruses, relatively little is known about the primary structure of the RNA in animal tumor viruses. Oncornaviruses contain single-stranded RNA, the major component of which sediments in the vicinity of 60–70 S (13–17). When the 60–70S RNA is denatured by dimethylsulfoxide (Me_2SO) or heat (15, 16, 18), it is converted in part to a slower moving component that sediments in the vicinity of 35 S. It is believed that 60–70S RNA with a molecular weight of about 10×10^6 is made up of three to four 35S moieties with a molecular weight in the range of 3×10^6 each (15, 17, 19, 20).

In an effort to determine the 3′-OH-terminal nucleoside of the 70S and 35S RNA of avian myeloblastosis virus (AMV), we have used the periodate oxidation–tritiated borohydride reduction procedure perfected for microanalytical analyses by the Randeraths (21, 22) following earlier procedures (23–25) and more recently used by others (26–29). In this method the exposed *cis*-hydroxyl groups on the 3′-terminal nucleoside of an RNA chain are oxidized to dialdehydes with periodate and subsequently reduced with tritium-labeled borohydride, yielding an RNA containing labeled 3′-termini. Alkaline hydrolysis of the intact RNA yields the terminal nucleoside as a labeled trialcohol, thus identifying the terminal 3′-OH-residue of the chain. We have applied this technique to several AMV RNA samples isolated from sucrose gradients.

Both the 60–70S RNA fraction and RNA from the 35S area fraction derived from the 60–70S RNA by heat treatment have adenosine as the major terminal nucleoside. Cytidine accounts for the next highest percentage of terminal nucleoside. While this work was in progress, Erikson *et al.* (30) reported finding uridine as the predominant terminal nucleoside of AMV 35S RNA. We have not as yet been able to confirm this finding in the virus preparations so far examined, under conditions where control RNA samples gave the expected 3′-OH-end nucleosides and correct molecular weight.

Abbreviations: AMV, avian myeloblastosis virus; DEP, diethylpyrocarbonate.

MATERIALS AND METHODS

Preparation of Virus. Avian myeloblastosis virus (AMV) BAI Strain A was used in all experiments. In addition to our own virus, we are indebted to Dr. and Mrs. Joseph Beard for supplies of AMV from both chicken plasma from infected chicks and from myeloblast tissue cultures. Both fresh plasma and plasma stored at −80° were used. Cell debris was removed by centrifugation at $600 \times g$, followed by two 20-min centrifugations at $5000 \times g$. The $5000 \times g$ supernate was centrifuged for 1.5 hr in the no. 30 Spinco rotor at $78{,}000 \times g$ to pellet the virus.

Preparation of RNA. RNA was extracted by modifications of the method of Duesberg (31, 32). The virus pellet was suspended in about 10 volumes of the following solution: STE buffer [0.1 N NaCl; 0.01 M Tris (pH 7.3); mM ethylene diamine tetraacetic acid (EDTA)]; 1% dithiothreitol; 10% phenol; 0.1% (w/v) bentonite; and 2 μl/ml diethylpyrocarbonate (DEP). The mixture was stirred for 20 min with an equal volume of 90% phenol (washed and saturated with buffer) in an ice bath. Following this, one volume of chloroform:isoamylalcohol (99:1) was added, and mixing was continued for 10 min. The extract was centrifuged for 30 min at $12{,}000 \times g$ at 4°. The aqueous layer was removed and stirred with 1 volume of 90% phenol for 10 min and again centrifuged to eliminate further traces of protein. The aqueous layer from this step was removed, and RNA was precipitated by the addition of 1/20 volume of 2 M Na acetate (pH 5) and 2 volumes of ethanol. The mixture was allowed to stand overnight at −20° before centrifugation. The RNA was dissolved

in a minimal volume of 0.1 M Na acetate, pH 5 (containing 1 μl/ml DEP) and was precipitated as before with alcohol. The RNA pellet was lyophilized to remove residual alcohol and was redissolved in 0.01 M NaCl, 0.001 M Tris, 0.1 mM EDTA (pH 7.3) containing 1 μl/ml of DEP. All glassware was treated with heat for 2 hr at 275° to inactivate ribonucleases, while other pieces of equipment were soaked in DEP at 2 μl/ml of H_2O and rinsed with DEP-treated H_2O (H_2O stirred with DEP for 1 hr, then boiled to eliminate the DEP).

Sucrose Gradients. All sucrose solutions were stirred with 2 μl/ml DEP for 1 hr and then heated to 95° for 15 min to destroy residual DEP. Linear gradients of 5–30% (w/w) sucrose in STE buffer were layered over a pad of 0.3-ml 50% sucrose. For isolation of 60–70S RNA, centrifugation was performed for 2.5 hr and for isolation of 35S material, centrifugation was performed for 5 hr at 41,000 rpm (R_{av} = 191,000 × g) in the Spinco SW41 rotor. An Isco model 640 density gradient fractionator with recorder was used for the collection of the fractions from the gradients.

The RNA was recovered from pooled tubes by the addition of 2.5 volumes of alcohol. It was allowed to precipitate overnight at −20° before centrifugation. The RNA samples were reprecipitated from alcohol, lyophilized, and redissolved in a small volume of H_2O that had been treated with DEP.

Determination of 3'-OH-Terminal Nucleoside (21) with Minor Modifications. The following conditions are given for about 15 pmol of terminal nucleoside. For viral high-molecular weight RNA of assumed molecular weight of 3×10^6, 15 pmol is the equivalent of about 45 μg or about 1 A_{260} unit. Samples of tRNA of molecular weight of 3×10^4 would require only about 0.01 A_{260} units for the same amount of terminal nucleoside. About 1 A_{260} unit of high-molecular weight RNA in 80 μl/H_2O was incubated with 20 μl of 0.01 M $NaIO_4$ in the dark at room temperature for 5 hr. This was about a 13,000-fold excess of periodate. After incubation the tube containing the solution was chilled, 10 μl of 1 M Na phosphate buffer (pH 7.0) was added, and immediately thereafter 20 μl of 0.1 M [^{3}H]$NaBH_4$ (Amersham-Searle, diluted to about 2.5 Ci/mmol, in 0.1 M NaOH) was added. The tubes were stoppered and incubated at room temperature overnight in a hood in the dark. Eighty μl of 5 N acetic acid were added to destroy the excess borohydride, and the unstoppered tubes were left 30 min in the hood. The samples were dried in a stream of filtered air, and were taken up in 100 μl H_2O and dried again. This step was repeated. In order to eliminate most of the nonnucleotide tritiated material, we reprecipitated the RNA four times from a small volume (50–100 μl) of 0.1 N Na acetate (pH 5), with 2 volumes of alcohol. The RNA was redissolved in a final volume of 50 or 100 μl H_2O.

The A_{260} value of an aliquot of the RNA solution was determined, and about 0.8 A_{260} of RNA in 50 μl was taken for hydrolysis. One-tenth volume of piperidine (Fisher) was added, and the small tube was stoppered securely. The RNA was hydrolyzed for 2 hr at 95° in an oven. After hydrolysis the piperidine was evaporated with a stream of filtered air. The samples were dissolved in 50–100 μl H_2O and the drying process repeated three times. The hydrolysate was dissolved in 20 μl H_2O. About one-third was used for each two-dimensional chromatogram.

The hydrolysate was applied to a 20 × 20 cm thin-layer cellulose plate (Eastman no. 6064, cellulose on plastic backing). About 8 μl of the hydrolysate (corresponding to about 0.2 A_{260} of the original RNA) was premixed with the four unlabeled nucleoside trialcohol markers (2 μl of a mixture of 0.001 M each), and all was applied in 1-μl aliquots to a single spot.

The chromatogram was eluted in two dimensions in tanks about 11 × 11 × 4 in. The first dimension was run in Solvent System F (21). The solvent was run about 2 cm onto a wick (Whatman no. 1 paper) at the top of the plate, or a total of about 20 cm from the origin. The wick was removed, the plates were air dried, and another wick was applied for the second dimension, which was eluted in Solvent System G (21). This was developed about 23 cm from the origin onto the wick.

The plates were air dried and the marker spots were made visible and outlined under ultraviolet light. Each spot, plus blank areas of similar size completely surrounding the known marker, was cut out. Each piece of plastic plate was put in a 6-ml counting vial and was wet with 100 μl 4 N NH_4OH. After 0.50 hr, 5 ml of Kinard's scintillation fluid were added (33), the samples were shaken to provide mixing of the contents, and they were counted on a Nuclear Chicago Unilux II scintillation counter. Duplicate chromatograms were run. On one we located the radioactive compounds by radioautography with low temperature solid scintillation fluorography (34) to ascertain that the cpm coincided with the carrier trialcohols.

Preparation of [^{3}H]Trialcohols. For the determination of the specific activity of the labeled 3'-OH-terminal nucleosides of the RNA, it was necessary to prepare labeled trialcohols with each batch of [^{3}H]borohydride solution with known nucleosides. These were prepared by a modification (21) of the procedure of Khym and Cohn (24). Stock aqueous solutions, 1 mM each of adenosine, cytidine, guanosine, and uridine, were made and the concentrations were determined by spectrophotometry in 0.067 M phosphate buffer at pH 6 at the maximum of each. These were: adenosine, $\epsilon_{259\ nm}$ = 15.4 × 10^3; cytidine, $\epsilon_{270\ nm}$ = 9.1 × 10^3; guanosine, $\epsilon_{253\ nm}$ = 13.6 × 10^3; and uridine, $\epsilon_{262\ nm}$ = 10.1 × 10^3 (35). 25 μl Of 1 mM nucleoside were oxidized with 25 μl of 2 mM Na periodate for 30 min in the dark at room temperature. 10 μl Of 0.1 M K[^{3}H]borohydride in 0.1 N KOH (2.5 Ci/mmol) were added. After 30 min in the dark at room temperature, 10 μl of 5 M acetic acid were added, the tubes were left unstoppered in a hood for 20 min, and then the samples were evaporated to dryness. They were taken up in 100 μl H_2O and dried twice. The final samples were dissolved in 50 μl H_2O, thus making 0.5-mM [^{3}H]trialcohol solutions. We applied 1- and 2-μl aliquots to cellulose plates along with the mixture of known markers in order to be able to recognize the spots. Solvent System G (21) was used in one direction only; the UV spot and surrounding blank areas were cut out and counted as before. The trialcohols formed in the batch of borohydride used for most of the experiments described herein had the following specific activities: adenosine = 275, guanosine = 188, uridine = 225, and cytidine = 245 cpm/pmol trialcohol. The average value of 233 cpm/pmol was used for calculation of the molecular weights of the RNA fractions.

Gel Electrophoresis. A slab gel electrophoresis unit (E-C Apparatus Co.) was used for analysis of various RNA fractions. The Tris-borate-EDTA buffer system of Peacock and

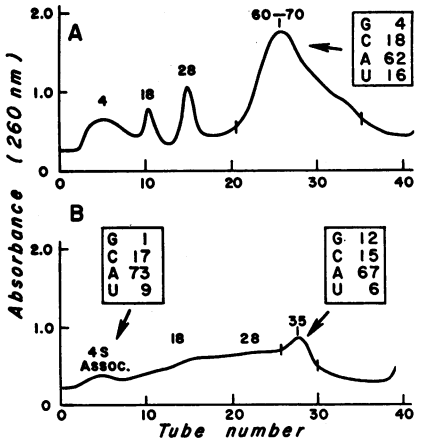

FIG. 1. Sucrose gradients of AMV RNA. (*A*) AMV RNA insoluble in 2 M NaCl, centrifuged 2.5 hr as discussed under *Methods*. (*B*) Pooled 60–70S peak from *A* heated to 70° for 3 min and centrifuged for 5 hr.

The numbers in boxes refer to the % cpm in the trialcohols of G = guanosine, C = cytidine, A = adenosine, and U = uridine. See *text* and Table 1 for details. The numbers near the peaks refer to the approximate S values. These were determined in separate gradients of each run with an ascites cell cytoplasmic RNA containing 18 and 28S RNA. The *vertical lines* at the 60–70S RNA and 35S RNA regions indicate where the fractions were pooled.

Dingman was used with a gel of 1.75% total acrylamide, with 0.09% bisacrylamide, and 0.5% agarose (36).

RESULTS

The techniques perfected by the Randeraths allow one to reduce periodate-oxidized nucleosides with tritium-labeled borohydride, and to isolate them by two-dimensional thin-layer chromatography (21). These methods, applied to digests of RNA, have enabled this laboratory to isolate and identify the minor bases of AMV tRNA (37). Similar procedures applied to intact RNA, followed by alkaline hydrolysis, yield the terminal base as a labeled trialcohol, identifying the terminal 3′-OH-nucleoside of the chain. Since the borohydride method used for labeling the 3′-OH-terminal nucleoside will react with any 3′-OH-terminal nucleoside, the samples should be homogeneous and free from degraded RNA with unphosphorylated 3′-OH-groups.

The main impediment to progress in sequencing this RNA has been the highly heterogeneous character of the RNA as isolated. We, as others, have found RNA isolated from AMV by conventional procedures to yield two main fractions on sucrose gradients: one sedimenting at about 4 S and the other at 60–70 S, plus small amounts of RNA sedimenting in the 18S and 28S areas. Others have shown that a portion of 60–70S RNA will, after treatment with heat or Me_2SO, sediment at about 35 S (15) and that additional RNA sedimenting at 4 S is released (38, 39). The latter RNA has been termed the "70S-associated 4S." In our experiments we have found that upon heating, or treating the pooled 60–70S RNA with Me_2SO, a variable picture is obtained either by gel electrophoresis or repeated sucrose gradient centrifugation. In general we find the heated 60–70S material to yield a more or less discrete peak at about 35 S (variable from one preparation to another) that merges with a broad, continuous band of RNA of lower sedimentation velocity extending down to the 4–5S region. This is shown in Fig. 1*B*. The molecules of RNA contained in the peak sedimenting at 60–70S (Fig. 1*A*) would appear to be held together by noncovalent forces as a single hydrodynamic unit. When RNA, extracted from virus that had been frozen, was compared with RNA from fresh virus, there was an increased amount of low molecular weight RNA from the frozen preparation even though the 60–70S RNA appeared to be intact until heated.

We have isolated 60–70S RNA from sucrose gradients of several viral preparations from both fresh and frozen plasma and have heated the pooled 60–70S material and applied it to a second gradient in order to collect the RNA in the 35S area of the gradient. (See Fig. 1.) We have found the 3′-OH-terminal nucleoside from 60–70S RNA, the 35S RNA, and the tRNA fractions to be adenosine, as is the 4S material obtained by heating the 60–70S RNA, as indicated in Fig. 1 and Table 1.

We have used a purified tRNA from *Escherichia coli* and a purified 28S cytoplasmic ribosomal RNA from mouse ascites tumor cells as known controls with respect to 3′-OH-termini and molecular weight. The results are shown in Table 1.

Although a control sample of $tRNA_f^{met}$ has the correct end group (adenosine), the 28S cytoplasmic ribosomal RNA from ascites cells was a more suitable control. This ribosomal RNA is likely to end in uridine and is in the molecular size range of the 35S viral material in contrast with the purified

TABLE 1. *3′-OH terminal nucleosides of various RNA samples*

RNA	Mol.	% cpm in trialcohols			
Regions from sucrose gradients*	Wt.† × 10^6	Guanosine	Cytidine	Adenosine	Uridine
AMV-RNA					
60–70S pooled					
A. Frozen plasma	2.1	13	15	67	5
B. Frozen tissue culture	2.0	11	21	62	6
C. Fresh plasma	2.0	4	18	62	16
35S region from 70S (heated)					
C. Fresh plasma	2.2	12	15	67	6
D. Frozen plasma	1.6	9	16	63	12
tRNA area					
C. Fresh plasma	0.06	5	17	75	3
70S associated 4S area					
C. Fresh plasma	0.23	1	17	73	9
$tRNA^{met}_{f,coli}$	0.03	1	15	81	3
28S ascites ribosomal RNA	2.0	6	3	13	78
		3	3	10	84

The various AMV RNA fractions were isolated from sucrose gradients similar to and including those shown in Fig. 1, as described under *Methods*. $tRNA_f^{met}$ was obtained through the courtesy of Oak Ridge National Laboratories. The 28S RNA was isolated from a sucrose gradient of cytoplasmic RNA from ascites cells.

* Approximate S values.

† Calculated as A_{260} units in sample $\times \frac{1}{21.4} \times 10^{-3} \times \frac{1}{\text{moles of terminal nucleosides in sample}}$.

tRNA sample, which is much smaller. Although there are no published data for the 3'-OH-nucleoside of the 28S RNA of ascites tumor cells, Lane *et al.* (40) have reported 28S RNA from L cells (mouse origin) to end in uridine, and similar results have been reported for rabbit reticulocyte ribosomal 28S RNA by Hunt *et al.* (41). A plant 28S RNA ending in uridine was also cited in a review by Attardi and Amaldi (42).

We have determined the specific activities of known nucleoside trialcohols prepared with our [H^3]borohydride solutions. The molecular weights of these RNA fractions calculated directly from the pmol of trialcohols formed per unit mass are in the correct order of magnitude, giving molecular weights of about 2×10^6 for the 35S RNA. By the borohydride method the molecular weight for the 60–70S and the 35S RNA fractions were similar, which is consistent with the suggestion of a subunit structure for 60–70S material found by others (15, 17, 19, 43).

DISCUSSION

While this investigation was in progress, Erikson reported finding the 3'-OH-terminal nucleoside of AMV 35S RNA to be at least 80% uridine (30), using the borohydride technique of Leppla *et al.* (27); and Maruyama *et al.* (43), using the borohydride method described here (21), found uridine to be the predominant terminal base in 70S RNA in several tumor viruses (AKR mouse leukemia, feline leukemia, and viper). In addition, Lewandowski *et al.* (44) have also found uridine at the 3'-hydroxyl end of several subunits of RNA from influenza virus.

To date, we have not been able to confirm the finding that the 3'-OH-terminal residue of the high molecular weight RNA from AMV is predominantly uridine. Even under conditions where the "70S associated" 4S RNA is no longer associated with the larger molecule, adenosine followed by cytidine has been the major end group, while uridine has consistently been found in less than 15% of the terminal residues.

The fact that we find the 28S RNA of mouse ascites tumor cell to end in uridine indicates that the method is capable of identifying uridine and of yielding an estimate of molecular weight in the correct range. Fluorograms of the two-dimensional plates showed highly radioactive areas in addition to the trialcohol markers. These unidentified areas may confuse the counting of labeled trialcohols following a one-dimensional separation system. We have often seen such nonnucleotide components, particularly in RNA samples isolated from sucrose gradients. These spots are many times more radioactive than the trialcohols, and great care must be taken to ensure that the radioactivity coincides with the trialcohols added as carrier. It has been pointed out (21, 27, 43, 44) that [^{3}H]borohydride reacts with many compounds that may appear as reaction byproducts. The origin spot on the chromatograms, especially of the high molecular weight RNA, always contains many times more counts than are found in the trialcohols. This radioactivity remaining at the origin is not bound to the nucleotides of the digest, which also remain at the origin in this chromatographic system. When "origin" material was transferred to polyethyleneimine cellulose plates and developed with 1 M LiCl, the mononucleotides moved away from the origin, and radioautographs indicated that the counts were not in the mononucleotide spots identified in the ultraviolet light. The process of reprecipitating the RNA several times after treatment with borohydride is essential for lowering of the "blank" areas.

Diethylpyrocarbonate (DEP) has been used extensively as a ribonuclease inhibitor (45, 46). We have used a relatively low concentration (0.2%, v/v) when isolating RNA, while others sometimes have used much higher concentrations (3.3%) (47). DEP is known to react with adenine at concentrations greater than those used in this study (48). We therefore treated an aliquot of total viral RNA (containing all four end groups) with 3.3% DEP for 5 min at 37°, followed by 30 min at 0°, and then precipitation of the RNA with alcohol in the manner described by Hatanaka *et al.* (47). The trialcohols liberated after borohydride treatment of the control and treated RNA were identical, indicating that under these conditions DEP has no observable effect on the terminal nucleosides of RNA. One should incidentally remain aware of the possibility of reaction of DEP with other nucleotides in the RNA molecule.

It has been shown with RNA viruses from plants and bacteria that the 3'-OH-terminal adenosine of the RNA is involved in the binding of the RNA synthetase (10). By analogy it would be reasonable to speculate that the 3'-OH terminal sequences of the high molecular weight RNA of AMV are logical sites for attachment of the RNA:DNA polymerase or perhaps for other enzymatic activities.

Some years ago, using ascites tumor cells, we first demonstrated that the 3'-OH terminal nucleosides of transfer RNAs ended in -CCA (49), and that the amino acid was attached to the terminal adenosine of the molecule (50). The reason for this oligonucleotide sequence in the -CCA handle common to all tRNA molecules remains obscure to this day.

Since, in our viral preparations, we find the terminal 3'-OH-nucleoside to end predominantly in adenosine (70%) and cytidine (15%), we are interested in the report of Chapeville *et al.* (12) that the terminal sequence of the turnip yellow mosaic virus can serve as the site for the esterification of valine. This report states that the 3'-OH-end of the large molecular weight RNA is $tRNA^{val}$. The biological function of such a segment of RNA is not known at present. Because of the common -CCA terminal ending of all tRNAs and in the light of Chapeville *et al.*'s finding of $tRNA^{val}$ as a terminal piece of the high molecular weight RNA of turnip yellow mosaic virus, it will be of interest to determine whether or not these high molecular weight RNA fractions from oncogenic viruses will accept amino acids. It is equally intriguing to examine our findings in relation to very recent reports from several laboratories (51–54) of the presence of sequences of adenylyl residues in viral RNAs.

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1. Weith, J. C. & Gilham, P. T. (1967) *J. Amer. Chem. Soc.* **89**, 5473–5474.
2. Weith, J. C. & Gilham, P. T. (1969) *Science* **166**, 1004–1005.
3. Dahlberg, J. E. (1968) *Nature* **220**, 548–552.
4. DeWachter, R. & Fiers, W. (1967) *J. Mol. Biol.* **30**, 507–527.
5. Cory, S., Adams, J. M. & Spahr, P. F. (1972) *J. Mol. Biol.* **63**, 41–56.

6. Min Jou, W., Contreras, R. & Fiers, W. (1970) *FEBS Lett.* **9**, 222–224.
7. Mandeles, S. (1967) *J. Biol. Chem.* **242**, 3103–3107.
8. Glitz, D. G., Bradley, A. & Fraenkel-Conrat, H. (1968) *Biochim. Biophys. Acta* **161**, 1–12.
9. Glitz, D. G. & Eichler, D. (1971) *Biochim. Biophys. Acta* **238**, 224–232.
10. Staves, R. L. & August, J. T. (1970) *Annu. Rev. Biochem.* **39**, 527–560.
11. Cory, S., Sphar, P. F. & Adams, J. M. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 1–11.
12. Yot, P., Pinck, M., Haenni, A.-L., Duranton, H. M. & Chapeville, F. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 1345–1352
13. Robinson, W. S., Pitkanen, A. & Rubin, H. (1965) *Proc. Nat. Acad. Sci. USA* **54**, 137–144.
14. Bonar, R. A., Sverak, L., Bolognesi, D. P., Langlois, A. J., Beard, D. & Beard, J. W. (1967) *Cancer Res.* **27**, 1138–1157.
15. Duesberg, P. H. (1968) *Proc. Nat. Acad. Sci. USA* **60**, 1511–1518.
16. Erikson, R. L. (1969) *Virology* **37**, 124–131.
17. Duesberg, P. H. (1970) *Curr. Top. Microbiol. Immunol.* **51**, 79–104.
18. Montagnier, L., Golde, A. & Vigier, P. (1969) *J. Gen. Virol.* **4**, 449–452.
19. Bader, J. P., Steck, T. H. & Kakefuda, T. (1970) *Curr. Top. Microbiol. Immunol.* **51**, 105–113.
20. Duesberg, P. H. & Vogt, P. K. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 1673–1680.
21. Randerath, K. & Randerath, E. (1971) *in Procedures in nucleic acid research*, eds. Cantoni, G. L. & Davies, E. R. (Harper and Row, New York), Vol. II, pp. 796–812.
22. Randerath, K. & Randerath, E. (1969) *Anal. Biochem.* **28**, 110–118.
23. Whitfeld, P. R. & Markham, R. (1953) *Nature* **171**, 1151–1152.
24. Khym, J. X. & Cohn, W. E. (1960) *J. Amer. Chem. Soc.* **82**, 6380–6386.
25. Zamecnik, P. C., Stephenson, M. L. & Scott, J. F. (1960) *Proc. Nat. Acad. Sci.* **46**, 811–822.
26. Rajbhandary, U. L., Stuart, A., Faulkner, R. D., Chang, S. H. & Khorana, H. G. (1966) *Cold Spring Harbor Symp. Quant. Biol.* **31**, 425–434.
27. Leppla, S. H., Bjoraker, B. & Bock, R. M. (1968) *Methods Enzymol.* **12**, 236–240.
28. Kamen, R. (1969) *Nature* **221**, 321–325.
29. Banerjee, A. K., Ward, R. & Shatkin, A. J. (1971) *Nature New Biol.* **232**, 114–115.
30. Erikson, R. L., Erikson, E. & Walker, T. A. (1971) *Virology* **45**, 527–528.
31. Duesberg, P. H. & Robinson, W. S. (1965) *Proc. Nat. Acad. Sci. USA* **54**, 794–890.
32. Duesberg, P. H. (1968) *Proc. Nat. Acad. Sci. USA* **59**, 930–937.
33. Kinard, F. E. (1957) *Rev. Sci. Instrum.* **28**, 293–294.
34. Randerath, K. (1970) *Anal. Biochem.* **34**, 188–205.
35. Nat. Acad. Sci.-Nat. Res. Counc., Publ. no. 719, 1960, Suppl. 1, 1963.
36. Peacock, A. C. & Dingman, C. W. (1968) *Biochemistry* **7**, 668–674.
37. Randerath, K., Rosenthal, L. J. & Zamecnik, P. C. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 3233–3237.
38. Erikson, E. & Erikson, R. L. (1971) *J. Virol.* **8**, 254–256.
39. McCain, B., Biswal, N. & Benyesh-Melnick, M. (1971) *Bacteriol. Proc. Abstr.* p. 221.
40. Lane, B. & Tamaoki, T. (1967) *J. Mol. Biol.* **27**, 335–348.
41. Hunt, J. A. (1970) *Biochem. J.* **120**, 353–363.
42. Attardi, G. & Amaldi, F. (1970) *Annu. Rev. Biochem.* **39**, 183–212.
43. Maruyama, H. B., Hatanaka, M. & Gilden, R. V. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 1999–2001.
44. Lewandowski, L. J., Content, J. & Leppla, S. H. (1971) *J. Virol.* **8**, 701–707.
45. Solymosy, F., Fedorcsak, L., Gulyas, A., Farkas, G. L. & Ehrenberg, L. (1968) *Eur. J. Biochem.* **5**, 520–527.
46. Summers, W. C. (1970) *Anal. Biochem.* **33**, 459–463.
47. Hatanaka, M., Huebner, R. J. & Gilden, R. V. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 10–12.
48. Leonard, N. J., McDonald, J. J. & Reichmann, M. E. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 93–98.
49. Hecht, L. I., Zamecnik, P. C., Stephenson, M. L. & Scott, J. F. (1958) *J. Biol. Chem.* **233**, 954–963.
50. Hecht, L. I., Stephenson, M. L. & Zamecnik, P. C. (1959) *Proc. Nat. Acad. Sci. USA* **45**, 505–518.
51. Bellamy, A. R. & Joklik, W. K. (1967) *Proc. Nat. Acad. Sci. USA* **58**, 1389–1395.
52. Johnston, R. E. & Bose, H. R. (1972) *Biochem. Biophys. Res. Commun.* **46**, 712–718.
53. Lai, M. M. C. & Duesberg, P. H. (1972) *Nature* **235**, 383–386.
54. Gillespie, D., Marshall, S. & Gallo, R. C. (1972) *Nature*, in press.