*Acta Paediatrica Academiae Scientiarum Hungaricae, Vol. 22 (1–2), pp. 33–42 (1981)*

# Virus-specific reverse transcriptase in acute leukaemia

S. KISS, D. SCHULER

Second Department of Paediatrics, Semmelweis University Medical School, Budapest

(Received November 27, 1980)

A simple cell homogenate fractionation procedure was used to enrich for virus-specific reverse transcriptase. Animal cells infected with Rauscher leukaemic viruses provided the positive controls necessary to establish guidelines for fractionation, enzyme preparation and assay of human malignant cells. In the peripheral blood of five acute lymphoblastic leukaemic children we found no evidence to support the presence of any oncorna viruses. The results obtained with acute leukaemic lymphoblasts and with healthy thymocytes were similar.

The unique properties of RNA tumour virus reverse transcriptase, especially its ability to transcribe natural or synthetic RNA, have suggested that this enzyme activity might provide a sensitive assay for the detection of RNA tumour viruses in suspected biological materials. In this respect, much effort has been directed towards detecting human tumour viruses by assaying malignant cells for virus specific reverse transcriptase (RT) activity [1, 4, 10, 11, 12, 13, 21, 23, 24, 26, 29, 30]. There were, however, difficulties in detecting a viral enzyme, because it was expected to be present in low concentrations in malignant cells, and the presence of cellular polymerases can also respond in certain assays that were earlier thought to be specific for viral RT [3, 9, 32, 33, 37, 38].

For the time being, heteropolymer RNA remains the most specific template for virus RT, but the enzyme activity is much lower with natural RNA than with the synthetic template primers [20, 21]. In addition, the natural RNA is serving as template for synthesis of products, which are difficult to demonstrate [6, 10, 34]. In view of the limited amount of clinical material, synthetic template-primer complex (poly(2'-O-methylcytidylate) oligodeoxyguanylate (poly/rCm/ · oligo/dG/) has been used by us for the detection of virus related RT in human acute leukaemic cells. Poly/rCm/ · oligo/dG/$_{12-18}$ is a specific template primer for virus RT, but it is much less active with virus enzymes than with poly/rA/ · oligo/dT/ or poly/rC · oligo/dG/ [14, 15, 16, 17, 20, 21, 37]. It has, however, recently been reported that polyethylene glycol 6000 (PEG 6000) enhances reverse transcription, augmenting both the rate and the duration of the polymerization [7]. We could in fact demon-

strate that polymerization is higher in PEG 6000 with poly/rCm/ · oligo/ /dG/$_{12-18}$, than in bovine albumin with poly/rA/ · oligo/dT/$_{12}$.

The use of poly/rCm/ · oligo/dG/$_{12-18}$ for identification of virus related RT activity from human leukaemic tissue may be complicated by the presence of the template independent terminal deoxynucleotidyltransferase activity (TdT), which is present in thymus, bone marrow and especially in certain types of acute leukaemic cells [5, 28, 29, 25, 30, 37]. The primer portion of poly/rCm/ · oligo/dG/$_{12-18}$ serves as an efficient primer for the terminal addition of dGMP by means of TdT. TdT activity, however, differs from all template-dependentDNA polymerases in its sensitivity to complete inhibition by high levels of ATP [5, 29]. For this reason, RT activity has been investigated by us in the presence and absence of ATP.

This paper describes in detail our studies for the detection of viral RT in human acute leukaemic cells. Animal cells infected with Rauscher leukaemic viruses provided the positive controls necessary to establich guidelines for enzyme preparation and assay of human malignant cells.

## Materials and Methods

*Chemicals and enzymes.* Poly(rCm), oligo-(dG)$_{12-18}$, Terminal deoxynucleotidyl Transferase (TdT) were obtained from PL-Biochemicals, Milwaukee, Wis., USA. Poly-(A)· oligo(dT)$_{12}$ and Reverse Transcriptase (RT) were bought from Boehringer Mannheim GmbH, FRD. $^3$H/TTP, specific activity, 28 Ci/mmol was bought from the Radiochemical Centre, Amersham, England. ($^3$H)dGTP — specific activity, 28 Ci/mmol was suplied by the Isotope Institute, Moscow, Soviet Union. All the other chemicals were bought from Reanal Inc.-Budapest, Hungary.

*Viruses.* Rauscher Murine Leukaemia Virus preparations were obtained as a gift from Dr. F. J. Rauscher, National Institutes of Health, Bethesda, Md., USA. The virus titre was $10^5$ ID 50/ml I. P. in adult mice, the Code No., was ATCC VR294.

*Mice.* Balb/c mice were bought from "Human" Inc., Gödöllő, Hungary.

*Spleen.* The Rauscher Murine Leukaemia Virus preparation was inoculated I. P. into 3-week-old Balb/c mice, as described previously [22]. The animals were sacrificed 3 weeks later; mean wet spleen weight was 3 g per animal. Spleens were also removed from 6-week-old healthy Balb/c mice after sacrifice. Both the enlarged leukaemic spleens and the normal ones were stored at —70 °C.

*Human blood.* Ten ml peripheral blood was taken from each of 5 acute lymphoblastic leukaemic children before treatment, immediately after having diagnosed the disease. The bloods were stored at —70 °C.

*Human thymuses.* A piece of thymus gland has been removed from each of 6 children suffering from congenital heart disease, immediately after the thorax has been opened for cardiac surgery. The thymus glands were stored at —70 °C.

*Enzyme preparations.* All preparation procedures were done at 4°C. Frozen 10 ml human blood was finely minced and disrupted in a Braun type glass-Teflon homogenizer in 10 ml homogenization buffer containing 50 mM Tris HCl pH 7.8, 1.0 mM dithiothreitol, and 20% v/v glycerol. Three g frozen leukaemic or normal spleen was also disrupted in 20 ml homogenization buffer. After homogenization the materials were centrifuged in an MSE High Speed 18 centrifuge at 5000× $g$ for 10 minutes, then the supernatants at 12,000× $g$ for 15 minutes. Solid KCl and

concentrated NP-40 were added to the mitochondrium-free supernatants to a final concentration of 0.4 M for KCl, and of 0.5% v/v for NP-40. The solution was stirred up several times and after 25 minutes, 80 ml homogenization buffer and 1.0 g phosphocellulose were added under continuous stirring with a glass rod for 20 minutes, then the phosphocellulose was sedimented out by centrifugation at 5000× *g* for 10 minutes. The sedimented phosphocellulose was washed 3 times with 80 ml of a solution containing 50 mM Tris HCl, pH 7.8, 1.0 mM dithiothreitol, 0.1 M KCl, 0.1% v/v NP-40, and 20% v/v glycerol. After the last wash, 3.5 ml of 1.0 M KCl, in homogenization buffer was added under stirring for 20 minutes. After centrifugation, the KCl concentration of the phosphocellulose-free supernatant was measured; it amounted to 0.4 M. The enzyme-containing solution was then concentrated to 0.5 ml with Minicon S 125 concentrator (Amicon Corporation, Lexington, USA), and from the concentrated solution three 0.1 ml samples were used for enzyme activity determination.

*DNA polymerase assay.* Reactions were carried out in volumes of 0.4 ml, including the 0.1 ml phosphocellulose purified sample. The reaction mixture contained one of the additional components, thus

a) 1.0 unit poly(rCm) · oligo/$dG_{12-18}$ + + 10 $\mu$Ci $^3$H-dGTP,

b) 0.5 unit oligo$(dG)_{12-18}$ + 10 uCi $^3$H-dGTP,

c) 1.0 unit poly(A)• oligo$(dT)_{12}$ + + 10 $\mu$Ci $^3$H-TTP,

and as a constant component,

50 mM Tris HCl, pH 7.8, 100 mM KCl, 0.5 mM $MnCl_2$, and either 50 $\mu$g bovine serum albumin or 10% v/v polyethylene glycol 6000.

Before use, 5 units of poly(rCm) and 5 units of oligo$(dG)_{12-18}$ freshly prepared by the method of Marcus [27, 31] were added.

After incubation at 37°C, the reactions were terminated by direct absorption by Whatman GF/C glass fibre paper. Then the papers were washed consecutively once in 10% trichloroacetic acid (TCA) containing sodium pyrophosphate, twice in 5% TCA and finally twice in 95% ethanol at 4 °C, each time for 15 minutes. TCA precipitable activity was estimated by liquid scintillation counting in a PPO-POPOP-toluene system.

Statistical analysis of the results was performed by the *t* test.

## Results

Figure 1 shows that the activity of reverse transcriptase prepared from the spleens of Rauscher leukaemic mice is template dependent.

PEG 6000 enhances reverse transcription, augmenting both the rate and duration of polymerization.

In PEG 6000 with poly/rCm/ · · oligo/dG the yield of complementary $^3$H-DNA is greater than in bovine serum albumin with poly/A/ · · oligo/dT/.

By means of our method, RT activity can be measured in as few as 2—3 mg spleen of Rauscher leukaemic mice.

With poly/rCm/ · oligo/dG/ the maximum yield of complementary $^3$H-DNA is obtained if incubation is continued for 90 minutes. The results presented in the Tables are given accordingly.

In Fig. 2 it is seen that the activity of RT is not influenced by the presence or absence of 0.1 mM ATP in the reaction mixture. Terminal deoxynucleotidyl transferase activity decreases considerably in the presence of 0.1 mM ATP. ATP in a dose

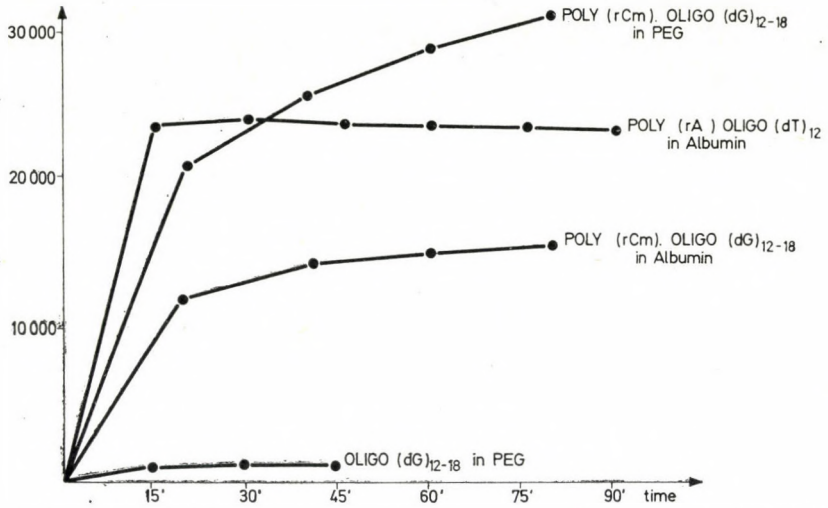


FIG. 1. Enzyme activity prepared from spleen of Rauscher leukaemic mouse. Assays were carried out in reaction mixtures containing either 50 $\mu$g bovine serum albumin without ATP or 10% (v/v) polyethylene glycol 6000 (PEG) without ATP. The values given are counts per minute per 50 mg wet weight spleen

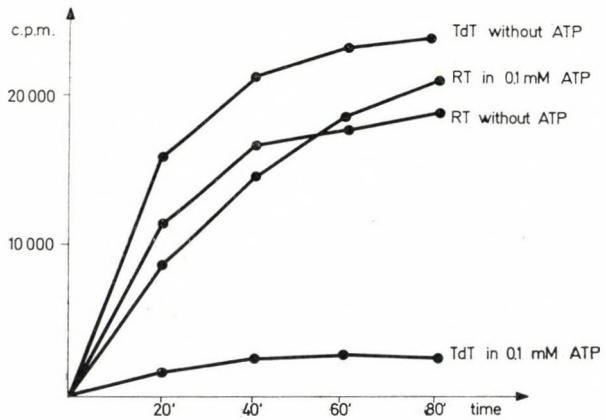


FIG. 2. Terminal deoxynucleotidyl transferase (TdT) and reverse transcriptase (RT) activities measured in the presence or absence of 0.1 mM ATP. The values given are counts per minute per 1 unit of TdT and of RT equal to 50 mg wet weight leukaemic spleen

of 0.2 mM inhibited TdT even more, but then RT was also inhibited.

Tables I and II show that template dependent enzyme activity can only be obtained from the spleens of Rauscher leukaemic mice. There is no template dependent enzyme activity in the spleen of healthy mice.

Tables III and IV show that template dependent enzyme activity was obtained from the leukaemic bloods and also from the healthy thymuses.

TABLE I

Enzyme activity prepared from spleen of Rauscher leukaemic mice. Assays were carried out in reaction mixtures containing 50 μg bovine serum albumin without ATP. The values given are counts per minute per 50 mg wet weight spleen

| | — | $OLIGO(dG)_{12-18}$ | POLY(rCm) $OLIGO(dG)_{12-18}$ |
|---|---|---|---|
| | 348 | 602 | 17 015 |
| | 342 | 588 | 17 255 |
| | 317 | 598 | 18 056 |
| Average | 335 | 596 | 17 442 |
| Standard deviation | ±16 | ±7 | ±544 |

TABLE II

Enzyme activity prepared from spleens of healthy mice. Assays were carried out in reaction mixtures containing 50 μg bovine serum albumin without ATP. The values given are counts per minute per 50 mg wet weight spleen

| | — | $OLIGO(dG)_{12-18}$ | POLY(TCm). $OLIGO(dG)_{12-18}$ |
|---|---|---|---|
| | 402 | 499 | 394 |
| | 438 | 465 | 437 |
| | 444 | 393 | 407 |
| Average | 428 | 452 | 412 |
| Standard deviation | ±23 | ±54 | ±22 |

TABLE III

Enzyme activity prepared from the blood of acute lymphoblastic leukaemic children. Assays were carried out in reaction mixtures containing 10% (v/v) PEG with 0.1 mM ATP. The values given are counts per minute per 2 ml of blood

| Name | Sex | Age in years | Leukocyte count G/l. | Blasts, per cent | — | $OLIGO(dG)_{12-19}$ | POLY(rCm). $OLIGO(dG)_{12-18}$ |
|---|---|---|---|---|---|---|---|
| K. M. | girl | 10 | 66 | 97 | 6104 | 7096 | 12 060 |
| T. T. | girl | 2 | 40 | 95 | 6184 | 7864 | 14 164 |
| M. F. | boy | 13 | 26 | 95 | 6052 | 7424 | 8 668 |
| V. G. | boy | 4 | 32 | 100 | 5732 | 6344 | 11 588 |
| G. Z. | boy | 15 | 30 | 100 | 6792 | 7808 | 12 200 |
| | | Average | | | 6172 | 7307 | 11,736 |
| | | Standard deviation | | | ±381 | ±621 | ±1978 |

$\rightarrow P < 0.01 \leftarrow \rightarrow P < 0.01 \leftarrow$

TABLE IV

Enzyme activity prepared from thymuses of healthy children. Assays were carried out in reaction mixtures containing 10% (v/v) PEG with 0.1 mM ATP. The values given are counts per minute per 50 mg wet weight thymus

| | — | $OLIGO(dG)_{12-18}$ | $POLY(rCm).OLIGO(dG)_{12-18}$ |
|---|---|---|---|
| | 6762 | 9072 | 13 307 |
| | 7188 | 9861 | 13 778 |
| | 6931 | 9072 | 13 227 |
| Average | 6960 | 9335 | 13 437 |
| Standard deviation | ±214 | ±456 | ±297 |

$P < 0.01$ ⟷ $P < 0.01$

TABLE V

Enzyme activity prepared from thymuses of healthy children. Assays were carried out in reaction mixtures containing 10% (v/v) PEG with 0.1 mM ATP. The values given are counts per minute per 50 mg wet weight thymus

| | — | $OLIGO(dG)_{12-18}$ | $POLY(Cm).OLIGO(dG)_{12-18}$ |
|---|---|---|---|
| | 6762 | 9839 | 20 161 |
| | 6782 | 9093 | 20 070 |
| | 6850 | 9474 | 21 253 |
| Average | 6808 | 9468 | 20 494 |
| Standard deviation | ±34 | ±373 | ±657 |

$P < 0.01$ ⟷ $P < 0.01$

Template dependent synthesis amounted to 4429 ± 191 c.p.m. per 2 ml of acute leukaemic blood, and to 4102 ± ± 165 c.p.m. per 50 mg healthy thymuses; the difference was not significant ($p > 0.50$).

With template + primer the total synthetized $^3$H-DNA was 11 736 ± ± 1978 c.p.m. and 13 437 ± 297 c.p.m. per 2 ml of acute leukaemic blood and per 50 mg of healthy thymuses, respectively; the difference was not significant ($0.20 > p > 0.10$).

Under the influence of oligo/$dG/_{12-18}$ without template, significantly increased $^3$H-DNA synthesis was observed both in the investigated acute leukaemic lymphoblasts and in the healthy thymocytes, because of the incomplete inhibition of TdT activity in both cases.

It can be seen in Table V that the template dependent enzyme activity obtained from healthy thymuses was considerably increased if 2 units of poly/rCm/ were annealed with 1 unit of

oligo/dG/$_{12}^{-}{}_{18}$. With such a type of template primer complex, the synthetized amount of $^{3}$H-DNA increased from 13 437 $\pm$ 297 c.p.m. to 20 494 $\pm$ $\pm$ 657 c.p.m. The template dependent $^{3}$H-DNA increased from 4102 $\pm$ $\pm$ 165 c.p.m. to 11 026 $\pm$ 717 c.p.m. The increase was significant ($p < 0.01$).

We have taken peripheral blood from healthy children and prepared enzymes from it, but the results were not comparable to those prepared from leukaemic blood, because the leukocyte count was very low, and the leukocytes of healthy children were mature, whereas those of leukaemic children were immature. Further, the leukocytes of healthy children are TdT negative whereas in the blood of leukaemic children there are TdT positive blasts.

## Discussion

In the past, the possible viral aetiology of leukaemia could not be investigated efficiently in humans because one could not apply the tests used in laboratory animals. Now there are biochemical methods which have proved useful for the purpose.

The oncogenic RNA viruses exhibit two biochemical properties unique to them [24, 36]. They possess a large single-stranded RNA molecule with a sedimentation coefficient of 60 S to 70 S and they also contain reverse transcriptase, an enzyme capable of using the viral 70 S RNA as a template to generate a DNA complementary copy [2, 24, 36, 39].

Our aim was to test the presence of RT in human leukaemia by the most sensitive and specific enzymatic procedures available and to compare them with the results obtained in non-malignant cells. This required an appraisal of the sensitivity and specificity of currently available assay procedures for viral RT in cell homogenates.

The described cell homogenate fractionation procedure was used to enrich viral enzymes. Our results with animal cells infected with tumour viruses provided the positive controls necessary to establish guidelines for cell fractionation, enzyme preparation and assay of human malignant cells. On the basis of the results presented in the first two Tables and two Figures we proved that our method is sensitive, template and virus specific. Still, in the peripheral blood of five acute leukaemic children we found no evidence to support the presence of any oncorna viruses, as acute leukaemia cells and healthy thymocytes gave similar results. The observed increase of template specific enzyme activity may have been the consequence of cellular polymerases being present in our assay system. Although poly/rCm/· · oligo/dG/ has been reported to be the most specific synthetic template-primer complex for the detection of viral enzyme, even these templates can to some extent be used by cellular DNA polymerases [35, 38]. Moreover, it has recently been shown that this template could even stimulate some cellular enzymes [35].

Spiegelman et al worked out a spe-

cial technique to detect reverse transcriptase and high molecular weight (70 S) RNA in a functional complex to demonstrate the expression of viral components in human malignancies [24, 36]. Positive results were obtained in more than 95% of the leukaemic patients, irrespective of their illness being acute or chronic, lymphoid or myelogenous [24]. With the same simultaneous detection technique they found 95% positivity from $10^9$ leukocytes. The amount of product formed in the simultaneous detection technique is approximately 2 orders of magnitude lower than with the use of poly/A/ · · oligo/dT [20, 21, 37]. Moreover, it can be seen from Fig. 2 that polyethylene glycol 6000 enhances reverse transcription, augmenting both the rate and duration of polymerization. Investigating the enzyme reaction in polyethylene glycol 6000 with poly/rCm/ · oligo/dG/ increased the yield of complementary $^3$H-DNA in excess to that produced in bovine albumin with poly/A/ · oligo/dT/. Thus, the number of the leukaemic cells used in our experiments was 5—10 times more than the number sufficient to demonstrate virus positivity.

In a large series of leukaemic patients, Gallo et al. [10] found, by utilizing more extensive purification methods and rigid criteria, that approximately 10% of the human leukaemic cells examined were positive for reverse transcriptase activity, and most of these were of myelogenous origin. The fact that 90% of human leukaemias may be negative for virus reverse transcriptase indicates the possibility, that most human leukaemias are transformed, but virus non-producer cells.

Consequently, one may conclude that human leukaemias, at least in part, could be caused by viruses, but after having infected and transformed some stem cells in the bone marrow, the viruses disappear from the organism. The situation is similar in the virus-negative cases of leukaemia in cats. In Glasgow, up to 50% of the feline lymphomas are negative for FeLV [8, 19].

If the viruses, as the causative agents, have disappeared from the organism by the time when the leukaemia is diagnosed, the disease could be cured by an eradication of transformed cell clones.

## References

1. Ackerman, W., Murphy, W., Miller, B.: RNA-dependent DNA synthesis in cell free preparations of human leukemia cells. Biochem. biophys. Res. Commun. **42**, 723 (1971).
2. Baltimore, D.: RNA-dependent DNA polymerase in virions of RNA tumour viruses. Nature (Lond.) **226**, 1209 (1970).
3. Bauer, G., Hofschneider, P. H.: An RNA-dependent DNA polymerase, different from the known viral reverse transcriptase, in the chicken system. Proc. nat. Acad. Sci. (Wash.) **73**, 3025 (1976).
4. Baxt, W., Hehlman, R., Spiegelman, S.: Human leukaemic cells containing reverse transcriptase associated with a high molecular weight viral-related RNA. Nature New Biol. **240**, 72 (1972).
5. Bhalla, R. B., Schwartz, M. K., Modak, M. J.: Selective inhibition of terminal deoxynucleotidyl transferase (TdT) by adenosine ribonucleoside triphosphate (ATP) and its application

in the detection of TdT in human leukemia. Biochem. biophys. Res. Commun. **76**, 1056 (1977).
6. Bhattacharyya, J., Xuma, M., Reitz, M., Sarin, P. S., Gallo, R. C.: Utilization of mammalian 70S RNA by a purified reverse transcriptase from human myelocytic leukemia cells. Biochem. biophys. Res. Commun. **54**, 324 (1973).
7. Chan, E. W., Phylis, J., Dale, Isabel, L. Greco, G., James, Rose Timothy, E. O'Connor: Effects of polyethylene glycol on reverse transcriptase and other polymerase activities. Biochim. biophys. Acta (Amst.) **606**, 353 (1980).
8. Essex, M., Grant, C. K., Cotter, S. M., Sliski, A. H., Hardy, W. D. Jr: Leukemia specific antigens: FOCMA and immune surveillance. In: Modern Trends in Human Leukemia III (ed. by R. Neth, R. C. Gallo, P. H. Hofschneider, K. Mannweiler) Springer-Verlag, Berlin—Heidelberg—New York 1979, p. 453.
9. Friedlander, B., Fry, M., Bolden, A., Weissbach, A.: A new synthetic RNA-dependent DNA polymerase from human tissue culture cells. Proc. nat. Acad. Sci. (Wash.) **69**, 452 (1972).
10. Gallo, R. C., Gallagher, R. E., Miller, N. R., Mondal, H., Saxinger, W. C., Mayer, R. J., Smith, R. G., Gillespie, D. H.: Relationships between components in primate RNA tumor viruses and in the cytoplasm of human leukemic cells; implications to leukemogenesis. Cold Spr. Harb. Symp. quant. Biol. **34**, 933 (1974).
11. Gallo, R. C., Miller, N. R., Saxinger, W. W., Gillespie, D. H.: DNA related to a primate RNA tumor virus genome which is synthetised endogenously by reverse transcriptase in viruslike particles from human acute leukemia cells. Proc. nat. Acad. Sci. (Wash.) **70**, 3219 (1973).
12. Gallo, R. C., Sarin, P. S., Allen, P. T., Newton, W. A., Priori, E. S., Bowen, J. M., Dmochowski, L.: Reverse transcriptase in type C virus particles of human origin. Nature new Biol. **232**, 140 (1971).
13. Gallo, R. C., Yang, S., Ting, R.: RNA-dependent DNA polymerase of human acute leukaemic cells. Nature (Lond.) **228**, 927 (1970).
14. Gerard, G. F.: Poly(2'-o-methylcytidylate). oligodeoxyguanylate, a template-primer specific for reverse transcriptase, is not utilized by HeLa cell DNA polymerase. Biochem. biophys. Res. Commun., **63**, 706 (1975).
15. Gerard, G. F., Lowenstein, P. M., Green, M.: Detection of reverse transcriptase in human breast tumours with poly(rCm).oligo(dG). Nature (Lond.) **256**, 140 (1975).
16. Gerard, G. F., Rottman, R., Green, M.: Poly(2'-o-methylcytidylate).oligodeoxyguanylate as a template for the directed DNA polymerase in RNA tumor virus particles and a specific probe for the RNA directed enzyme in transformed murine cells. Biochemistry **13**, 1632 (1974).
17. Gerard, G. F., Lowenstein, P. M., Green, M.: Detection in human ovary and prostate tumors of DNA polymerase activity that copies poly (2'-o-methylcytidylate).oligodeoxyguanylate. Cancer Res. **38**, 1008 (1978).
18. Gulati, S., Axel, R., Spiegelman, S.: Detection of RNA instructed DNA polymerase and high molecular weight RNA in malignant tissue. Proc. nat. Acad. Sci. (Wash.) **69**, 2020 (1972).
19. Sarrett, O.: Infectious leukemias in domestic animals. In: Modern Trends in Human Leukemia III, eds: R. Neth, R. C., Gallo, P. H. Hofschneider, K. Mannweiler, Springer-Verlag, Berlin—Heidelberg—New York 1979. p. 439.
0. Kiessling, A. A., Goulian, M.: A comparison of the enzymatic responses of the DNA polymerases from four RNA tumor viruses. Biochem. biophys. Res. Commun., **71**, 1069 (1976).
21. Kiessling, A. A., Goulian, M.: Detection of reverse transcriptase activity in human cells. Cancer Res. **39**, 2062 (1979).
22. Kiss, S., Astaldi, G.: Simple method for isolation of virus specific nucleic acid from leukemic tissue. Wadley med. Bull. **3**, 54 (1973).
23. Kotler, M., Weinberg, E., Haspel, O. T., Olshevsky, U., Becker, Y.: Particles released from arginine-deprived human leukaemic cells. Nature new Biol. **244**, 197 (1973).
24. Kufe, D. W., Spiegelman, S.: Molecular probing for viral etiology of human leukemias and lymphomas, including Burkitt's disease. Wadley med. Bull. **3**, 83 (1973).
25. Kung, P. C., Long, J. C., McCaffrey, R. P., Ratliff, R. L., Harrison, T. A., Baltimore, D.: Terminal deoxynucleotidyl transferase in the diagnosis of leukemia and malignant lymphoma. Amer. J. Med. **64**, 788 (1978).
26. Mak, T., Aye, M., Messner, H., Sheinin, R., Till, J., McCulloch, E.: Reverse transcriptase activity: Increase in marrow cultures from leukemic pa-

tients in relapse and remission. Brit. J. Cancer **29**, 433 (1974).
27. Marcus, S. L.: Resolution and characterization of intracytoplasmic forms of reverse transcriptase from Rauscher leukemia virus-producing cells. J. Virol. **26**, 1 (1978).
28. Marcus, S. L., Smith, S. W., aarowski, C. J. and Modak, M. J.: Terminal deoxyribonucleotidyl transferase activity in acute undifferentiated leukemia. Biochem. biophys. Res. Commun. **70**, 37 (1976).
29. Marcus, S. L., Kopelman, R.: Poly-(2'-o-methylcytidylate)-directed DNA polymerase and terminal deoxynucleotidyltransferase activities in particulate fractions obtained from human spleens. Cancer Res. **40**, 1014 (1980).
30. McCaffrey, R., Smoler, D. F., Baltimore, D.: Terminal transferase in a case of childhood ALL. Proc. nat. Acad. Sci. (Wash.) **70**, 737 (1973).
31. Modak, M. J., Marcus, S. L.: Purification and properties of Rauscher murine leukemia virus DNA polymerase and selective inhibition of mammalian viral reverse transcriptase by inorganic phosphate. J. biol. Chem. **252**, 11 (1977).
32. Penner, R., Cohen, L., Loeb, L.: RNA-dependent DNA polymerase: Presence in normal human cells. Biochem. biophys. Res. Commun. **42**, 1228 (1971).
33. Penner, R., Cohen, L., Loeb, L.: RNA-dependent polymerase in human lymphocytes during gene activation by PHA. Nature (Lond.) **232**, 58 (1971).
34. Reitz, M., Smith, R. G., Roseberry, E. A., Gallo, R. C.: DNA-directed and RNA-primed DNA synthesis in microsomal and mitochondrial fractions of normal human lymphocytes. Biochem. biophys, Res. Commun. **57**, 934 (1974).
35. Robert-Guroff, M., Gallo, R. C.: DNA polymerase gamma of human lymphoblasts. Biochemistry **16**, 2866 (1977).
36. Schlom, J., Spiegelman, S.: Simultaneous detection of reverse transcriptase and high molecular weight RNA unique to oncogenic RNA viruses. Science **174**, 840 (1971).
37. Sarngadharan, M. G., Robert-Guroff, M., Gallo, R. C.: DNA polymerases of normal and neoplastic mammalian cells. Biochim. biophys. Acta (Amst.) **516**, 419 (1978).
38. Spadari, S., Weissbach, A.: HeLa cell R-deoxyribonucleic acid polymerases. J. biol. Chem. **249**, 5809 (1974).
39. Temin, H., Baltimore, D.: RNA-directed DNA synthesis and RNA tumor viruses. Advanc. Virus Res. **17**, 129 (1972).

S. Kiss, M. D.
Tűzoltó u. 7.
1094 Budapest, Hungary