DNA POLYMERASES IN LYMPHOID CELLS

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Introduction

Three major DNA polymerases have been identified in soluble extracts from eukaryotic cells (1). One is a high molecular weight enzyme (about 150,000 daltons), found predominantly in cytoplasmic fractions, which we refer to as DNA polymerase C (1–4). The second is a lower molecular weight form (about 40,000 daltons), which is recovered primarily from nuclei (but also present in cytoplasm), which we refer to as DNA polymerase N (1–4). A third activity has also been seen, which can copy the adenylate-containing strand of the synthetic homopolymers poly(A) · oligo(dT) or poly(dA) · poly(dT), in the presence of Mn^{++} (1, 5, 6). We call this enzyme DNA polymerase A.

We have developed a method which allows us to rapidly separate, quantitate, and identify these activities in crude cell or tissue extracts. In the course of screening a variety of cells and tissues both for these normal DNA polymerases and for the unique reverse transcriptase of RNA tumor viruses (7), we have discovered that certain lymphoid cells have unique DNA metabolizing enzymes.

Normal Mouse Bone Marrow Fibroblasts

Figure 1 shows the three major polymerase recovered from a monolayer culture of mouse fibroblasts called JLS-V9 cells, which are not producing detectable C-type particles (8). Cells are prepared for these assays as follows: cell disruption is accomplished by repeated freeze-thawing. The crude homogenate is treated with non-ionic detergent to solubilize membrane-bound enzymes. High speed centrifugation yields a particle-free supernatant containing the solubilized enzymes, which is analyzed directly by chromatography on a phosphocellulose column. A salt gradient is passed through the column, and fractions are assayed for DNA synthetic activity.

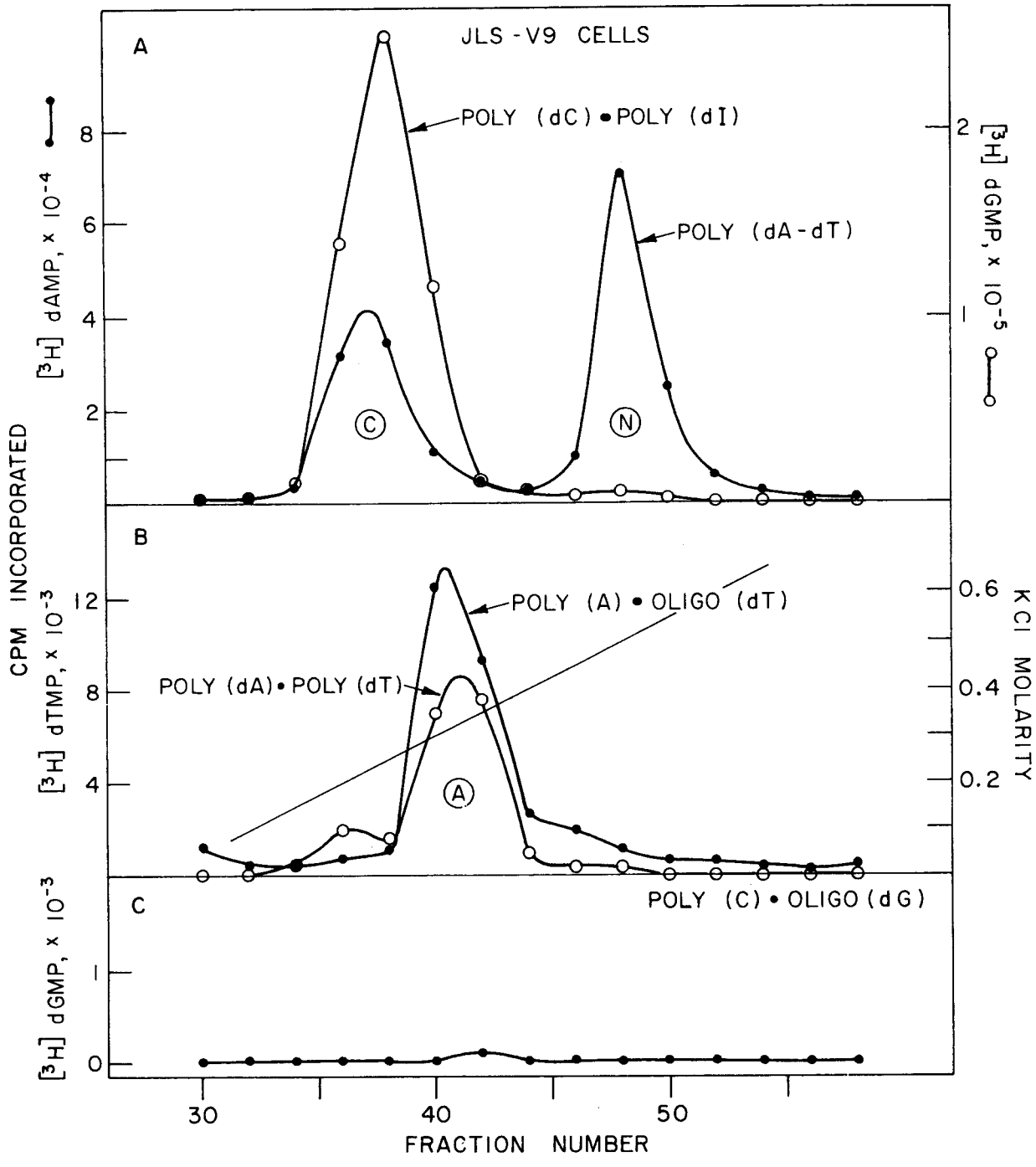

Fig. 1: DNA polymerases of uninfected murine fibroblasts, (JLS-V9 cells).
The individual panels represent assays of the fractions from a single phosphocellulose column using various templates, primers, and substrates. Assay conditions were as defined in reference 1.

Figure 1A shows two activities able to copy the alternating co-polymer, poly(dA-dT). These activities correspond to DNA polymerases C and N. Polymerase C is also evident when dGMP incorporation stimulated by poly(dC) • poly(dI) is measured. Figure 1B shows the activity of DNA polymerase A. It elutes as a separate peak, identifiable by its ability to synthesize poly(dT) when stimulated by poly(A) • oligo(dT) or poly(dA) • poly(dT). Figure 1C shows that there is no enzyme in this tumor virus-free mouse cell line which can copy poly(C) • oligo(dG). This will become a critical negative finding as discussed below.

Polymerases in Virally Infected Cells

A culture of JLS-V9 cells was infected with Moloney murine leukemia virus (MLV), and then became known as JLS-V11 cells (8). Figure 2 shows some of the DNA polymerase activities present in these infected cells. Figure 2A demonstrates polymerases C and N, as defined by activity with poly(dA-dT). Figure 2B shows that a major new activity is demonstrable in these cells, which we call DNA polymerase V. This enzyme polymerizes dGTP onto an oligo(dG) primer, directed by a poly(C) template. No such activity is present in the uninfected JLS-V9 cells (Fig. 1C). Polymerase V (termed V because of its restriction to the virus-infected cells) could be completely separated from polymerase C by re-chromatography on DEAE-Sephadex. Viral polymerase isolated directly from Moloney leukemia virions behaves exactly as does polymerase V on phospho-cellulose and DEAE-Sephadex. In fact, much of polymerase V probably comes from recently-made virions on the cell surface.

Specificity of Poly(C) • Oligo(dG)

We have screened a variety of viruses, cells, and tissues for an enzyme activity able to synthesize poly(dG) in response to poly(C) • oligo(dG). Such activity is demonstrable in virions of all RNA tumor viruses we have tested (7). It is also present in all cells known to be infected by, and producing oncogenic RNA viruses. Cells from 12

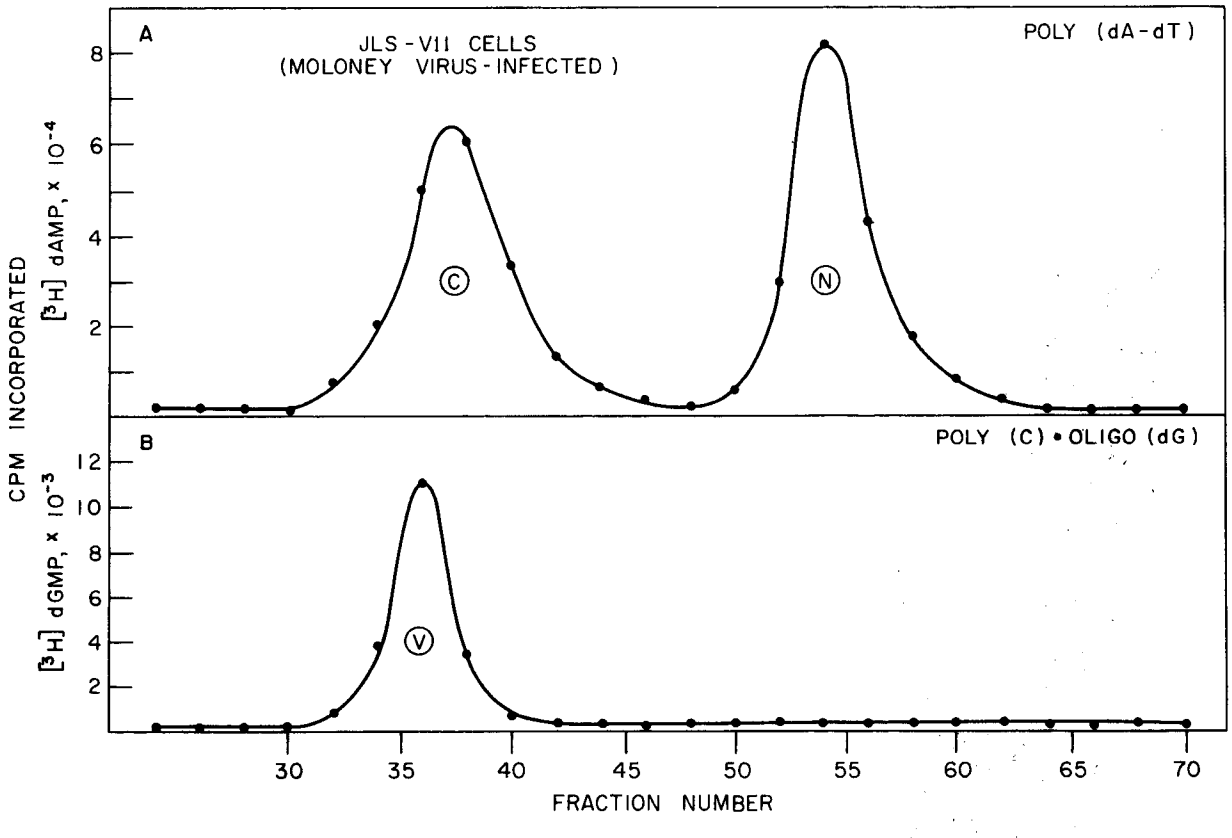

Fig. 2: DNA polymerases of murine fibroblasts productively infected with Moloney murine leukemia virus (JLS-V11 cells).

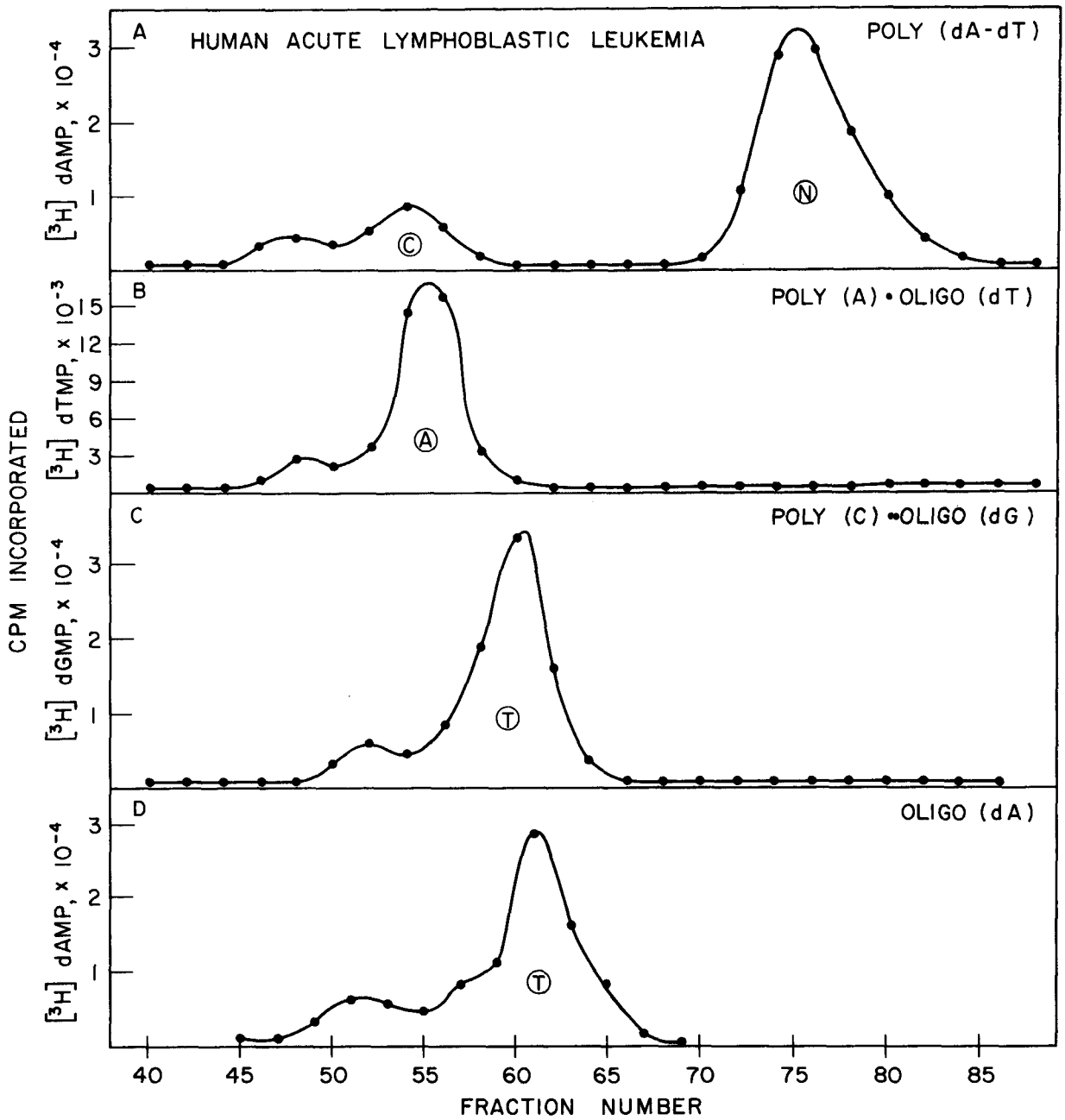

Fig. 3: DNA polymerases of circulating leukemic cells from a case of childhood acute lymphoblastic leukemia.

Reaction conditions are contained in reference 1. Taken from reference 1 by permission of the publishers.

cases of human leukemia (lymphoblastic, myeloblastic, lymphosarcoma, and chronic lymphatic), when assayed in this manner were all negative for polymerase V activity while they all contained polymerases C, N, and A.

Polymerases in Childhood Acute Lymphoblastic Leukemia

In the circulating leukemic cells from three out of five cases of childhood acute lymphoblastic leukemia we have noted a unique DNA polymerase in addition to the polymerases C, N and A which are evident in Figures 3A and 3B. This new enzyme

we have labeled DNA polymerase T (Figs. 3C and 3D). It scored on our initial screen for polymerase V in these cells, using poly(C) · oligo(dG). (Fig. 3C). However, as shown on Table 1 and in Figure 3D, it is completely template-independent. It can polymerize a deoxynucleoside triphosphate when supplied only an oligomer as initiator. In this respect it behaved similarly to calf-thymus terminal transferase, previously described by Bollum and co-workers (9, 10), an enzyme later shown to be unique to thymus tissue by Chang (11). Her study showed that in several animal species, only thymus had activity while the following tissues contained no such end-addition enzyme: bone marrow, spleen, lymph nodes, circulating lymphocytes, liver, lung, and bursa of Fabricius (from chickens).

Comparison with Human Thymus

We compared the lymphoblastic leukemia cell polymerases with the polymerases recovered from normal human infant thymus processed in the same manner. Figure 4 shows this data. The three normal enzymes are present (Figs. 4A and 4B), plus a fourth (Fig. 4C) activity which elutes from the phosphocellulose column at the same salt concentration and has the same primer requirement as does the terminal transferase from the leukemic cells.

Comparison with Other Human Cells

A variety of other human cells have been assayed using these same procedures. Only in ALL and normal thymus has an oligomer-stimulated end-addition enzyme (terminal transferase) been noted. Negative cells include three cases of lymphosarcoma cell leukemia, two cases of chronic lymphotic leukemia, two cases of acute myelo-blastic leukemia, normal spleen, normal circulating lymphocytes, infectious mononucleosis cells in culture, lymphosarcoma cells in culture, a line of lymphocytes derived from a patient with Burkitt's lymphoma, and HeLa cells.

DNA Polymerase in Chick Bursa

The occurrence of a DNA synthetic enzyme unique to the thymus, the organ which gives rise to T-lymphocytes, led us to ask whether the organ which processes

Table 1. Dependence of dGMP incorporation on poly(C) and oligo(dG)

Additions	Avian myeloblastosis virus DNA polymerase	Moloney leukemia virus DNA polymerase V	All polymerase T	Terminal transferase
	(pmol of dGMP incorporated)			
Poly(C) oligo(dG)	140	5.5	4.0	137
Poly(C) alone	< 0.1	< 0.4	0.4	–
Oligo(dG) alone	< 0.1	< 0.4	4.8	112

Adapted from reference 1. Data on avian myeloblastosis virus taken from reference 14.

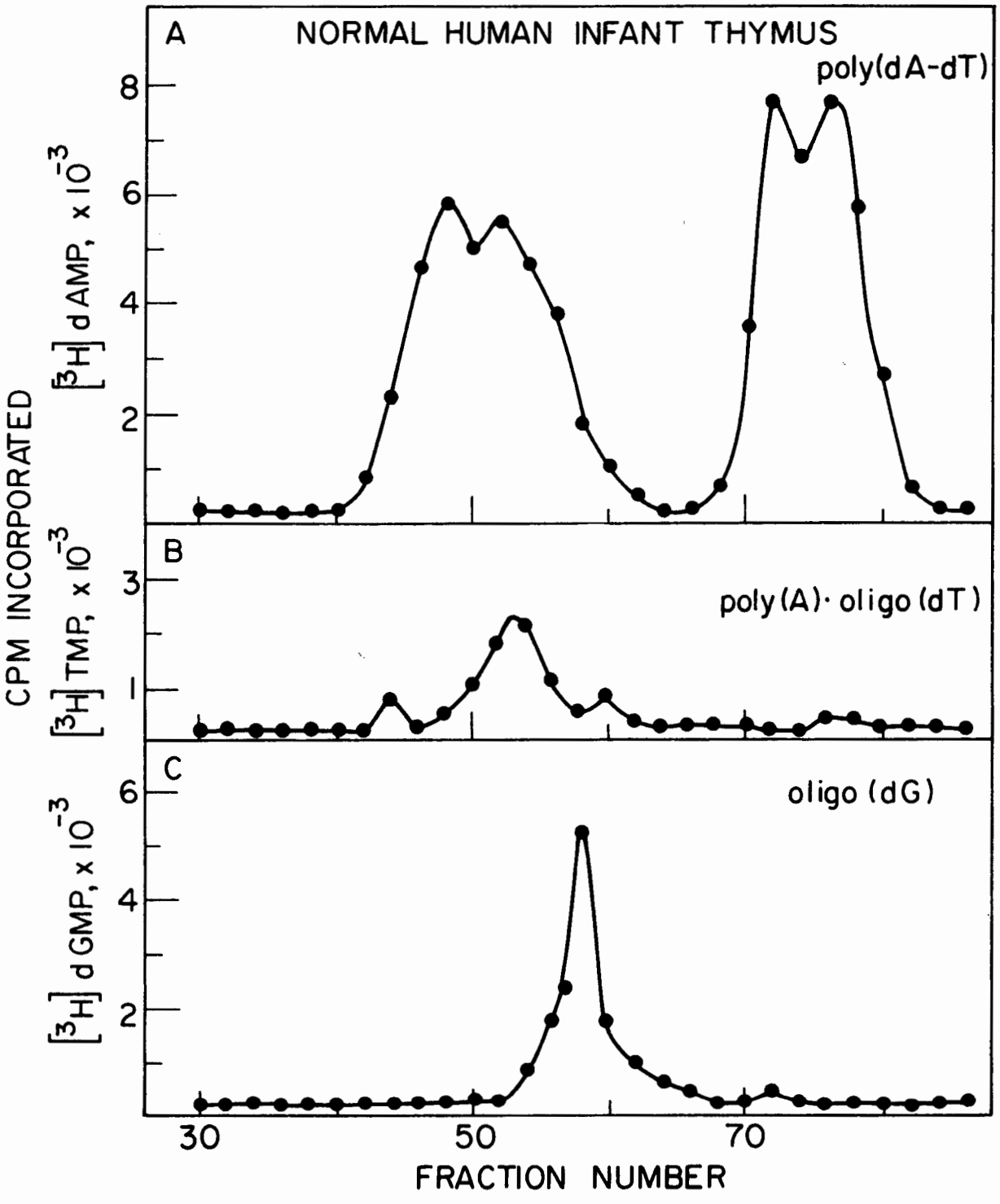

Fig. 4: DNA polymerases of normal human infant thymus.
Taken from reference 1 by permission of the publishers.

B-lymphocytes might similarly have a unique DNA polymerase. In chickens such an organ exists, and is called the bursa of Fabricius. Figure 5 shows the DNA synthetic activities present in 3–4 week old chick bursa tissue processed as described for other tissues here. A variety of activities are present. Figure 5A shows two major peaks with poly(dA-dT), which presumably are polymerase C and N from bursal cells. A third, new activity stimulated by poly(dA-dT) is also noted eluting at 0.6 M KCl. Figure 5B

shows that when activated DNA and a single deoxynucleoside triphosphate (here dTTP) are supplied to the various column fractions, a very significant peak of incorporation is noted eluting at about 0.6 M KCl. At lower salt concentrations a broad band of similar activity is seen. When a second deoxynucleoside triphosphate (here dCTP) is added, the enzyme eluting at 0.6 M KCl is dramatically inhibited; the broad low salt peak is inhibited to a lesser degree. Figure 4C shows a small peak of activity eluting at 0.25 M KCl when the bursal fractions are assayed for thymic-type terminal transferase. Under these conditions, i. e., oligo$(dA)_{14}$-stimulated incorporation of dGTP, the chick thymus shows an extremely active enzyme eluting from

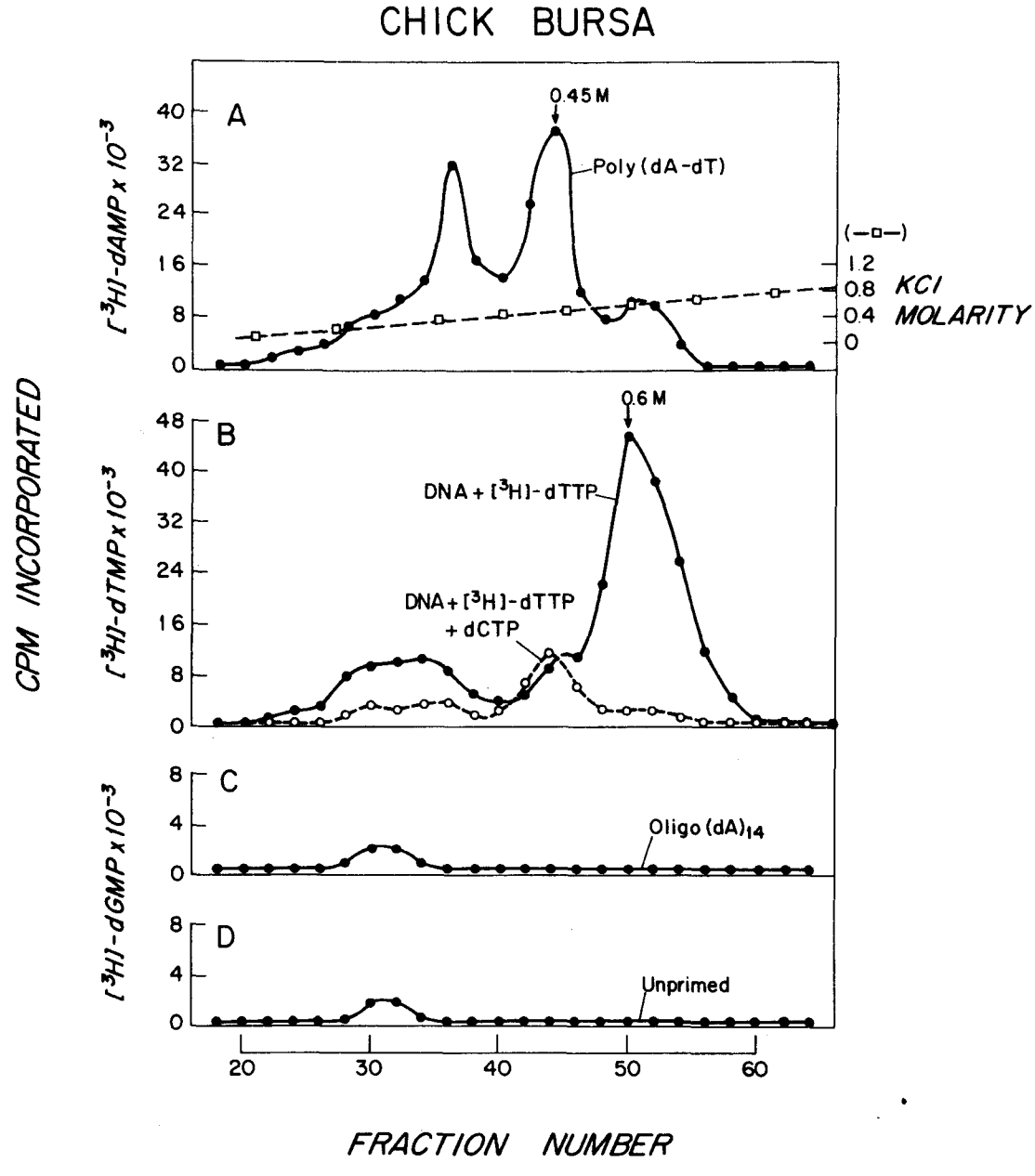

Fig. 5: DNA polymerases of chick bursa.

For panel B, 5 μg of DNase I treated calf thymus DNA, 200 picomoles of ^{3}H-dTTP (8000 cpm/pmole), 2000 picomoles of dCTP, and 0.6 mM $MnCl_2$, were used under reaction conditions noted in reference 1.

Table 2. DNA polymerases of chick tissues

Tissue	Terminal Transferase	Enzymes 0.6M „Bursal Enzyme"	Low Salt „Bursal Enzyme"	Replicative DNA Polymerases
Thymus	+++	–	±	+
Bursa	–	+++	++	+
Spleen (young)	–	++	+	+
Spleen (adult)	–	+	±	+
Liver	–	–	±	+
Muscle	–	–	±	+

Tissues were processed as described in text. Minus(–) signs indicate little or no ability to function under the assay conditions as defined in the text and reference 1. Plus(+) signs indicate increasing levels of activity. "Replicative DNA polymerases" refers to DNA polymerases C and N.

phosphocellulose at 0.4 M salt. Although this activity in the bursa has not yet been studied in any detail, the fact that we see essentially as much activity in the absence of oligo$(dA)_{14}$ (Fig. 4D), suggests that it is not related to terminal transferase. Further study of this activity is contemplated.

We have screened a number of tissues from 3–4 week old chicks for these activities, as shown in Table 2. Thymus contains the oligomer-stimulated terminal transferase. It lacks the activity seen in the bursa which elutes at 0.6 M KCl. All chick tissues contain the low salt broad peak, maximally active with nicked DNA, and a single deoxynucleoside triphosphate. Beside the bursa of Fabricius, only the spleen, an organ thought to be populated in part by lymphocytes from the bursa (12, 13), contains the enzyme that elutes at 0.6 M KCl. Our tentative conclusion is that this particular enzyme is unique to developing bursal (B-) lymphocytes.

Conclusions

We have developed a method which allows us to separate the three major soluble DNA polymerases present in eukaryotic cells by a single step purification from a crude cell extract. Using this methodology we can also detect viral reverse transcriptase as an additional fourth activity in cells which are producing virions.

In studying a variety of cells and tissues, we have noted that lymphocytes from different sources contain unique DNA polymerases. Certain cases of human acute lymphoblastic leukemia have circulating leukemic cells which have terminal transferase, an enzyme previously known to be present only in thymus. Cells (presumably lymphocytes) from the bursal system of chicks contain a unique, and hitherto undescribed enzyme, which is maximally active with nicked DNA and a single triphosphate.

The role of these unique enzymes in B-lymphocyte and T-lymphocyte precursor cells remains to be defined.

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