DNA POLYMERASE γ OF RABBIT INTESTINAL EPITHELIAL CELLS*

Institute of Oncology,
ul. Wawelska 15; 02-034 Warszawa, Poland

1. DNA-directed DNA polymerase γ was isolated from epithelial cells of the rabbit small intestine, and characterized.
2. The molecular weight of the enzyme, determined by Sephadex G-200 filtration, was 105,000 ± 15%.
3. The enzyme showed preference for poly(A) replication on poly(A)-poly(dT) or poly(A)-oligo(dT)_{12-18} templates, as compared with activated DNA. Poly(C)-oligo(dG)_{12-18} replication was not observed.
4. The Michaelis constants for dTTP in replication of activated DNA and poly(A)-oligo(dT)_{12-18} were 3.3 and 2.0 μM, respectively.

Reports from various laboratories indicate that animal cells contain at least four DNA polymerases (EC 2.7.7.7, DNA nucleotidyl transferase): high-molecular DNA polymerase α, low-molecular DNA polymerase β, DNA polymerase γ, and mitochondrial DNA polymerase. Preliminary findings regarding the presence of DNA polymerases α and β, and of the mitochondrial DNA polymerase in the rat intestinal epithelial cells have been reported by Leung & Zbarsky (1970), Poulson et al. (1974) and Poulson & Zbarsky (1973). In the present study we have attempted to demonstrate and characterize DNA polymerase γ in epithelial cells of the small intestine. On account of the generally low level of this enzyme in animal material, we used small intestine of rabbit. For detachment of epithelial cells the method of Weiser (1973) was adopted.

MATERIALS AND METHODS

Substrates. Deoxyribonucleoside triphosphates were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.); \(^7\)H-labelled deoxyribonucleoside triphosphates were obtained from the Radiochemical Centre (Amersham, England).

* This investigation was supported by grant no. 1302 from the Polish Governmental Research and Development Programme PR-6 "Control of Malignant Neoplasms".
Templates and primers. Native calf thymus DNA (Sigma Co.) was activated with DNase I according to Fansler & Loeb (1974). Oligo (dT)_{12-18} and oligo(dG)_{12-18} were obtained from P-L Biochemicals Inc. (Milwaukee, Wis., U.S.A.), and poly(A), poly(C) and poly(dT), from Miles Laboratories (Elkhart, Ind., U.S.A.). Poly 2'-O-methylcytidylic acid was prepared according to Žmudzka et al. (1969). Synthetic templates primers were prepared by mixing two complementary polynucleotides or a polynucleotide and an oligonucleotide at concentrations of 200 μg/ml in 5 mM Tris-HCl buffer, pH 7.8, containing 10 mM NaCl, followed by heating to about 80°C and annealing at room temperature for 1 h, and at 4°C for 18 h.

Chemicals: DEAE-cellulose (DE-52) from Whatman Co., CM-Sephadex (C-50 medium) and Sephadex G-200 from Pharmacia Co., DNase I from Worthington Co., and polyethylene glycol 20,000 from Fluka Co.

Buffers. The following buffers were used: buffer A: 2.1 mM KH₂PO₄, 1.2 mM Na₂HPO₄, pH 7.4, 3.8 mM KCl, 138 mM NaCl, 1.5 mM EDTA and 0.5 mM mercaptoethanol; buffer B: 1 mM potassium phosphate, pH 7.5, 10 mM NaCl and 0.5 mM mercaptoethanol; buffer C: 0.4 mM potassium phosphate, pH 7.5, 0.5 mM mercaptoethanol; buffer D: 20 mM potassium phosphate, pH 7.5, 0.5 mM mercaptoethanol; buffer E: 50 mM Tris-HCl, pH 7.5, 10% glycerol and 1 mM mercaptoethanol; buffer F: 50 mM Tris-HCl, pH 7.5, 0.2 mM KCl, 1 mM EDTA, 10% glycerol and 1 mM mercaptoethanol.

Isolation of intestinal epithelial cells. Detachment of epithelial cells from the rabbit small intestine was performed according to Weiser (1973). A non-fasting rabbit (Popieliańska Biała) weighing 3 kg was killed by cervical dislocation. The small intestine was removed, separated from duodenum, large intestine and mesenterium, washed with cold 154 mM NaCl containing 1 mM dithiothreitol (DTT), and turned inside out. Then it was placed for 15 min at 37°C in 8 mM KH₂PO₄ - 5.6 mM Na₂HPO₄ buffer, pH 7.3, containing 1.5 mM KCl, 96 mM NaCl and 27 mM sodium citrate. The washing solution was removed. To detach the epithelial cells, the intestine was kept for 5 min, with occasional stirring, in 50 ml of buffer A. Subsequently, the intestine was removed, rinsed by immersion in 25 ml of the same buffer, and again soaked in buffer A to release the next batch of epithelial cells. This procedure was repeated several times; the incubation time varied as indicated in Table 1. The 50-ml and 25-ml portions of the buffer from each treatment were pooled and centrifuged at 900 g for 5 min at 0°C. The pellets were washed twice with buffer A and centrifuged each time at 900 g for 5 min at 0°C. The successive pellets (1-11) were stored at -28°C.

Isolation of DNA polymerase γ. Frozen cells (46 g) derived from batches 4 and 5 (Table 1), were thawed and suspended in 150 ml of buffer B. All further steps were performed at 4°C. The cell suspension was homogenized manually (twenty-five strokes) and centrifuged at 1000 g for 10 min. The pellet was suspended in 20 ml of 1 mM potassium phosphate, pH 7.5, containing 320 mM sucrose, 1 mM MgCl₂ and 0.3% Triton X-100, then it was homogenized and centrifuged at 1000 g for 10 min; the sediment obtained was resuspended and the homogenization procedure was repeated. Three 1000 g supernatants were pooled and centrifuged at 15,000 g for 10 min.
The 1000 g pellet was suspended in 27 ml of 1 mm-potassium phosphate, pH 7.5, containing 320 mm-sucrose, 2 mm-MgCl₂ and 0.5 mm-mercaptoethanol; the phosphate concentration of the solution was adjusted to 0.4 m with 1 m-potassium phosphate, pH 7.5. The solution was then sonicated with the use of MSE sonicator, three times for 15 sec, at 1.8 V, and centrifuged at 20 000 g for 20 min to remove lysosomes and mitochondria.

The 15 000 g and 20 000 g supernatants were pooled and loaded onto a DEAE-cellulose column (2 x 31 cm) equilibrated with buffer C. The column was eluted with the same buffer, the effluent and eluate were pooled (480 ml), concentrated to 180 ml in the presence of 30% polyethylene glycol (20 000) in buffer D, and dialysed against the same buffer.

The dialysed material was loaded onto a DEAE-cellulose column (1.8 x 16 cm) equilibrated with buffer D. The column was washed with 75 mm-potassium phosphate, pH 7.5, containing 0.5 mm-mercaptoethanol until free of material absorbing at 280 nm, then the elution was continued with buffer C. The fractions absorbing at 280 nm were concentrated in the presence of 25% polyethylene glycol (20 000) in buffer C, dialysed against buffer E, and adsorbed on a CM-Sephadex column (1.5 x 39 cm) equilibrated with buffer E.

The CM-Sephadex column was washed with buffer E until free of material absorbing at 280 nm, followed by 150 ml of a linear gradient from 100 mm to 800 mm-KCl in buffer E, and 1.4-ml fractions were collected. The material not adsorbed on CM-Sephadex and the eluted fractions were assayed for DNA polymerase activity. Fractions eluted with 570-600 mm-KCl were pooled (12.5 ml), concentrated to 1.3 ml in the Amicon standard stirred cell with UM 10 Diaflo membrane, and dialysed against buffer F.

The dialysed sample (0.8 ml) was loaded onto a Sephadex G-200 column (1.9 x 70 cm) equilibrated with buffer F and calibrated with cytochrome c, lysozyme, ovomucoid, bovine serum albumin and catalase as protein markers with molecular weights of 12 000, 17 000, 45 000, 69 000 and 250 000, respectively. The column was eluted with buffer F, at a flow rate of 3 ml/h/cm². A total of 170 fractions (1.2 ml) were collected and DNA polymerase was assayed therein using synthetic polynucleotide template. Fractions with the highest activity were pooled (47 ml), concentrated to 1.8 ml in an Amicon standard stirred cell, dialysed against buffer F with glycerol content adjusted to 50%, and stored at -27°C.

Microscopic examination. Single drops of the suspension of the freshly detached epithelial cells were smeared on microscopic slides, air-dried, fixed with methanol for 5 min, and stained with aceto-orcein, hematoxylin and eosin, or periodic acid leucofuchsina (PAS) for examination with light microscope.

Protein determination. Protein was assayed by Lowry's method (1951).

Alkaline phosphatase determination. Alkaline phosphatase was assayed using p-nitrophenylphosphate as a substrate (Lowry, 1957). The reaction mixture contained in a final volume of 1.1 ml: 500 mm-Tris-HCl buffer, pH 9.3, containing 0.3 mm-ZnCl₂ and 0.4 mm-p-nitrophenylphosphate, and the enzymatic fraction investigated; the mixture was incubated for 30 min at 37°C. Then, 0.5 ml of 0.5
m-NaOH was added, and the absorption at 410 nm was measured. One unit of the enzymatic activity is defined as the amount of the enzyme required to hydrolyze 1 micromole of p-nitrophenylphosphate at 37°C during 30 min.

**DNA polymerase assay.** DNA polymerase was assayed using as template either activated calf thymus DNA (system I) or synthetic polynucleotide complexed with oligonucleotide (system II). In system I the standard reaction mixture contained in a final volume of 50 μl: the amount of enzyme as indicated for each experiment, 40 mM-potassium phosphate, pH 7.0, 7 mM-KCl, 8 mM-MgCl₂, 0.8 mM-DTT, crystalline bovine serum albumin (1 mg/ml), activated calf thymus DNA (0.3 mg/ml), dCTP, dATP, dGTP and [³H]dTTP (40 c.p.m./pmole), each at concentration of 0.1 mM. In system II the reaction mixture contained in a final volume of 50 μl: the amount of enzyme as indicated in each experiment, 40 mM-Tris-HCl, pH 8.0, 100 mM-KCl, 0.4 mM-MnCl₂, 1.6 mM-DTT, crystalline bovine serum albumin (1 mg/ml), poly(A) (20 μg/ml) with oligo (dT)₁₂₋₁₈ (10 μg/ml) and 0.1 mM-[³H]dTTP (40 c.p.m./pmole). If in system II poly(dA) (20 μg/ml) with oligo(dT)₁₂₋₁₈ (1 μg/ml); or poly(C) (20 μg/ml) with oligo(dG)₁₂₋₁₈ (20 μg/ml); or poly(Cm) (20 μg/ml) with oligo(dG)₁₂₋₁₈ (20 μg/ml), were used as templates, then 0.1 mM-[³H]dTTP (40 c.p.m./pmole) or 0.1 mM-[³H]dGTP (40 c.p.m./pmole) were applied as substrate. Incubations were carried out at 37°C for 30 min. Any deviations from standard conditions and procedure are indicated in the descriptions of the particular experiments. The radioactive acid-precipitable material was collected and washed on Whatman no. 1 filters (diameter 24 mm) according to Bollum (1966). One unit of enzyme activity is defined as the amount catalysing incorporation of 1 nmole of [³H]dTTP into the acid-precipitable material at 37°C during 60 min with activated DNA template.

**RESULTS**

**Collection and evaluation of intestinal epithelial cells.** Incubation of the rabbit small intestine devoid of intestinal juice and mucus, in the metal-chelating buffer (Weiser, 1973) resulted in a gradual detachment of epithelial cells from the mucosal stroma. Data given in Table 1 show that the cells were released abundantly between 25 and 135 min of incubation. The number of the cells collected before 25 min and after 135 min of incubation accounted only for 6 - 8% of the total number of collected cells. An attempt at shortening the time of incubation at 37°C by pre-incubation of the intestine in the "releasing" buffer A at 0°C for 30 min was unsuccessful.

On light-microscope examination, the first two or three batches of the cellular material comprised singly dispersed cells with pale cytoplasm and poorly defined boundaries. Many of these cells gave a positive PAS reaction, and all were considered as epithelial cells of the villi. Further batches contained increasing amounts of tightly packed, regular, oval or rounded clumps of basophilic, PAS-negative cells representing epithelium of the crypts. These conglomerates predominated in the last three or four batches. No supportive connective tissue was seen in the material obtained.
Table 1

Characteristics of batches of the epithelial cells detached from rabbit small intestine

Epithelial cells were detached successively in a step-wise procedure according to Weiser (1973). Alkaline phosphatase was assayed, as described in Materials and Methods, in cytoplasmic and nuclear cell fractions obtained as follows: from the thawed cells a 30% homogenate in 50 mM-Tris-HCl buffer, pH 7.4, containing 154 mM-NaCl, was prepared and centrifuged at 2500 g for 10 min. The supernatant was considered to be the cytoplasmic fraction; the precipitate was homogenized, filtered through cheese-cloth and treated as the membrane-nuclear fraction. The activity of the sum of cellular DNA polymerases was determined in the cytoplasmic fraction using standard system 1 described in Materials and Methods. NT, not tested.

<table>
<thead>
<tr>
<th>Batch no.</th>
<th>Time of release (min)</th>
<th>Wet mass (g)</th>
<th>Alkaline phosphatase (u/g wet mass)</th>
<th>DNA polymerases (u/g wet mass)</th>
<th>(u./batch)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 - 5</td>
<td>0.88</td>
<td>70.0</td>
<td>2.30</td>
<td>2.00</td>
</tr>
<tr>
<td>2</td>
<td>5 - 15</td>
<td>1.41</td>
<td>79.55</td>
<td>3.75</td>
<td>5.30</td>
</tr>
<tr>
<td>3</td>
<td>15 - 25</td>
<td>3.02</td>
<td>122.90</td>
<td>4.45</td>
<td>13.45</td>
</tr>
<tr>
<td>4</td>
<td>25 - 40</td>
<td>5.85</td>
<td>95.66</td>
<td>3.95</td>
<td>23.10</td>
</tr>
<tr>
<td>5</td>
<td>40 - 60</td>
<td>9.75</td>
<td>93.37</td>
<td>4.45</td>
<td>43.40</td>
</tr>
<tr>
<td>6</td>
<td>60 - 80</td>
<td>10.04</td>
<td>73.42</td>
<td>4.55</td>
<td>45.75</td>
</tr>
<tr>
<td>7</td>
<td>80 - 100</td>
<td>9.93</td>
<td>49.29</td>
<td>5.35</td>
<td>52.95</td>
</tr>
<tr>
<td>8</td>
<td>100 - 120</td>
<td>10.71</td>
<td>32.25</td>
<td>6.54</td>
<td>69.30</td>
</tr>
<tr>
<td>9</td>
<td>120 - 135</td>
<td>11.80</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>10</td>
<td>135 - 150</td>
<td>4.67</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>11</td>
<td>150 - 180</td>
<td>0.25</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

Microscopic evaluation was supplemented by the assays, in cell homogenates, of alkaline phosphatase (Lowry, 1957) known to occur abundantly in the epithelial cells of villi. This enzyme can be regarded as a marker differentiating between the epithelial cells of the villi and crypts. As can be seen from Table 1, the phosphatase activity was the highest in the cells released within 25 min. Consistently with the data of Weiser (1973), the alkaline phosphatase activity was decreased in the batches released on more prolonged incubation, in parallel with the release of crypt cells.

Data presented in Table 1 refer to the total DNA polymerase activity (α+β+γ+ +mitochondrial DNA polymerase), assayed in the cellular cytoplasmic fraction which contains most of this activity (Weissbach, 1975). In contrast to alkaline phosphatase, the DNA polymerase activity was the highest in the crypt cells, and was about three times higher than that found in the villi cells released within the first 5 min of incubation.

A parallel experiment, in which the epithelial cells were collected by mechanical scraping of the small intestine at 4°C, afforded fewer cells (40 g) with lower DNA polymerase activity (4 u./g wet mass), as compared with the technique used. Moreover, the scraped material contained connective tissue derived from the mucosal stroma. Batches 4 - 9 were chosen for preparation of DNA polymerases basing on the yield of cells released from epithelium (Table 1) and DNA polymerase activity.
Preparation of DNA polymerase γ. DNA polymerase γ was isolated from the postmitochondrial fraction pooled with the nuclear extract. From this material, nucleic acids were removed on the first DEAE-cellulose column, and DNA polymerase β, not adsorbed from 20 mm-potassium phosphate, on the second DEAE-cellulose column (cf Methods) under conditions described by Weissbach et al. (1971), Lewis et al. (1974) and Siedlecki et al. (1976).

The DNA polymerase activity retained on DEAE-cellulose and eluted with 0.4 M-potassium phosphate buffer, pH 7.5, is expected to be due to polymerases α and γ (Spadari & Weissbach, 1974; Matsukage et al., 1975; Siedlecki et al., 1976). To remove DNA polymerase α, the eluate was chromatographed on CM-Sephadex. The two peaks exhibiting the DNA replication activity, were eluted with 470 and 580 mm-KCl. The first peak accounted for 75% of the total activity measured after the removal of nucleic acids; it showed no poly(A)·oligo(dT)_{12-18} replication activity. This indicates that the first peak corresponds to DNA polymerase α; under the same conditions also DNA polymerase α of murine LBN/b leukaemic cells was eluted from CM-Sephadex (Siedlecki et al., 1976).

The DNA polymerase eluted with 580 mm-KCl (second peak), in addition to the DNA replication ability, actively synthesized poly(dT) on poly(A)·oligo(dT)_{12-18} template, and thus should be identified as DNA polymerase γ. The most active fractions of this peak, after filtration through Sephadex G-200 (Fig. 1) and concentration, were used in further experiments as a preparation of DNA polymerase γ.

![Fig. 1. Sephadex G-200 gel filtration of DNA polymerase γ. A total of 122 enzyme units from the CM-Sephadex column was filtered as described in Materials and Methods. The DNA polymerase activity was assayed in standard system II. Insert: calibration of Sephadex G-200 column with cytochrome c (C), lysozyme (Lys), ovomuculin (Ov), bovine serum albumin (BSA) and catalase (Cat). The first four proteins were determined spectrophotometrically at 280 nm, and catalase with \( \text{H}_2\text{O}_2 \) according to Chance & Maehly (1955). Void volume \( (V_0) \) was measured with dextran blue. ●, DNA polymerase γ.](image)

Molecular weight of DNA polymerase γ, determined by Sephadex G-200 filtration (Fig. 1) in the presence of protein markers, was 105,000 ± 15%.

DNA replication by DNA polymerase γ. DNA replication was linear within the first 50 min of incubation (Fig. 2) with activated calf thymus DNA as a template. Presence of four deoxyribonucleoside triphosphates was indispensable for maximum DNA replication. The Michaelis constant, determined for dTTP during DNA
replication, calculated from the Lineweaver-Burk plot, was 3.3 μM. The replication was not inhibited by 1 mM or 10 mM N-ethylmaleimide.

Fig. 2

Fig. 2. Kinetics of [3H]dTMP incorporation into the acid-precipitable material. Replication of activated DNA (○) and (A)₆·(dT)₁₂·₁₈ (●) with DNA polymerase γ (0.15 unit) was carried out in standard systems I and II, respectively, as described in Materials and Methods.

Fig. 3

Fig. 3. Effect of concentration of the enzyme on [3H]dTMP incorporation into the acid-precipitable material. Replication of poly(A)·oligo(dT)₁₂·₁₈ by DNA polymerase γ was carried out in standard system II as described in Materials and Methods.

Replication of synthetic templates by DNA polymerase γ. The DNA polymerase γ preparation obtained exhibited a marked replication activity with synthetic templates: poly(A)·poly(dT), poly(A)·oligo(dT)₁₂·₁₈, as well as poly(dA)·oligo(dT)₁₂·₁₈, in the presence of Mn²⁺. The replication was linear within the first 40 min of incubation at 37°C (Fig. 2), and incorporation of radioactivity was proportional.

Table 2

Template characteristics of DNA polymerase γ

<table>
<thead>
<tr>
<th>Template</th>
<th>Incorporated deoxynucleotide (pmoles)</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated DNA</td>
<td>25.5</td>
<td>1</td>
</tr>
<tr>
<td>(A)₆·(dT)₁₈</td>
<td>150.0</td>
<td>5.8</td>
</tr>
<tr>
<td>(A)₆·(dT)₁₂·₁₈</td>
<td>172.0</td>
<td>6.2</td>
</tr>
<tr>
<td>(dA)₆·(dT)₁₂·₁₈</td>
<td>80.5</td>
<td>3.1</td>
</tr>
<tr>
<td>(C₆)₆·(dG)₁₂·₁₈</td>
<td>2.5</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>(C₆)₆·(G₆)₁₂·₁₈</td>
<td>1.5</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>(dT)₁₂·₁₈</td>
<td>5.0</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Assays were performed as described in Materials and Methods, using 0.05 unit of DNA polymerase γ, and standard system I for measuring DNA replication, and system II for replication with a synthetic polynucleotide template.
to the concentration of the enzyme (Fig. 3). The $K_m$ value for dTTP in the reaction with poly(A)-oligo(dT)$_{12-18}$ was 2 µM. The relative activities with various templates are recorded in Table 2.

DISCUSSION

In normal animal organism the epithelium of the alimentary tract, uterus and skin is, in addition to the bone marrow, thymus and regenerating liver, one of the few tissues in which the cells divide rapidly. The intense DNA synthesis in epithelium is associated with a high total DNA polymerase activity (Loeb, 1975). DNA polymerases $\alpha$ and $\beta$, and the mitochondrial DNA polymerase of rat intestinal epithelium, have been the subject of a few studies (Poulson & Zbarsky, 1973; Poulson et al., 1973, 1974). In the present work, polymerase $\gamma$ was isolated from the rabbit intestinal epithelium. For detection of this activity, the removal of DNA polymerases $\gamma$ and $\beta$, and mitochondrial DNA polymerase, was found to be an indispensable preliminary step.

For separation of the cells of various differentiation level, i.e. from the scaling cells of the upper villi to the dividing crypt cells, we have used the technique described by Weiser (1973). In this way it was possible to obtain a material which was more abundant, histologically purer, and richer in DNA polymerase as compared with the epithelial cells scraped from the intestine.

The molecular weight of the final preparation of DNA polymerase $\gamma$ was found to be 105 000, which is consistent with the most often reported values (Spadari & Weissbach, 1974; Srivastava, 1974; Lewis et al., 1974). However, two extremely divergent values: 230 000 - 315 000 (Matsukage et al., 1975), and 45 000 (Maia et al., 1971; Evans et al., 1976; Siedlecki et al., 1976) were also found.

The enzyme from rabbit small intestine, similarly as DNA polymerases $\gamma$ from other tissues (Spadari & Weissbach, 1974; Lewis et al., 1974; Srivastava, 1974; Matsukage et al., 1975) exhibits relatively high activity of replication of poly(A)-poly(dT) and poly(A)-oligo(dT)$_{12-18}$, as compared with DNA replication. In this respect it differs distinctly from DNA polymerase $\beta$ which replicates poly(A)-poly(dT) at a lower rate (Chang, 1974). Moreover, the intestinal DNA polymerase $\gamma$ replicates poly(dA)-oligo(dT)$_{12-18}$ with the activity lower by half than with the poly(A)-oligo(dT)$_{12-18}$ template. DNA polymerase $\gamma$ of the highest purity attained so far (Matsukage et al., 1975) exhibits the same template characteristics. On the other hand, Lewis et al. (1974) and Srivastava (1974) are of the opinion that the very low activity on poly(dA)-oligo(dT)$_{12-18}$ template is typical of DNA polymerase $\gamma$.

The Michaelis constant of the DNA polymerase $\gamma$ isolated by us from epithelial cells, is 3.3 µM, i.e. distinctly lower than the $K_m$ values about 10 and 13 µM of DNA
polymerases $\alpha$ and $\beta$ (Loeb, 1975; Bollum, 1975). This low value of $K_m$ seems to be typical for DNA polymerases $\gamma$ from various tissues (Spadari & Weissbach, 1974; Matsukage et al., 1975; Siedlecki et al., 1976).

The adapted method for selective release of epithelial cells can be applied for studies on the fate of DNA polymerase $\gamma$ in the cells irreversibly leaving the life-reproductive cycle.

We thank Prof. Dr. A. Michalowski for helpful discussion and microscopic evaluation of the material.

REFERENCES


DNA POLIMERAZA γ Z KOMÓREK NABŁONKA JELITA KRÓLKA

Streszczenie

1. Wyizolowano DNA zależną DNA polimerazę γ z komórek nabłonka jelita cienkiego królika.

2. Ciężar mlekularny enzymu określony metodą filtracji na Sephadex G-200 ustalono na 105 000± 15% daltonów.

3. Enzym wykazywał większą preferencję do replikacji poli(A) na matrycy poli(A)-poli(dT) lub poli(A)-oligo(dT)₁₂₋₁₈ niż do replikacji aktywowanego DNA. Nie obserwowano replikacji poli(C) na matrycy poli(C)-oligo(dG)₁₂₋₁₈.

4. Stała Michaelis dla substratu dTTP mierzona w reakcji replikacji aktywowanego DNA i poli(A)-oligo(dT)₁₂₋₁₈ wynosi, odpowiednio, 3,3 i 2,0 μM.

Received 20 October, 1976.