THE EFFECTS OF AGE
AND OESTRONE TREATMENT ON DNA POLYMERASE
ACTIVITY IN ANTERIOR PITUITARY GLANDS
OF MALE RATS

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(Received 22 January 1973)

SUMMARY

DNA polymerase activity was found in the cytoplasmic fraction and in isolated nuclei from anterior pituitary glands of male rats. The enzyme activity was assayed by measuring the incorporation of [3H]dTTP into DNA in a medium containing Tris–HCl buffer (pH 8.5), the four deoxyribonucleoside triphosphates, Mg2+, ATP and activated calf thymus DNA. The DNA polymerase activity decreased with age in glands from animals aged 25 days to over a year but increased after oestrone treatment in vivo. These changes in activity, more pronounced in the cytoplasmic fraction than in the isolated nuclei, were similar to changes in DNA synthesis measured in anterior pituitary glands under the same physiological conditions.

Isolated nuclei also retained endogenous DNA synthetic activity in the absence of added template. Addition of a cytoplasmic fraction to the reaction medium stimulated activity by as much as 1.9-fold but the degree of stimulation was the same whether the cytoplasm was from young, old or oestrone-treated animals.

INTRODUCTION

Cell turnover in the anterior pituitary gland is extremely low, but there is good evidence that it varies with the physiological state of the animal. From the results of a number of recent autoradiographic studies (Crane & Loomes, 1967; Mastro, Hymer & Therrien, 1969; Korfsmeyer & Davidoff, 1970; Städtler, Stöcker, Dhom & Tietze, 1970) it has been estimated that 1–2% of the pituitary cells in the glands of young adult rats are actively synthesizing DNA at any one time. Furthermore, 1–2% of the cells can be stimulated to divide in response to hormonal treatment; specific cells are affected depending on the type of hormone administered. For example, hormone imbalance resulting from injection of oestrogens stimulates DNA synthesis in acidophilic mammatrophs (Wolfe, 1935; Clifton & Meyer, 1956; Stevens

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& Helfenstein, 1966), whereas castration stimulates DNA synthesis in basophilic gonadotrophs (Pomerat, 1941; Hymer, Mastro & Griswold, 1970). In addition to these changes, DNA synthesis in the anterior pituitary exhibits diurnal fluctuation and also varies with the oestrous state of the animal (Hunt & Hunt, 1966) or the development of pregnancy and lactation (Hunt, 1949). Finally, frequency of mitosis in the pituitary decreases with the age of the rat; mitoses are frequent in the neonatal rat and decline as the animal progresses through adulthood into old age (Pomerat, 1941; Hunt, 1943).

The in-vivo fluctuations in the rates of DNA synthesis following physiological stimuli are also reflected in the ability of isolated pituitary glands to incorporate $[^3]H$thymidine into cellular DNA in vitro (Mastro et al. 1969a; Hymer et al. 1970). In an attempt to explain these changes at a subcellular level, we have measured the activity and localization of DNA polymerase (deoxynucleoside triphosphate: DNA deoxynucleotidyltransferase, E.C. 2.7.7.7), during oestrone treatment and natural ageing of the animals. These studies show that the level of cytoplasmic DNA polymerase which utilizes an activated DNA template (see Materials and methods), changes in parallel with the level of endogenous DNA synthetic activity.

A preliminary report of this work has already been published (Mastro & Hymer, 1970).

**MATERIALS AND METHODS**

**Animals**

Male Sprague–Dawley rats were obtained from the Holtzman Company, Madison, Wisconsin, at least 1 week before use. They were kept in conditions of 12 h light, 12 h darkness with food *ad libitum*. Most experiments were carried out on animals ranging in age from 35 to 55 days; in certain studies young (25–28 days) or old (8–15 months) rats were used. The old animals were described by the Holtzman Company as retired breeders.

Rats (46–51 days) were injected subcutaneously with oestrone (Nutritional Biochemicals Corp., Cleveland, Ohio, 10 $\mu$g in 0.15 ml corn oil) or corn oil only as a control. Injections were given on alternate days over a period of 7 days; the rats were killed on the day after the final injection.

**Preparation of cellular fractions**

Rats were killed by cervical dislocation between 08.30 and 09.30 h. The pituitary glands were removed quickly, and the pars distalis was freed from the neural lobe and from most of the pars intermedia. This preparation of pars distalis (hereafter referred to as anterior pituitary gland) was homogenized in 0.25 m-sucrose and 2 mM-MgCl$_2$ (hereafter referred to as homogenizing medium) in a ground glass homogenizer. One to four pituitaries were homogenized in 0.1 ml homogenizing medium depending on the age of the animal and the size of the pituitary. All operations were carried out at 0–4 °C unless stated otherwise.

The homogenates were centrifuged for 15 min (1000 g, SS-34 rotor) in a Sorval Superspeed RCS Refrigerated Centrifuge. The supernatant fraction was withdrawn and was used in the assay for cytoplasmic DNA polymerase activity.

Nuclei were isolated according to the method of Hymer & Kuff (1964). The
nuclear pellet was resuspended in 1 ml homogenizing medium containing 0·1 % Triton X-100 (Rhom and Hass Co.), was centrifuged and washed twice in homogenizing medium. The isolated nuclei were resuspended in homogenizing medium and used immediately for assay. Recovery of nuclei was approximately 70 %.

Other methods for isolating nuclei by centrifugation through different concentrations of sucrose were tried (Widnell & Tata, 1964; Blobel & Potter, 1966), but the yields of nuclei from the small amounts of pituitary tissue were too small (30–50 %) to warrant their use.

Assay of DNA polymerase activity

The incubation mixture was composed of 0·04 m-Tris–HCl (pH 8·5), 5 mm-MgCl₂, 10 mm-KCl, 1 mm-2-mercaptoethanol, 5 mm-ATP, 0·2 mm each dATP, dCTP, dGTP (sodium salts, Sigma Chemical Co.), 0·15 mm-[³H]dTTP (0·8–3·0 Ci/mmol, obtained from New England Nuclear Corporation as 11·4–18·3 Ci/mmol), 35 μg calf thymus DNA, and cytoplasmic fraction (100–600 μg protein) in a total volume of 0·3 ml. These conditions were determined to be optimal for pH, and Mg²⁺ concentration, and showed a linear incorporation of [³H]dTTP with respect to time and enzyme concentration. The cytoplasmic fraction showed maximal DNA polymerase activity in the standard incubation medium in the presence of activated DNA (see Preparation of DNA) and all four deoxyribonucleotides. Incorporation of [³H]dTTP with the activated DNA was 60 times that of the cytoplasm alone and about four times that of the activity with either native or heat-denatured DNA. Omission of three of the triphosphates from the standard medium decreased DNA polymerase activity by 55 %. The enzyme activity increased linearly with the concentration of cytoplasmic protein up to 600 μg and remained linear from 10 to 60 min. Under standard conditions, the cytoplasmic fraction from anterior pituitary glands of 38- to 49-day-old animals incorporated 40 pmol [³H]dTTP/mg protein during 10 min of incubation.

Incubations were carried out in 12 ml conical glass tubes at 37 °C with shaking. The reactions were terminated by placing the tubes on ice and adding ice-cold 10 % trichloroacetic acid and 3 mg of Celite 545 (Fisher Scientific Co., Pittsburgh, Pa.) as a carrier. After standing for 30 min the tubes were centrifuged (1000 g, 15 min) and the precipitates resuspended in 2 ml of an aqueous solution containing 1 % sodium pyrophosphate (w/v) and 5 % trichloroacetic acid (w/v). The precipitate was collected by filtration on to Gelman metricel membranes (0·45 μ pore size) layered with 5 mg Celite. After washing with ice-cold 5 % trichloroacetic acid the precipitates were hydrolysed in 5 % trichloroacetic acid at 80 °C for 30 min and aliquots of the hydrolysate were added directly to 5 ml Aquasol liquid scintillation fluid (New England Nuclear Corporation) or were neutralized with NH₄OH and mixed with 10 ml Bray’s solution (Bray, 1960). Radioactivity was determined in a Unilux 11, Nuclear Chicago Liquid scintillation spectrometer with a ¹³⁷Ba external standard. Counting efficiency was 25 % in Aquasol and 16 % in Bray’s solution. All counts were corrected for background and converted to disintegrations per minute (d.p.m.). Results are expressed as pmol [³H]dTTP incorporated per mg protein or nuclear DNA.

The protein content in aliquots of the cytoplasmic fraction was measured after extraction of the nucleic acids by a modified Schneider (1945) procedure. The protein
content was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951), using bovine serum albumin (fraction V, Nutritional Biochemical Corporation, Cleveland, Ohio) as a standard.

The reaction mixtures with the nuclei were treated similarly except that 6% perchloric acid (v/v) was used instead of trichloroacetic acid as precipitant and the residues were washed sequentially with 95% ethanol and ethanol/ether (1/1). DNA content was determined by the method of Burton (1956) as modified by Giles & Myers (1965). Salmon sperm DNA (Calbiochem, La Jolla, California, highly polymerized) was used as a standard.

**Preparation of DNA**

Native DNA was prepared by dissolving calf thymus DNA (Sigma Chemical Co., type 1, highly polymerized) in 0.02 M-Tris-HCl, 5 mM-MgCl₂ (pH 8.5) to a final concentration of 350 μg DNA/ml. A portion of this DNA was denatured by heating at 100 °C for 15 min followed by rapid cooling on ice. Another part was activated (Aposhian & Kornberg, 1962) by incubation at 16 °C for 3 min with 1 μg pancreatic DNAase/ml (Worthington Biochemical Corp., Freehold, N.J., code DPFF, electrophoretically purified from RNAase activity) and then heated at 90 °C for 15 min to destroy enzyme activity. Hydrolysis of the DNA was monitored at different times by precipitating samples with 6% perchloric acid (v/v) and measuring the absorbance of the supernatant at 260 nm (Oleson & Koerner, 1964). In this manner, the preparation of activated primer DNA was determined to be degraded approximately 25%.

**CsCl gradient sedimentation of DNA extracted from isolated nuclei of anterior pituitary glands**

DNA was extracted from isolated nuclei according to the procedure described by Thomas, Berns & Kelley (1966). To nuclei which had been incubated for 20 min in the reaction mixture, was added 2 ml cold homogenizing medium containing 4.5 x 10⁻⁷ M-dTTP. Nuclei were centrifuged (1000 g, 15 min) and resuspended to a concentration of 10⁸ nuclei per ml in saline–citrate–Tris buffer (SC-Tris : 0.15 M-sodium chloride, 0.015 M-trisodium citrate in 0.01 M-Tris–HCl, pH 8.0). Nuclei were lysed with 0.5% (w/v, final concentration) sodium lauryl sulphate. Deproteinization was effected by a 12 h incubation at 37 °C with pronase (Calbiochem) final concentration 2 mg/ml.

DNA was extracted by shaking the lysate at 50 °C for 15 min with an equal volume of phenol saturated with 0.01 M-Tris–HCl, pH 8.0. The aqueous phase was re-extracted with phenol (37 °C, 30 min); the phenol interface was re-extracted with one-half volume of fresh buffer. The resulting aqueous phases were combined, re-extracted with phenol, and dialysed extensively against SC–Tris to remove the remaining phenol. Ribonuclease (Worthington Biochemical Corporation), previously heated to 80 °C for 15 min to destroy DNAase activity, was added to the DNA to a final concentration of 100 μg per ml. The DNA was finally dialysed against SC–Tris. Recovery of DNA from the nuclei was approximately 60%.

Preliminary analytical centrifugation experiments were carried out to determine the buoyant density of the extracted DNA (Vinograd & Hearst, 1962). These
DNA polymerase in anterior pituitary gland

analytical CsCl equilibrium density-gradient centrifugations showed that the DNA had a buoyant density of 1.693 g/cm³ relative to that of OX 174 DNA taken as 1.718 g/cm³.

For preparative CsCl density gradient centrifugation (Vinograd & Hearst, 1962), samples of the extracted DNA (20 μg) were mixed with a CsCl solution to obtain a final density of 1.70 g DNA/cm³ in 3.0 ml. The solution was overlayed with mineral oil and centrifuged at 100,000 g for 67 h at 8 °C (SW 39 motor, Spinco Model L preparative ultracentrifuge). Tubes were punctured and ten drop fractions were collected and diluted with 0.1 ml SC–Tris. The absorbance of each sample was measured at 230, 260 and 280 nm. The radioactivity in each fraction was determined as described under the assay of DNA polymerase activity.

Authoradiography

For autoradiography, isolated nuclei were incubated for 20 min, washed, re-suspended in homogenizing medium and centrifuged (Shandon Cytocentrifuge, Shandon Southern Instruments, Sewickley, Pennsylvania) on to gelatin coated microscope slides. Preparations were air dried, fixed with ethanol: acetic acid (3:1) at 0 °C for 15 min, and washed with distilled water. Slides were dipped in Kodak NTB-3 emulsion (3 parts emulsion: 1 part distilled water) and stored at 4 °C for 12 weeks. After development of the autoradiographs, the nuclei were stained with 0.5% toluidine blue. Approximately 8000 nuclei from five slides were counted. Background counts ranged from 0.6-3.0 grains per nuclear area. Nuclei with four or more silver grains were considered to be labelled.

RESULTS

Effect of ageing on the level of cytoplasmic DNA polymerase activity

To determine the effects of age on DNA polymerase activity the cytoplasmic fractions from anterior pituitary glands of animals ranging in age from 25 to 450+ days, were assayed. DNA polymerase activity declined steadily during the first year of life (Fig. 1). The average activity in cytoplasm of 25- to 32-day-old rats was 64 pmol [3H]dTTP incorporated per mg protein in 10 min; this was about four times the activity found in the pituitaries of the retired-breeder rats. Mixtures of cytoplasmic fractions from young and old rats were exactly additive (Fig. 2) thereby precluding that activators or inhibitors of the enzyme were concentrated in either preparation.

Effect of oestrone treatment on DNA polymerase activity in the anterior pituitary

In earlier studies it was found that the administration of oestrone (10 μg on alternate days) to 46- to 51-day-old male rats for a week caused a two- to threefold increase in [3H]thymidine incorporation into intact pituitary glands incubated in vitro (A. Mastro, unpublished observation). To measure if DNA polymerase activity was also stimulated by the hormone treatment, cytoplasmic fractions were prepared from pituitaries of oestrone-treated male rats and assayed. The results (Fig. 3) show that the DNA polymerase activity of the oestrone-treated animals increased 50% over that of the corresponding control animals.
Text-fig. 1. The incorporation of [³H]dTTP by cytoplasmic fractions from anterior pituitary glands of rats of various ages. These assays were routinely carried out for 10 and 20 min periods when kinetics of incorporation were linear for all ages studied. The number of determinations is given in parentheses. The activity of each group is significantly different from the others ($P < 0.001$) except for the two oldest groups ($P < 0.05$). The vertical bars represent ±s.e.m.

Text-fig. 2. The incorporation of [³H]dTTP by cytoplasmic fractions from anterior pituitary glands of 28-day-old (●) and retired-breeder (○) male rats, and a mixture of the two fractions (▲). The incubations were carried out for 10 min with three concentrations of cytoplasm, as described in the text.
DNA polymerase in anterior pituitary gland

Text-fig. 3. The incorporation of [H³]dTTP by cytoplasmic fractions from anterior pituitary glands of male rats treated with oestrone (squares) or the corn oil vehicle (circles). Rats (46-56 days old) were injected subcutaneously with 10μg oestrone in 0.15 ml corn oil on alternate days for 7 days and killed on the 8th day. The preparation of the enzyme and assay procedure is described in Materials and methods. Closed figures represent assays using 700 μg cytoplasmic protein, open figures represent 300 μg cytoplasmic protein.

DNA polymerase activity associated with isolated nuclei

Although the major fraction of DNA polymerase from mammalian tissue is usually found in the soluble protein fraction, nuclei isolated in low ionic media contain a measurable level of DNA polymerizing enzymes (Keir, 1965). It was of interest, therefore, to test for DNA polymerase activity in nuclei from anterior pituitary glands of animals of various ages and after oestrone treatment. For these studies the nuclei were incubated in the standard incubation medium in the presence of activated calf thymus DNA. The activity with the added DNA was 3-7 times that of the nuclei in the absence of added template. Native or heat denatured DNA was relatively ineffective as a template. When nuclei from pituitary glands of young and old rats were incubated with the activated DNA, the DNA polymerase activity of 25-day-old animals was found to be twice that of the retired-breeder rats (Table 1). In these experiments the exogenous template was not rate limiting since three concentrations of nuclei (30, 50 and 70 μg DNA) from young and old animals showed linear activity with the same amount of added DNA (35 μg). In similar experiments, nuclei from anterior pituitary glands of oestrone-treated animals were compared with nuclei from anterior pituitary glands of corresponding control animals. Although the
activity of the nuclei of the oestrone-treated animals was always greater than that of the controls, the average increase of activity, 13%, was not statistically significant (Table 1).

Table 1. DNA polymerase activity of nuclei isolated from anterior pituitary glands of 25-day-old and retired-breeder rats and from rats treated with oestrone

<table>
<thead>
<tr>
<th>Treatment</th>
<th>[3H]dTTP incorporated during 10 min incubation (pmol/mg nuclear DNA ± s.e.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-day-old rats</td>
<td>112.0 ± 4.1*</td>
</tr>
<tr>
<td>Retired-breeder rats</td>
<td>52.3 ± 2.2*</td>
</tr>
<tr>
<td>Oestrone-treated rats</td>
<td>116.6 ± 6.2</td>
</tr>
<tr>
<td>Vehicle-treated rats</td>
<td>103.1 ± 6.2</td>
</tr>
</tbody>
</table>

Isolated nuclei were incubated in the standard incubation medium in the presence of activated calf thymus DNA. The components of the reaction mixture and the assay procedure are described in the Materials and methods. The values are the average of seven determinations.

* Values are significantly different (P < 0.001) as determined by Student’s t-test.

Text-fig. 4. CsCl density gradient profile of DNA extracted from isolated nuclei incubated with [3H]dTTP. After nuclei were incubated for 20 min in the standard medium, DNA was extracted and centrifuged to equilibrium in CsCl. Fractions were collected from the bottom of the tube. The left hand abscissa is absorbancy at 260 nm (○—□—□); the right is radioactivity in c.p.m. (O—□—O).

DNA synthesis in isolated nuclei

Isolated nuclei have been shown to synthesize DNA in the presence of added substrates and co-factors using DNA of nuclei as the template (Friedman & Mueller, 1968; Thompson & McCarthy, 1968; Lynch, Brown, Umeda, Langreth & Lieberman,
DNA polymerase in anterior pituitary gland

1970). To assess whether or not nuclei from isolated pituitary glands in different hormonal states varied in their DNA synthesizing activity, studies were carried out in ageing and oestrone-treated rats. Isolated nuclei (40–80 μg DNA) were incubated in the standard incubation medium without added DNA or cytoplasmic fraction. The DNA synthetic activity of nuclei remained relatively constant during ageing and oestrone treatment in contrast to observed changes in the DNA polymerase activity assayed with template DNA. The average value was 34 pmol $[^3]$H]dTTP incorporated per mg DNA per 10 min incubation.

Table 2. DNA polymerase activity of isolated anterior pituitary gland nuclei from male rats in the presence and absence of calf thymus DNA

<table>
<thead>
<tr>
<th>Assay number</th>
<th>Components</th>
<th>$[^3]$H]dTTP incorporated (d.p.m./10 min incubation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nuclei + DNA</td>
<td>6325</td>
</tr>
<tr>
<td>2</td>
<td>Nuclei</td>
<td>3352</td>
</tr>
<tr>
<td>3</td>
<td>Activity due to added DNA</td>
<td>2943</td>
</tr>
<tr>
<td>4</td>
<td>DNA + supernatant from a previous nuclear incubation</td>
<td>2835</td>
</tr>
<tr>
<td>5</td>
<td>Nuclei previously incubated for 10 min + fresh medium for additional 10 min</td>
<td>3147</td>
</tr>
</tbody>
</table>

Nuclei were incubated in standard medium for 10 min, placed on ice and centrifuged (1000 g, 20 min). The supernatant was incubated with activated calf thymus DNA for an additional 10 min (no. 3). Fresh medium containing $[^3]$H]dTTP was added to the nuclear pellet and the incubation was continued for 10 min (no. 4). Two controls (nos 1, 2) were incubated in medium containing $[^3]$H]dTTP for 10 min and were then stopped.

The incorporation appeared to be due to actual synthesis of new DNA for the following reasons: Firstly, the $[^3]$H]dTTP incorporation dropped to 25 and 14% of the maximum when one or three of the deoxyribonucleoside triphosphates were omitted from the reaction mixture. This result suggested that incorporation was not due only to terminal addition of triphosphates. Secondly, omission of ATP from the incubation mixture decreased activity to 55% of the maximum. A specific role for ATP in the initiation of DNA synthesis has been suggested (Kidwell & Mueller, 1969). Thirdly, the incorporated $[^3]$H]dTTP was sensitive to pancreatic DNAase (100 μg caused release of 96% of the radioactivity) but not RNAase. Fourthly, in a CsCl density gradient sedimentation of DNA extracted from isolated nuclei after incubation, the radioactivity coincided exactly with the pituitary DNA (Fig. 4). Finally, autoradiographs of nuclei after a 20 min incubation showed the grains to be associated with the nuclei (Plate); 3.4 ± 0.08 (S.E.M.)% of the nuclei were labelled. This value agrees well with the 1–2% labelling of nuclei in pituitary glands incubated in vitro (Mastro et al. 1969a; Mastro, Shelton & Hymer, 1969b).

It has been reported previously that DNA synthesis in isolated nuclei is stimulated by soluble factors in the cytoplasm (Friedman & Mueller, 1968; Thompson & McCarthy, 1968; Lynch et al. 1970; Long & Garren, 1972). To test for possible effects of cytoplasmic factors on DNA synthesis in isolated nuclei we incubated nuclei with several concentrations of the pituitary cytoplasmic fraction from young, old or oestrone-treated rats. The incorporation of $[^3]$H]dTTP during a 5 min assay with 50 μg protein was stimulated 1.9-fold (187.7 ± 18.5%) over the endogenous activity.
Maximal stimulation was obtained with 50 µg of the cytoplasmic protein fraction. The stimulating activity of the cytoplasmic fraction was the same whether it was obtained from young, old or oestrone-treated animals.

The effect of the cytoplasm did not appear to be due to the large amount of polymerase in the cytoplasmic fraction because the preparations of nuclei were found to contain excess DNA polymerase (Table 2). The results shown in Table 2 were from nuclei incubated in standard medium for 10 min, centrifuged and the supernatant withdrawn. The nuclei were resuspended in fresh radioactive medium and incubated for an additional 10 min. Activated DNA was added to the supernatant fraction. This was also incubated for an additional 10 min. In other tubes nuclei alone or nuclei plus DNA were incubated for 10 min. The calculations shown in Table 2 indicate that calf thymus DNA incubated alone had as much activity as when it was incubated with the nuclei directly (2835 d.p.m. v. 2943 d.p.m.). The exogenous DNA must therefore act with polymerase that passed out from the nuclei into the medium during the incubation. Even after removal of the medium containing these enzymes, the nuclei continued to synthesize DNA at the original rate (3147 d.p.m. v. 3382 d.p.m.). This result strongly suggests that DNA polymerases which can use an activated template are in excess in isolated pituitary nuclei.

**DISCUSSION**

In many mammalian systems, higher levels of the soluble DNA polymerase activity have been correlated with increased cell proliferation during normal development and ageing (Mukundan, Devi & Sarker, 1963; Brasel, Ehrenkranz & Winick, 1970; Stockdale, 1970), hormonal stimulation (Coffey, Shimazki & Williams-Ashman, 1968; Masui & Garren, 1970), and chemically (Barka, 1965; Lockwood, Voytovich, Stockdale & Topper, 1965), or mechanically induced tissue growth (Bollum & Potter, 1959; Ove, Jenkins & Laszlo, 1969).

**Table 3. Total DNA polymerase activity of nuclear and cytoplasmic fractions of anterior pituitary glands of male rats**

<table>
<thead>
<tr>
<th>Condition of rats</th>
<th>[³H]dTTP incorporated (pmol/mg nuclear DNA ± s.e.m.)</th>
<th>Ratio activities A : B</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 25-day-old</td>
<td>525 ± 48</td>
<td>2·6</td>
</tr>
<tr>
<td>B Retired-breeder</td>
<td>204 ± 34</td>
<td></td>
</tr>
<tr>
<td>A Oestrone-treated</td>
<td>559 ± 2</td>
<td>1·5</td>
</tr>
<tr>
<td>B Control</td>
<td>374 ± 1</td>
<td></td>
</tr>
</tbody>
</table>

Isolated nuclei or cytoplasmic fractions were assayed with activated calf thymus DNA as described in the text. [³H]dTTP incorporation was calculated as the sum of the activities of equivalent amounts of nuclear and cytoplasmic fractions, and expressed as pmol incorporated/mg nuclear DNA per 10 min. The values shown are the average of four experiments with the 25-day-old and retired-breeder rats, and three experiments with the oestrone.-treated animals.

The present study shows that overall DNA polymerase activity in the anterior pituitary gland of the male rat decreases with the age of the animal and can be increased by oestrogen treatment *in vivo* (Table 3). These alterations in activity are similar to the changes in [³H]thymidine incorporation into DNA under the same physiological conditions (Crane & Loomes, 1967; Mastro *et al.* 1969a).
DNA polymerase in anterior pituitary gland

About 75% of the DNA polymerase of the pituitary gland was found in the cytoplasmic fraction while the remaining 25% was retained by the nuclei. The changes in polymerase activities in both sites correlate with the changes in rates of DNA synthesis in vivo under the various physiological states (Clifton & Meyer, 1956; Crane & Loomes, 1967). The nuclear associated polymerase, however, was less influenced. These data are in agreement with those of Masui & Garren (1970) and Coffey et al. (1968) who report that other tissues show the nuclear DNA polymerase level to be less responsive than the cytoplasmic enzyme to different states of growth after hormone treatment.

DNA synthesizing activity passed out of the nuclei during the course of a normal incubation (Table 2). Nevertheless, isolated nuclei exhibited an ability to synthesize DNA in the absence of added template. This activity did not correlate with the changes in DNA synthesis as measured in the intact glands, implying that the template activity of the endogenous DNA did not change in these various physiological states. Recently Wallace, Hewish, Venning & Burgoyne (1971) have reported that nuclei isolated from regenerating liver cells show a similar lack of activity with endogenous template even though the nuclei retain DNA polymerase detectable by an added DNA. The changes in DNA synthesis observed in vivo (Hunt & Hunt, 1966; Crane & Loomes, 1967) in the pituitary gland must result from changes in DNA polymerase activity of a fragile nuclear DNA synthetic apparatus or the presence of some controlling factors not apparent under the conditions of these experiments. If the cytoplasm of the 2 or 3% of the pituitary cells which have been stimulated to synthesize DNA contains specific nuclear regulating factors, as has been shown for HeLa cell cytoplasm (Friedman & Mueller, 1968), it is conceivable that their effects may either be overlooked in the presence of degradative enzymes or be diluted by the relatively large amount of cytoplasmic material from non-dividing cells.

In conclusion, the results from the present study have shown that concomitant with the changes in DNA synthesis in the anterior pituitary gland of the male rat (Mastro et al. 1969a; Hymer et al. 1970) there is a corresponding change in DNA polymerase activity. The activity, found both in the cytoplasm and associated with nuclei, decreases with increasing age over a relatively long time in the life span of the rat but can be increased significantly in the cytoplasm and to a lesser degree in the nuclei of the anterior pituitary gland of rats after a week of oestrone injections.

The authors thank Dr William D. Taylor for his help with the CsCl gradient sedimentations of DNA.

This study was supported in part by grants from the National Science Foundation (GB-33686), the National Institutes of Health (NCI-G-72-3863) and a Public Health Service research career development award (1-K04-AM-15808) to W.C.H. A.M. was a National Science Foundation Pre-doctoral fellow.

REFERENCES


DNA polymerase in anterior pituitary gland


DESCRIPTION OF PLATE

Autoradiograph of isolated nuclei from anterior pituitary gland after 20 min incubation with [3H]dTTP. Silver grains can be seen over the nucleus in the centre of the field. The marker line denotes 10 μm.