

# CHANGES IN CHROMATIN TEMPLATE ACTIVITY AND THEIR RELATIONSHIP TO DNA SYNTHESIS IN MOUSE PAROTID GLANDS STIMULATED BY ISOPROTERENOL

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## ABSTRACT

Chromatin template activity of mouse parotid glands increases after a single injection of isoproterenol (IPR), a procedure that causes, after a lag period of 20 hr, a marked stimulation of DNA synthesis and cell division in salivary glands of rodents. The increase in chromatin template activity occurs as early as 1 hr and peaks between 6 and 10 hr after IPR, paralleling previously reported changes in the incorporation of uridine-<sup>3</sup>H into total cellular RNA of mouse parotids. Template activity was measured *in vitro* in a system in which parotid gland chromatin was incubated with an exogenous RNA polymerase isolated from *Escherichia coli*. Similar results were obtained when template activity of parotid gland chromatin was assayed using an homologous RNA polymerase from mouse liver. Chromatin template activity in mouse parotids was also studied after the administration of drugs capable of inducing in salivary glands both DNA synthesis and secretion or secretion alone. The results indicate that the increased chromatin template activity occurring 6 hr after IPR is related to the subsequent onset of DNA synthesis. Furthermore, the increased chromatin template activity caused by IPR is inhibited by the previous administration of puromycin, an inhibitor of IPR-stimulated DNA synthesis.

## INTRODUCTION

Several lines of evidence indicate that gene activation may play an important role in stimulating G<sub>0</sub> cells to enter DNA synthesis and cell division (Allfrey, 1969; Church and McCarthy, 1970; Lieberman, 1970; Baserga and Stein, 1971), and prominent among these is the increase in chromatin template activity that occurs in several models of stimulated DNA synthesis. An increased chromatin template activity in the very early prereplicative phase has been reported in lymphocytes stimulated to divide by phytohemagglutinin (Hirschhorn et al., 1969), in rat liver cells stimulated to divide by partial hepatectomy (Thaler and Vिलее, 1967; Bannai and Terayama, 1969; Mayfield and Bonner, 1972), in estrogen-stimulated rat uterus

cells (Barker and Warren, 1966; Teng and Hamilton, 1968), and in WI-38 fibroblasts stimulated to divide by a change of medium (Farber et al., 1971; Rovera et al., 1971). In WI-38 fibroblasts, the template activity of isolated chromatin increased as early as 1 hr after stimulation, remained elevated for another 8 hr, and increased further at 12 hr. The results were interpreted as indicating that, shortly after the cells are stimulated to proliferate, there is activation of the genome.

However, the dependence of subsequent DNA synthesis on a previous increase in chromatin template activity has not been demonstrated beyond a mere temporal relationship. The isopro-

terenol-(IPR) stimulated salivary gland offers the possibility, through experimental manipulations, to define this relationship in more precise terms. A single intraperitoneal injection of IPR results, after a lag period of 20 hr, in a marked stimulation of DNA synthesis in the salivary gland cells of rodents (Barka, 1965; Baserga, 1966), followed a few hours later by a burst of mitosis (Baserga and Heffler, 1967). Several biochemical changes have been described during the prereplicative phase preceding the onset of DNA synthesis (Baserga, 1970), and their relevance to cell proliferation can be studied by using other sialagogues and analogs of IPR (Baserga and Stein, 1971).

The aim of this study was to investigate changes in chromatin template activity occurring in mouse parotids stimulated to proliferate by the administration of IPR and to relate these changes to the subsequent stimulation of DNA synthesis.

#### MATERIALS AND METHODS

Fels A male mice bred in this laboratory and weighing approximately 30 g were used. *dl*-Isoproterenol (0.3  $\mu$ mole/g of body weight) and pilocarpine (50  $\mu$ g/g of body weight) were injected, dissolved in water. Puromycin (50  $\mu$ g/g of body weight) was injected, dissolved in 0.9% NaCl. 1-Phenyl-2-isopropylamino-ethanol (0.3  $\mu$ mole/g of body weight) was dissolved either in 0.1 N HCl or 40% ethanol. All drugs were injected intraperitoneally. The mice were killed by cervical dislocation, and the parotid was dissected out, freed of lymph nodes and fat tissue and processed as described below. When liver was needed, mice were bled by decapitation.

#### Isolation of Chromatin

Chromatin was prepared from a purified nuclear fraction, with slight modifications, by the method of Paul and Gilmour (1968). Nuclei were first isolated by homogenizing the minced tissue in 0.25 M sucrose, 0.05 M KCl, 0.005 M MgCl<sub>2</sub>, 0.05 M Tris-HCl, pH 7.4, using a Potter-Elvehjem homogenizer with a loose-fitting pestle. The homogenate was passed through a steel screen filter to remove connective tissue, then centrifuged at 600 g in an International refrigerated centrifuge, model PR-6, for 3 min (International Equipment Co., Needham Heights, Mass.). The pellet was washed three times with 10 volumes of Earle's balanced salt solution, and nuclei were isolated by disrupting the cells in 80 mM NaCl, 20 mM ethylenediaminetetraacetate (EDTA), and 1% Triton X-100, pH 7.2. The nuclei were washed twice with 10 volumes of 0.15 M NaCl, 0.01 M Tris-HCl, pH 7.5, resuspended in distilled water, and chilled for 20 min in an ice bath. The nuclei were then

lysed in distilled water by gentle homogenization with several strokes of a Teflon pestle, and the chromatin was pelleted by centrifugation at 20,000 g in a Sorvall, model RC-2, refrigerated centrifuge for 15 min (Ivan Sorvall, Inc., Norwalk, Conn.). DNA as chromatin was measured from the absorbancy at 260 m $\mu$ . The absorption spectrum of parotid gland chromatin (not shown) did not differ from that reported in the literature for other tissues (Marushige and Bonner, 1966; Shaw and Huang, 1970). The yield of chromatin from purified nuclei was 76%.

#### Preparation of RNA Polymerases

*Escherichia coli* RNA polymerase was prepared from early exponential-phase *E. coli*, strain B, purchased from General Biochemicals Div., Mogul Corp., Chagrin Falls, Ohio, by the method of Chamberlin and Berg (1962) as modified by Bonner et al. (1968). The enzyme was purified up to and including the DEAE-cellulose column chromatography step (fraction 4). Mouse liver RNA polymerase was prepared with slight modifications by the method of Roeder and Rutter (1970).

#### Assay of Template Activity

The complete incubation mixture for *E. coli* RNA polymerase was the one described by Bonner et al. (1968), with the modification (50  $\mu$ mole NaCl) by Bekhor and Bavetta (1971). Incubation was carried out according to Farber et al. (1971), and the radioactivity incorporated in RNA was determined in 15 ml of Cellosolve-toluene (1:3, v/v) scintillation cocktail (Union Carbide Corp. New York) (Gilman, 1970) in a Packard liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.) at 80% efficiency. The template activity assay for mouse liver RNA polymerase was the one described by Roeder and Rutter (1969). The radioactivity incorporated into RNA was counted in an Omnifluor-toluene-NCS (4 g of Omnifluor [Pilot Chemicals Inc., Div., Watertown, Mass.] in 975 ml toluene plus 25 ml NCS) scintillation cocktail in a Packard liquid scintillation spectrometer at 33% efficiency.

#### RNAse Activity of Chromatin

RNAse activity of chromatin preparations was determined by incubating <sup>3</sup>H-labeled ribosomal RNA (rRNA) with chromatin in the incubation mixture for template activity described above. rRNA with a specific activity of 23,400 dpm/ $\mu$ g was obtained from logarithmically growing HeLa S-3 cells labeled with uridine-5-<sup>3</sup>H (0.1  $\mu$ Ci/ml) for 48 hr. rRNA was isolated by the method of Warner et al. (1966). The purity of the ribosomal preparation was assessed by the ratio, A<sub>260</sub> nm/A<sub>280</sub> nm and A<sub>260</sub> nm/A<sub>235</sub> nm (Peterman, 1964).

RNAse activity was determined by incubating  $^3\text{H}$ -labeled rRNA for 10 min at  $37^\circ\text{C}$  with or without chromatin in the complete assay mixture described above for the assay of template activity with *E. coli* RNA polymerase, minus ATP- $^{14}\text{C}$ .

### Proteolytic Activity of Chromatin

Chromosomal proteins were prelabeled for 60 min with leucine- $^3\text{H}$  ( $1.6 \mu\text{Ci}/\mu\text{g}$  body weight) and the chromatin was prepared as described above. Of the chromatin suspension (control chromatin 190 dpm/ $\mu\text{g}$  DNA; IPR-stimulated chromatin 180 dpm/ $\mu\text{g}$  DNA), 0.6 ml was incubated at  $37^\circ\text{C}$  in the incubation mixture for chromatin template activity described above, minus RNA polymerase and ATP- $^{14}\text{C}$ , in a final volume of 1.5 ml. Samples (0.4 ml) were removed at zero time, and at 10 and 30 min. The samples were precipitated with 10% trichloroacetic acid, collected on Millipore filters (Millipore Corp., Bedford, Mass.), and their radioactivities were counted in the Cellosolve-toluene mixture described above. Proteolytic activity was expressed as the percentage of acid-insoluble radioactive protein recovered.

### Chemicals

*dl*-Isoproterenol was purchased from Winthrop Laboratories, New York. 1-Phenyl-2-isopropylaminoethanol was synthesized in our laboratories by Dr. Daniel Swern according to the method described by Kirby et al. (1969). Pilocarpine was from Sigma Chemical Company, St. Louis, Missouri, and puromycin dihydrochloride from Nutritional Biochemicals Corporation, Cleveland, Ohio. All other chemicals were of reagent grade. ATP- $^{14}\text{C}$ , sp act 49.8 mCi/mmole; ATP- $^3\text{H}$ , sp act 9.53 Ci/mmole; uridine-5- $^3\text{H}$ , sp act 27.8 Ci/mmole; and leucine- $^3\text{H}$ , sp act 31.9 Ci/mmole, were purchased from New England Nuclear Corporation, Boston, Massachusetts.

## RESULTS

### Template Activity of Chromatin Isolated from Mouse Parotid Glands at Various Intervals after Administration of IPR

The template activity of chromatin of mouse parotid glands (using an exogenous *E. coli* RNA polymerase) was investigated at various intervals after a single intraperitoneal administration of IPR. The effect of chromatin concentration on template activity of control or IPR-stimulated chromatin is shown in Fig. 1 *a*, and a double reciprocal plot of the data is shown in Fig. 1 *b*. All

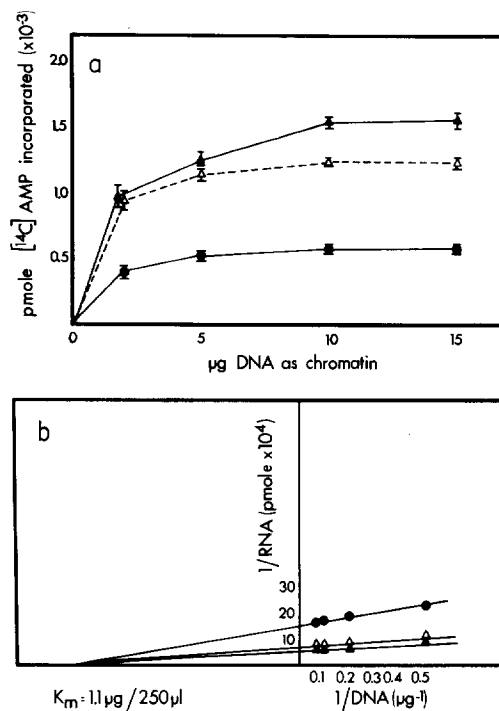


FIGURE 1 (a) Template activity of chromatin from control and IPR-stimulated parotid glands of mice. Chromatin was isolated and its template activity determined as detailed in the Materials and Methods section. Each point was assayed with 40  $\mu\text{g}$  of fraction 4 *E. coli* RNA polymerase. (●—●) controls; ( $\Delta$ — $\Delta$ ) 1 hr and ( $\blacktriangle$ — $\blacktriangle$ ) 6 hr after IPR. Bars represent the standard deviation. (b) Double reciprocal plot of the results in (a).

enzyme reactions with either control or IPR-stimulated chromatin as substrate have the same  $K_m$ , suggesting that the affinity of *E. coli* RNA polymerase for the enzyme-binding sites on chromatin is similar in parotid gland chromatin of control and IPR-stimulated mice.

Fig. 2 shows the chromatin template activity at saturating amount of template ( $V_{\text{max}}$ ) of mouse parotid glands at various times after a single intraperitoneal administration of IPR. There is an increase in chromatin template activity within 1 hr after IPR administration, with a peak between 6 and 10 hr. Such an increase in  $V_{\text{max}}$  suggests the presence of more sites available for RNA synthesis in the parotid chromatin of mice injected with IPR. Notice that by 48 hr the template activity has returned to control levels. In this system, DNA synthesis begins 20 hr after IPR, and by 40 hr it has returned to control levels.

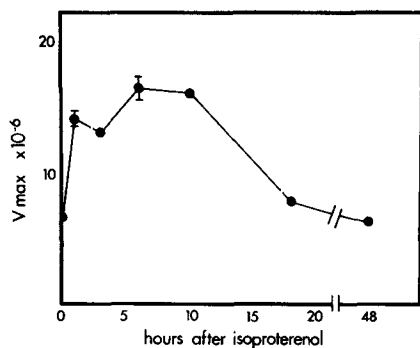


FIGURE 2 Chromatin template activity at saturating amount of template ( $V_{max}$ ) of mouse parotid glands at various hours after a single injection of IPR. Each  $V_{max}$  calculation is based on the amount of AMP- $^{14}$ C incorporated into RNA at four different concentrations of parotid gland chromatin. Chromatin was isolated and its template activity determined as detailed in Materials and Methods. 40  $\mu$ g of fraction 4 *E. coli* RNA polymerase were used for each determination. Where given, bars represent standard deviation.

#### *Template Activity of Chromatin from Control and IPR-Stimulated Parotid Glands of Mice using an Homologous Polymerase*

The template activity of chromatin of mouse parotid glands in control and IPR-treated mice was also measured using an homologous RNA polymerase isolated from mouse liver. Figs 3 *a* and 3 *b* show that even with the homologous polymerase there is an increase in template activity of chromatin isolated from parotid glands of mice 1 hr after administration of IPR. Whether the parotid gland chromatin used is from control or IPR-stimulated mice, the  $K_m$  of the reaction is the same.

If the increase in chromatin template activity observed in parotids of IPR-stimulated mice can be considered real, the question must be raised whether such an increase is related to the subsequent stimulation of DNA synthesis and cell division or is purely coincidental. A series of experiments were carried out to distinguish between these two alternatives.

#### *Effect of Pilocarpine and 1-Phenyl-2-Isopropylaminoethanol on Chromatin Template Activity of Mouse Parotid Glands*

Since IPR is a powerful stimulant of salivary gland secretion (Byrt, 1966; Robinovitch and

Sreebny, 1969; Whitlock et al., 1968), the increased chromatin template activity observed after administration of IPR could be related to secretion rather than to stimulation of DNA synthesis. We, therefore, investigated the effect of an analog of IPR and of pilocarpine on chromatin template activity of mouse parotid glands.

1-Phenyl-2-isopropylaminoethanol is an analog of IPR from which it differs by the absence of the two hydroxyl groups in the phenyl ring. It was originally synthesized by Kirby et al. (1969) and was shown to stimulate both DNA synthesis and secretion in mouse salivary glands when injected, dissolved in 0.1 N HCl. However, this analog of IPR does not stimulate DNA synthesis, although it stimulates salivary glands' secretion, when injected, dissolved in 40% ethanol (Labows et al., 1971). The effect of 1-phenyl-2-isopropylaminoethanol, dissolved in either 0.1 N HCl or 40% ethanol, on chromatin template activity of mouse parotid glands is shown in Figs. 4 *a* and 4 *b*. When dissolved in 0.1 N HCl, this analog of IPR stimulates chromatin template activity both at 1 hr (98%) and at 6 hr (101%) after its administration. When it was injected, dissolved in 40% ethanol, an increase in chromatin template activity was found at 1 hr (95%) but not at 6 hr after the analog administration. The lack of stimulation of chromatin template activity at this time cannot be related to a toxic effect of 40% ethanol, because IPR dissolved in 40% ethanol is able to stimulate chromatin template activity 6 hr after its administration (Fig. 4 *b*), as well as DNA synthesis 20 hr later (Novi and Baserga, 1972). These results suggest that the increase in template activity occurring 1 hr after IPR or its analog can be related to both DNA synthesis and secretion, but that the genome activation occurring 6 hr later is selectively related to the subsequent stimulation of DNA synthesis.

Pilocarpine is known to induce secretion in salivary glands but no increase in DNA synthesis (Ohlin, 1966). Observations made in this laboratory (unpublished) confirm that pilocarpine does not appreciably stimulate the synthesis of DNA in mouse parotid glands, although a little increase (about 30%) can be observed. The results in Fig. 5 show a slight increase in chromatin template activity at 1 hr (15%) and 6 hr (26%) after pilocarpine administration. However, such a slight difference above control values is of questionable significance without statistical evaluation.

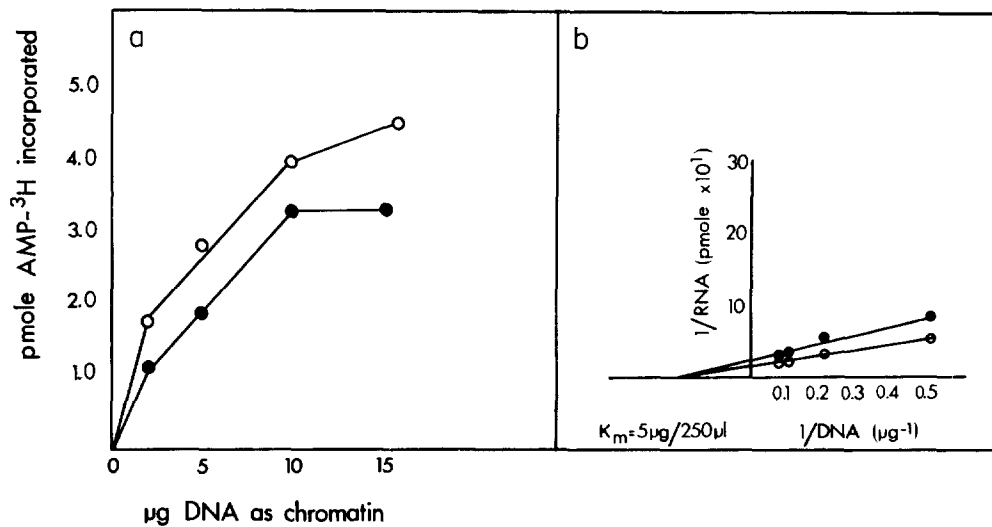


FIGURE 3 (a) Template activity of chromatin from control and IPR-stimulated parotid glands of mice using homologous RNA polymerase. Chromatin was isolated and its template activity determined as detailed in the Materials and Methods section. Each point was assayed with 1.3 mg of fraction 3 mouse liver RNA polymerase. (●—●) controls; (○—○) 1 hr after IPR. (b) Double reciprocal plot of the results in (a).

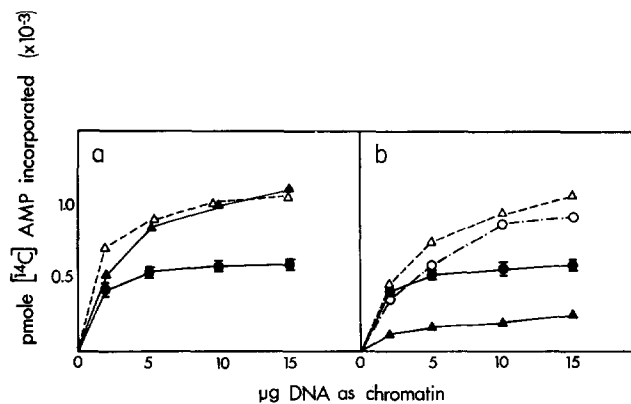


FIGURE 4 Effect of 1-phenyl-2-isopropylaminoethanol dissolved in either 0.1 N HCl (a) or in 40% ethanol (b) on template activity of chromatin from mouse parotid glands. Chromatin was isolated and its template activity determined as detailed in the Materials and Methods section. Each point was assayed with 40 µg of fraction 4 *E. coli* RNA polymerase. (●—●) control; (△—△) 1 hr and (▲—▲) 6 hr after 1-phenyl-2-isopropylaminoethanol administration; (○—○) 6 hr after IPR in 40% ethanol.

#### *Effect of Puromycin on the Increased Chromatin Template Activity of Mouse Parotid Glands Caused by IPR*

Puromycin, an inhibitor of protein synthesis (Nathans, 1964), has been demonstrated to suppress the stimulation of DNA synthesis in mouse parotid glands when injected immediately before IPR (Sasaki et al., 1969). Puromycin does not

modify gene function per se (Fig. 6), unlike cycloheximide (Rovera et al., 1971). However, when injected 30 min before IPR, it causes a complete inhibition of the increase in chromatin template activity occurring 1 or 6 hr after IPR (Fig. 6). These results further support the correlation between increased chromatin template activity and the subsequent onset of DNA synthesis in parotids of mice.

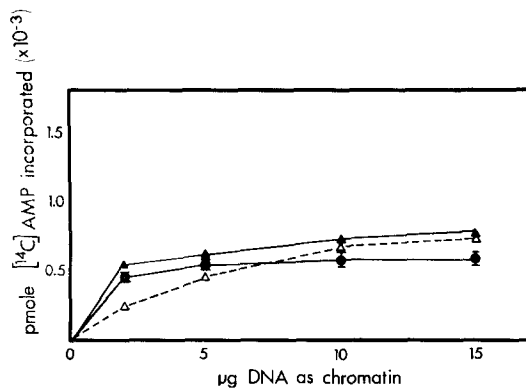


FIGURE 5 Template activity of chromatin isolated from mouse parotid glands after administration of pilocarpine. Chromatin was isolated and its template activity determined as detailed in the Materials and Methods section. Each point was assayed with 40  $\mu\text{g}$  of fraction 4 *E. coli* RNA polymerase. (●—●) control; ( $\Delta$ — $\Delta$ ) 1 hr and ( $\blacktriangle$ — $\blacktriangle$ ) 6 hr after pilocarpine.

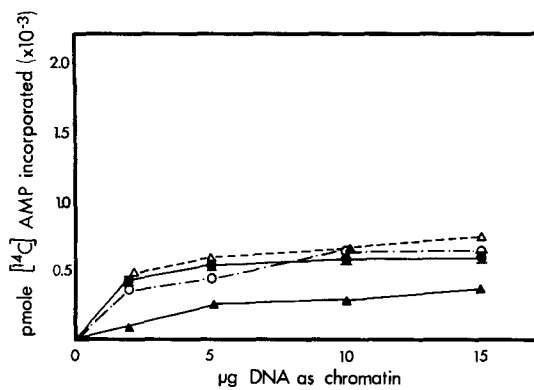


FIGURE 6 Effect of puromycin on the increase in chromatin template activity of mouse parotid glands after administration of IPR. Chromatin was isolated and its template activity determined as detailed in the Materials and Methods section. Each point was assayed with 40  $\mu\text{g}$  of fraction 4 *E. coli* RNA polymerase. Puromycin was given 30 min before IPR and the mice were killed 1 hr ( $\Delta$ — $\Delta$ ) or 6 hr ( $\blacktriangle$ — $\blacktriangle$ ) after IPR. (●—●) control; (○—○) puromycin in untreated mice.

#### Effect of IPR on Mouse Liver Chromatin Template Activity

Previous observations (Novi and Baserga, 1971) have demonstrated that under the conditions in which IPR stimulates DNA synthesis in mouse salivary glands, no stimulation of DNA synthesis

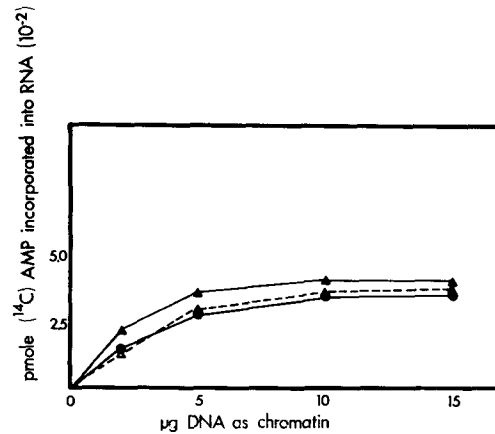


FIGURE 7 Template activity of liver chromatin from control and IPR-treated mice. Chromatin was isolated and its template activity determined as detailed in the Materials and Methods section. Each point was assayed with 40  $\mu\text{g}$  of fraction 4 *E. coli* RNA polymerase. (●—●) control; ( $\Delta$ — $\Delta$ ) 1 hr and ( $\blacktriangle$ — $\blacktriangle$ ) 6 hr after IPR administration.

occurred in mouse liver. In order to demonstrate that the stimulus for genome activation by IPR is organ specific, i.e., selectively restricted to the salivary glands, the ability of liver chromatin to act as template for *E. coli* RNA was investigated 1 and 6 hr after IPR. As shown in Fig. 7, no appreciable difference could be observed between control livers and livers from mice injected with IPR.

#### RNAse Activity of Mouse Parotid Gland Chromatin

Table I shows that the RNAse activity present in parotid gland chromatin of IPR-stimulated mice is the same as that observed in control parotids. These results rule out the possibility that the increase in template activity observed after IPR could reflect differences in RNAse concentrations. Furthermore, the ability of IPR-stimulated chromatin to act as template for RNA polymerase was not affected when bentonite (500  $\mu\text{g}$ ) was added to the complete chromatin template assay mixture (not shown).

#### Proteolytic Activity of Mouse Parotid Gland Chromatin

Table II shows that there are no appreciable differences in the amount of proteolytic activity

TABLE I  
RNase Activity of Parotid Gland Chromatin of  
Control and IPR-Stimulated Mice

	Acid-soluble radio- activity (dpm $\times$ $10^{-3}$ )
Minus chromatin	110
Control chromatin (16.25 $\mu$ g of DNA)	145
Chromatin 1 hr after IPR administration (16.32 $\mu$ g of DNA)	151

RNase activity is measured as acid-soluble radioactivity recovered from a 10 min incubation containing  $^3\text{H}$ -labeled rRNA (18.8  $\mu$ g;  $440 \times 10^{-3}$  dpm), chromatin and the complete template activity incubation mixture minus ATP- $^{14}\text{C}$ .

TABLE II  
Proteolytic Activity of Parotid Gland Chromatin of  
Control and IPR-Stimulated Mice

Time of incubation	Proteolytic activity (dpm/ $\mu$ g DNA)	
	Control chromatin	Chromatin 1 hr after IPR administration
<i>min</i>		
0	100	100
10	100	100
30	79	73

Proteolytic activity was measured as acid-insoluble radioactivity recovered from an incubation mixture containing leucine- $^3\text{H}$  labeled chromatin and the complete template activity incubation mixture minus ATP- $^{14}\text{C}$  and RNA polymerase. Changes in proteolytic activity after 10-30 min of incubation are shown as percentage of values at zero time (100%). Results are means of triplicates.

between chromatin preparations. No loss of radioactivity was observed in the first 10 min of incubation. After 30 min, the loss of radioactivity from the insoluble pellet was 21% from control chromatin and 27% from IPR-stimulated chromatin.

#### DISCUSSION

Our results demonstrate that after IPR administration the ability of parotid gland chromatin to act as template for exogenous (*E. coli*) RNA polymerase is increased.

The present experiments are based on the assumption that there is fidelity of transcription in

the isolated chromatin, that is, that isolated chromatin transcribes in vitro the same RNA that it synthesizes in vivo. A number of reports in the literature have, indeed, indicated that the program for tissue-specific transcription is retained in the isolated chromatin (Paul and Gilmour, 1968; Bekhor et al., 1969; Marushige and Dixon, 1969; Smith et al., 1969; Chetsanga et al., 1970; Tan and Miyagi, 1970; Chytil and Spelsberg, 1971). In addition, it has been repeatedly reported that chromatin template activity is stimulated whenever quiescent  $G_0$  cells are stimulated to proliferate (Baserga and Stein, 1971). An exception to this rule has been recently reported by Dati and Maurer (1971) who found no increase in chromatin template activity in the uterus of mice treated with estrogen. However, this report only measured one single point, 10 hr after the administration of estrogen and, in addition, showed that the assays were heavily dependent on the RNase activity of the mixtures. Previous results from our laboratory have indicated that the increased chromatin template activity observed in stimulated WI-38 fibroblasts does not depend on any change in either RNase or proteolytic activity of the chromatin preparation.

Several data indicate that the increase in template activity of chromatin from IPR-stimulated parotids really reflects the increased availability of sites for transcription. The kinetics of polymerase-template interaction show that *E. coli* RNA polymerase has the same affinity for control and IPR-stimulated chromatin. There are no appreciable differences in RNase and proteolytic activity of unstimulated and IPR-stimulated chromatin. Barka and Van der Noen (1969) had already reported that IPR did not cause changes in the amount of proteolytic activity in whole homogenates of rat submandibular glands.

Recent observations have indicated that RNA polymerases isolated from different sources bind to and transcribe from different sites on purified chromatin (Butterworth et al., 1971). This criticism does not apply to our experimental conditions because we found that with either *E. coli* or mouse liver RNA polymerase there was an increase in chromatin template activity of parotid glands from IPR-stimulated mice over control levels.

The difference in  $K_m$  observed when the template activity of parotid gland chromatin was assayed with exogenous (*E. coli*) RNA polymerase

or homologous mouse liver polymerase (cf. Figs. 1 b and 3 b) suggests a different affinity of each RNA polymerase for parotid gland chromatin. It cannot, however, be established in our experimental conditions, considering the different degree of purification of each RNA polymerase. These results seem to indicate that both RNA polymerases might be able to bind to specific sites of parotid gland chromatin and seem to validate the use of an exogenous (*E. coli*) RNA polymerase for measuring quantitative changes in chromatin template activity in mammalian cells. However, these experiments give no indication on possible qualitative changes occurring in the transcriptional activity of chromatin.

Our results also indicate that the increase in chromatin template activity occurring 6 hr after the stimulation is correlated to the subsequent onset of DNA synthesis. Only drugs inducing both DNA synthesis and secretion are able to stimulate chromatin template activity at 1 and 6 hr after their administration. When only secretion is induced, there is no increase in chromatin template activity at 6 hr. Puromycin has been demonstrated to have an inhibitory effect on IPR-stimulated DNA synthesis in mouse salivary glands (Sasaki et al., 1969), when administered before IPR, and it is also able to inhibit the increase in chromatin template activity induced by IPR. These data further support the correlation between the activation of the genome by IPR and the subsequent onset of DNA synthesis in parotid glands of mice. In our experiments, puromycin was used as an inhibitor of protein synthesis because cycloheximide has been demonstrated to increase chromatin template activity per se (Rovera et al., 1971; and unpublished observations in salivary glands).

The changes in chromatin template activity of mouse parotid glands after IPR parallel previously reported changes in the incorporation of uridine-<sup>3</sup>H into total cellular RNA of mouse parotids (Novi and Baserga, 1972). Furthermore, there is a temporal relationship between the peak of chromatin template activity (6–10 hr after IPR) and the peak of cytoplasmic rRNA synthesis (8–12 hr after IPR) (Novi and Baserga, 1972). It suggests that the RNA molecules transcribed 6–10 hr after IPR could be ribosomal. We have already demonstrated (Novi and Baserga, 1972) that the synthesis of rRNA is also correlated with the subsequent stimulation of DNA synthesis caused in parotid glands by IPR, and consequently have

postulated that the signal initiating DNA synthesis in our system has to operate through the control of rRNA synthesis.

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