Effects of Tumor-promoting Agents 12-O-Tetradecanoylphorbol-13-acetate and Phenobarbital on DNA Synthesis of Rat Hepatocytes in Primary Culture

Norimasa Sawada, Jeffrey L. Staecker, and Henry C. Pitot

ABSTRACT

Both 12-O-tetradecanoylphorbol-13-acetate (TPA) and phenobarbital (PB) enhanced hepatocyte DNA synthesis stimulated with epidermal growth factor (EGF) by 60 to 80% in primary culture when measured by the incorporation of [3H]thymidine. This apparent increase was not due to changes in the specific activity of the deoxythymidine triphosphate (dTTP) pool. The enhancement occurred even at relatively high cell densities, but this was not found with the PB treatment. Although both TPA and PB enhanced DNA synthesis significantly, TPA was most effective when added during the late G1 and/or S phase of the hepatocyte cell cycle, whereas PB treatment was least effective in this period. The binding of EGF was transiently down-regulated by TPA, then restored to control values 6 h later, whereas the binding of this factor was significantly increased at both the 12th and 24th h after PB addition.

These results suggest that EGF binding to hepatocytes is not correlated with the enhancement of DNA synthesis by TPA or PB and that the down-regulation of EGF binding is not causally related to the enhancement of DNA synthesis by TPA.

INTRODUCTION

The development of a number of histogenetic types of neoplasms occurs in several stages; the first two have been termed initiation and promotion (1, 2). Currently, initiation is considered to be the result of irreversible alterations in the DNA structure (1, 2). In contrast, the mechanisms of promotion and of tumor promoters have not been fully clarified. Recently, however, it has become apparent that one or more of the effects of tumor promoters in carcinogenesis are on cell membrane structures and functions: in particular, cell-cell communication (3–6); morphological changes (7–9); and cell surface receptors involved in growth control mechanisms (10–18).

The acquisition by initiated cells of resistance to cytotoxic agents (19–22) may also be a possible mechanism of promotion during hepatocarcinogenesis (2). However, using primary cultures of preneoplastic liver cells, Kitagawa et al. (23–25) demonstrated that tumor promoters act on initiated hepatocytes directly to increase their mitotic activation. Furthermore, Schulte-Hermann et al. (26) have demonstrated that promoting agents elicit DNA synthesis of both preneoplastic and normal tissues in vivo, though to a lesser degree in the latter. More recently Edwards and Lucas (27) also showed an effect of liver tumor promoters on DNA synthesis using primary cultures of rat hepatocytes. Furthermore, Armato et al. (27–29) have shown that tumor promoters enhance DNA synthesis of neonatal hepatocytes in primary culture by affecting the cell surface mechanisms. Thus, the mitogenic activity of liver tumor promoters may be their most important action in effecting tumor promotion. Recent efforts to improve the culture of rat hepatocytes have enabled us to stimulate proliferation of these cells in primary culture with EGF as an initiator of hepatocyte DNA synthesis (30). In this study, we focused on the interaction between EGF and the tumor promoters, TPA and PB, in their relation to hepatocyte DNA synthesis and demonstrated that both TPA and PB enhance DNA synthesis in cultured hepatocytes, although the mechanism(s) of the enhancement of DNA synthesis appears to differ between the two agents.

MATERIALS AND METHODS

Reagents. DMEM with high glucose, Ham’s F-12 medium (F-12), penicillin, and streptomycin were obtained from KC Biological, Inc. (Lenexa, KS). Insulin, transferrin, selenium, and EGF were purchased from Collaborative Research, Inc. (Lexington, MA). Albumin (crystallized and lyophilized), collagenase (type I), TPA, and deoxynucleotide standards were obtained from Sigma Chemical Co. (St. Louis, MO). Dexamethasone was obtained from Organon, Inc. (W. Orange, NJ). HEPES was purchased from Research Organics, Inc. (Cleveland, OH). Collagen type I was obtained from Collagen Corporation (Palo Alto, CA), and Percoll from Pharmacia (Upsalla, Sweden).

Isolation and Culture of Rat Hepatocytes. Hepatocytes were isolated by a collagenase perfusion technique (31) from male Harlan Sprague-Dawley rats (Madison, WI) weighing about 200 to 240 g, and the hepatocytes were then purified by Percoll (32). These cells were suspended in a mixture of DMEM and F-12 (33) supplemented with 5 µg insulin/ml, 5 µg transferrin/ml, 5 ng selenium/ml, 10⁻⁷ M dexamethasone, 100 µg streptomycin/ml, 100 units penicillin/ml, and 15 mg proline/liter. In the present study, the cell viability as determined by the trypan blue exclusion test was greater than 95%. The cells (2 × 10⁶ viable cells in 2 ml of the medium) were plated in 35-mm plastic culture dishes which had been coated with 20 µg collagen type I and were dried at room temperature. For experiments in which the specific activity of the dTTP pool was determined, 4 × 10⁶ cells were plated in 20 ml of medium in 150-mm plastic culture dishes coated with 300 µg of type I collagen. The medium was renewed at 2 and 24 h after plating.

Measurement of DNA Synthesis. EGF treatment of the cultures was from the 12th h to the 24th h in order to induce hepatocyte DNA synthesis. TPA, PB, or DMSO was added to the cultures at the second h, except for the cycle experiments. Medium was renewed at the 24th h. For determination of the time course of DNA synthesis, the cultures were treated for 2 h with 0.5 µCi of [3H]thymidine at the 22nd, 28th, 34th, 39th, 45th, or 50th h after plating. Since the results of the time course of DNA synthesis (Fig. 1) showed that the peak time was near the 44th h, DNA synthesis was measured by the addition of [3H]thymidine to the cultures from the 44th to 46th h in all subsequent experiments. Subsequent assays for replicative DNA synthesis were done according to Nakamura et al. (34).

For autoradiography, the cultures were treated with 1.0 µCi of [3H]thymidine from the 36th h to the 52nd h. Then, the cultures were...
was washed with PBS 3 times, fixed with cold absolute ethanol, and covered with emulsion (Kodak Co.). After 1-wk exposure, the autoradiographs were developed and stained with hematoxylin and eosin. The percentage of labeled hepatocytes was evaluated by calculating the percentage of hepatocytes with labeled nuclei in five fields in five different dishes (200 to 500 cells).

**RESULTS**

The effects of different concentrations of TPA and PB on hepatocyte DNA synthesis were studied. Of the concentrations used, 50 ng TPA/ml and 1 mM PB enhanced DNA synthesis to the greatest extent (Table 1), although all the concentrations except 2 mM PB enhanced DNA synthesis somewhat. In this study, DNA synthesis was expressed as dpm/h/culture, because PB and TPA caused an increase in the amount of protein content in the cultures. Even though we have shown that fibronectin as a substrate for hepatocytes enhances DNA synthesis more than does collagen type I (38), collagen type I was used as a substrate in these experiments, because we observed no or little enhancement of DNA synthesis by TPA and PB when the cells were cultured on fibronectin. This might be related to the observation that cell surface fibronectin is decreased by treatment with TPA (39).

**Time Course of Hepatocyte DNA Synthesis.** The time course of DNA synthesis was examined to determine the effect of the promoting agents on the peak time of DNA synthesis. As shown in Fig. 1, active DNA synthesis was detected from the 34th h after plating, with the maximum DNA synthesis at the 44th h. The time course and peak time of DNA synthesis with TPA treatment were no different from those with PB treatment; however, the total amount of DNA synthesis was increased by 60 to 80% by the treatment with either PB or TPA. Thus, [3H]thymidine incorporation into DNA was measured from the 44th to the 46th h in all subsequent experiments.

**Table 1 Effects of various concentrations of TPA and PB on hepatocyte DNA synthesis**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Control</th>
<th>DMSO (0.1%)</th>
<th>TPA</th>
<th>PB</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/ml</td>
<td>100</td>
<td>25</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>± SD</td>
<td>92.8 ± 5.1</td>
<td>100.3 ± 5.0</td>
<td>81.2 ± 6.8</td>
<td>108.8 ± 5.9</td>
</tr>
</tbody>
</table>

Fig. 1. Time course of hepatocyte DNA synthesis in primary culture with or without PB or TPA. Each point represents the average of at least three determinations. SD did not exceed 15% of the mean for any individual point. See **Materials and Methods** for the details of additions.
ENHANCEMENT OF HEPATOCYTE DNA SYNTHESIS BY TUMOR PROMOTERS

Higher rate of DNA synthesis in TPA-treated cells, no significant differences in labeling indices could be obtained, possibly due to the large standard deviations of the means of the labeling indices (data not shown).

**EGF Binding.** Since EGF binding may be influenced by tumor promoters (10), we examined the effect of TPA and PB on EGF binding to hepatocytes in primary culture. The presence of TPA resulted in a down-regulation of EGF binding, but PB enhanced EGF binding by the 12th h (Fig. 4). Two h after TPA addition, EGF binding of TPA-treated cells was only half that of control cultures. However, by the 10th h, EGF binding of TPA-treated cultures recovered to the level of control cultures, while the EGF binding of PB-treated cultures was significantly higher than the control value. Since EGF was routinely added to the cultures at the 12th h after plating, down-regulation of EGF receptors by TPA or the amount of EGF binding did not appear to be directly related to DNA synthesis.

**Action of TPA and PB on the Cell Cycle.** Since treatment with TPA or PB did not result in a change of the peak time of DNA synthesis (Fig. 1), and since EGF binding was not related to the enhancement of DNA synthesis by the promoting agents (Fig. 4), we postulated that the promoters enhanced DNA synthesis by acting on the cells at the G1 or S phase of the cell cycle. Hence, we treated the cultures with TPA or PB in three different ways: treatment from (a) the second to the 24th h; (b) the 24th to the 46th h; (c) the 36th to the 46th h (late G1 and/or S phase). Of these three treatments, TPA treatment from the 36th to the 46th h had the greatest effect in enhancing DNA synthesis, whereas PB treatment during the same time had little effect on enhancement (Table 2). Autoradiographic examination of the hepatocytes in culture during the period from the 36th to 46th h of TPA treatment showed that labeling indices of control cultures and TPA-treated cultures were 6.6 ± 1.1% and 10.3 ± 1.4%, respectively. These results suggest that TPA acts on the cells in G0 or early G1 and stimulates the cells to progress from G1 to S phase, while PB acts on the cultured hepatocytes only in G0 to G1 to enhance DNA synthesis.

**Effects of TPA and PB on dTTP-specific Activity.** The incorporation of [3H]thymidine into acid-insoluble material is a widely used method for measuring DNA synthesis. However, this method cannot be used to discriminate between treatments that actually affect DNA synthesis as compared to those that increased in the same way, although the amount of DNA synthesis in the treated cells was larger than that of control cultures at all the concentrations of EGF examined. In particular, at between 0.6 and 2.5 ng of EGF/ml, the enhancement of DNA synthesis by TPA or PB was equal to or greater than that at either 10 ng/ml or 20 ng/ml of EGF alone. Since cell density serves to regulate hepatocyte DNA synthesis (40, 41), the effect of promoters on this regulation was examined. As shown in Fig. 3, TPA enhanced hepatocyte DNA synthesis at all cell densities examined, whereas PB enhanced DNA synthesis only at the lower cell densities. On the other hand, there was no difference in labeling indices between the promoter-treated cells and control cells at cell densities of 6 and 8 x 10⁵, though there was a significant difference at the two lower cell densities tested. At the two highest cell densities, despite the
ENHANCEMENT OF HEPATOCYTE DNA SYNTHESIS BY TUMOR PROMOTERS

Table 2 Effects of TPA and PB on hepatocyte DNA synthesis in different phases of culture

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control, EGF, 12-24 h</th>
<th>TPA 24-46 h</th>
<th>PB 24-46 h</th>
<th>TPA 36-46 h</th>
<th>PB 36-46 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.9 ± 100 (100)</td>
<td>36 ± 24 (150)</td>
<td>10.1 ± 100 (170)</td>
<td>10.5 ± 177 (177)</td>
<td>7.9 ± 133 (133)</td>
</tr>
<tr>
<td>2</td>
<td>8.4 ± 100 (100)</td>
<td>56 ± 24 (128)</td>
<td>12.5 ± 148 (148)</td>
<td>12.1 ± 144 (144)</td>
<td>9.6 ± 114 (114)</td>
</tr>
<tr>
<td>3</td>
<td>8.5 ± 100 (100)</td>
<td>50 ± 24 (146)</td>
<td>15.7 ± 185 (185)</td>
<td>14.6 ± 173 (173)</td>
<td>11.4 ± 134 (134)</td>
</tr>
<tr>
<td>4</td>
<td>4.6 ± 100 (100)</td>
<td>18 ± 126 (144)</td>
<td>9.9 ± 214 (126)</td>
<td>7.3 ± 159 (159)</td>
<td>5.2 ± 112 (112)</td>
</tr>
<tr>
<td>5</td>
<td>6.8 ± 100 (100)</td>
<td>15 ± 111 (151)</td>
<td>7.6 ± 192 (124)</td>
<td>10.1 ± 147 (147)</td>
<td>9.4 ± 138 (138)</td>
</tr>
</tbody>
</table>

| % Incorporation | 100% | 128 ± 8.5 | 182 ± 22.0 | 160 ± 13.0 | 165 ± 16.2 | 121 ± 10.1 |

*a Mean of triplicate cultures except for Experiment 5.
*b Numbers in parentheses, percentages.
# Mean of five cultures.
*° Significantly different from the other PB treatments (P < 0.05).
#° Significantly different from the other TPA treatments (P < 0.01).

Table 3 Effects of TPA and PB on the specific activity of the dTTP pool

<table>
<thead>
<tr>
<th>Sample</th>
<th>3H dpm in dTTP peak</th>
<th>nmol dTTP</th>
<th>Specific activity of dTTP</th>
<th>3H incorporation into acid-insoluble material</th>
<th>nmol dTTP incorporated</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF</td>
<td>17,272</td>
<td>182.0</td>
<td>94.9</td>
<td>41,434</td>
<td>437</td>
<td>100</td>
</tr>
<tr>
<td>TPA, EGF</td>
<td>17,180</td>
<td>188.4</td>
<td>91.2</td>
<td>65,921</td>
<td>723</td>
<td>165</td>
</tr>
<tr>
<td>PB, EGF</td>
<td>16,272</td>
<td>172.4</td>
<td>94.4</td>
<td>61,044</td>
<td>646.7</td>
<td>148</td>
</tr>
</tbody>
</table>

* Cells were cultured as described in "Materials and Methods," with EGF (10 ng/ml) included from 12 to 24 h of culture; [3H]thymidine labeling was carried out from 44 to 46 h. When added, TPA (50 ng/ml) was present from 36 to 46 h, and PB (1 mM) was present from 24 to 46 h. The dTTP peak was collected, and 3H content was measured by liquid scintillation counting of 0.5-min fractions obtained from 16 to 20 min. The maximum amount of radioactivity always occurred in the 18.0- to 19.0-min fractions (corresponding to the dTTP peak), although radioactivity was present in the fractions from 17.5 to 19.5 min, and the dpm from these fractions were included in calculations for determining dTTP-specific activity.
#° Specific activity is expressed as dpm/nmol dTTP.
¢ The amount of [3H]thymidine incorporated into acid-insoluble material.
$ The amount of dTTP incorporated into acid-insoluble material was calculated by subtracting the 3H counts in the acid-insoluble material by the specific activity of the dTTP pool.

Table 4 Effect of TPA and PB on the spreading of rat hepatocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cell area of single hepatocytes (μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 h</td>
<td>828 ± 222 (n = 98)</td>
</tr>
<tr>
<td>Control</td>
<td>1142 ± 276 (n = 120)</td>
</tr>
<tr>
<td>Control (0.1% DMSO)</td>
<td>1160 ± 280 (n = 83)</td>
</tr>
<tr>
<td>PB, 1 mM</td>
<td>1058 ± 237 (n = 103)</td>
</tr>
<tr>
<td>TPA, 50 ng/ml</td>
<td>1618 ± 369 (n = 104)</td>
</tr>
</tbody>
</table>

* PB or TPA was added to cultures 2 h after plating.

Effects of TPA and PB on Cell Spreading. Since it has been

Fig. 5. High-pressure liquid chromatography of TCA-soluble extracts from cultured hepatocytes stimulated to divide as described for Table 3. The buffers used for chromatography were: Buffer A, 7 mM KH₂PO₄ (pH 4.2); and Buffer B, 1.0 M KH₂PO₄ (pH 4.5). The flow rate was 2.0 ml/min, and compounds were eluted by increasing the proportion of Buffer B as indicated. The absorbance profile (A₂₅₄) at 0.05 absorbance units full scale is shown, and the positions of dTTP and ATP are indicated.

affect the specific activity of the dTTP pool (e.g., a treatment that affects thymidine uptake or metabolism into dTTP). To ensure that the observed increases in DNA synthesis seen with TPA or PB treatment were not the result of changes in precursor pools, we determined the specific activity of the dTTP pool under conditions that maximize TPA- and PB-induced increases in DNA synthesis. Measurable amounts of dTTP could not be detected in freshly isolated hepatocytes, but exogenously added dTTP could be resolved from other components (including other exogenously added deoxynucleotides) in these samples (data not shown). A surprisingly large amount of dTTP was observed in cells that were cultured under conditions that stimulate DNA synthesis (Fig. 5). The specific activity of the dTTP pool and the incorporation of [3H]thymidine were concurrently measured in one experiment in which cells were treated with EGF alone or in conjunction with TPA or PB. The increases in incorporation of [3H]thymidine in cultures treated with TPA or PB (Table 3) are in agreement with the results in Table 2 and reflect real increases in DNA synthesis as measured by dTTP incorporation. However, under the condition of increasing lengths of time that hepatocytes are exposed to EGF, the incorporation of [3H]thymidine into acid-insoluble material greatly overstates the actual increase in DNA synthesis because of a concomitant increase in the specific activity of the dTTP pool (results not shown).
ENHANCEMENT OF HEPATOCYTE DNA SYNTHESIS BY TUMOR PROMOTERS

reported that tumor promoters caused morphological changes in cultured cells (7–9), and also that cell spreading is closely related to DNA synthesis in vitro (42), we measured cell areas of isolated hepatocytes on dishes at the second h and the tenth h after addition of TPA or PB. As shown in Table 4, cell spreading of hepatocytes was enhanced by TPA, but not by PB. As shown in Fig. 6, TPA induced pseudopodial activity of hepatocytes. Although proline, norepinephrine, EGF, and cyclic AMP, all of which enhance hepatocyte DNA synthesis (34, 43–46), also enhance cell spreading, cell spreading associated with prominent pseudopodial activity as seen in TPA-treated hepatocytes was not readily observed in the presence of these factors.

DISCUSSION

Cell proliferation is essential for the formation of preneoplastic lesions derived from a single initiated hepatocyte. From this point of view, we examined in the present experiments the effects of the tumor-promoting agents, TPA and PB, on hepatocyte DNA synthesis in primary culture. These studies have demonstrated the following three effects of tumor promoters on replicative DNA synthesis in primary cultures of rat hepatocytes: (a) hepatocyte DNA synthesis was enhanced by treatment with tumor promoters following EGF treatment; (b) the promoters altered to varying degrees the effect of cell density on DNA synthesis in hepatocytes; and (c) the promoters potentiated the sensitivity of hepatocytes to EGF. In addition, EGF-binding experiments showed that TPA transiently down-regulated the number of EGF receptors and also that PB up-regulated or maintained the number of the receptors. At the time EGF was added to cultures, the number of EGF receptors of the cells treated with TPA was not different from that of the control cultures; this strongly suggests that postreceptor events are important in the observed enhancement of DNA synthesis. In this context, it was critical for the most effective enhancement of DNA synthesis by TPA that treatment with TPA be at the late G1 phase. On the other hand, PB seems to be effective when added simultaneously with EGF or shortly after EGF treatment, but not at late G1 and/or S phase. Thus, TPA and PB may act differently during the cell cycle to enhance DNA synthesis.

A major effect of tumor promoters, i.e., enhancement of DNA synthesis of cells in the cell cycle, but not in G0 stage, seems to be more advantageous for proliferation of preneoplastic cells than for normal cells, possibly because the percentage of preneoplastic cells in the cell cycle, not in a quiescent phase, is much greater than that of noninitiated cells (47). In addition, Farber has suggested that hepatocytes in preneoplastic lesions fail to return to a resting (G0) phase (2). Thus, although it is difficult to explain by this effect alone why initiated cells proliferate more than normal cells in response to tumor promoters, this enhancement of DNA synthesis may account for the better response of preneoplastic than of normal cells to certain growth stimuli, if the initiated cells fail to return to the G0 phase or easily traverse from G0 to G1.

A second effect of promoters, i.e., release of hepatocytes from the regulation of DNA synthesis by cell density, may be related to inhibition of cell-cell communication (3–6). Ogawa et al. (48) demonstrated by electron microscopic techniques the significant widening of intercellular spaces between hepatocytes in preneoplastic lesions; this finding suggests that hepatocyte replication in the lesions is to some extent regulated by cell density. Thus, this effect of promoters may permit the cells in the lesions to proliferate more easily than the cells in normal tissues.

A third effect of these tumor promoters on DNA synthesis, potentiation of the effects of EGF, may account for the in vivo mitogenic activity of PB on hepatocytes (49), because EGF exists in serum at a concentration of about 1 ng/ml (50). In addition, almost all liver tumor promoters cause hyperlasia (49) with hypertrophy. Thus, it is likely that the promoters induce hepatocyte DNA synthesis in vivo by the potentiation of serum EGF on the synthesis. Although we demonstrated in this paper three different effects of tumor promoters on the enhancement of DNA synthesis in cultured hepatocytes, these three mechanisms may cooperate with one another to promote carcinogenesis.

The reported effects of TPA on serum-induced or EGF-induced cell proliferation have been inconsistent; inhibition (51–53) and enhancement (54, 55) have both been reported. For rat hepatocytes, we have demonstrated an enhancing effect of TPA on DNA synthesis, although TPA was reported to abolish the effect of EGF on Ca²⁺ mobilization (56) and on the

Fig. 6. Four-h cultured hepatocytes. At the second h after plating, the cells were refed control (C) medium (upper), TPA (50 ng/ml)-containing medium (middle), or PB (1 mM)-containing medium (lower). × 50.
phosphorylation of proteins in hepatocytes (56). In addition, we have shown that TPA down-regulated EGF receptors transiently, as has been reported for HeLa cells (57). This down-regulation may result from phosphorylation of the 654th amino acid of the receptor by protein kinase C (58). Since a 12-h exposure to EGF was required to induce DNA synthesis and since, 24 h after the initiation of EGF, treatment was required to reach the maximum DNA synthesis (59), it is possible that some of the early responses of hepatocytes to TPA are not related directly to initiation of DNA synthesis.

PB is a potent tumor promoter in hepatocarcinogenesis (1, 2), and PB feeding of rodents results in liver hypertrophy and hyperplasia (49). Armato et al. (28, 29), Edwards and Lucas (27), and Kitagawa et al. (23–25) demonstrated the enhancing effects on DNA synthesis or cell growth, with primary cultures of neonatal rat hepatocytes, adult rat hepatocytes, and preneoplastic liver cells, respectively. On the other hand, Armato et al. (28, 29) and our studies (this paper) demonstrated an enhancing effect of TPA on hepatocyte DNA synthesis, while Kayano et al. (24) failed to show the effect. Kayano et al., however, cultured the cells with TPA over several weeks and then determined colony formation. We treated the cells with the agent for only 1 or 2 days. Since Wälder and Lützelschwab (60) reported that the effect of TPA on intercellular communication of rat liver epithelial cells was transient, the discrepancy of the TPA effect on hepatocyte DNA synthesis and colony formation might result from the relative transient effect of TPA on the former promoter under the conditions of our experiments.

In our studies, 125I-EGF was bound to the cell surface of PB-treated hepatocytes in vitro more than to the cell surface of control cells. In vivo, however, Hwang et al. (61) reported that PB feeding resulted in a decrease in EGF binding to microsomal or Golgi fractions. Since they expressed EGF binding per protein content and since PB induces proliferation of many of the components of the microsome fraction (49), it is difficult to interpret the results of Hwang et al. (61) regarding the effects of PB on EGF binding per cell (versus per protein content).

ACKNOWLEDGMENTS

The authors wish to thank Gerald L. Sattler for technical expertise, and we also thank Dr. Ilse L. Riegel for editorial assistance with the manuscript, Susan Moran for preparing the figures, and Mary Jo Markham and Kristen Luick for typing the manuscript.

REFERENCES


Effects of Tumor-promoting Agents 12-O-Tetradecanoylphorbol-13-acetate and Phenobarbital on DNA Synthesis of Rat Hepatocytes in Primary Culture

Norimasa Sawada, Jeffrey L. Staecker and Henry C. Pitot


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/47/21/5665

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.