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# Stimulation of the Acute-Phase Response in Simian Virus 40-Hepatocyte Cell Lines

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**Seven simian virus 40 (SV40)-hepatocyte cell lines were characterized with respect to the ability to express eight liver acute-phase genes. cDNA clones corresponding to albumin, serum amyloid A, $\alpha_1$-acid glycoprotein, haptoglobin, α-, β-, and γ-fibrinogen, and $\alpha_1$-major-acute-phase protein mRNAs were used in Northern (RNA) or slot blot analyses. In the noninduced state, six of the seven cell lines showed significant (i.e., liverlike) levels of constitutive expression of all genes examined except that expression of haptoglobin mRNA was considerable lower than in the normal liver. To examine whether these immortalized liver cells can respond appropriately to inflammatory mediators, cells were treated with conditioned medium from activated human monocytes or mixed lymphocyte cultures. Results showed that these SV40-hepatocyte cell lines responded to the conditioned media in culture by down-regulating albumin gene expression and up-regulating other acute-phase genes in a time- and dose-dependent manner. These results indicate that the SV40-hepatocytes retained not only the ability to express a number of acute-phase genes but also the ability to respond to external stimuli. The usefulness of these cell lines for analysis of the molecular mechanisms involved in the regulation of these acute-phase genes is discussed.**

The acute-phase response is a systemic response accompanying an inflammatory response caused by tissue injury, infection, or neoplasia (15, 21, 22). During the acute-phase response, expression of a heterogeneous group of proteins synthesized preferentially in the liver, collectively called acute-phase proteins, is altered. Expression of $\alpha_1$-acid glycoprotein ($\alpha_1$-AGP), C-reactive protein, hemopexin, fibrinogen, haptoglobin, $\alpha_1$-major-acute-phase protein ($\alpha_1$-MAP; thiostatin or $\alpha_1$-cysteine proteinase inhibitor), serum amyloid A (SAA), $\alpha_2$-macroglobulin, and the complement components C3 and factor B increases; the magnitude of the increase varies from a fewfold to more than several hundredfold, depending on the specific acute-phase protein (15, 21). On the other hand, expression of albumin and $\alpha_1$-inhibitor III decreases (9, 25). The acute-phase response of the liver has been experimentally studied in whole animals by injection with turpentine, bacterial endotoxin, etc. (32, 38; for a review, see reference, 24), and in liver slices from animals treated with turpentine (42).

The acute-phase response is biologically significant and represents an excellent system for studying how gene expression is regulated in fully differentiated tissue in response to external stimuli. To analyze coordinate regulation of expression of the acute-phase genes at a molecular level, it is beneficial to have in vitro systems in which the acute-phase genes are expressed and in which the acute-phase response can be triggered. Recently, primary hepatocytes of mouse or rat origin and human and rodent hepatoma cell lines have been successfully used to study regulation of expression of a variety of acute-phase genes (1, 5, 11, 23). Hepatic cells in culture have proved effective for analyzing the effects of specific agents that trigger the acute-phase response (18, 19, 37). Similarly, transfection of hepatoma cells with DNA fragments containing upstream sequences of acute-phase genes has been used successfully to identify *cis*-acting regulatory elements mediating the acute-phase response (3, 36).

We recently developed a series of cell lines derived from normal adult rat hepatocytes transfected with simian virus 40 (SV40) DNA (46–48), designated SV40-hepatocyte cell lines (48). We previously demonstrated that these cell lines secrete albumin at high levels, that many of the cell lines also secrete high levels of the serum proteins transferrin, hemopexin, and C3, and that albumin synthesis in these cells can be regulated by cell density and exposure to dexamethasone (46). We have also shown that five of the SV40-hepatocyte cell lines at low passage express albumin, transferrin, $\alpha_1$-antitrypsin, and phosphoenolpyruvate carboxykinase RNAs at levels approaching those found in normal adult liver (47). We have demonstrated that these SV40-hepatocyte cell lines can express several liver-specific genes at high levels. This property makes these cell lines extremely useful for studying constitutive expression of these genes at the molecular level. It is also important to determine whether changes in expression of liver-specific genes in response to external stimuli occur in SV40-hepatocyte cells in the same fashion as in hepatocytes in vitro. To address this question, we chose to determine whether the acute-phase response can be triggered in these SV40-hepatocyte cell lines in culture. Specifically, this study was designed to determine whether SV40-hepatocyte cell lines retain the capacity to express specific acute-phase mRNAs, how the level of expression compares with that in normal liver, and whether the expression of these genes is altered in the manner expected for the acute-phase response when the

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cells are treated with conditioned medium from activated monocytes or mixed lymphocyte cultures.

## MATERIALS AND METHODS

**Hepatocyte cultures.** Primary cultures of adult rat hepatocytes were isolated by collagenase perfusion of male Fischer F344 rats (180 to 200 g) as previously described (7) and modified (14, 20). Hepatocytes were washed in RPCD medium (48) supplemented with 5% fetal calf serum (FCS) and plated at a density of approximately $10^6$ cells per 60-mm-diameter plastic cell culture dish coated with rat tail collagen (12). At 5 to 6 h after plating, hepatocyte monolayers were fed fresh serum-supplemented RPCD, and at 24 h after plating the cells were fed RPCD medium supplemented with 2% dimethyl sulfoxide and 25 ng of epidermal growth factor (Collaborative Research, Inc., Lexington, Mass.) per ml. Hepatocytes were fed fresh RPCD plus epidermal growth factor plus dimethyl sulfoxide every 2 days and were harvested at 18 days after isolation for preparation of RNA.

**Cell lines.** SV40-hepatocyte cell lines were maintained in 100-mm-diameter plastic tissue culture dishes and fed fresh RPCD medium (46) every 3 days. When cultures became confluent, the cells were trypsinized and plated at a 1:10 dilution in fresh dishes, using RPCD supplemented with 5% FCS to aid cell attachment. After attachment (1 to 4 h after plating), fresh RPCD medium (without FCS) was added.

**Preparation of conditioned media from monocyte and mixed lymphocyte cultures.** Monocyte- and mixed lymphocyte culture-conditioned media were prepared according to the methods of Mehta et al. (31) and Maizel et al. (29), respectively. Pure populations of human peripheral blood monocytes were obtained by countercurrent centrifugal elutriation of mononuclear leukocyte-rich fractions obtained from normal donors who were undergoing routine plateletpheresis (27). Monocytes were washed with medium (RPMI 1640 supplemented with L-glutamine, 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer, 20 μg of gentamicin per ml, and 5% human AB serum) and plated at $10^6$/ml in RPMI 1640 with 10% FCS. After cells were attached for 2 h, monocytes were stimulated with 10 μg of lipopolysaccharide (Difco Laboratories, Detroit, Mich.) per ml for 72 h. Monocyte-conditioned medium was filter sterilized and stored at −20°C. Mixed lymphocyte culture-conditioned medium was obtained by incubating human peripheral blood lymphocytes ($10^6$/ml) from four healthy donors in RPMI 1640 containing 0.25% bovine serum albumin and 0.75% phytohemagglutinin (PHA M form; GIBCO Laboratories, Grand Island, N.Y.). Cells were cultured for 72 h at 37°C, at which time the conditioned medium was separated from the cells by centrifugation and filtration.

**Treatment of SV40-hepatocyte cell lines with conditioned medium.** CWSV1 or CWSV16 cells were grown to 60 to 70% confluence in 100-mm-diameter plastic tissue culture dishes. The cells were then washed and refed control, drug-treated, or conditioned medium. Unless otherwise stated, control medium was prepared by mixing equal volumes of medium used for plating lymphocyte or monocyte cultures (RPMI 1640 supplemented with 10% FCS, antibiotics, etc.) and medium used for growing SV40-hepatocyte cell lines (RPCD medium) (48). Drug-treated medium contained equal volumes of (i) RPMI 1640 supplemented with either 10% FCS, antibiotics and lipopolysaccharide (10 μg/ml), or 0.75% phytohemagglutinin (ii) RPCD medium. Conditional medium contained equal volumes of medium from stimulated monocytes or lymphocytes and RPCD medium. In one set of experiments, the ratio of control, drug-treated, or conditioned medium to RPCD medium was varied to determine whether the effect of conditioned medium was concentration dependent. After the CWSV1 or CWSV16 cells were incubated with control, drug-treated, or conditioned medium for the indicated lengths of time, the cells were harvested for preparation of RNA. Cell morphology was monitored throughout the experiments.

**DNA probes.** Rat liver cDNAs corresponding to albumin, SAA, haptoglobin, $\alpha_1$-AGP, α-, β-, and γ-fibrinogen, and $\alpha_1$-MAP mRNAs were initially isolated from a λgt10 cDNA library by differential cDNA hybridization and then subcloned into pBR325 (26). The identities of cDNAs were determined by DNA sequence comparison with published sequences. Plasmid DNAs were isolated by standard procedures and used as hybridization probes.

**Isolation of RNA and measurement of mRNA levels.** Total cellular RNA was isolated by the procedure of Chirgwin et al. (10) from livers of normal Sprague-Dawley rats (250 to 300 g) and rats treated with turpentine (0.5 ml/100 g) to induce the acute-phase response. Cellular RNA from SV40-hepatocyte cell lines was prepared by the guanidinium thiocyanate procedure of Chirgwin et al. (10) as modified by Setzer et al. (44). Total RNA concentration was determined spectrophotometrically.

After treatment with glyoxal, 10 μg of total RNA was separated by electrophoresis (30) in a 0.8% agarose gel in 10 mM sodium phosphate buffer, pH 7.0. RNA was stained with ethidium bromide to ensure that the amounts in each track were equal and then transferred to diazotized aminothiophenol paper (43). Prehybridization and hybridization with nick-translated probes (39) were carried out as described by Wahl et al. (45) except that dextran sulfate was not used.

The slot blot method of Gasser et al. (16) was used to determine mRNA levels more accurately. Samples of total RNA (4 μg per slot) were applied to nitrocellulose filters and hybridized with nick-translated cDNA probes (specific activity, $10^8$ cpm/μg or higher). After washing, the filters were autoradiographed with Kodak XAR-5 film (Eastman Kodak Co., Rochester, N.Y.) and a Cronex Lightning-Plus screen (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) at −70°C.

## RESULTS

**Expression of acute-phase RNAs in CWSV cells.** A series of SV40-hepatocyte cell lines that continue to express liver-specific functions were previously derived in our laboratory (46). Many of these cell lines retain the capacity to synthesize a variety of serum proteins or messages for these proteins at liverlike or near-liverlike levels. To determine whether any of the SV40-hepatocyte cell lines could be used to study the acute-phase response, we screened seven SV40-hepatocyte cell lines for expression of eight acute-phase mRNAs. Six of the seven cell lines expressed albumin, SAA, $\alpha_1$-AGP, α-, β-, and γ-fibrinogen, and $\alpha_1$-MAP mRNAs at measurable levels and, in some cases, at liverlike or near-liverlike levels (Fig. 1). All seven cell lines expressed much lower levels of haptoglobin mRNA. As was expected, the cell lines expressed considerably higher levels of actin RNA than did the liver. The seventh cell line, CWSV8, which was previously shown not to express albumin beyond passages 8 to 10 and expressed $\alpha_1$-AGP only at low levels, was included as a negative control. We previously reported that CWSV1 and CWSV16 cells at low passage express high levels of

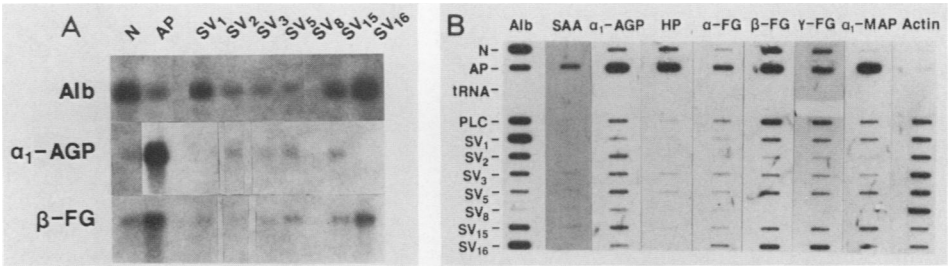


FIG. 1. Expression of liver-specific genes in SV40-hepatocyte cell lines. (A) Seven SV40-hepatocyte cell lines were cultured in RPCD. Total RNA (10 μg) was prepared and analyzed by Northern (RNA) hybridization with nick-translated albumin (Alb), $\alpha_1$-AGP, and β-fibrinogen (β-FG) cDNAs. RNA from normal (N) and turpentine-treated (AP) rat liver was included for comparison. (B) Total cellular RNA (4 μg per slot) was applied to nitrocellulose filters (16) and hybridized with albumin (Alb), SAA, $\alpha_1$-AGP, haptoglobin (HP), α-, β-, or γ-fibrinogen (α-, β-, or γ-FG), $\alpha_1$-MAP, and actin cDNAs. RNA samples: N, normal liver; AP, acute-phase liver; tRNA, yeast tRNA; PLC, 18-day primary hepatocytes cultured in RPCD plus epidermal growth factor plus dimethyl sulfoxide. $SV_1$, $SV_2$, $SV_3$, $SV_5$, $SV_8$, $SV_{15}$, and $SV_{16}$ are the seven individually derived SV40-hepatocyte cell lines.

albumin mRNA (46, 47). These findings were confirmed in this study. In addition, the CWSV1 and CWSV16 cell lines also expressed high levels of α-, β-, and γ-fibrinogen, $\alpha_1$-AGP, and $\alpha_1$-MAP mRNAs and, for this reason, were used for subsequent studies.

RNA extracted from 18-day primary adult rat hepatocytes cultured in dimethyl sulfoxide-supplemented, chemically defined medium was also analyzed for expression of the same acute-phase RNAs. Comparison of the slot blot analyses (Fig. 1B) showed that expression of the acute-phase mRNAs by CWSV cells was generally similar to expression in 18-day hepatocytes. Haptoglobin mRNA levels in 18-day hepatocytes and CWSV cells were considerably lower than haptoglobin mRNA levels in the liver, and β- and γ-fibrinogen mRNA levels were slightly lower in the CWSV1 cells than in 18-day primary hepatocytes.

**Effects of treating CWSV1 cells with conditioned medium from activated monocytes or mixed lymphocyte cultures on expression of acute-phase RNAs.** CWSV1 cells were treated with 50% conditioned medium from monocytes or mixed lymphocytes for 36 h to determine whether the cells retained the ability to respond to cytokines present in the conditioned medium and whether this ability to respond depended on whether the cells were treated with medium exposed to activated monocytes or to mixed lymphocyte cultures. RNA was extracted from treated and control cells and analyzed for albumin and $\alpha_1$-AGP mRNAs (Fig. 2). The albumin gene was chosen for this study because its expression diminishes during the acute-phase response, and the $\alpha_1$-AGP gene was selected because its expression is enhanced in the acute-phase response. Quantitation of the slot blot analyses (Fig. 2B) showed that treatment of CWSV1 cells with conditioned medium resulted in a 4- to 5-fold decrease in albumin mRNA levels and a concomitant 10- to 15-fold increase in $\alpha_1$-AGP mRNA levels. Thus, we conclude from the slot blot analyses that direct treatment of lipopolysaccharide on CWSV1 cells did not alter the expression of albumin and $\alpha_1$-AGP; treatment of CWSV1 cells with conditioned medium from either activated monocytes or mixed lymphocyte cultures triggered the down-regulation of albumin gene expression and the dramatic up-regulation of $\alpha_1$-AGP; the effect was identical whether conditioned medium from activated monocytes or from mixed lymphocyte cultures was used; the effect of albumin expression was similar quantitatively to the in vivo response; and the effect of conditioned medium on $\alpha_1$-AGP expression was marked, but the level of expression of $\alpha_1$-AGP mRNA in treated CWSV1 cells was approximately 30% of the level observed in vivo in liver from treated animals.

Alterations in albumin and $\alpha_1$-AGP mRNA levels in CWSV1 cells were indistinguishable when the cells were treated with conditioned medium from either monocytes or mixed lymphocyte cultures (Fig. 2). Since large quantities of conditioned medium from mixed lymphocyte cultures can be prepared more readily, this medium was used for all subsequent studies.

**Effect of exposure time of CWSV1 cells to conditioned medium on acute-phase mRNA levels.** CWSV1 cells were treated for various lengths of time with conditioned medium from mixed lymphocyte cultures to examine the kinetics of induction of these acute-phase genes. RNA was extracted from CWSV1 cells treated for 6, 12, and 24 h with 50% conditioned medium from phytohemagglutinin-treated mixed lymphocyte cultures and analyzed for the expression of acute-phase RNAs (Fig. 3A). The effects of treatment

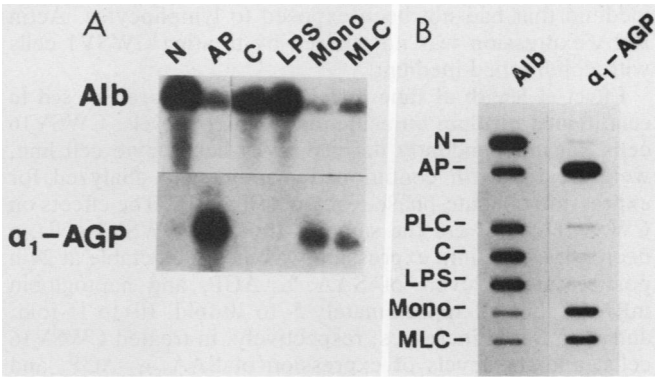


FIG. 2. Effects of activated monocyte and mixed lymphocyte culture supernatants on the expression of albumin and $\alpha_1$-AGP in CWSV1 cells. CWSV1 cells were cultured with 1:1 RPCD culture medium and activated monocyte supernatant or 1:1 RPCD and activated mixed lymphocyte culture supernatant for 36 h. Total RNA was prepared and analyzed by Northern blot (10 μg per lane) (A) or slot blot (4 μg per slot) (B) with albumin (Alb) and $\alpha_1$-AGP cDNAs. RNA samples: N, normal liver; AP, acute-phase liver; PLC, 18-day primary hepatocytes. Culture conditions for CWSV1 cells: C, medium control; LPS, drug-treated control (containing 5 μg of lipopolysaccharide per ml); Mono, conditioned medium from stimulated monocytes; MLC conditioned medium from stimulated mixed lymphocyte cultures.

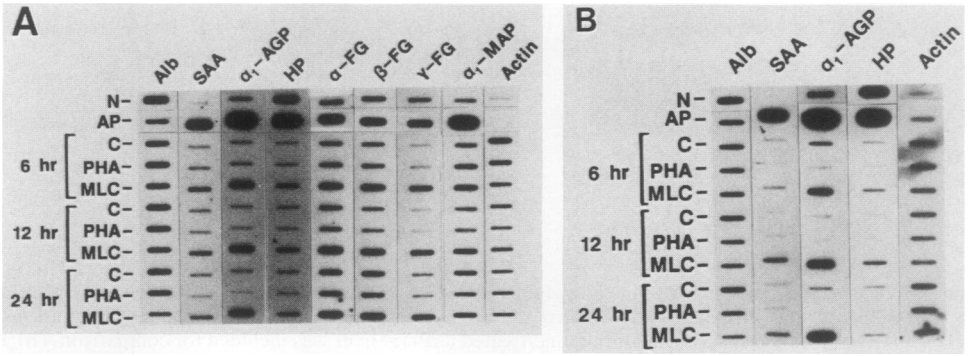


FIG. 3. Stimulation of the expression of acute-phase genes in SV40-hepatocyte cell lines with mixed lymphocyte culture-conditioned medium. (A) CWSV1 cells treated with 1:1 RPCD medium and mixed lymphocyte culture-conditioned medium for 6, 12, and 24 h. Culture conditions: C, medium control; PHA, drug-treated control containing 0.37% phytohemagglutinin; MLC, conditioned medium from stimulated mixed lymphocyte cultures. RNA samples from normal (N) and acute-phase (AP) liver were run in parallel for comparison. (B) Experiments performed as described for panel A except that the CWSV16 cell line was used. Total RNA was examined by slot blot analysis (4 μg of RNA per slot) for expression of acute-phase genes. The genes are as described for Fig. 1B. Abbreviations for the samples are as for panel A.

with conditioned medium paralleled the response observed in vivo in animals treated with turpentine; that is, the levels of SAA, $\alpha_1$-AGP, haptoglobin, α-, β-, and γ-fibrinogen, and $\alpha_1$-MAP mRNAs increased, and the level of albumin mRNA decreased. The time course of the effect was specific for each acute-phase reactant. At 6 and 12 h posttreatment, there was no significant decrease in albumin expression. At 24 h posttreatment, however, a fourfold reduction in albumin mRNA was detected. The level of expression of all three species of fibrinogen mRNAs by treated CWSV1 cells was similar in magnitude to that observed in vivo, ranging from two- to threefold for α- and β-fibrinogen expression to five- to eightfold for γ-fibrinogen expression. Although the constitutive level of haptoglobin mRNA in CWSV1 cells was considerably lower than in normal liver, a consistent three- to fourfold increase in haptoglobin mRNA was seen in treated CWSV1 cells versus CWSV1 control cells. No alteration in the expression of acute-phase RNAs was observed in cells treated with phytohemagglutin-containing medium that had not been exposed to lymphocytes. Actin RNA expression was not altered by treating CWSV1 cells with conditioned medium.

**Effect of length of time that CWSV16 cells are exposed to conditioned medium on acute-phase mRNA levels.** CWSV16 cells, an independently derived SV40-hepatocyte cell line, were treated with conditioned medium and analyzed for expression of acute-phase reactants (Fig. 3B). The effects on CWSV16 cells were the same as those on CWSV1 cells: a decrease in albumin expression was most detectable at 24 h posttreatment; levels of SAA, $\alpha_1$-AGP, and haptoglobin mRNAs showed approximately 5- to 10-fold, 10- to 15-fold, and 3- to 5-fold increases, respectively, in treated CWSV16 cells; and the levels of expression of SAA, $\alpha_1$-AGP, and haptoglobin were much lower than those observed in vivo. These results suggested that the ability of CWSV hepatocyte cell lines to elicit the acute-phase response is not unique to CWSV1 cells.

**Effect of the concentration of conditioned medium used to treat CWSV1 cells on the acute-phase response.** In the preceding experiments, the medium used to treat CWSV1 and CWSV16 cells consisted of control, drug-treated, or conditioned medium diluted equally with the standard culture medium used for growing CWSV1 cells (RPCD), as described in Materials and Methods. Failure to detect the same magnitude of stimulation for some of the acute-phase RNAs as was seen in vivo may simply reflect the fact that the amount of cytokine present in the volume of conditioned medium was not sufficient to produce a maximal effect. To determine whether the effect of conditioned medium on the acute-phase response in CWSV1 cells was concentration dependent, cells were treated with medium containing 1 part control, drug-treated, or conditioned medium to 9 parts RPCD (10%), the equal mixture used in previous experiments (50%), or 9 parts control, drug-treated, or conditioned medium to 1 part RPCD (90%). The effect of conditioned medium concentration was most apparent for the albumin and $\alpha_1$-AGP RNAs and when 50% conditioned medium was compared with 10% medium (Fig. 4). A three- to fourfold increase in β-fibrinogen RNA levels was observed at 12 but not 36 h after treatment, and this increase was not dose dependent.

**Morphology of CWSV1 cells cultured in mixed lymphocyte culture-conditioned medium.** CWSV1 cells are epithelial cells that grow in patches at first but eventually fill the majority of the surface of the culture plate. Treatment with conditioned medium caused CWSV1 cells to elongate, become more refractile, and form long, stringy groupings of cells such that

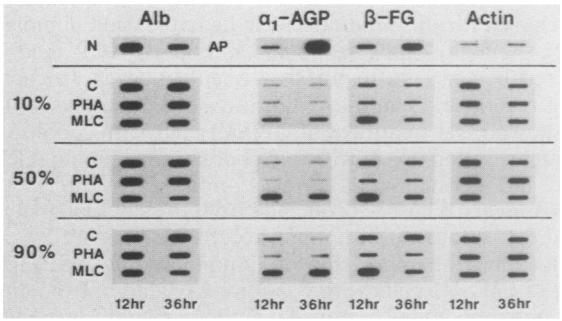


FIG. 4. Dose-dependent stimulation of acute-phase genes in CWSV1 cells. CWSV1 cells were treated for 12 and 36 h with medium containing 1 part control, drug-treated, or conditioned medium to 9 parts RPCD (10%), the equal mixture used in previous experiments (50%), or 9 parts control, drug-treated, or conditioned medium to 1 part RPCD (90%). Total RNA was analyzed by slot blot with albumin (Alb), $\alpha_1$-AGP, and β-fibrinogen (β-FG) cDNAs. Rat liver RNAs from normal (N) or 36-h acute-phase (AP) rats were included for comparison.

large open spaces appeared in the culture dish (Fig. 5). The effect was readily apparent by 12 h. No alterations in cell morphology occurred when CWSV1 cells were treated with control or drug-treated medium. Similarly, the effect on cell morphology was more evident when the amount of conditioned medium used to treat the cells was increased.

## DISCUSSION

The two goals of this study were (i) to continue to characterize the SV40-hepatocyte cell lines as a model system for studying liver-specific gene expression and (ii) to determine whether these cell lines have potential value for studies of regulation of acute-phase gene expression. We conclude that (i) SV40-hepatocyte cell lines retain the capacity to express at least eight acute-phase genes (albumin, SAA, $\alpha_1$-AGP, haptoglobin, $\alpha$-, $\beta$-, and $\gamma$-fibrinogen, and $\alpha_1$-MAP); (ii) the level of expression for some of these genes approximates the levels found for normal rat liver, while for other genes the levels are lower but within the same magnitude of expression; and (iii) the expression of these genes can be altered (up- or down-regulated) in the same manner as in liver. These findings (i) support our previous conclusion that SV40-hepatocyte cell lines function at the biochemical and molecular levels like the liver and (ii) strongly suggest that the cell lines will be of value for molecular analysis of regulation of acute-phase gene expression in rat liver.

Acute-phase gene expression has been studied in several species (4, 11, 28) by using whole animals, primary hepatocytes in culture, and a series of animal and human hepatoma cell lines. One of the disadvantages of using the former approach is that the agent triggering the response is administered to the whole animal; hence, the actual agent acting at the level of the liver may be a secondary or even tertiary signal. In addition, the tissue being examined contains several different cell types. To circumvent these problems, in vitro models for studying the acute-phase response by using primary hepatocytes in culture or established hepatoma cell lines have recently been described. The advantage of using primary hepatocytes, at least in some culture systems, is that the cells represent the functional normal parenchymal cells of the liver and therefore tend to better resemble liver than do most cell lines. It is possible by using primary hepatocytes in culture to investigate directly the agents that trigger the acute-phase response in hepatocytes. Fetal (41) and adult (40) rat hepatocytes could be used to study the ability of substances produced by activated leukocytes to stimulate the acute-phase response. Interleukin-1 (IL-1) induces acute-phase protein synthesis in rat and mouse hepatocytes (18); IL-6/interferon-$\beta_2$ (IFN-$\beta_2$) induces the acute-phase response in primary rat hepatocytes (17); a series of hepatocyte-stimulating factors (HSFs) can induce the acute-phase response (5); and HSF and dexamethasone interact to induce fibrinogen production in rat hepatocytes (34).

Disadvantages of using primary hepatocyte cultures, including primarily that the cell population is not totally homogeneous and that the cells do not lend themselves well to molecular studies, can be avoided by using cell lines. The acute-phase response has been studied in a variety of rat and human hepatoma cell lines. A subline of Reuber H-35 hepatoma cell lines has been used to study induction of the acute-phase response by two human HSFs (6). The rat hepatoma cell line FAZA has been used to study regulation of fibrinogen production by HSF and dexamethasone (34) and by other factors (13). The human hepatoma cell line Hep-G2 (17) and the rat hepatoma cell line Fao-9 (2) have been used to study the effects of IL-6/IFN-$\beta_2$ on the acute-phase response. The human hepatoma cell line Hep-3B2 has been used to study increased production of fibrinogen, $\alpha_1$-AGP, haptoglobin, and C3 and the decreased production of albumin after treatment with IL-1 or with conditioned medium from activated monocytes (8, 11). The human hepatoma cell lines Hep-G2 and Hep-3B2 have been used to demonstrate that tumor necrosis factor has a role in inducing the acute-phase response (35).

In vitro studies of acute-phase gene regulation have made it apparent that the effects observed vary among cell lines and between cell lines and primary hepatocytes. For example, the mechanism by which dexamethasone contributes to induction of fibrinogen production differs in the FAZA rat hepatoma cell line from what has been observed in primary rat hepatocytes (34). Similarly, the haptoglobin gene is expressed in Hep-G2 cells but not in Hep-3B cells (33), and the effects of two squamous cell HSFs on a series of acute-phase proteins are different in rat hepatoma cell lines than in human hepatoma cell lines and primary hepatocytes (6).

Many transformed hepatoma cell lines express a limited battery of liver-specific genes and in this regard differ from primary hepatocytes and normal liver. At early passage, SV40-hepatocyte cell lines do not produce tumors when inoculated into test animals and therefore are immortalized but are not transformed (48). The data accumulated to date on liver-specific gene expression by the SV40-hepatocyte cell lines indicates that these cells at early passages more closely resemble primary hepatocytes and liver than do the hepatoma cell lines tested (46, 48). However, it is important to note that the SV40-hepatocyte cell lines contain and express SV40 genetic information and thus differ from hepatocytes in normal liver. Furthermore, they are cell lines and, as such, have properties of cell lines in culture, including the capacity to replicate and to express higher levels of actin than are expressed by cells in tissue. Since one of the goals of this study was to determine whether the SV40-hepatocyte cell lines were an appropriate in vitro model for normal liver, direct comparisons were made between the levels of acute-phase gene RNAs in control and treated SV40-hepatocyte cell lines and in normal and acute-phase livers. This type of direct comparison of mRNA levels has not been carried out previously in many studies. The levels of expression of albumin RNA by the CWSV1 and CWSV16 cells were equal to or greater than the levels found in normal liver. In general, the levels of the other acute-phase gene RNAs were highest in the CWSV1 and CWSV16 cells than in the other cell lines examined. Levels of SAA and $\alpha_1$-MAP RNAs in these two cell lines were equal to or greater than those in liver. $\alpha_1$-AGP RNA levels were slightly lower in the cell lines than in liver or in primary hepatocytes. The $\alpha$-, $\beta$-, and $\gamma$-fibrinogen RNA levels were slightly lower than in liver; however, in normal liver, the primary hepatocytes, and the two cell lines, the RNA for the $\beta$ chain of fibrinogen was expressed at the highest level, suggesting that a normal pattern of expression of the fibrinogens was observed in the cell lines. Haptoglobin was expressed by CWSV1 and CWSV16 cells but at a level considerably lower than in liver. The data reported in this study suggest that the steady-state levels of constitutive and induced acute-phase gene RNAs in SV40-hepatocyte cell lines more closely resemble the levels in primary hepatocytes and liver than has been previously shown for many hepatoma cell lines. The H-35 subline expresses a large battery of acute-phase genes, but it has been reported that

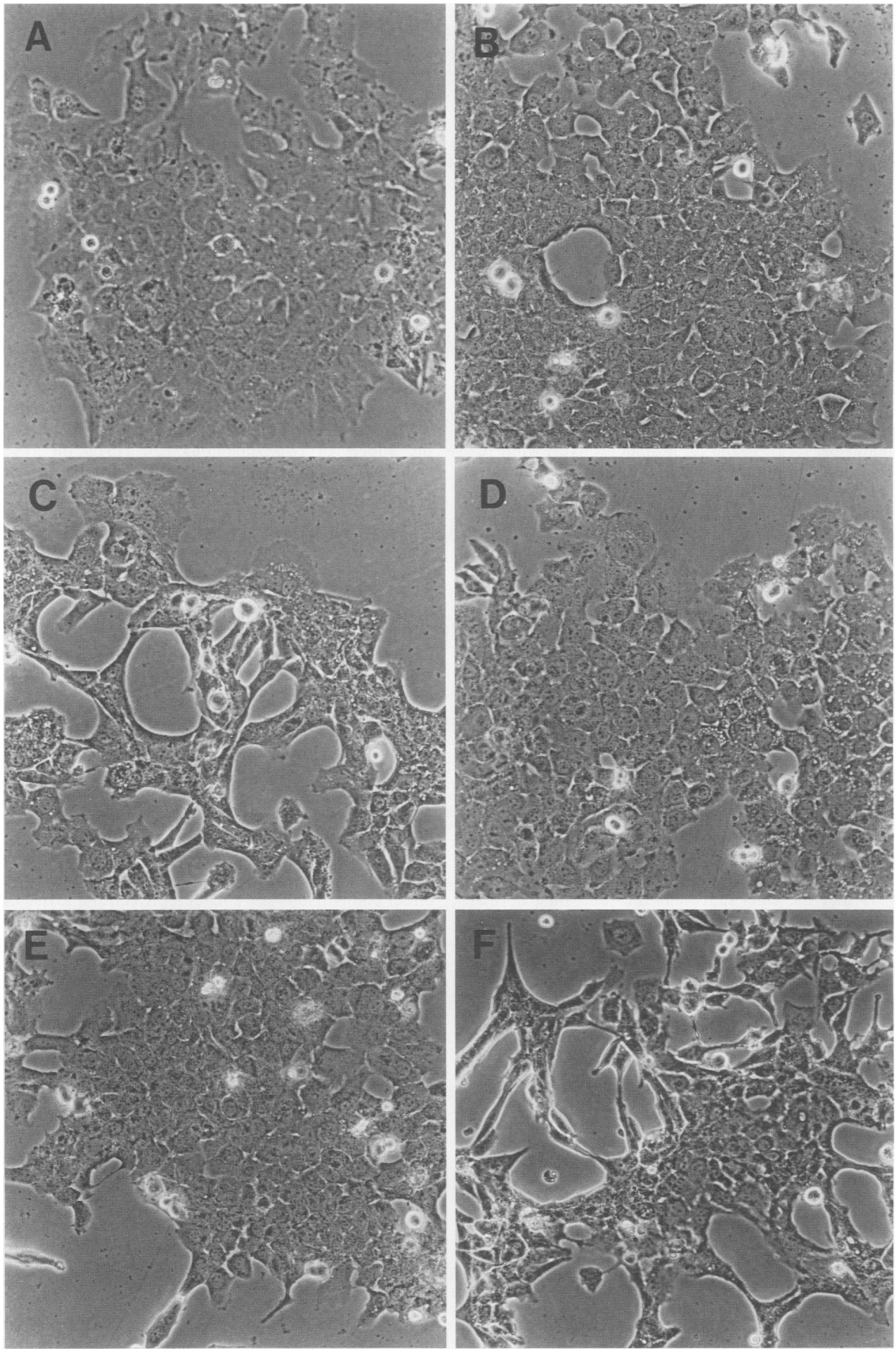


FIG. 5. Morphology of CWSV1 cells cultured in medium containing activated mixed lymphocyte culture supernatants. CWSV1 cells were cultured with the designated medium for 12 h. Cells were cultured in 50% control medium (A), 50% drug-treated medium (B), 50% conditioned medium (C), 90% control medium (D), 90% drug-treated medium (E), and 90% conditioned medium (F).

the relative amounts of each plasma protein secreted are 10- to 100-fold less than in primary hepatocytes (6). The SV40-hepatocyte cell lines express all of the acute-phase genes that were tested and for this reason may be an excellent choice for in vitro studies of the acute-phase response. In future studies, it will be necessary to expand the number of acute-phase genes examined to determine whether these cell lines are universally of value for studying expression of the acute-phase genes.

The SV40-hepatocyte cell lines also respond to stimulation by the conditioned medium from monocyte or mixed lymphocyte cultures to regulate the expression of the acute-phase genes in the same fashion as is seen in acute-phase liver. Albumin RNA levels decreased markedly, and levels of SAA, $\alpha_1$-AGP, haptoglobin, and the three fibrinogen mRNAs increased. The magnitude of response for the albumin and fibrinogen genes was similar to what was observed in vivo, the response for the $\alpha_1$-AGP and haptoglobin genes was somewhat less than that observed in vivo, and the increase in $\alpha_1$-MAP RNA levels was minimal. The amount of time required for alteration in gene expression to occur after treatment of the cells with conditioned medium varied among the genes. For example, the increased levels of $\alpha_1$-AGP occurred rapidly, whereas the decrease in albumin RNA levels was not easily detected until 24 h after treatment. The magnitude of change in expression of the acute-phase genes in the SV40-hepatocyte cell lines depended on the amount of conditioned medium used to treat the cells. It is possible that the magnitude of the response for some of the genes would be even greater if the cells were exposed to higher concentrations of the factors present in the conditioned medium. This can be accomplished by treating the cells with higher concentrations of purified factors (as discussed below). The fact that the SV40-hepatocytes cell lines maintained the ability to respond to factors that trigger the acute-phase response was of particular interest because it demonstrated that the SV40-hepatocyte cell lines not only maintained the ability to express genes normally expressed in vivo by the liver but also maintained the ability to respond to specific external stimuli in the same fashion as the liver.

It is apparent from recent studies that (i) many genes in the liver are up-regulated and others are down-regulated when the acute-phase response is triggered, (ii) multiple factors present in crude reagents (conditioned medium from monocyte and mixed lymphocyte cultures, culture medium from human squamous carcinoma cells, etc.) act to induce these changes in gene expression, and (iii) each specific factor appears to act on subsets of these acute-phase genes. Many of the factors present in conditioned medium that induce the acute-phase response have been defined and are now available in reasonably pure form. One of our next goals will be to treat the SV40-hepatocyte cell lines with purified factors (IL-1$\alpha$, IL-1$\beta$, IL-6/IFN-$\beta_2$, tumor necrosis factor, etc.) to determine the effects of these individual components, alone and in combination, on expression of specific acute-phase genes.

The SV40-hepatocyte cell lines grow in a chemically defined medium in the absence of serum, which means that the effect of adding purified or partially purified substances to the cells can be measured without interference from serum. It is important to note that the chemically defined medium used for growing SV40-hepatocyte cell lines contains dexamethasone. Since it has been shown that dexamethasone regulates the expression of some acute-phase genes, this fact must be taken into consideration. However, dexamethasone can be removed from the medium, and studies can be carried out to test directly the effect of dexamethasone on expression of acute-phase genes in these cells.

Changes in the acute-phase gene expression are regulated primarily at the level of gene transcription; therefore, the acute-phase genes are excellent models for studying coordinated transcriptional regulation of gene expression. cDNAs and genomic DNAs have been isolated for many of the acute-phase genes, and for some of these genes the upstream *cis*-acting regulatory DNA sequences have been identified (3, 36). Since many of the acute-phase genes are regulated by several hormones or cytokines, each external stimulus may work by a different mechanism, and those that work at the transcriptional level may recognize a variety of *cis*-acting regulatory DNA sequences. The SV40-hepatocyte cell lines may prove useful for molecular analysis of *cis*-acting DNA sequences of the acute-phase genes.

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## LITERATURE CITED

1. **Andus, T., T. Geiger, T. Hirano, T. Kishimoto, T. A. Tran-Thi, K. Decker, and P. C. Heinrich.** 1988. Regulation of synthesis and secretion of major rat acute-phase proteins by recombinant human interleukin-6 (BSF-2/IL-6) in hepatocyte primary cultures. Eur. J. Biochem. **173**:287–293.
2. **Andus, T., T. Geiger, T. Hirano, H. Northoff, U. Ganter, J. Bauer, T. Kishimoto, and P. C. Heinrich.** 1987. Recombinant human B cell stimulatory factor 2 (BSF-2/IFN-$\beta_2$) regulates $\beta$-fibrinogen and albumin mRNA levels in Fao-9 cells. FEBS Lett. **221**:18–22.
3. **Arcone, R., G. Gualandi, and C. Ciliberto.** 1988. Identification of sequences responsible for acute-phase induction of human C-reactive protein. Nucleic Acids Res. **16**:3195–3207.
4. **Baumann, H., R. E. Hill, D. N. Sauder, and G. P. Jahreis.** 1986. Regulation of major acute-phase plasma proteins by hepatocyte-stimulating factors of human squamous carcinoma cells. J. Cell Biol. **102**:370–383.
5. **Baumann, H., G. P. Jahreis, D. N. Sauder, and A. Koj.** 1984. Human keratinocytes and monocytes release factors which regulate the synthesis of major acute-phase plasma proteins in hepatic cells from man, rat, and mouse. J. Biol. Chem. **259**: 7331–7342.
6. **Baumann, H., V. Onorato, J. Gauldie, and G. P. Jahreis.** 1987. Distinct sets of acute-phase plasma proteins are stimulated by separate human hepatocyte-stimulating factors and monokines in rat hepatoma cells. J. Biol. Chem. **262**:9756–9768.
7. **Berry, M. N., and D. S. Friend.** 1969. High-yield preparation of isolated rat liver parenchymal cells: a biochemical and fine structural study. J. Cell Biol. **43**:506–520.
8. **Beuscher, H. U., R. J. Fallon, and H. R. Colten.** 1987. Macrophage membrane interleukin-1 regulates the expression of acute-phase proteins in human hepatoma Hep 3B cells. J. Immunol. **139**:1896–1901.
9. **Braciak, T. A., W. Northemann, G. O. Hudson, B. R. Shiels, M. R. Gehring, and G. H. Fey.** 1988. Sequence and acute phase regulation of rat $\alpha_1$-inhibitor III messenger RNA. J. Biol. Chem. **263**:3999–4012.
10. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**:5294–5299.
11. **Darlington, G. J., D. R. Wilson, and L. B. Lachmann.** 1986.

Monocyte-conditioned medium, interleukin-1, and tumor necrosis factor stimulate the acute-phase response in human hepatoma cells *in vitro*. J. Cell Biol. **103**:787–793.
12. **Elsdale, T., and J. Bard.** 1972. Collagen substrata for studies on cell behavior. J. Cell Biol. **54**:624–637.
13. **Evans, E., G. M. Courtois, P. L. Kilian, G. M. Fuller, and G. R. Crabtree.** 1987. Induction of fibrinogen and a subset of acute-phase response genes involves a novel monokine which is mimicked by phorbal esters. J. Biol. Chem. **262**:10850–10854.
14. **Feldhoff, R. C., J. M. Taylor, and L. S. Jefferson.** 1977. Synthesis and secretion of rat albumin *in vivo*, in perfused liver, and in isolated hepatocytes: effects of hypophysectomy and growth hormone treatment. J. Biol. Chem. **252**:3611–3616.
15. **Fey, G. H., and G. M. Fuller.** 1987. Regulation of acute-phase gene expression by inflammatory mediators. Mol. Biol. Med. **4**:323–338.
16. **Gasser, C. S., C. C. Simonsen, J. W. Schilling, and R. T. Schimke.** 1982. Expression of abbreviated mouse dihydrofolate reductase genes in cultured hamster cells. Proc. Natl. Acad. Sci. USA **79**:6522–6526.
17. **Gauldie, J., C. Richards, D. Harnish, P. Lansdorp, and H. Baumann.** 1987. Interferon $\beta_2$/B-cell stumulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. Proc. Natl. Acad. Sci. USA **84**:7251–7255.
18. **Gauldie, J., D. N. Sauder, K. P. W. J. McAdam, and C. A. Dinarello.** 1987. Purified interleukin-1 (IL-1) from human monocytes stimulates acute-phase protein synthesis by rodent hepatocytes *in vitro*. Immunology **60**:203–207.
19. **Geiger, T., T. Andus, J. Klapproth, H. Northoff, and P. C. Heinrich.** 1988. Induction of $\alpha_1$-acid glycoprotein by recombinant human interleukin-1 in rat hepatoma cells. J. Biol. Chem. **263**:7141–7146.
20. **Isom, H. C.** 1980. DNA synthesis in isolated hepatocytes infected with herpes viruses. Virology **103**:199–216.
21. **Koj, A.** 1974. Acute-phase reactants. Their synthesis, turnover and biological significance, p. 73–125. *In* A. C. Allison (ed.), Structure and function of plasma proteins, vol. 1. Plenum Publishing Corp., London.
22. **Koj, A.** 1986. Biological functions of acute-phase proteins and the cytokines involved in their induced synthesis, p. 331–342. *In* W. Reutter and H. Popper (ed.), Modulation of liver cell expression. Falk Symposium 43. MTP Press Ltd., Lancaster, England.
23. **Koj, A., J. Gauldie, E. Regoeczi, D. N. Sauder, and G. D. Sweeney.** 1984. The acute-phase response of cultured rat hepatocytes: system characterization and the effect of human cytokines. Biochem. J. **224**:505–514.
24. **Kushner, I.** 1982. The phenomenon of the acute-phase response. Ann. N.Y. Acad. Sci. **389**:39–48.
25. **Liao, W. S. L., L. S. Jefferson, and J. M. Taylor.** 1986. Changes in plasma albumin concentration, synthesis rate, and mRNA level during acute inflammation. Am. J. Physiol. **251**:C928–C934.
26. **Liao, W. S. L., and G. R. Stark.** 1985. Cloning of rat cDNAs for eight acute-phase reactants and kinetics of induction of mRNAs following acute inflammation. Inflammation Res. **10**:220–222.
27. **Lopez-Berestein, G., J. Reuben, E. M. Hersh, R. Kilbourn, J. P. Hester, M. Bielski, M. Talpaz, and G. M. Mavligit.** 1983. Comparative functional analysis of lymphocytes and monocytes from plateletapheresis. Transfusion **23**:201–206.
28. **MacIntyre, S. S., D. Schultz, and I. Kushner.** 1983. Synthesis and secretion of C-reactive protein by rabbit primary hepatocyte cultures. Biochem. J. **210**:707–715.
29. **Maizel, A. L., S. R. Mehta, S. Hauft, D. Franzini, L. B. Lachman, and R. J. Ford.** 1981. Human T lymphocyte/monocyte interaction in response to lectin: kinetics of entry into the S-phase. J. Immunol. **127**:1058–1064.
30. **McMaster, G. K., and G. G. Carmichael.** 1977. Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. Proc. Natl. Acad. Sci. USA **74**:4835–4838.
31. **Mehta, K., J. Turpin, and G. Lopez-Berestein.** 1987. Induction of tissue transglutaminase in human peripheral blood monocytes by intracellular delivery of retinoids. J. Leuk. Biol. **41**:341–348.
32. **Morrow, J. F., R. S. Stearman, C. G. Peltzman, and D. A. Potter.** 1981. Induction of hepatic synthesis of serum amyloid A protein and actin. Proc. Natl. Acad. Sci. USA **78**:4718–4722.
33. **Oliviero, S., G. Morrone, and R. Cortese.** 1987. The human haptoglobin gene: transcriptional regulation during development and acute-phase induction. EMBO J. **6**:1905–1912.
34. **Otto, J. M., H. E. Grenett, and G. M. Fuller.** 1987. The coordinated regulation of fibrinogen gene transcription by hepatocyte-stimulating factor and dexamethasone. J. Cell Biol. **105**: 1067–1072.
35. **Perlmutter, D. H., C. A. Dinarello, P. I. Punsal, and H. R. Colten.** 1986. Cachectin/tumor necrosis factor regulates hepatic acute-phase gene expression. J. Clin. Invest. **78**:1349–1354.
36. **Prowse, K. R., and H. Baumann.** 1988. Hepatocyte-stimulating factor, $\beta_2$ interferon, and interleukin-1 enhance expression of the rat $\alpha_1$-acid glycoprotein gene via a distal upstream regulatory region. Mol. Cell. Biol. **8**:42–51.
37. **Ramadori, G., J. D. Sipe, C. A. Dinarello, S. B. Mizel, and H. R. Colten.** 1985. Pretranslational modulation of acute-phase hepatic protein synthesis by murine recombinant interleukin 1 (IL-1) and purified human IL-1. J. Exp. Med. **162**:930–942.
38. **Ricca, G. A., R. W. Hamilton, J. W. McLean, A. Conn, J. E. Kalinyak, and J. M. Taylor.** 1981. Rat $\alpha_1$-acid glycoprotein mRNA: cloning of double-stranded cDNA and kinetics of induction of mRNA levels following acute inflammation. J. Biol. Chem. **256**:10362–10368.
39. **Rigby, P. W., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase. I. J. Mol. Biol. **113**:237–251.
40. **Ritchie, D. G., and G. M. Fuller.** 1981. An *in vitro* bioassay for leukocytic endogenous mediator(s) using cultured rat hepatocytes. Inflammation **5**:275–287.
41. **Rupp, R. G., and G. M. Fuller.** 1979. The effects of leukocytic and serum factors on fibrinogen biosynthesis in cultured hepatocytes. Exp. Cell. Res. **118**:23–30.
42. **Schreiber, G., G. Howlett, M. Nagashima, A. Millership, H. Martin, J. Urban, and L. Kotler.** 1982. The acute-phase response of plasma protein synthesis during experimental inflammation. J. Biol. Chem. **257**:10271–10277.
43. **Seed, B.** 1982. Diazotizable arylamine cellulose paper for the coupling and hybridization of nucleic acids. Nucleic Acids Res. **10**:1799–1810.
44. **Setzer, D. R., M. McGrogan, J. H. Nunberg, and R. T. Schimke.** 1980. Size heterogeneity in the 3′ end of dihydrofolate reductase messenger RNAs in mouse cells. Cell **22**:361–370.
45. **Wahl, G. M., R. A. Padgett, and G. R. Stark.** 1979. Gene amplification causes over production of the first three enzymes of UMP synthesis in *N*-(phosphonacetyl)-L-aspartate-resistant hamster cells. J. Biol. Chem. **254**:8679–8689.
46. **Woodworth, C. D., and H. C. Isom.** 1987. Regulation of albumin gene expression in a series of rat hepatocyte cell lines immortalized by simian virus 40 and maintained in chemically defined medium. Mol. Cell. Biol. **7**:3740–3748.
47. **Woodworth, C. D., J. W. Kreider, L. Mengel, T. Miller, V. Meng, and H. C. Isom.** 1988. Tumorigenicity of simian virus 40-hepatocyte cell lines: effect of in vitro and in vivo passage on expression of liver-specific genes and oncogenes. Mol. Cell. Biol. **8**:4492–4501.
48. **Woodworth, C. D., T. Secott, and H. C. Isom.** 1986. Transformation of rat hepatocytes by transfection with simian virus 40 DNA to yield proliferating differentiated cells. Cancer Res. **46**:4018–4026.