MOLECULAR PHARMACOLOGY, 14, 672-681

Effects of Polyriboinosinic acid·polyribocytidylic acid and a Mouse Interferon Preparation on Cytochrome P-450-Dependent Monooxygenase Systems in Cultures of Primary Mouse Hepatocytes

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(Received December 12, 1977)
(Accepted February 27, 1978)

SUMMARY

RENTON, KENNETH W., DELORIA, LAUREL B. & MANNERING, GILBERT J. (1978) Effects of polyriboinosinic acid·polyribocytidylic acid and a mouse interferon preparation on cytochrome P-450-dependent monooxygenase systems in cultures of primary mouse hepatocytes. *Mol. Pharmacol.*, 14, 672-681.

Previous studies from our laboratory have shown that a variety of interferon-inducing agents depress cytochrome P-450-dependent monooxygenase systems when administered to rats. With the expectation that the use of cultured hepatocytes would provide a more accessible means of studying the mechanism by which interferon-inducing agents depress these enzyme systems, measurements were made of the effects of the interferon inducer, poly rI·rC and a crude preparation of mouse interferon on the cytochrome P-450 content and aminopyrine *N*-demethylase and benzo[*a*]pyrene hydroxylase activities of primary, nonreplicating mouse hepatocytes maintained on floating collagen membranes. During the first 24 hr of culture, hepatocytes lost about 80% of their cytochrome P-450, 97% of their aminopyrine *N*-demethylase activity, and 90% of their benzo[*a*]pyrene hydroxylase activity. The specific activity of cytochrome P-450 (nanomoles of [^{14}C]formaldehyde formed from the *N*-demethylation of aminopyrine per nanomole of P-450 per minute) was lowered from 4.6 to as little as ⅒ this value. Exposure of the cultures to poly rI·rC (5 μg/ml of culture) during the second 24 hr of culture caused a 40% increase in the cytochrome P-450 content of the hepatocytes. The specific activity of cytochrome P-450 *de novo* relative to the *N*-demethylation of aminopyrine was restored to that of the cytochrome P-450 of freshly isolated hepatocytes or the cytochrome P-450 of microsomes isolated from liver homogenates. The mouse interferon preparation (1000 units/ml of culture medium) was considerably less potent as an inducer of cytochrome P-450 in cultured hepatocytes, but the specific activity of the cytochrome P-450 *de novo* induced by mouse interferon was as high as that of the cytochrome P-450 induced by poly rI·rC. Relative to benzo[*a*]pyrene hydroxylase activity, the specific activity of the cytochrome P-450 that survived the first 24 hr of culture was about the same as that for the cytochrome P-450 of microsomes isolated from liver homogenates. Both poly rI·rC and mouse interferon preparation induced the hydroxylase activity in 24-hr-old cultures of hepatocytes. Poly rI·rC did not induce the hydroxylase activity in cultured Reuber hepatoma cells. Poly rI·rC was shown to induce interferon activity in cultured hepatocytes. Methods are described for the determination of aminopyrine *N*-demethylase and

This work was supported by Grant GM 15477 from the National Institutes of Health.

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0026-895X/78/0144-0672$02.00/0

benzo[*a*]pyrene hydroxylase activities and cytochrome P-450, cytochrome b_5, and DNA contents of cultured hepatocytes from one mouse.

INTRODUCTION

In previous publications from this laboratory (1, 2) we showed that many interferon-inducing agents depress hepatic cytochrome P-450-dependent monooxygenase systems when administered to rats. Because postulated mechanisms of interferon induction and antiviral activity of interferon have been derived largely from studies of cell cultures, it seemed likely that mechanisms by which interferon-inducing agents depress hepatic monooxygenase systems might best be revealed by studying their effects on cultured hepatocytes. Studies on monooxygenase systems of freshly isolated rat hepatocytes have been published (3–5), but only recently has it been possible to maintain isolated hepatocytes for the time deemed necessary to demonstrate the effect that interferon or an interferon inducer might exert on the monooxygenase systems of these cells. Michalopoulos and Pitot (6, 7) have recently shown that nonreplicating rat hepatocytes can be maintained on floating collagen membranes for as long as 3 weeks. The viability of these cells, as determined by the measurement of several biochemical functions, was similar to that of uncultured hepatocytes. However, as had been experienced by others (5, 6) who had used hepatocytes suspended or attached to plastic, severe loss of cytochrome P-450 occurred during the first 24 hr after the cells were isolated (7). Michalopoulos and associates (8) showed that, despite this loss, monooxygenase systems in these cells could be induced by phenobarbital or 3-methylcholanthrene in a manner similar to that seen *in vivo*.

In the present study, measurements were made of the effects of the interferon inducer poly rI·rC[2] and a crude preparation of mouse interferon on the cytochrome P-450 content and monooxygenase activity of primary, nonreplicating mouse hepatocytes maintained on floating collagen membranes.

[2] The abbreviations used are: poly rI·rC, polyribonosinic acid·polyribocytidylic acid; TCA, trichloroacetic acid.

MATERIALS AND METHODS

Isolation of mouse hepatocytes. Mouse rather than rat hepatocytes were used because, with some exceptions, interferon is species-specific and because a mouse interferon preparation was commercially available and a rat preparation was not. One of the usual methods for preparing rat hepatocytes (8) was modified to accommodate the small liver of the mouse. Per gram of liver, yields of viable hepatocytes were about 3 times greater than those obtained with rat livers using the same modified procedure, and even greater when the customary procedure (8) was used with rat livers. Male, randomly bred Swiss-Webster mice (25–35 g) were anesthetized with sodium pentobarbital (100 mg/kg, intraperitoneally), the abdomen was opened, the intestines were displaced to the left, and the portal vein was exposed. The chest was opened, and the inferior vena cava was cannulated in retrograde fashion via an incision in the right atrium with a 20-gauge plastic cannula connected to a variable-speed pump that was delivering Seglen's calcium-free perfusion medium (9) at a rate of 10 ml/min before the cannula was inserted. The hepatic portal vein was cut to allow the perfusion fluid to escape. In mice of this size, the 20-gauge cannula fits tightly enough that a ligature is not required. After 2 min, Seglen's perfusion medium was switched to Seglen's perfusion medium containing 25 mg of collagenase per 50 ml, and the perfusion was continued for another 5 min at the same rate. The perfusion was switched back to Seglen's medium for 1 min to flush collagenase from the tissue. The perfusion cannula was disconnected, and the liver was excised and transferred to a 100-mm culture dish containing perfusion medium. The liver was broken apart gently with forceps and raked several times. As the cells were released, the fluid was transferred to a beaker and fresh medium was

added to the tissue. Three to four changes (total volume, about 100 ml) gave the best yield of cells. The pooled fluid was swirled gently and transferred to centrifuge tubes. The cells were sedimented by centrifugation at 50 × *g* and suspended in 25 ml of L-15 medium (6). Cells were counted in a hemocytometer; viability was determined by the uptake of trypan blue. Cells were diluted to the desired concentration with L-15 medium. The typical yield of cells from a 30-g mouse (liver weight, 1.6 g) was 100×10^6; the viability was about 95%. The typical yield from a liver (12 g) of a 200-g rat, perfused in the same way, was 250×10^6; the viability was about 90%.

The isolation of cells was performed at room temperature under aseptic conditions, using sterilized equipment and solutions.

Culture of mouse hepatocytes. Isolated hepatocytes were suspended in L-15 medium (1×10^6 cells/ml) containing 1 g of bovine albumin, 50 mg of insulin, 100 ml of fetal calf serum, 200 μg of penicillin, and 200 μg of streptomycin per liter (6). Three milliliters of the cell suspension were layered gently over a rat tail collagen membrane (6) attached to 15 × 60 mm plates and placed immediately in an incubator at 37°. The cells were allowed to remain on the membrane for 4 hr, after which the medium was removed along with unattached cells, most of which were nonviable. Fresh medium (2.5 ml) was added, and the collagen membrane was detached from the plate using a transfer pipette. Medium was exchanged at 24-hr intervals throughout the duration of the experiment. Better than 70% of the mouse cells became attached to the membrane. When rats were used, only about 50% of hepatocytes were attached to the membrane.

The time between the anesthetization of the mouse and the plating of the hepatocytes was 30–40 min.

Preparation of hepatocyte cultures for assays. The membranes with attached hepatocytes from two or three culture plates were transferred to a test tube, and 0.4 ml of 1 M phosphate buffer and 0.1 ml of collagenase solution (5.0 mg/ml) were added. Then 0.9% NaCl was added to give a volume of 4.0 ml. The mixture was homogenized with 10 strokes of a tightly fitting pestle in an all-glass Dounce homogenizer and incubated at 37° for 5 min. Aliquots of the homogenate were used for cytochrome P-450, cytochrome b_5, [*methyl*-^{14}C]aminopyrine *N*-demethylase, benzo[*a*]pyrene hydroxylase, and DNA assays.

Assays. [*methyl*-^{14}C]Aminopyrine *N*-demethylase and benzo[*a*]pyrene hydroxylase activities were assayed by the methods of Poland and Nebert (10) and Wattenberg and Leong (11), respectively, using 0.5 ml of the homogenate of the cultured hepatocytes in each assay. Cytochromes b_5, P-450, and P-420 were assayed by the methods of Omura and Sato (12), using undiluted homogenate in 1.0-ml cuvettes. After the cuvettes had been spectrally balanced in an Aminco DW-2 spectrophotometer, a few crystals of NADH were added to the sample cuvette and the reduced cytochrome b_5 difference spectrum was recorded. A few crystals of dithionite were added to both cuvettes, the contents of the sample cuvette were saturated with carbon monoxide, and the reduced cytochrome P-450·CO spectrum was recorded. The cytochrome P-420 content of the hepatocytes was calculated by a method that corrects for the negative contribution of the cytochrome P-450 spectrum to the cytochrome P-420 spectrum (13). The contents of these cuvettes were prepared for the DNA assay by the method of Bonney *et al.* (14): 0.5 ml of the contents was mixed with 0.5 ml of 1 N NaOH and 1.0 ml of a TCA solution (15% TCA in 2 N HCl) and centrifuged at 500 × *g*. The pellet was dissolved in 1.0 ml of 5% TCA solution and heated in a bath for 20 min at 90°. The DNA was quantified by the method of Dische (15).

The decision to use whole homogenates for assays of cytochrome P-450 and monooxygenase activities of hepatocytes was made after examining the distribution of cytochrome P-450 in subcellular fractions (Table 1). The microsomal fraction (100,000 × *g* pellet) contained only 19% of the cytochrome P-450 found in the whole homogenate. Seventy per cent of the cytochrome P-450 sedimented with the 10,000 × *g* pel-

let. The sedimentation of cytochrome P-450 in cultured hepatocytes from adult mice resembles that seen with homogenates of fetal human (16) and fetal rat (17) livers.

Interferon assays. Medium decanted from hepatocyte culture plates (about 10 ml) was dialyzed against 2400 ml of 0.01 M HCl–0.1 MNaCl solution, pH 2.0, at 4° for 4 days. The solution was replaced with 2400 ml of 0.1 M phosphate buffer in 0.9% NaCl and dialyzed for 12 hr; the buffer was replaced with another 2400 ml of phosphate buffer, and the dialysis was repeated for 12 hr. The dialyzed medium was assayed for its interferon activity by the plaque reduction method (18, 19), using L-929 cells challenged with vesicular stomatitis virus and NIH reference mouse interferon as the standard. A unit of interferon is the amount of interferon that causes 50% plaque reduction.

Materials. Mouse L-929 cells and rat Reuber hepatoma cells (H-4-IIE) were gifts from Dr. M. Darnell, 3M Company, and Dr. D. Nebert, National Institute of Child Health and Human Development, respectively. The following materials were obtained from the specified sources: L-15 and MEM-E media, Associated Biomedic Systems, Buffalo, N. Y.; fetal calf serum (heat-inactivated), Grand Island Biological Company; insulin, collagenase, DNA, poly rI·rC, benzo[*a*]pyrene, $NADP^+$, NADH, and NADPH, Sigma Chemical Company; aminopyrine, Aldrich Chemical Company; [*methyl*-^{14}C]aminopyrine, Amersham; and mouse interferon standard, National Institute of Allergy and Infectious Diseases.

The mouse interferon preparation was made by Bionetics Laboratory Products by the method of Oie *et al.* (20), which uses C-243-3 mouse cells infected with Newcastle disease virus. The virus is inactivated by dialyzing the preparation for 5 days at pH 2. We assayed the preparation for its interferon activity and found it to contain 200,000 and 175,000 units of interferon per milliliter in two separate assays.

TABLE 1

Distribution of cytochrome P-450 in subcellular fractions of freshly isolated mouse hepatocytes

Data are representative of the results obtained with a typical hepatocyte preparation. Each value is the mean of two determinations on the same hepatocyte preparation.

Subcellular fraction	Cytochrome P-450
	nmole/10^6 cells
Whole homogenate	0.69
10,000 × *g* pellet	0.49
10,000 × *g* supernatant	0.15
100,000 × *g* pellet (microsomes)	0.13
100,000 × *g* supernatant	0.00

RESULTS

Loss of cytochrome P-450 and monooxygenase activity in cultured hepatocytes. Twenty-four hours after isolation, cultured hepatocytes had lost about 80% of their cytochrome P-450, 90% of their benzo[*a*]pyrene hydroxylase activity, and 97% of their aminopyrine *N*-demethylase activity (Fig. 1). After 72 hr, 92% of cytochrome P-450 was lost, only 3% of the benzo[*a*]pyrene activity remained, and aminopyrine *N*-de-

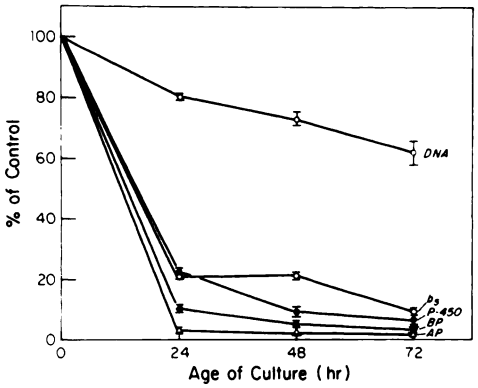

FIG. 1. *Losses of cytochrome P-450 and monooxygenase activity in cultured mouse hepatocytes*

Data are expressed as percentages of values obtained with isolated mouse hepatocytes immediately before culture. One hundred per cent values (per culture): DNA, 385 μg; cytochrome P-450, 1.84 nmoles; cytochrome b_5, 1.12 nmoles; benzo[*a*]pyrene (BP) hydroxylase activity, 0.46 nmol of 3-hydroxybenzo[*a*]pyrene formed per minute; [*methyl*-^{14}C]aminopyrine (AP) *N*-demethylase activity, 8.5 nmoles/min. These experiments were repeated 10–12 times, using hepatocytes from different mice for one or the other monooxygenase reaction (cytochromes P-450, P-420, and b_5 and DNA were always measured). The data were obtained from the same batch of hepatocytes. Each point is the mean ± standard of values obtained from individual observations on four cultures.

methylase activity was negligible. A decline in DNA content of about 20% occurred during the first 24 hr of culture; another 20% disappeared during the next 48 hr. Loss of cytochrome b_5 paralleled that of cytochrome P-450.

The loss of cytochrome P-450 approximated that observed by Michalopoulos, Sattler, and Pitot (7) in cultured rat hepatocytes. In their study, cytochrome P-450 levels fell from 4.5 pmoles/μg of DNA at zero time to 1.1 at 24 hr and to 0.5 at 72 hr; corresponding values derived from Fig. 1 are 4.8, 1.5, and 1.0.

The temporal relationship between the disappearance of cytochrome P-450 and the appearance of cytochrome P-420 is shown in Fig. 2. At 24 hr, cytochrome P-450 levels had dropped to 27% of the initial value, but the amount of cytochrome P-420 formed was only about one-third of the loss of cytochrome P-450. This suggests either that not all the cytochrome P-450 is converted to cytochrome P-420 when it disappears, or, if this is not the case, that a large amount of cytochrome P-420 as well as cytochrome P-450 is lost during the first 24 hr of culture. On the other hand, between 24 and 48 hr of culture, the cytochrome P-420 level increased more rapidly than the cytochrome P-450 level declined. This may suggest that cytochrome P-450 was being synthesized *de novo* and converted to cytochrome P-420 during the interval between 24 and 72 hr. However, it would seem more likely that a cytochrome P-420 spectrum was being formed by the combination of albumin or other proteins with the heme released from cytochrome b_5 (21), which declined in the hepatocytes at about the same rate as cytochrome P-450 (Fig. 1).

Induction of cytochrome P-450 and monooxygenase activity in cultured hepatocytes with poly rI·rC and mouse interferon preparation. Figure 3 shows the inductive effect of several concentrations of poly rI·rC on the cytochrome P-450 content of cultured hepatocytes. A maximum inductive effect of about 40% was reached with 5 μg of poly rI·rC per milliliter.

Table 2 summarizes the effects of poly rI·rC and mouse interferon preparation on components and activities of monooxygenase systems of cultured mouse hepatocytes. Levels of cytochromes P-450 and b_5 were both increased by about 45% by poly rI·rC; aminopyrine *N*-demethylase and benzo[*a*]pyrene hydroxylase activities were increased by 544% and 67%, respectively. Mouse interferon preparation increased the cytochrome P-450 level by 25%, and demethylase activity by 65%. Neither poly rI·rC nor mouse interferon preparation had

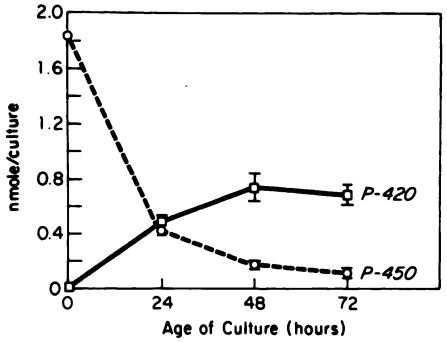

FIG. 2. *Formation of cytochrome P-420 in cultured mouse hepatocytes*

Data were obtained from the same hepatocyte cultures used in Fig. 1. Each point is the mean ± standard error of values obtained from four cultures.

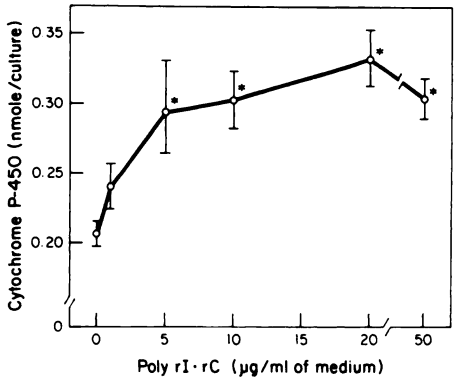

FIG. 3. *Induction of cytochrome P-450 in cultured mouse hepatocytes with poly rI·rC*

Twenty-four-hour cultures were exposed to the indicated amounts of poly rI·rC and examined 24 hr later for their content of cytochrome P-450. Each point and bar represent the mean ± standard error of values obtained from three cultures; all cultures were obtained from the same mouse. The cytochrome P-450 content of the cells before culture was 1.58 nmoles/culture.

* Significantly different from the corresponding control ($p < 0.05$). The stimulatory effect of poly rI·rC (20 μg/ml) was demonstrated seven times in other experiments.

TABLE 2

Induction of cytochromes P-450, P-420, and b_5 and of aminopyrine N-demethylase and benzo[a]pyrene hydroxylase activities in cultured mouse hepatocytes with poly rI·rC and mouse interferon preparation

Hepatocytes from one mouse were used for the poly rI·rC experiment, and hepatocytes from another mouse, for the mouse interferon experiment. Twenty-four-hour cultures were exposed to poly rI·rC (20 μg/ml of medium) or to mouse interferon preparation (1000 units/ml of medium) and examined 24 hr later for their cytochrome contents and monooxygenase activities. The data are means ± standard errors per culture obtained from four cultures.

	Control	Poly rI·rC	Control	Interferon
DNA (μg)	143 ± 26	146 ± 27	220 ± 26	206 ± 16
P-450 (nmole)	0.17 ± 0.02	0.24 ± 0.03[a]	0.19 ± 0.01	0.24 ± 0.02[a]
P-420 (nmole)	0.55 ± 0.06	0.59 ± 0.09	0.49 ± 0.05	0.50 ± 0.03
b_5 (nmole)	0.14 ± 0.02	0.20 ± 0.02[a]		
Aminopyrine *N*-demethylation (nmoles HCHO formed/min)	0.079 ± 0.04	0.430 ± 0.05[a]	0.363 ± 0.05	0.610 ± 0.03[a]
Benzo[a]pyrene hydroxylation (nmoles 3-OH-BP formed/min)[b]	0.077 ± 0.001	0.115 ± 0.014[a]		

[a] Significantly different from the corresponding control ($p < 0.05$). Similar results were obtained when the experiment was repeated.

[b] Although it is recognized that several hydroxylation products of benzo[a]pyrene are produced, results are based on fluorescent units standardized with 3-hydroxybenzo[a]pyrene (3-OH-BP).

a significant effect on the DNA or cytochrome P-420 content of the hepatocytes. The wavelength of maximal absorbance (450 nm) of reduced cytochrome P-450–carbon monoxide complex was not changed by these agents. Although the cytochrome P-450 contents of the two control cultures were very similar (0.17 and 0.19 nmole), aminopyrine *N*-demethylase activities were very different (0.079 vs. 0.363). While variations of this order among different batches of hepatocytes were not uncommon, little variation was seen among cultures from the same batch of hepatocytes.

The specific activity of the cytochrome P-450 of freshly isolated, uncultured cells with respect to aminopyrine *N*-demethylase activity was the same as that of the cytochrome P-450 in microsomes prepared from mouse liver homogenates (Table 3). The specific activity of 48-hr control cultures was only about 40% of this value in one experiment, and about 10% in a second experiment. In both experiments the calculated cytochrome P-450 induced *de novo* by poly rI·rC or mouse interferon preparation in 48-hr cultures was equal to that observed in microsomes or in freshly isolated, uncultured hepatocytes.

The specific activity of the cytochrome P-450 of freshly isolated, uncultured hepatocytes with respect to benzo[a]pyrene hydroxylase activity was only half that observed in microsomes prepared from mouse liver homogenates (Table 3). However, the specific activity of the cytochrome P-450 in 48-hr control cultures returned to that observed in microsomes. The specific activity of the cytochrome P-450 of hepatocyte cultures that had been exposed to poly rI·rC was about the same as that of the control culture. The low specific activity of the cytochrome P-450 of freshly isolated uncultured hepatocytes, compared with that seen in microsomes, is not readily explained. However, comparable results were obtained with hepatocytes isolated from a mouse treated with 3-methylcholanthrene (20 mg/kg/day for 4 days). The specific activity of the cytochrome P-450 of 48-hr cultures of cells obtained from this animal was 4.16, whereas that of the freshly isolated hepatocytes from the same mouse was only 2.26.

Effects of repeated exposure of cultured hepatocytes to poly rI·rC or mouse interferon preparation on cytochrome P-450 levels. Three established features of the antiviral action of interferon-inducing agents are that (a) the effect is short-lived, (b) the cells become refractive to interferon for several days after the initial antiviral response, and (c) the cells remain refractive if they are continuously exposed to the agent (22). An experiment was designed to determine whether cultured hepatocytes would also become refractive to the induc-

TABLE 3

Specific activity of cytochrome P-450 relative to aminopyrine N-demethylase and benzo[a]pyrene hydroxylase activities in cultured hepatocytes induced with poly rI·rC or mouse interferon preparation

Conditions	Specific activity[a]	
	Aminopyrine	Benzo[a]pyrene
Experiment 1		
Freshly isolated, uncultured hepatocytes	4.6[b]	0.25[b]
48-hr cultured hepatocytes (CH)	0.48[b]	0.47[b]
48-hr CH + poly rI·rC (20 μg/ml)	4.7[c]	0.55[c]
Experiment 2		
Freshly isolated, uncultured hepatocytes	4.6[b]	
48-hr cultured hepatocytes (CH)	1.9[b]	
48-hr CH + interferon preparation (1000 units/ml)	4.9[c]	
Hepatic microsomes	4.8 ± 0.2[d]	0.58 ± 0.04[d]

[a] Calculated from data presented in Table 2, except for data obtained with hepatic microsomes.

[b] Specific activity of noninduced cytochrome P-450 = (nmoles aminopyrine demethylated or benzo[a]pyrene hydroxylated/min/culture) + (nmoles P-450/culture).

[c] Specific activity of induced P-450 = (nmoles aminopyrine demethylated or benzo[a]pyrene hydroxylated/min/induced culture) − (nmoles aminopyrine demethylated or benzo[a]pyrene hydroxylated/min/noninduced culture) + (nmoles P-450/induced culture) − (nmoles of P-450/noninduced culture).

[d] Specific activity of microsomal cytochrome P-450 = nmoles aminopyrine demethylated or benzo[a]pyrene hydroxylated/mole P-450. Values are means ± standard errors of results obtained from four mice. These were not the same mice from which the hepatocytes were obtained.

tive effect of poly rI·rC or mouse interferon preparation on cytochrome P-450 levels of cultured hepatocytes. Twenty-four-hour cultures of hepatocytes were exposed to poly rI·rC or mouse interferon preparation for 1 day or repetitively for 5 days with daily exchange of the medium. Increases in cytochrome P-450 levels of 50% or 30%, respectively, were seen in the cultures that had been exposed once to poly rI·rC or mouse interferon preparation (Table 4). After 5 days of continuous exposure to these agents, levels of cytochrome P-450 returned to control values. While these results indicate that the hepatocytes had become refractive to the effects of interferon, the possibility existed that the ability of hepatocytes to respond to interferon had been lost when they were cultured for more than 48 hr. This was shown not to be the case by including a group of hepatocytes in the study which were exposed to mouse interferon on the fourth and fifth days of culture rather than on the second day. It can be seen in Table 4 that these hepatocytes were still capable of responding to the inductive effect of the mouse interferon preparation on cytochrome P-450.

Induction of interferon in cultured hepatocytes with poly rI·rC. If the induction of interferon is to be considered a possible mechanism for the induction of monooxygenase systems in cultured hepatocytes, it would seem necessary to demonstrate that cultured hepatocytes are capable of producing interferon in response to poly rI·rC. Medium from cultured hepatocytes exposed to poly rI·rC for 24 hr was shown to contain 103 units of interferon activity per milliliter. When poly rI·rC was omitted, the medium contained 30 units of interferon activity per milliliter. Medium incubated with poly rI·rC in the absence of hepatocytes contained no interferon activity.

Absence of inductive effect of poly rI·rC on benzo[a]pyrene hydroxylase activity of Reuber hepatoma cells. Reuber hepatoma cells (H-4-II-E) were studied in an attempt to find a cytochrome P-450-containing cell that could be cultured more readily than the primary hepatocyte and still respond to the inductive effect of poly rI·rC on monooxygenase systems. The cells were cultured in MEM-E medium plus 10% fetal calf serum for 24 hr with and without the addition of poly rI·rC (20 μg/ml). The cells exposed to poly rI·rC and the unexposed cells possessed benzo[a]pyrene activities of 1.16 ±

TABLE 4

Effect of repeated exposure of cultured hepatocytes to poly rI·rC or mouse interferon preparation on cytochrome P-450 levels

All values were obtained using hepatocytes from one mouse. After 24 hr in culture, cells were exposed to poly rI·rC (20 μg/ml of medium) or mouse interferon preparation (1000 units/ml of medium) for 1 or 5 days with daily exchange of the medium. Assays were performed 24 hr after the last addition of the agent. In one case, cultures were not exposed to mouse interferon until the fifth day of culture; these hepatocytes were examined for their cytochrome P-450 content after exposure to mouse interferon (1000 units/ml of medium) for 2 days. The data are means ± standard errors from four cultures, except as noted.

Agent	Day of culture		P-450
	Addition of agent	P-450 assay	
			nmole/culture
None	2	3	0.41 ± 0.03
Poly rI·rC	2	3	0.61 ± 0.03[a]
None	2, 3, 4, 5, 6	7	0.18 ± 0.03
Poly rI·rC	2, 3, 4, 5, 6	7	0.17[b]
None	2	3	0.41 ± 0.03
Interferon preparation	2	3	0.53 ± 0.03[a]
None	2, 3, 4, 5, 6	7	0.18 ± 0.03
Interferon preparation	2, 3, 4, 5, 6	7	0.23 ± 0.03
Interferon preparation	5, 6	7	0.34 ± 0.04[a]

[a] Significantly different from the corresponding control ($p < 0.05$).

[b] Average of data from two cultures.

0.24 and 1.11 ± 0.11 nmoles of hydroxylated benzo[*a*]pyrene formed per 10^6 cells/hr, respectively.

DISCUSSION

When cultured on floating collagen membranes, hepatocytes lost about 80% of their cytochrome P-450 after 24 hr, and 90% after 48 hr (Fig. 1). The interferon inducer poly rI·rC restored the amount of cytochrome P-450 lost between 24 and 48 hr (Fig. 3). The loss of cytochrome P-450-dependent monooxygenase (aminopyrine *N*-demethylase) activity was even more drastic than that of cytochrome P-450. Thus the specific activity of the surviving cytochrome P-450 was only 10–40% of that of freshly isolated hepatocytes (Table 3). Not all hepatic cytochrome P-450 is involved in monooxygenase reactions (23, 24). While the surviving cytochrome P-450 in cultured cells may represent inactivated hemoprotein, the possibility should also be considered that it may represent much of the cytochrome P-450 that does not normally participate in monooxygenase reactions. In either event, a comparison of the specific activities of cytochrome P-450 relative to aminopyrine *N*-demethylase and benzo[*a*]pyrene hydroxylase activities shows that a selective loss of monooxygenase activity occurred; i.e., maintenance of hepatocytes in culture greatly reduced the specific activity of cytochrome P-450 relative to aminopyrine *N*-demethylase activity, but the specific activity relative to benzo[*a*]pyrene hydroxylation was as great as, if not greater than, that observed with freshly isolated hepatocytes (Table 3). Guzelian and associates (25) have also observed selective losses of monooxygenase activity relative to loss of cytochrome P-450 in cultured rat hepatocytes; e.g., losses of aminopyrine *N*-demethylase and aniline hydroxylase activities paralleled the loss of cytochrome P-450, but there was little decline in *p*-nitroanisole *O*-demethylase activity.

Poly rI·rC induced partial recovery of the cytochrome P-450 lost from hepatocytes during the first 24 hr of culture (Fig. 3). The mouse interferon preparation was less effective than poly rI·rC as an inducer of cytochrome P-450, possibly because much of it may be destroyed by the cells (26). The cytochrome P-450 induced *de novo* by either poly rI·rC or the interferon

preparation possessed a specific activity relative to aminopyrine *N*-demethylation equal to that of the cytochrome P-450 of hepatic microsomes or freshly isolated, uncultured hepatocytes. This induction of cytochrome P-450 and monooxygenase activity by poly rI·rC or mouse interferon could occur in several ways: (a) by reactivating arrested synthesis of cytochrome P-450, (b) by slowing the rate of breakdown of cytochrome P-450 *de novo*, or (c) by increasing the rate of synthesis of cytochrome P-450. The current studies do not reveal which mechanism or combination of mechanisms is functioning.

The observation that poly rI·rC induces cytochrome P-450-dependent monooxygenase systems in hepatocytes was unexpected in view of our earlier observation that interferon-inducing agents depress these sytems in intact rats and mice (1, 2). However, paradoxical results have been described previously for the activities of some other agents on cytochrome P-450 systems. For example, biogenic amines depress cytochrome P-450 systems *in vivo*, but induce aryl hydrocarbon hydroxylase activity markedly in cultures of fetal rat liver cells (27). Dibutyryl cyclic AMP decreases cytochrome P-450-dependent monooxygenase systems in intact animals, perfused livers, and liver slices (28–30), but induces aryl hydrocarbon hydroxylase activity in a variety of cultured mammalian cells (31, 32). Paradoxical results have also been described for the activities of interferon on a variety of biological systems (33). For example, interferon inhibits the division of both normal cells and tumor cells *in vivo* and *in vitro* (34, 35), the regeneration of mouse liver after partial hepatectomy (36), primary antibody response (37, 38), and induced DNA synthesis in mouse lymphocytes (39), but enhances the phagocytic capacity of macrophages (40), the expression of histocompatibility antigens of lymphoid cells in mice (41), and the toxic effect of sensitized lymphocytes to target cells (42). Depending on the amount used, interferon may decrease or increase interferon production on subsequent exposure of cells to an interferon inducer (23); low doses of interferon stimulate and high doses inhibit the number of spleen antibody-forming cells (37). Numerous examples of inhibition of viral oncogenesis by exogenous interferon have been cited (33), but mouse serum interferon inoculated locally 1 day prior to injection of murine sarcoma virus accelerates the appearance of tumors (43). Interferon induces aryl hydrocarbon hydroxylase in cultured primary hepatocytes (Table 2), but not in fetal mouse secondary cultures (44). However, the induction of aryl hydrocarbon hydroxylase by benz[*a*]anthracene is enhanced by interferon in cultured fetal cells (44). These diverse and sometimes opposite activities of interferon suggest that any given effect may represent the sum of the effects of a variety of processes influenced by interferon, in which case the observed effect might be expected to vary from one biological system to another.

The inductive effect of poly rI·rC on hepatic monooxygenase systems need not necessarily be due to the induction of interferon. Components of the process that leads to the induction of interferon might also be involved in the process that leads to the induction of cytochrome P-450-dependent monooxygenase systems in cultured hepatocytes without the involvement of interferon. The mouse interferon preparation used in this study was not highly purified, and the possibility exists that an impurity induced the monooxygenase systems in hepatocytes rather than interferon. An answer to this question awaits the time when a homogeneous preparation of mouse interferon becomes available.

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