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EFFECTS OF THE INTERFERON INDUCING AGENTS TILORONE AND POLYRIBOINOSINIC ACID · POLYRIBOCYTIDYLIC ACID (POLY IC) ON THE HEPATIC MONOOXYGENASE SYSTEMS OF PREGNANT AND FETAL RATS*

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Abstract—Interferon inducing agents, including tilorone and polyriboinosinic acid · polyribocytidylic acid (poly IC), are known to depress hepatic cytochrome P-450-dependent monooxygenase systems and the induction of these systems by phenobarbital (PB) and 3-methylcholanthrene (MC) in mature male rats. The current study investigated the effects of tilorone and poly IC on the cytochrome P-450 systems of non-induced, PB-induced, MC-induced and pregnenolonecarbonitrile (PCN)-induced pregnant rats and their fetuses. Pregnant rats received either tilorone or poly IC and saline, PB, MC or PCN, and microsomes from their livers and those of their fetuses were examined for cytochrome P-450 content, aminopyrine (AP) *N*-demethylase activity and benzo[*a*]pyrene (BP) hydroxylase activity. The generalization can be made from these studies that, when the interferon inducing agents caused changes in cytochrome P-450 content or monooxygenase activities of either induced (PB, MC or PCN) or non-induced (saline) animals, decreases were seen in maternal livers and increases in fetal livers. Thus, in maternal livers tilorone depressed cytochrome P-450 and AP *N*-demethylase activity in non-induced and PB-, MC- and PCN-induced rats and BP hydroxylase activity in the induced animals; BP hydroxylase activity was not depressed in non-induced maternal livers. Poly IC depressed cytochrome P-450 and AP *N*-demethylase activity in non-induced and PB-induced rats but not in PCN-induced animals. BP hydroxylase was depressed by poly IC in both PB- and PCN-induced animals. Fetal hepatic cytochrome P-450 and monooxygenase activities were increased by tilorone in PB- and PCN-induced rats but not in non-induced or MC-induced animals. Poly IC increased cytochrome P-450 and both monooxygenase activities in PB- and PCN-induced fetal livers, whereas only BP hydroxylase activity was increased in the fetuses of non-induced rats. Several possible explanations are offered for the opposite effects produced by interferon inducing agents in maternal and fetal livers. Unlike maternally administered tilorone, which induced fetal cytochrome P-450 and monooxygenase activities in the liver, intrauterine tilorone depressed cytochrome P-450 and had no effect on AP *N*-demethylase or BP hydroxylase activities in the fetal liver. Intrauterine poly IC was without effect on the cytochrome P-450 systems of the fetal liver. Treatment of pregnant rats with tilorone on days 17–20 of gestation inhibited normal maternal weight gain and produced overt signs of toxicity. A dose of 10 mg/kg of poly IC was very toxic in pregnant rats but produced no overt signs of toxicity in virgin female rats. Time courses of the depressant effects of a single injection of poly IC were observed in mid-term pregnant, late-term pregnant, lactating and adult virgin females. Maximum losses of cytochrome P-450 and ethylmorphine (EM) *N*-demethylase activity were seen 48 hr after poly IC administration to pregnant and virgin rats, and recoveries were complete within 96 hr. Similar results were observed in lactating rats except that the nadir occurred at 24 rather than at 48 hr. The response of BP hydroxylase activity to poly IC was qualitatively similar except that this activity was not depressed in the mid-term pregnant rats.

The cytochrome P-450-dependent monooxygenase systems of rats are almost nonexistent in the fetus until the end of gestation, at which time low levels of activity are detectable [1–6]. After parturition, these systems develop rapidly and are highly inducible by phenobarbital (PB)§ and 3-methylcholanthrene (MC) [3, 4]. The administration of MC induces cytochrome P-450 systems in the fetus but PB does not [4, 6–12], even though PB achieves significant concentrations in fetal livers [4]. One hypothesis that has been offered to explain the inability of the fetal hepatocyte to respond to induction by PB-type agents is that an unidentified repressor of enzyme synthesis in the fetus prevents the inducer

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§ Abbreviations: PB, phenobarbital: MC, 3-methylcholanthrene; PCN, pregnenolonecarbonitrile; poly IC, polyriboinosinic acid · polyribocytidylic acid; AP, aminopyrine; BP, benzo[*a*]pyrene; and EM, ethylmorphine.

from exerting its effect in the nucleus [13–15]. Direct evidence for the existence of this hypothetical repressor was offered by Klinger *et al.* [16], who found that the induction of hexobarbital metabolism in weanling rats by PB was inhibited by a supernatant fraction of fetal rat liver. Drug metabolism is depressed in maternal livers during pregnancy [17–19]. Maternal cytochrome P-450 systems can be induced with PB, but not to the extent seen in non-pregnant female rats [20]. On the other hand, induction in pregnant rats by MC is not depressed [20]. Because both the patterns of suppression of drug metabolism and the selective repression of induction of drug metabolism are similar in maternal and fetal livers, Guenther and Mannering [20] suggested that the same repressor substance is involved in both the maternal and the fetal livers. Because the repressor effect on the maternal liver is seen within 3 days of conception, they proposed that the repressor substance is of maternal rather than of fetal origin and that it communicates freely between fetal and maternal circulations.

Renton and Mannering [21] observed that a wide variety of chemically unrelated interferon inducing agents markedly depress hepatic cytochrome P-450 systems of mature rats and mice (see Ref. 22 for review). The induction of cytochrome P-450 systems by PB or MC was also shown to be depressed in mature rats by interferon inducing agents [23]. The current study deals with the effects of the interferon inducing agents tilorone and polyriboinosinic acid · polyribocytidylic acid (poly IC) on the hepatic cytochrome P-450 systems of fetal, pregnant, lactating and virgin female rats.

MATERIALS AND METHODS

Animals and injection schedules. Pregnant Sprague–Dawley strain rats, 8–12 weeks of age, were received from the Biolab Co., St. Paul, MN, 12–14 days after being bred. Animals were bred overnight; the following day was considered day 1 of pregnancy. Pregnant rats received sodium PB in saline (40 mg/kg/day, i.p.), MC in corn oil (20 mg/kg/day, i.p.), or pregnenolonecarbonitrile (PCN) (20 mg/kg/day, i.p.) in Tween 80 solution (0.15 ml Tween 80/ml water) on days 17–20 of gestation. Control rats received 2.0 ml/kg of saline (0.9%, w/v, i.p.) on days 17–20 of gestation. Some of these animals received tilorone in saline (50 mg/kg/day, p.o.) on days 17–20 of gestation or poly IC in saline (10 mg/kg, i.p.) on day 20 of gestation. Rats were killed 24 hr after the last injection. Virgin female (200–225 g), lactating (2–7 days postpartum), mid-term pregnant rats (killed on day 14 of gestation) or late-term pregnant rats (killed on day 21 of gestation) received single doses of saline (1 ml/kg, i.p.) or poly IC (10 mg/kg, i.p.). These animals were killed 24, 48, 72 or 96 hr after the final injections.

Intrauterine injections of tilorone or poly IC were performed as described by Mendelson *et al.* [24]. The peritoneal cavity of 19- or 20-day pregnant rats was opened under ether anesthesia, and both uterine horns were exposed. A 27 gauge needle was used to inject 0.1 ml of saline, tilorone (20 mg) or poly IC (3.5 mg) in saline into each uterine horn. Abdomens were sutured, and the mature rats and their fetuses were killed 12, 24 or 48 hr later.

Assays. An Aminco DW-2 spectrophotometer was used to determine the cytochrome P-450 content of the liver preparations, including whole homogenates, as described by Estabrook *et al.* [25]. AP *N*-demethylase activity of liver preparations was determined by measuring the formation of [^{14}C]formaldehyde as described by Poland and Nebert [26]. BP hydroxylase activity was determined by the method of Wattenberg *et al.* [27]. EM *N*-demethylase activity was determined as described previously [28]. Tilorone levels of maternal and fetal liver homogenates were determined by the method of Hoenig and Preteux [29]. Succinate cytochrome *c* reductase activity of liver preparations was determined by the method of Sottocasa *et al.* [30]. Protein concentrations of the liver preparations were determined as described by Lowry *et al.* [31].

Liver preparations. A conventional centrifugal method [28] was used to prepare hepatic microsomes from adult female rats. However, because Ackermann *et al.* [32] showed that human fetal cells resist homogenization, it seemed likely that homogenates of fetal or early neonatal livers would contain unbroken cells causing a loss of cytochrome P-450 to the 200 *g* fraction. In fact, the 200 *g* pellet from 1- and 56-day-old rats contained about 30 and 6%, respectively, of the cytochrome P-450 originally present in the whole homogenates. Phase contrast microscopy revealed numerous whole hepatocytes in the 200 *g* fraction of the 1-day-old rats but few in that of 56-day-old rats. The problem of unbroken cells was resolved by homogenizing the liver in distilled water to lyse the cells and then adjusting the suspension to isotonicity with a hypertonic buffer solution. A 25% homogenate of liver in water was allowed to stand for 5 min in ice before it was diluted to a 20% (w/v) suspension with a cold solution of 5.75% KCl in 0.5 M sodium phosphate, pH 7.4, added slowly with agitation. This modified homogenization procedure lowered the loss of cytochrome P-450 to the 200 *g* pellet from 30 to 8%. Unruptured hepatocytes were not detected in the 200 *g* pellet. Sixty-four percent of the cytochrome P-450 in the 200 *g* supernatant fraction obtained in this manner was lost to the 9000 *g* pellet, whereas only about 50% of the cytochrome P-450 in the 200 *g* supernatant fraction, prepared from 56-day-old rats in the conventional manner, was lost to this fraction. The greater loss of cytochrome P-450 to the 9000 *g* pellet from 1-day-old rats was not unexpected in view of the observation of Chatterjee *et al.* [33] that the 9000 *g* pellets from fetal rats contain much larger numbers of microsomes than those from 14-day rats.

Tangen *et al.* [34] used gel filtration to remove hemoglobin from the hepatic 13,000 *g* supernatant fraction. It therefore seemed probable that gel filtration might be used to circumvent the loss of cytochrome P-450 to the 9000 *g* pellet. The 20% lysed homogenates of nonperfused rat livers obtained by the modified homogenization procedure were centrifuged at 200 *g* for 20 min. Five milliliters of the 200 *g* supernatant fraction was carefully layered over a Sepharose 2B column (2.5 × 10 cm) which had been thoroughly washed with distilled water and equili-

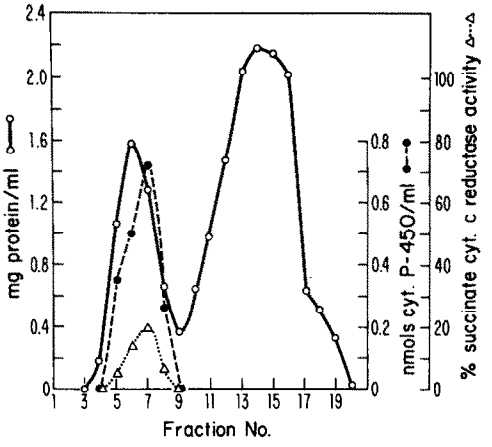

Fig. 1. Elution profile of 200 *g* hepatic supernatant fraction from a Sepharose 2B column. Five milliliters of 200 *g* supernatant fraction equivalent to 1 g of liver from an adult male rat was applied to a Sepharose 2B column (2.5 × 10 cm). Approximately 10 ml fractions were collected and assayed for protein, cytochrome P-450 and succinate-cytochrome *c* reductase activity.

brated with 1.15% KCl in 0.1 M phosphate buffer, pH 7.4. Column flow was maintained by gravity, and 10 ml fractions were collected. The microsomal fraction was eluted in the column void volume. Approximately 50% of the hepatic mitochondria, estimated by using succinate-cytochrome *c* reductase activity as a marker, was eluted with the microsomal fraction. Eluant fractions 5–8 (Fig. 1) were pooled. The pooled microsomal fraction (S-microsomes) contained about 1.2 mg protein/ml. The specific activity of the cytochrome P-450 was about 0.4 nmole/mg protein; no hemoglobin was detectable. The recovery of microsomes was estimated by using cytochrome P-450 as a marker enzyme. Recovery of S-microsomes from fetal liver was about 90%. Recovery of microsomes from adult liver, obtained by differential centrifugation, was about 45%, which agrees with the results of Eriksson *et al.* [35]. Values given in the figures and tables are corrected to accommodate these losses of microsomes.

Chemicals. The following chemicals were purchased from the indicated suppliers: ethylmorphine HCl, Mallinckrodt, Inc., St. Louis, MO; aniline HCl, Eastman Organic Chemicals, Rochester, NY; benzo[*a*]pyrene, Aldrich Chemical Co., Milwaukee, WI; sodium phenobarbital (PB), Merck Sharp & Dohme, Rahway, NJ; aminopyrine, K & K Laboratories, Inc., Plainview, NY; [dimethylamino-^{14}C]-aminopyrine (AP), Amersham Searle Corp., Arlington Heights, IL; glucose-6-phosphate dehydrogenase, Boehringer Mannheim Corp., New York, NY; aquasol 2, New England Nuclear Corp., Boston, MA; glucose-6-phosphate, NADPH, $NADP^+$, NADH, 3-methylcholanthrene (MC), polyriboinosinic acid · polyribocytidylic acid (poly IC), cytochrome *c*, disodium succinate and Sepharose 2B, Sigma Chemical Co., St. Louis MO. Pregnenolonecarbonitrile (PCN) was a gift of the Upjohn Co., Kalamazoo, MI. Tilorone dihydrochloride was a gift of Richardson-Merrell, Inc., Cincinnati, OH.

Statistical analysis. Data were evaluated by using a 2^n factorial analysis of variance and Duncan's new multiple range test.

RESULTS

Effects of phenobarbital, 3-methylcholanthrene and pregnenolonecarbonitrile on the hepatic cytochrome P-450 content and aminopyrine N-demethylase and benzo[a]pyrene hydroxylase activities of late-term pregnant and fetal rats. The effects of inducers of cytochrome P-450 systems on the cytochrome P-450 content and AP *N*-demethylase and BP hydroxylase activities of livers from 21-day pregnant rats and their fetuses are summarized in Tables 1 and 2. PB induced 43 and 87% increases in maternal and fetal cytochrome P-450 respectively. Increases of 88 and 107% in AP *N*-demethylase and BP hydroxylase activities, respectively, were induced by PB in maternal livers. No induction of either monooxygenase activity occurred in fetal livers even though cytochrome P-450 was induced.

Administration of MC to 21-day pregnant rats induced cytochrome P-450, AP *N*-demethylase and BP hydroxylase levels in maternal livers by 209, 65 and 1851% respectively. Corresponding increases in the livers of their fetuses were 359, 121 and 6064%. The blue shift in the spectrum of the reduced cytochrome P-450 · CO complex from 450 to 448 nm was seen in the microsomal preparations from maternal livers but not in those from fetal livers.

PCN induced 71 and 178% increases in maternal and fetal cytochrome P-450, respectively, and corresponding increases in AP *N*-demethylase and BP hydroxylase activities of 204 and 740% and 495 and 2815% respectively.

It is to be noted that although the percentages of induction of cytochrome P-450 content and monooxygenase activities in maternal and fetal livers by PB, MC or PCN were often quite similar, absolute values seen in fetal livers after induction were still several-fold lower than those seen in non-induced maternal livers.

Effects of tilorone and poly IC on induced and non-induced hepatic cytochrome P-450 content and monooxygenase activities of livers from late-term pregnant and fetal rats. The effects of tilorone on the cytochrome P-450 content and AP *N*-demethylase and BP hydroxylase activities of maternal and fetal livers from 21-day pregnant rats treated with saline, PB, MC or PCN are summarized in Table 1. Tilorone depressed cytochrome P-450 levels of maternal livers by 35, 42, 36 and 47%, respectively, in rats treated with saline, PB, MC and PCN. Corresponding losses of AP *N*-demethylase and BP hydroxylase activities were 47, 56, 47 and 40% and 11 (not significant), 25, 32 and 44%. In contrast, cytochrome P-450 systems of fetal livers were either unaffected or induced tilorone. Thus, cytochrome P-450 levels and AP *N*-demethylase and BP hydroxylase levels of fetal livers from saline- and MC-treated pregnant rats were not altered significantly by tilorone; tilorone induced by cytochrome P-450, AP *N*-demethylase and BP hydroxylase levels 37 and 38%, 64 and 43% and 280

Table 1. Effects of administration of phenobarbital (PB), 3-methylcholanthrene (MC), pregnenolonecarbonitrile (PCN), and tilorone on maternal and fetal hepatic cytochrome P-450 and monooxygenase levels*

	P-450 (nmoles/g liver)		AP *N*-demethylase (nmoles CH_2O/g liver/min)		BP hydroxylase (nmoles 3-OH BP/g liver/min)	
Treatment	Maternal	Fetal	Maternal	Fetal	Maternal	Fetal
Saline	18.00 ± 0.80	0.66 ± 0.06	70.4 ± 6.7	1.34 ± 0.20	2.91 ± 0.67	0.11 ± 0.03
Tilorone	11.60 ± 0.80†	0.78 ± 0.12	37.0 ± 4.1†	1.79 ± 0.39	2.59 ± 0.33	0.22 ± 0.06†
PB	25.31 ± 2.64†	1.21 ± 0.12†	120.4 ± 17.0†	1.64 ± 0.16	6.06 ± 0.68†	0.15 ± 0.02
PB + tilorone	14.73 ± 1.51‡	1.66 ± 0.12‡	40.7 ± 7.8‡	2.69 ± 0.54‡	3.77 ± 0.49‡	0.57 ± 0.17‡
MC	55.56 ± 13.33†	3.03 ± 0.49†	115.9 ± 11.1†	2.96 ± 0.28†	56.76 ± 8.61†	6.78 ± 1.65†
MC + tilorone	35.56 ± 5.56§	3.31 ± 0.86	61.1 ± 8.5§	2.34 ± 0.37	38.89 ± 3.33§	7.83 ± 1.57
PCN	31.56 ± 2.67†	1.84 ± 0.17†	198.1 ± 10.0†	12.31 ± 2.02†	16.15 ± 0.89†	3.00 ± 0.35†
PCN + tilorone	16.89 ± 1.78‖	2.56 ± 0.22‖	118.5 ± 19.3‖	17.57 ± 3.28‖	9.11 ± 1.56‖	3.95 ± 0.34‖

* Pregnant rats received 50 mg of tilorone/kg/day, p.o., or 40 mg of PB/kg/day, 20 mg 3-MC/kg/day or 20 mg PCN/kg/day, i.p., with or without the same dose of tilorone, on days 17–20 of gestation. Rats were killed 24 hr after the last drug administration, maternal microsomes were prepared by differential centrifugation, S-microsomes were prepared from pooled fetal livers, and the cytochrome P-450 content and aminopyrine (AP) *N*-demethylase and benzo[*a*]-pyrene (BP) hydroxylase activities of these liver preparations were determined. Values represent mean ± S.E. after adjustment for 45 and 90% recoveries of microsomes from maternal and fetal livers respectively. N = 8 for each drug treatment.

† Significantly different from saline-treated rats ($P < 0.05$).

‡ Significantly different from PB-treated rats ($P < 0.05$).

§ Significantly different from MC-treated rats ($P < 0.05$).

‖ Significantly different from PCN-treated rats ($P < 0.05$).

Table 2. Effects of administration of phenobarbital (PB), pregnenolonecarbonitrile (PCN), and poly IC on maternal and fetal hepatic cytochrome P-450 and monooxygenase levels*

	P-450 (nmoles/g liver)		AP *N*-demethylase (nmoles CH_2O/g liver/min)		BP hydroxylase (nmoles 3-OH BP/g liver/min)	
Treatment	Maternal	Fetal	Maternal	Fetal	Maternal	Fetal
Saline	19.64 ± 1.51	0.59 ± 0.04	61.5 ± 5.2	1.50 ± 0.21	2.89 ± 0.41	0.09 ± 0.02
Poly IC	13.22 ± 0.38†	0.57 ± 0.09	31.1 ± 6.3†	1.58 ± 0.35	2.47 ± 0.72	0.24 ± 0.09†
PB	28.33 ± 1.13†	1.13 ± 0.09†	125.9 ± 11.5†	1.67 ± 0.17	5.94 ± 0.62†	0.11 ± 0.02
PB + poly IC	22.67 ± 1.51‡	1.68 ± 0.12‡	92.6 ± 9.6‡	2.72 ± 0.45‡	4.69 ± 0.49‡	0.62 ± 0.08‡
PCN	32.89 ± 2.67†	1.64 ± 0.16†	203.3 ± 15.2†	11.54 ± 1.58†	18.37 ± 1.11†	2.83 ± 0.34†
PCN + poly IC	27.56 ± 1.78	2.58 ± 0.22§	174.1 ± 9.6	15.69 ± 1.23§	14.74 ± 0.96§	4.38 ± 0.19§

* Pregnant rats received 10 mg of poly IC/kg, i.p., on day 20 of gestation, or 40 mg of PB/kg/day, i.p., or 20 mg PCN/kg/day, i.p., with or without the same dose of poly IC on days 17–20 of gestation. Rats were killed 24 hr after the last drug administration, maternal microsomes were prepared by differential centrifugation, and S-microsomes were prepared from pooled fetal livers; the cytochrome P-450 content and aminopyrine (AP) *N*-demethylase and benzo[*a*]-pyrene (BP) hydroxylase activities of these liver preparations were determined. Values represent mean ± S.E. after adjustment for 45 and 90% recoveries of microsomes from maternal and fetal livers respectively. N = 8 for each drug treatment.

† Significantly different from saline-treated rats ($P < 0.05$).

‡ Significantly different from PB-treated rats ($P < 0.05$).

§ Significantly different from PCN-treated rats ($P < 0.05$).

Table 3. Effect of intrauterine administration of poly IC or tilorone on fetal hepatic cytochrome P-450 content and monooxygenase activities*

Treatment	P-450 (nmoles/g liver)	AP *N*-demethylase (nmoles CH_2O/g liver/min)	BP hydroxylase (nmoles 3-OH BP/g liver/min)
Saline (24 hr)	0.97 ± 0.12	1.67 ± 0.15	0.10 ± 0.01
Saline (48 hr)	0.94 ± 0.11	1.80 ± 0.07	0.07 ± 0.005
Tilorone (24 hr)	0.69 ± 0.06†	1.33 ± 0.07†	0.07 ± 0.01
Tilorone (48 hr)	0.66 ± 0.11†	1.07 ± 0.04†	0.05 ± 0.005†
Poly IC (12 hr)	1.18 ± 0.08	1.66 ± 0.10	0.12 ± 0.01
Poly IC (24 hr)	1.03 ± 0.14	1.64 ± 0.16	0.08 ± 0.02

* Twenty-day pregnant rats were anesthetized with ether, their abdominal cavities were opened, and the uterine horns were exposed. Saline (0.1 ml), poly IC (3.5 mg total dose in 0.1 ml saline) or tilorone (20.0 mg total dose in 0.1 ml saline) was injected into each uterine horn. Abdomens were sutured. Rats were killed 12, 24 or 48 hr after drug administration, and fetal hepatic S-microsomes were prepared from the pooled fetuses from each pregnant rat. Values represent mean ± S.E. after adjustment for 90% recovery of microsomes from fetal livers. N = 6 for each drug treatment.

† Significantly different from saline-treated rats ($P < 0.05$).

and 32% in fetal livers from PB- and PCN-treated fetuses respectively.

The effects of poly IC on cytochrome P-450 systems of livers from saline-, PB- and PCN-treated maternal rats (Table 2) were similar to those produced by tilorone. It was not possible to determine the effect of poly IC on MC-induced systems because the co-administration of MC and poly IC was severely toxic to pregnant rats. Poly IC depressed cytochrome P-450 by 33, 20 and 16 (not significant) percent in saline-, PB- and PCN-treated maternal livers respectively. No decrease was seen in control fetal livers, and increases rather than decreases were observed in fetal livers from PB- and PCN-treated rats (49 and 57% respectively). Poly IC depressed AP *N*-demethylase activity in maternal livers by 49, 26 and 20% but activity was increased in fetal livers by 5 (not significant), 63 and 36%. The BP hydroxylase activity of maternal livers from saline-treated rats was not altered significantly by poly IC but that of PB- and PCN-treated animals was depressed about 20%. On the other hand, poly IC induced BP hydroxylase activity of fetal livers of saline-, PB- and PCN-treated rats by 167, 464 and 55% respectively.

Effects of the intrauterine administration of interferon inducing agents on fetal cytochrome P-450 and monooxygenase activities. Because the administration of interferon inducing agents to the pregnant rat produced paradoxical effects in maternal and fetal cytochrome P-450 systems (Tables 1 and 2), it seemed pertinent to determine what effects these agents might produce if they were made more directly available to the fetus via intrauterine injection. Twenty-four hours after the intrauterine administration of tilorone, fetal hepatic cytochrome P-450 and AP *N*-demethylase activity were depressed 27 and 26% respectively; BP hydroxylase activity was not affected significantly (Table 3). Forty-eight hours after intrauterine administration of tilorone, cytochrome P-450, AP *N*-demethylase activity and BP hydroxylase activity were depressed 31, 41 and 34% respectively. On the other hand, no effects of intra-

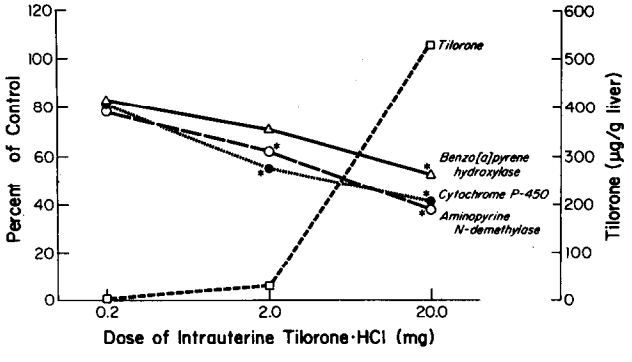

Fig. 2. Dose–response of intrauterine tilorone on fetal hepatic cytochrome P-450 systems. Pregnant rats were anesthetized, injected with 0.2, 2.0 or 20.0 mg of tilorone in saline, and killed 24 hr later. Their fetuses were removed, and homogenates of their pooled livers were assayed for their tilorone content. S-microsomes were prepared from these homogenates, and cytochrome P-450 and AP *N*-demethylase and BP hydroxylase activities were determined. One hundred percent control values for cytochrome P-450 content and AP *N*-demethylase and BP hydroxylase activities were: 1.06 ± 0.11 nmoles P-450/g liver, 1.47 ± 0.18 nmoles CH_2O/g liver/min and 0.059 ± 0.001 nmoles 3-OH BP/g liver/min respectively. These values are the mean ± S.E. after adjustment for 90% recovery of microsomes from fetal livers. N = 8 for each dose of tilorone. Key: (*) significantly different from control ($P < 0.05$).

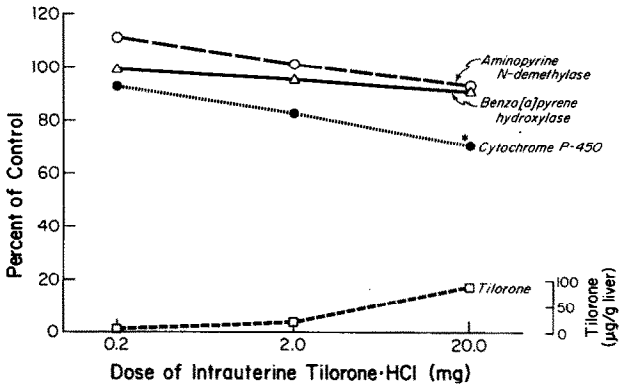

Fig. 3. Effect of intrauterine tilorone on hepatic cytochrome P-450 systems of the pregnant rat. Intrauterine administration of tilorone was made as described in Fig. 2. The pregnant rats were killed 24 hr later, hepatic microsomes were prepared by a conventional centrifugal method (Materials and Methods), and their cytochrome P-450 content and AP *N*-demethylase and BP hydroxylase activities were determined. One hundred percent control values for cytochrome P-450 content and AP *N*-demethylase and BP hydroxylase activities were: 16.4 ± 1.1 nmoles P-450/g liver, 37.0 ± 3.3 nmoles CH_2O/g liver/min and 0.89 ± 0.10 nmoles 3-OH BP/g liver/min respectively. These values are the mean ± S.E. after adjustment for 45% recovery of microsomes from maternal livers. N = 8 for each dose. Key: (*) significantly different from control ($P < 0.05$).

uterine poly IC on cytochrome P-450 systems were observed either at 12 or 24 hr after administration.

The depressant effect of intrauterine injection of tilorone on fetal drug metabolism was dose-related (Fig. 2). A hepatic tilorone concentration of 30 μg of tilorone/g depressed fetal hepatic cytochrome P-450 content and aminopyrine *N*-demethylase activity by 44 and 30% respectively. BP hydroxylase activity was not depressed significantly. An intrauterine dose of 20 mg of tilorone produced no significant loss of monooxygenase activities in the hepatic microsomes of the pregnant rat, although a 20% loss of cytochrome P-450 occurred (Fig. 3). This loss coincided with a tilorone concentration of 85 μg/g of maternal liver.

That tilorone from the maternal circulation crosses the placenta was demonstrated by measuring the tilorone content of livers of fetuses from pregnant rats that had received 50 mg of tilorone (p.o.)/kg on days 17–20 of gestation. These fetal livers contained 20 μg tilorone/g; the maternal livers contained 170 mg tilorone/g.

Time courses of depressant effects of poly IC on cytochrome P-450 content and monooxygenase activities of livers from adult virgin female, pregnant and lactating rats. Figure 4 shows the time courses of depression and recovery of hepatic cytochrome P-450 that occur after a single administration of poly IC to adult virgin female, mid-term pregnant, late-term pregnant and lactating rats. A maximum loss of cytochrome P-450 of about 50% was reached 48 hr after administration of poly IC in both virgin and 21-day pregnant rats; recoveries were complete by 96 hr. A maximum loss of cytochrome P-450 of about 35% occurred in 14-day pregnant rats and recovery was also complete by 96 hr. Similar results were observed in lactating rats except that the nadir occurred at 24 rather than 48 hr.

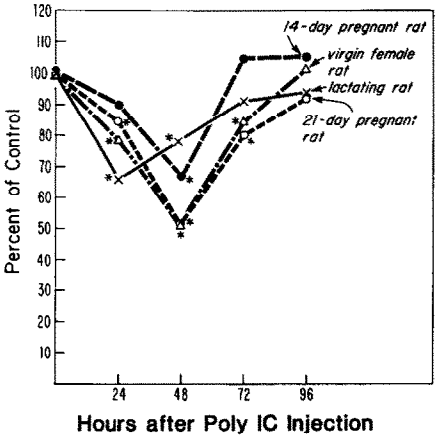

Fig. 4. Time courses of the depressant effects of poly IC on hepatic cytochrome P-450 from adult virgin female, pregnant and lactating rats. Rats received 10 mg poly IC/kg, i.p., and were killed 24, 48, 72 or 96 hr later. Hepatic microsomes were prepared by differential centrifugation, and their cytochrome P-450 contents were determined. One hundred percent control values for virgin female, 14-day pregnant, 21-day pregnant and lactating rats were: 29.9 ± 1.1, 24.0 ± 1.6, 26.4 ± 1.1, and 26.0 ± 1.6 nmoles P-450/g liver respectively. These values are the mean ± S.E. after adjustment for 45% recovery of microsomes from the livers. N = 6 for each point. Key: (*) significantly different from control ($P < 0.05$).

Figure 5 shows the time courses of depression and recovery of EM *N*-demethylase activity in the same animals that provided the data for Fig. 4. The loss and recovery of activity was similar to that for cytochrome P-450 shown in Fig. 3 except that the onset of recovery was somewhat delayed in virgin female and 21-day pregnant rats. The response of BP hydroxylase activity to poly IC administration (Fig. 6) was not always similar to that of cytochrome P-450 or EM *N*-demethylase activity, the major difference being that poly IC did not alter activity in the 14-day

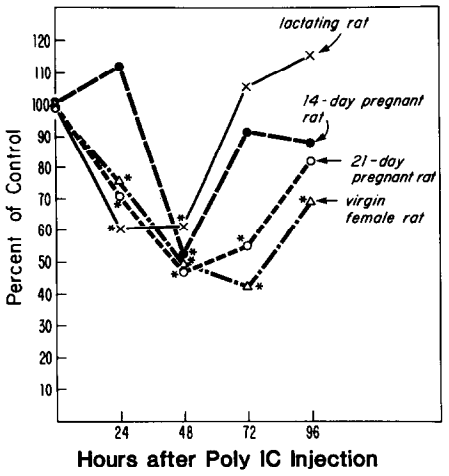

Fig. 5. Time courses of the depressant effects of poly IC on hepatic ethylmorphine (EM) *N*-demethylase activity from adult virgin female, pregnant and lactating rats. Rats received 10 mg poly IC/kg, i.p., and were killed 24, 48, 72 or 96 hr later. Hepatic microsomes were prepared by differential centrifugation, and their EM *N*-demethylase activities were determined. One hundred percent control values for virgin female, 14-day pregnant, 21-day pregnant and lactating rats were: 66.7 ± 4.4, 52.2 ± 9.3, 48.9 ± 4.1, and 60.0 ± 7.0 nmoles CH_2O formed/g liver/min respectively. These values are the mean ± S.E. after adjustment for 45% recovery of microsomes from the livers. N = 6 for each point. Key: (*) significantly different from control ($P < 0.05$).

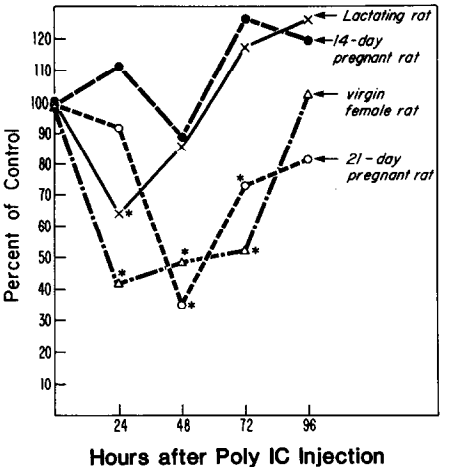

Fig. 6. Time courses of the depressant effects of poly IC on hepatic benzo[*a*]pyrene (BP) hydroxylase activity from adult virgin female, pregnant and lactating rats. Rats received 10 mg poly IC/kg, i.p., and were killed 24, 48, 72 and 96 hr later. Hepatic microsomes were prepared by differential centrifugation, and their BP hydroxylase activities were determined. One hundred percent control values of virgin female, 14-day pregnant, 21-day pregnant and lactating rats were: 3.35 ± 0.53, 1.80 ± 0.27, 2.47 ± 0.40, and 3.13 ± 0.43 nmoles 3-OH BP formed/g liver min respectively. These values are the mean ± S.E. after adjustment for 45% recovery of microsomes from the livers. N = 6 for each point. Key: (*) significantly different from control ($P < 0.05$).

pregnant rat whereas losses between 50 and 65% occurred in the other three groups of rats. As with EM *N*-demethylase activity, recovery of BP hydroxylase activity was somewhat delayed in virgin and 21-day pregnant rats.

A comparison of the 100% control values for the data presented in Figs. 4–6 shows the depressant effect of pregnancy on cytochrome P-450 systems. The cytochrome P-450 level was depressed 49 and 44% ($P < 0.05$) in 14- and 21-day pregnant females respectively. The level remained depressed by 45% ($P < 0.05$) in the lactating animals. EM *N*-demethylase activity was depressed 22 and 27% ($P < 0.05$) in 14- and 21-day pregnant rats, respectively, but no significant depression remained in the lactating animals. BP hydroxylase activity was depressed by 46 and 26% ($P < 0.05$) in 14- and 21-day pregnant rats, but values were normal in lactating animals.

Effects of the administration of tilorone, poly IC, phenobarbital, 3-methylcholanthrene and pregnenolonecarbonitrile to pregnant rats on gestational weight gain and fetal mortality. Treatment of pregnant rats

Table 4. Effects of tilorone, phenobarbital (PB), 3-methylcholanthrene (MC), and pregnenolone-carbonitrile (PCN) on gestational weight gain and fetal mortality*

Treatment	N	Weight gain (days 17–21 of gestation)	No. of fetuses/litter	No. of dead fetuses/litter
Saline	11	44 ± 5	10 ± 1	0
Tilorone	10	3 ± 4†	11 ± 1	0
PB	9	42 ± 5	8 ± 1	(0–1)
Tilorone + PB	8	−4 ± 7‡	10 ± 1	(0–1)
MC	9	26 ± 6	9 ± 1	(1–3)
Tilorone + MC	9	−19 ± 4§	10 ± 1	(0–2)
PCN	8	19 ± 6	9 ± 1	(0–2)
Tilorone + PCN	8	6 ± 3‖	8 ± 2	(1–3)

* Pregnant rats received 50 mg tilorone/kg/day, p.o., or 40 mg PB/kg/day, 20 mg MC/kg/day or 20 mg PCN/kg/day, i.p., on days 17–20 of gestation. Fetal death is reported as the range of dead fetuses observed per litter.

† Significantly different from saline-treated rats ($P < 0.05$).

‡ Significantly different from PB-treated rats ($P < 0.05$).

§ Significantly different from MC-treated rats ($P < 0.05$).

‖ Significantly different from PCN-treated rats ($P < 0.05$).

Table 5. Summary of effects of tilorone (T) and poly IC (IC) on induced and non-induced hepatic cytochrome P-450 systems of pregnant rats and their fetuses

	Direction of change*					
	Maternal			Fetal		
	P-450	AP	BP	P-450	AP	BP
T vs saline	↓	↓	NS	NS	NS	NS
IC vs saline	↓	↓	NS	NS	NS	↑
PB vs saline	↑	↑	↑	↑	NS	NS
MC vs saline	↑	↑	↑	↑	↑	↑
PCN vs saline	↑	↑	↑	↑	↑	↑
T + PB vs PB	↓	↓	↓	↑	↑	↑
T + MC vs MC	↓	↓	↓	NS	NS	NS
T + PCN vs PCN	↓	↓	↓	↑	↑	↑
IC + PB vs PB	↓	↓	↓	↑	↑	↑
IC + PCN vs PCN	NS	NS	↓	↑	↑	↑

* Data were derived from Tables 1 and 2. NS = no significant change ($P < 0.05$); arrows represent significant changes ($P < 0.05$). Abbreviations: AP = aminopyrine *N*-demethylase activity; and BP = benzo[*a*]pyrene hydroxylase activity.

with tilorone on days 17–20 of gestation inhibited the normal maternal weight gain observed at the end of gestation (Table 4). No increase in fetal mortality was observed. Co-administration of PB or PCN with tilorone did not further inhibit weight gain. Co-administration of tilorone with MC produced a severe weight loss. A similar study using poly IC was not possible because of the toxic effects suffered by pregnant rats (weight loss, ruffled fur, lethargy, vaginal discharge and eye infection). These effects are not seen in non-pregnant female rats. Pregnant rats could not tolerate more than a single injection of poly IC (10 mg/kg, i.p.); a second injection caused severe weight loss, lethargy and the death of some animals.

DISCUSSION

Table 5 is presented to facilitate a comparison of the effects of tilorone and poly IC on induced and non-induced hepatic cytochrome P-450 systems of pregnant rats and their fetuses. The generalization can be made from this table that the interferon inducing agents used in this study depress both induced and non-induced systems in maternal livers but either induce or have no effect on these systems in fetal livers. The stimulatory effect of interferon inducing agents on fetal cytochrome P-450 systems might be mediated by (a) maternal interferon transported to the fetus via the placenta, (b) fetal interferon induced by the interferon inducing agents transported via the placenta (c) reversing the effect of a hypothetical factor that depresses cytochrome P-450 systems in maternal and fetal livers [4, 20], or (d) effects of the interferon inducing agents other than those associated with the formation of interferon.

If (a) is the case, it is required that maternal interferon induced by tilorone or poly IC in the pregnant female be transported to the fetus and that the effect of this interferon on fetal cytochrome P-450 systems be opposite that produced in the maternal liver. High levels of interferon can be induced in pregnant animals and their fetuses [24, 36–38]. That interferon can be transported across the placenta is suggested by the observation that endogenous interferon administered to pregnant mice imparted antiviral activity to the fetuses [39]. Interferon is a normal component of the uterus, placenta and fetus [40]. The paradoxical effect of interferon on maternal and fetal cytochrome P-450 systems is not readily explained; however, numerous examples of paradoxical effects of interferon on a variety of biological systems are known [41].

If (b) is the case, the possibility can be considered that the type of interferon induced in the fetus may be different from that produced maternally and that the two interferons affect cytochrome P-450 systems differently. Another possibility that might be entertained is that maternal and fetal interferons are the same but that the several processes involved in the synthesis and degradation of cytochrome P-450 systems are affected to different extents by interferon in maternal and fetal livers. Assuming that interferon can depress both synthetic and degradative processes, the paradox could be explained if the net effect favours depression of synthesis of cytochrome P-450 systems in the maternal liver and depression of degradation of these systems in the fetal liver.

If (c) is the case, the effect of the interferon induced by poly IC may be that of reversing the depressant effect of the hypothetical substance that regulates hepatic cytochrome P-450 levels in both maternal and fetal livers (see beginning of paper). This concept requires that interferon does not depress fetal cytochrome P-450 systems or at least not enough to offset the opposite effect produced by reversal of the hypothetical inhibitory factor. The possibility that the interferon inducing agents *per se* may affect the hypothetical regulatory substance without the involvement of interferon is also to be considered.

If (d) is the case, the possibility must be considered that some or all of the observed effects may be due to actions of poly IC and tilorone not directly associated with interferon induction. The transport of poly IC from the pregnant rat to the fetus was not

examined, but it was shown that tilorone administered to the pregnant rat reached the fetus in appreciable quantity. The response, whether inhibition or stimulation, often depends on the biological system, the concentration of interferon, or temporal aspects of exposure of the system to interferon.

Unlike maternally administered tilorone, which induced cytochrome P-450 and monooxygenase activities in the liver, intrauterine tilorone depressed cytochrome P-450 and had no significant effect on AP *N*-demethylase or BP hydroxylase activities in the fetal liver. This apparent disparity might be explained if intrauterine tilorone does not induce interferon in the fetal liver and simply exerts a more general toxic effect on the fetus. In the former case, the inductive effect on fetal systems observed when tilorone was administered to the pregnant rat might then be attributed to induced maternal interferon transferred to the fetus via the placenta. An alternative possibility is that the hypothetical factor that may regulate cytochrome P-450 in the pregnant rat and fetus is generated in the pregnant rat, but not in the fetus, and that the intrauterine tilorone that is transported to the maternal liver from the uterine horns is not sufficient to alter the function of the hypothetical factor. Intrauterine poly IC was without effect on fetal cytochrome P-450 systems. This could mean that poly IC does not induce interferon in the fetus. It could also mean that poly IC is rapidly destroyed by nucleotidases in the fetus or its environment.

The depressant effects of a single intraperitoneal injection of poly IC on the cytochrome P-450 systems of pregnant (14- and 21-days of gestation), virgin female, and lactating rats were generally very similar but some minor differences were observed (Figs. 2–4), e.g. maximal depression of cytochrome P-450 and monooxygenase activities (EM *N*-demethylase, BP hydroxylase) occurred earlier in lactating rats (24 hr) than in pregnant or virgin rats (48 hr) and no significant depression of BP hydroxylase activity was observed in 14-day pregnant rats. The nadir of depression of BP hydroxylase activity also occurred earlier in the virgin female than in the 12-day pregnant rat. The reasons for these differences in the temporal aspects of depression of cytochrome P-450 systems by poly IC are not apparent.

Treatment of pregnant rats with tilorone inhibited normal maternal weight gain and produced overt signs of toxicity on days 17–20 of gestation (Table 4). The observation that a dose of 10 mg/kg of poly IC was toxic in pregnant rats was unexpected in view of its failure to elicit overt signs of toxicity in non-pregnant adult female rats. Gresser *et al.* (see Ref. 41 for review) observed that purified mouse interferon is highly toxic to newborn mice. Pups from pregnant rats treated with tilorone or poly IC exhibited low birth weights and a high incidence of neonatal mortality (Table 4). The possibility that interferon produced by the pregnant rat or its fetuses may be responsible for fetal and neonatal toxicity deserves consideration. The toxicity of poly IC might also be due to the presence of the interferon that occurs normally in the fetus and its surroundings [39] since interferon is known to markedly increase the cytotoxicity of poly IC in mouse embryo cells [42].

REFERENCES

1. J. R. Fouts and R. H. Adamson, *Science* **129**, 897 (1959).
2. J. R. Gillette and B. Stripp, *Fedn Proc.* **34**, 172 (1975).
3. A. H. Neims, M. Warner, P. M. Loughnan and J. V. Aranda, *A. Rev. Pharmac. Toxic.* **16**, 427 (1976).
4. T. M. Guenthner and G. J. Mannering, *Biochem. Pharmac.* **26**, 567 (1977).
5. D. K. Manchester and A. H. Neims, *Biol. Neonate* **31**, 213 (1977).
6. R. M. Welch, B. Gommi, A. P. Alvarez and A. H. Conney, *Cancer Res.* **32**, 973 (1972).
7. E. Schlede and J. H. Merker, *Naunyn-Schmiedeberg's Archs Pharmac.* **272**, 89 (1972).
8. C. R. Short, D. A. Kinden and R. Stith, *Drug Metab. Rev.* **5**, 1 (1976).
9. O. Pelkonen, P. Korhonen, P. Jouppila and N. T. Karki, in *Basic and Therapeutic Aspects of Perinatal Pharmacology* (Eds. P. L. Morselli, S. Garattini and F. Sereni), pp. 65–74. Raven Press, New York (1975).
10. J. K. Inscoe and J. Axelrod, *J. Pharmac. exp. Ther.* **129**, 128 (1960).
11. L. G. Hart, R. H. Adamson, R. L. Dixon and J. R. Fouts, *J. Pharmac. exp. Ther.* **137**, 103 (1962).
12. R. L. Dixon and V. J. Willson, *Archs int. Pharmacodyn. Thér.* **172**, 453 (1968).
13. G. Feuer and A. Liscio, *Nature, Lond.* **223**, 68 (1969).
14. J. R. Fouts, in *Fetal Pharmacology* (Ed. L. Boreus), pp. 305–20. Raven Press, New York (1973).
15. S. J. Yaffe and M. R. Juchau, *A. Rev. Pharmac.* **14**, 219 (1974).
16. W. Klinger, G. Zwacka and H. Ankermann, *Acta biol. med. Germ.* **20**, 137 (1968).
17. M. E. Dean and B. H. Stock, *Drug Metab. Dispos.* **3**, 325 (1975).
18. I. Gut, B. A. Becker and M. Gutova, *Archs Toxic.* **35**, 41 (1976).
19. G. Feuer, *Drug metab. Rev.* **9**, 147 (1979).
20. T. M. Guenthner and G. J. Mannering, *Biochem. Pharmac.* **26**, 577 (1977).
21. K. W. Renton and G. J. Mannering, *Biochem. biophys. Res. Commun.* **73**, 343 (1976).
22. G. J. Mannering, K. W. Renton, R. el Azhary and L. B. Deloria, *Ann. N.Y. Acad. Sci.* **350**, 314 (1980).
23. K. W. Renton, D. E. Keyler and G. J. Mannering, *Biochem. biophys. Res. Commun.* **88**, 1017 (1979).
24. J. Mendelson, R. Kapusta and V. Dick, *Am. J. Obstet. Gynec.* **107**, 902 (1970).
25. R. W. Estabrook, J. Peterson, J. Baron and A. Hildebrandt, in *Methods in Pharmacology* (Ed. C. F. Chignell), Vol. 2, pp. 303–50. Appleton-Century-Crofts, New York (1972).
26. A. P. Poland and D. W. Nebert, *J. Pharmac. exp. Ther.* **184**, 269 (1973).
27. L. W. Wattenberg, J. L. Leong and P. J. Strand, *Cancer Res.* **22**, 1120 (1962).
28. N. E. Sladek and G. J. Mannering, *Molec. Pharmac.* **5**, 174 (1969).
29. V. Hoenig and F. Preteux, *Xenobiotica* **7**, 339 (1977).
30. G. L. Sottocasa, B. Kuylenstierna, L. Ernster and A. Bergstrand, *J. Cell Biol.* **32**, 415 (1967).
31. O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
32. E. Ackermann, A. Rane and J. L. E. Ericsson, *Clin. Pharmac. ther.* **13**, 652 (1972).
33. I. B. Chatterjee, Z. H. Price and R. W. McKee, *Nature, Lond.* **207**, 1168 (1965).
34. O. Tangen, J. Jonsson and S. Orrenius, *Analyt. Biochem.* **54**, 597 (1973).

35. L. C. Eriksson, J. W. DePierre and G. Dallner, *Pharmac. Ther. (A)* **2**, 281 (1978).
36. J. C. Overall, Jr. and L. A. Glasgow, *Science* **167**, 1139 (1970).
37. C. R. Rinaldo, Jr., J. C. Overall, Jr. and L. A. Glasgow, *Infect. Immunity* **12**, 1070 (1975).
38. C. R. Rinaldo, Jr., D. W. Isackson, J. C. Overall, Jr., L. A. Glasgow, T. T. Brown, S. I. Bistner, J. H. Gillespie and F. W. Scott, *Infect. Immunity* **14**, 660 (1976).
39. D. M. Murasko and K. J. Blank, *Virology* **106**, 148 (1980).
40. A. K. Fowler, C. D. Reed and D. J. Giron, *Nature, Lond.* **286**, 266 (1980).
41. I. Gresser, in *Cancer—A Comprehensive Treatise* (Ed. F. Becker), pp. 521–71. Plenum Press, New York (1977).
42. W. E. Stewart, II, E. De Clercq, A. Billiau, J. Desmyter and P. De Somer, *Proc. natn. Acad. Sci. U.S.A.* **69**, 1851 (1972).