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ENHANCED PHENOBARBITAL INDUCTION OF LIVER MICROSOMAL DRUG-METABOLIZING ENZYMES IN MICE INFECTED WITH MURINE HEPATITUS VIRUS

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Abstract—The effect of an infection in mice with a murin hepatitis virus Buescher type (MHVB) on the liver microsomal drug metabolizing enzymes is investigated, alone, as well as in combination with phenobarbital pretreatment.

A diphasic effect of the infection with MHVB on the microsomal drug-metabolizing enzymes is observed: the enzyme activity is slightly increased 12 hr after the infection and is markedly depressed 48–60 hr after. The effect of phenobarbital pretreatment on the microsomal drug metabolizing enzymes is markedly enhanced in mice infected 12 or 24 hr previously.

These results suggest that the condition of stimulated biosynthesis of protein and RNA in the liver of infected mice may enhance the effect of phenobarbital.

THE important role of liver microsomal drug-metabolizing enzymes in the determination of intensity and duration of some drug actions has been widely recognized in recent years by several investigators.[1, 2] An increase or decrease of the drug effects may be observed through modifications of liver enzyme activities occurring after treatment with "inducers" or anabolic hormones or inhibiting drugs, and in some pathological conditions.[3–14]

Phenobarbital and other "inducers" produce, not only an increase of enzyme biosynthesis, but also an increase of liver weight, and of biosynthesis of microsomal and total liver protein and RNA.[6, 7, 15, 16] An accrued ability of phenobarbital to "induce" an increase of liver weight in rats bearing Walker carcinosarcoma 256 has been reported by Kato *et al.*[17]

On the other hand, Ackerman *et al.* obtained an increased biosynthesis of protein and RNA in Hela cells infected with type 1 poliovirus.[18] Green and Daesch also obtained an increased biosynthesis of protein and RNA in KB cells during exponential growth in a suspension infected with type 2 adenovirus.[19] This ribonucleic acid and protein increase is much larger than can be accounted by the virus growth.

The virus action on a cell is primarily a stimulatory one, as reported by Ackerman, and cell degeneration is a secondary effect to metabolic alteration related to the fact that critical materials, like nucleic acid and proteins, are not available to meet the needs of the normal cell turnover.[20, 21]

As for the virus-induced enzymes, the development of polysaccaridase in *Klebsiella* infected by a phage was observed by Park and Adams some years ago.[22, 23] Several similar phenomena have been observed in animal–virus systems; the appearance of neuraminidase in myxovirus-infected cells is well known. More recently, the

appearance of arginase in rabbit epithelium infected by rabbit papilloma virus was described by Rogers.[24]

The purpose of the present study is to find out if an infection with mouse hepatitis virus type Buescher (MHVB)[25] influences the microsomal drug-metabolizing enzymes, and if the induction with phenobarbital is modified in tissues of virus-infected animals.

In some experiments the influence of infection with MHVB, and of pretreatment with phenobarbital, and of both conditions together, on protein, RNA and DNA contents of liver cell is also being investigated.

Detailed experiments show that the injection with MHVB acts in opposite ways, initially stimulating and later reducing the activity of the microsomal drug-metabolizing enzymes, and that if the infection is produced 12 hr or 24 hr before the injection of phenobarbital, the effect of the drug on the enzyme activities is markedly enhanced.

EXPERIMENTAL

Female mice of the white Swiss strain, weighing about 9·5 g (17 days old) were used. Murine hepatitis virus of the Buescher strain (MHVB) was kindly supplied by Professor M. Kitaoka, of the National Institute of Health, Tokyo, Japan. Before using this virus, twenty-six passages were carried out in our Institute.

A centrifugation method is utilized to purify the MHVB. The livers of mice infected with MHVB are homogenized in 9 vols. of DIFCO's trypthose broth. The emulsion is rapidly frozen and thawed five times, then centrifuged at 4300 $\times$ g for 20 min. The supernatant is separated by decantation and recentrifugation, and then maintained at a temperature of —70 °C. The stock emulsion is diluted ten-fold just prior to use. The emulsion causing a 50 per cent mortality in 10 days in mice is about $10^{-4.6}$ of the stock emulsion (0·2 ml, i.p.).

In the present assay, an emulsion of 10^{-3} of the stock solution has been used. Mice are treated with 0·2 ml (i.p.) of emulsion and sacrificed 6, 12, 18, 24, 36, 48, 60, 72 and 96 hr later. The percentage of mortality in the infected mice 48 hr, 60 hr, 72 hr and 96 hr afterwards was, respectively, 7, 23, 40, 62. Phenobarbital (80 mg/kg) is given subcutaneously 36 or 48 hr before excision.

In all groups of mice (virus infected, treated with phenobarbital and controls), the activity of the microsomal drug-metabolizing enzymes has been determined by measuring the amount of hexobarbital, strychnine or pentobarbital metabolized by a liver microsomal preparation, during an incubation period of 1 hr.

Mice are killed by decapitation, and the pooled livers are homogenized in 3 parts (w/v) of isotonic KCl (1·15 per cent) with a Potter–Elvehjem type homogenizer. The nuclei and mitochondria are sedimented by centrifugation of the homogenate at 8500 $\times$ g for 15 min. The microsomal preparation contains for every 5 ml:3 ml of the microsome-containing supernatant, 0·1 ml with 20 μmole of glucose-6-phosphate, 0·1 ml with 0·4 μmole TPN, 0·1 ml with 50 μmole nicotinamide, 0·1 ml with 75 μmole $MgCl_2$, 0·1 ml with 1 mole KCl, 1·3 ml with 0·1 M sodium phosphate buffer pH 7·4 (or of 0·1 M Tris-buffer pH 8·2 for strychnine), and 0·2 ml of the substrate: hexobarbital, strychnine, pentobarbital (final concentration of hexobarbital, strychnine and pentobarbital 4×10^{-4}, 2×10^{-4} and 2×10^{-4}, respectively).

The microsomal preparation is incubated in 25 ml glass-stoppered Erlenmayer type flasks, which are shaken for 1 hr in the atmosphere at 37 °C. At the end of the

incubation period, 2 ml of the reaction mixtures are used for the determination of the amount of the remaining substrates.

Hexobarbital, pentobarbital and strychnine determinations are carried out according to the method of Cooper and Brodie, Brodie *et al.* and Kato *et al.*, respectively.[26–28]

The determination of protein and nucleic acids on livers of mice infected with MHVB or pretreated with phenobarbital is carried out according to Lowry *et al.* and Schneider, respectively.[29, 30]

RESULTS

The effect of MHVB infection

The infection with MHVB affects the activity of the hepatic microsomal drug-metabolizing enzymes diphasically (Fig. 1). The metabolism of hexobarbital is

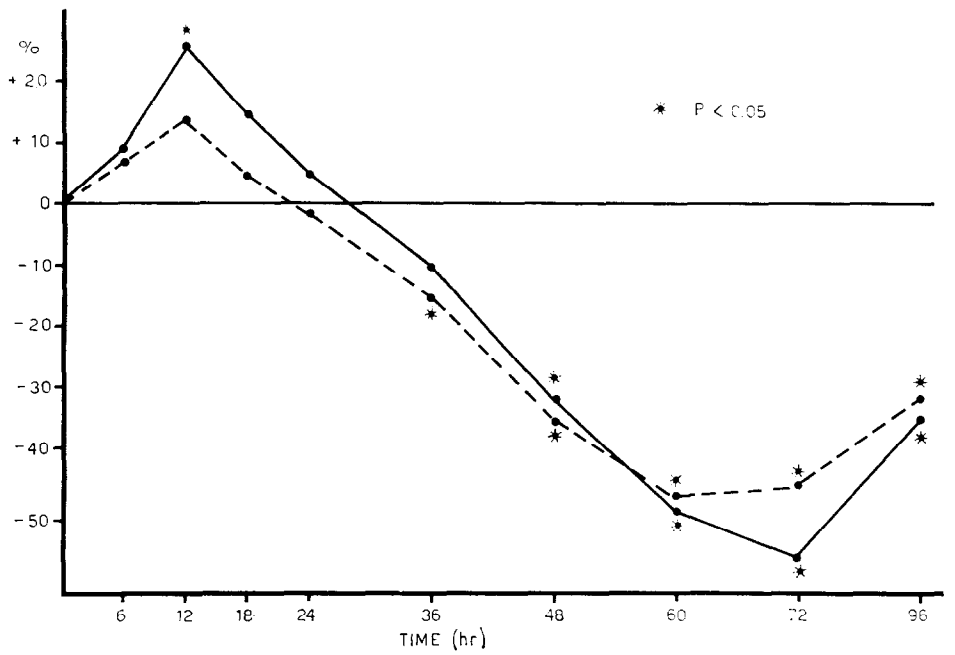

FIG. 1. *In vitro* metabolism of hexobarbital and strychnine in liver of mice infected with MHVB. The *solid line* represents hexobarbital metabolism, the *dotted* line strychnine metabolism. The values given represent averages of at least four determinations.

slightly stimulated in the first phase and a significantly increased enzyme activity is observed 12 hr after the infection. Later the enzyme activity decreases progressively. Seventy-two hours after the infection, the enzyme activity decreases by about 55 per cent. The values increase gradually later on in the surviving mice.

The metabolism of strychnine shows a similar variation but the early increase, after the infection, of the enzyme activity is less important and statistically not significant. Similar results are also observed in pentobarbital metabolism.

Phenobarbital inducing effect in mice infected with MHVB

Phenobarbital pretreatment markedly increases the metabolism of hexobarbital in normal mice, but in the joint pretreatment with phenobarbital and virus the

stimulating action of phenobarbital is less evident (Table 1). However, when phenobarbital is injected in mice previously infected with MHVB (12 or 24 hr earlier) its effect is greatly potentiated.

Thirty-six hours after phenobarbital pretreatment the enzyme activity increases by 72 per cent ($p < 0{\cdot}001$) while 36 hr after the infection it decreases by 11 per cent (no significance); but after joint pretreatment (36 hr afterwards) with phenobarbital and

TABLE 1. INFLUENCE OF VIRUS INFECTION ON THE INDUCTION OF HEXOBARBITAL METABOLIZING ENZYME BY PHENOBARBITAL

(The time interval in hours between virus infection or phenobarbital administration and the sacrifice is in brackets. The number of determinations is indicated in brackets. Pooled livers from two or three mice are used for each determination.)

	Pretreatment I		Pretreatment II		Hexobarbital metabolism (μg/g per hr)
(1)	—		—		176 ± 5 (12)
(2)	—		phenobarbital	(36 hr)	302 ± 12 (6)
(3)	—		phenobarbital	(48 hr)	308 ± 14 (6)
(4)	—		virus	(36 hr)	156 ± 7 (10)
(5)	—		virus	(48 hr)	120 ± 10 (10)
(6)	—		virus	(60 hr)	96 ± 13 (10)
(7)	—		virus	(72 hr)	84 ± 10 (10)
(8)	virus	(36 hr)	phenobarbital	(36 hr)	245 ± 12 (6)
(9)	virus	(48 hr)	phenobarbital	(36 hr)	352 ± 13 (6)
(10)	virus	(60 hr)	phenobarbital	(36 hr)	331 ± 18 (6)
(11)	homogenate*	(48 hr)	phenobarbital	(36 hr)	307 ± 14 (6)
(12)	virus	(48 hr)	phenobarbital	(48 hr)	218 ± 15 (6)
(13)	virus	(60 hr)	phenobarbital	(48 hr)	329 ± 21 (5)
(14)	virus	(72 hr)	phenobarbital	(48 hr)	278 ± 19 (5)
(15)	phenobarbital	(48 hr)	virus	(36 hr)	269 ± 10 (6)

2–1; 3–1; 5–1; 6–1; 7–1; 8–1; 8–2; 8–4; 9–1; 9–5; 9–8; 10–1; 10–6; 10–8; 12–3; 12–5; 13–1; 13–6; 13–12; 14–1; 14–7; 15–1; 14–4: $p < 0{\cdot}001$.
9–2; 12–1: $p < 0{\cdot}05$.
4–1; 10–2; 11–2; 11–9; 13–3; 14–3; 14–12; 15–3: n.s.

* The injection of an emulsion prepared from liver of control mice, using the same method as described for the liver of infected mice, does not produce any effect on the enzyme activity.

virus the enzyme activity is increased by 58 per cent in comparison with animals treated with virus only. In mice previously infected with MHVB, however, the phenobarbital injection causes an increase of 93 per cent, compared with mice pretreated with virus only. Similar results are observed when phenobarbital is injected in mice infected with virus 24 hours earlier: in this condition there is an increase of 246% in enzyme activity.

In another experiment phenobarbital was injected 48 hr before the determination of the enzyme activity, and in these conditions also the results are almost identical: the phenobarbital injection potentiates the effect in mice previously infected.

The same variations have been obtained using strychnine as a substrate.

Variation of liver protein, RNA and DNA contents

The liver weights and their contents in protein, RNA, and DNA in infected mice (MHVB) or in mice pretreated with phenobarbital, or submitted to both treatments are measured (Table 2).

The protein content and overall RNA show a decrease in livers of infected mice, but the injection of phenobarbital reverses this trend, at least partially. For example, protein and RNA contents of livers in mice infected with the virus 12 and 24 hr before pretreatment with phenobarbital are higher than those of mice infected with virus alone, and even the liver weight of these mice is increased. The ratio between liver and body weight is increased by 13 and 24 per cent in mice infected 12 or 24 hr, respectively, before the phenobarbital treatment.

TABLE 2. INFLUENCE OF VIRAL INFECTION OR PHENOBARBITAL OR JOINT TREATMENT ON LIVER WEIGHT AND LIVER PROTEIN, RNA AND DNA CONTENTS

(Phenobarbital pretreatment has been carried out 36 hr before the sacrifice. Time intervals between the viral infection and the sacrifice are indicated in brackets. The values represent average results obtained from ten mice.)

	Treatment		$\frac{\text{liver weight}}{\text{body weight}}$ (%)	Protein (mg/g)	RNA (mg/g)	DNA (mg/g)
(1)	Controls		4·88 ± 0·18	140 ± 1·7	7·53 ± 0·15	2·84 ± 0·08
(2)	Virus	(12 hr)	5·19 ± 0·19	134 ± 1·5	7·62 ± 0·17	2·87 ± 0·10
(3)	Virus	(36 hr)	5·23 ± 0·21	128 ± 2·7	7·06 ± 0·19	2·67 ± 0·17
(4)	Virus	(48 hr)	5·02 ± 0·30	125 ± 3·1	6·69 ± 0·26	2·88 ± 0·11
(5)	Virus	(60 hr)	4·64 ± 0·24	130 ± 3·3	5·50 ± 0·29	2·75 ± 0·13
(6)	Phenobarbital	(36 hr)	5·13 ± 0·23	142 ± 2·1	7·72 ± 0·23	2·66 ± 0·18
(7)	Virus +phenobarbital	(36 hr) (36 hr)	5·09 ± 0·15	136 ± 1·8	7·15 ± 0·19	2·57 ± 0·13
(8)	Virus +phenobarbital	(48 hr) (36 hr)	5·53 ± 0·21	141 ± 2·9	7·19 ± 0·30	2·59 ± 0·17
(9)	Virus +phenobarbital	(60 hr) (36 hr)	5·93 ± 0·29	132 ± 3·3	7·09 ± 0·39	2·95 ± 0·21

DISCUSSION

The slight increase of the enzyme activity, in the first phase of the virus infection may be due to the stimulating effect on the host cell. Microsomes are probably involved in this stage and, consequently, the drug-metabolizing enzymes may be activated. A great part of the later depression phase of the enzyme activity is related to a general decrease of all activity induced by the virus infection. The effects of jaundice and fasting, which cause a marked depression of the *in vitro* metabolism of hexobarbital and strychnine, must also be taken into account.[13, 14]

We observed in a previous work, that phenobarbital provokes an increase of the microsomal drug-metabolizing enzymes, even in rats pretreated with carbon tetrachloride, or pretreated with some blocking agents of the reticular–endothelial system, and in rats fed with proteinless or low protein diet.[15]

In the present work we observe a marked increase of phenobarbital effect in mice previously infected with MHVB (12 or 24 hr earlier). The increased effect of phenobarbital is not very likely to be due to the presence of activators or inhibitors, since an addition of the microsome-containing supernatant obtained from mice treated 36 or 48 hr earlier with phenobarbital to the microsome-containing supernatant of mice infected 12 hr previously with virus, causes only an additional result.

Even if the treatment with phenobarbital does not alter the mortality from viral infection, the phenobarbital injection (12 or 24 hr after viral infection) produces a marked increase of liver weight.

It has also been observed that in tumour-bearing rats there is an increase of liver weight together with protein and RNA biosynthesis, and this increase is greatly potentiated after phenobarbital administration.[17, 31–33]

Even here the effect of phenobarbital on the biosynthesis increase of microsomal drug-metabolizing enzymes, may be enhanced in the liver, where the biosynthesis of protein and RNA is stimulated by virus infection.

It may be that the viral infection produces a condition in which the biosynthesis of protein and RNA of the liver is stimulated, and thus an enhanced effect of the phenobarbital may be produced.

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