P-420 was measurable in the CCl_4-treated animals only after 24 hr, when a sharp peak at 420 mμ, was observed along with the peak at 450 mμ. At 3 and 12 hr, only a slight shoulder occurred at 420 mμ both in the controls and in the CCl_4-treated animals; this shoulder would account for less than 0·025 mμmole P-420.

No characteristic spectral changes were seen after addition of either CO or KCN to a microsomal suspension from control animals, indicating that the samples were free from hemoglobin or methemoglobin.

The sum of P-450, P-420 and cytochrome b_5 was less than the amount of total heme in the 3-hr or 12-hr experiments, but greater than the amount of total heme in the 24-hr experiment. Since these discrepancies cannot be explained by the presence of hemoglobin or methemoglobin, it is possible that the extinction coefficient of P-450 varies markedly and that the value (91 $mM^{-1}cm^{-1}$) derived from rabbit liver microsomes[10] is not always applicable. Despite these discrepancies, however, there is clearly a relationship between the decrease in the amount of P-450 and a loss of a heme component.

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Biochemical Pharmacology, Vol. 18, pp. 1533–1535. Pergamon Press. 1969. Printed in Great Britain

Cholesterol biosynthesis in the liver of experimentally induced porphyric mice*

(*Received* 11 *November* 1968; *accepted* 8 *January* 1969)

ACUTE porphyria is an inherited metabolic disease characterized by urinary excretion of excessive amounts of the porphyrin precursors, delta-aminolevulinic acid (ALA) and porphobilinogen as the result of an elevated activity of ALA synthetase in the liver.[1,2] Moreover, recent investigations have revealed the participation of changes of lipid metabolism in addition to those of porphyrin metabolism in acute human porphyria and experimental porphyria in animals. In 1961 Labbe, Hanawa and Lottsfeldt[3] reported more than two-fold increase in the synthesis of fatty acids by the liver of rats injected with the porphyrinogenic drug, allylisopropylacetamide (AIA). Recently Taddeini,

* This work was supported in part by National Institutes of Health Research Grant No. AM 06454 and in part by Mishima Kaiun Foundation Research Grant.

Nordstrom and Watson[4] have found a marked elevation of serum cholesterol in response to administration of porphyrinogenic substances such as AIA or 3,5-dicarbethoxy-1,4-dihydrocollidine (DDC). More recently DeMatteis[5] reported a stimulation of the incorporation of 2-^{14}C-acetate into liver cholesterol by griseofulvin, one of the porphyrinogenic drugs, postulating that an increased liver synthesis of cholesterol results in the production of hypercholesterolemia by porphyrinogenic drugs.

The present investigation was undertaken to study the effect of the porphyria-inducing chemicals and other drugs known to precipitate acute attacks in human hepatic porphyria without inducing porphyria in normal animals on the hepatic biosynthesis of cholesterol in mice.

The former chemicals studied include DDC,AIA,griseofulvin and hexachlorobenzene(C_6Cl_6) and the latter drug includes phenobarbital. Male mice of D-D strain, weighing 18–22 g, were obtained from the Research Institute of Infectious Disease in Tokyo. AIA (100 mg/kg), DDC (500 mg/kg), phenobarbital (80 mg/kg) were suspended in saline and injected every 12 hr. All animals were fed with a standard powder diet obtained from the Oriental Chemical Company in Tokyo. Mice were fed with griseofulvin and C_6Cl_6 each mixed in the standard powder diet (2·5%, w/w) *ad libitum*. Animals were sacrificed after 12 hr starvation at the end of a 3-day period of drug administration. Total plasma and liver cholesterol was determined according to the method of Sperry and Webb.[6] Incorporation of 1-^{14}C-acetate into cholesterol *in vitro* was measured by the method of Siperstein and Fagan,[7] using 700 *g* supernatant as enzyme solution. Incorporation of 1-^{14}C-acetate into liver cholesterol *in vivo* was assayed by injecting the isotope (4 μc/20 g body wt.) intraperitoneally and removing livers exactly 1 hr later. Cholesterol extraction and isotope counting were carried out in the same manner as *in vitro*.

As shown in Table 1, administration of phenobarbital resulted in an increased rate of cholesterol synthesis in the liver with a slightly decreased level of plasma cholesterol. This is in contrast to the DDC group, in which an increased level of plasma cholesterol and a decreased rate of hepatic cholesterol synthesis were observed. In AIA, griseofulvin and C_6Cl_6 treated groups increased plasma cholesterol levels were accompanied by increased rates of hepatic cholesterol synthesis. Cholesterol levels in the livers remain at control levels in almost all of treated groups. Though it must be taken into consideration that a change in a factor such as absorption rate or pool size could render results for acetate incorporation *in vivo* quite misleading as a measure of rate of synthesis of cholesterol, the remarkably good agreement between *in vivo* and *in vitro* results seems to reassure our interpretation of values for cholesterol labelling.

TABLE 1. CHOLESTEROL LEVELS IN PLASMA AND LIVER AND INCORPORATION OF 1-^{14}C-ACETATE INTO LIVER CHOLESTEROL *IN VITRO* AND *IN VIVO* IN EXPERIMENTAL PORPHYRIA

Treatment	No. of animals	Liver weight (g/100 g body wt.)	Cholesterol in Plasma (mg/100 ml)	Cholesterol in Liver (mg/100 g)	Specific activity of liver cholesterol *in vitro*† (counts/min/mg solid digitonide)	Specific activity of liver cholesterol *in vivo*‡ (counts/min/mg solid digitonide)
Control	6	4·96 ± 0·24	83·3 ± 16·2	250·5 ± 43·2	24·3 ± 5·4*	321·7 ± 52·4
Phenobarbital	6	6·30 ± 0·48	66·7 ± 11·3	243·2 ± 40·3	124·6 ± 21·6*	1254·8 ± 342·5*
DDC	6	6·82 ± 0·53	165·4 ± 28·8*	268·6 ± 38·5	14·8 ± 4·6*	182·5 ± 42·8*
Griseofulvin	6	6·73 ± 0·49	144·6 ± 22·5*	262·2 ± 45·3	97·3 ± 12·7*	1018·6 ± 212·6*
C_6Cl_6	6	5·56 ± 0·36	102·4 ± 14·2	260·5 ± 38·2	30·6 ± 6·2	427·3 ± 82·4*
AIA	6	5·83 ± 0·42	138·8 ± 19·5*	258·3 ± 46·6	78·8 ± 10·8*	968·6 ± 152·6*

* $P < 0·01$.

† Condition of additions: phosphate buffer (pH 7·4), 0·1 M; $MgCl_2$, $4·8 \times 10^{-3}$ M; nicotinamide, 30×10^{-3} M; NADP, $0·7 \times 10^{-3}$ M; NAD, $0·7 \times 10^{-3}$ M; glucose-6-phosphate, 18×10^{-3} M; 700 *g* supernatant of 30 per cent homogenate, 2·0 ml and 1-^{14}C-acetate, 4 μc in a total volume of 3·0 ml. Condition of incubation: one hr at 37° under 95 per cent O_2 and 5 per cent CO_2. After incubation, saponification, extraction with petroleum ether, precipitation as digitonide and counting in Packard Liquid Scintillation Counter were carried out as described by Siperstein and Fagan.[7]

‡ 1-^{14}C-acetate (4 μc/20 g body wt.) was injected i.p. 1 hr prior to sacrifice. Cholesterol purification and isotope counting were carried out in the same manner as *in vitro* examination. Each value represents the average and standard deviation.

As reported previously,[8] the porphyria-precipitating drugs such as phenobarbital and tolbutamide markedly stimulate, and DDC, one of the porphyria-inducing chemicals, strongly inhibits the activity of microsomal cytochromes (P-450 and b_5) and drug metabolizing enzyme system. Other porphyria-inducing chemicals such as AIA, griseofulvin and C_6Cl_6 have dual actions of stimulation and inhibition on the activity of microsomes. Furthermore, many steps in cholesterol biosynthesis and its catabolism are demonstrated to take place within the microsomes.[9] These facts and the present seemingly anomalous results seem to indicate that the rate of cholesterol biosynthesis could be regulated by the activity of the microsomes and that cholesterol catabolism in the microsomes might be more strongly influenced than its biosynthesis by both porphyria-precipitating and -inducing chemicals. In the latter chemicals the inhibition of cholesterol catabolism seems to play an important role in the production of hypercholesterolemia in the experimental porphyria.

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Biochemical Pharmacology, Vol. 18, pp. 1535–1538. Pergamon Press. 1969. Printed in Great Britain

Inhibition of the hepatic metabolism of amphetamine by desipramine*

(*Received* 12 *October* 1968; *accepted* 22 *November* 1968)

RECENT studies have shown that the enhanced action of amphetamine seen in rats after the administration of desipramine (DMI) is a consequence of the inhibition of the metabolism of amphetamine by DMI.[1, 2] This interaction, however, has not been demonstrated in liver preparations.The studies reported here were undertaken to investigate the inhibitory action of DMI on the metabolism of amphetamine both in hepatic microsomes and in the isolated perfused rat liver.

MATERIALS AND METHODS

Incubation studies. Male Sprague–Dawley rats weighing 200 g were used Animals were killed by cervical dislocation. Their livers were removed and chilled immediately on crushed ice All subse-

* Supported by National Institute of Health Research Grant MH 11468 from the U.S. Public Health Service.