It can be tentatively suggested that these changes are conformational in character and are caused by disturbances in the system maintaining stability of the DNA structure [9]. Together with other proteins, this role may be played in thymocytes by enzymes known as topoisomerases [3]. The possibility cannot be ruled out that Cd^{++}, by modifying topoisomerase function, modifies the postradiation repair of higher levels of DNA.

In these experiments the toxicity of Cd^{++} *in vitro* relative to unirradiated thymocytes was thus only weak. At the same time, it can be tentatively suggested that the significant antipycnotic effect and the enhanced preservation of the membranes of irradiated thymus cells are the result of inhibition of the endogenous interphase death program by Cd^{++} ions [8].

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EFFECT OF AMINOPYRINE ON THE DEGRADATION RATE OF CYTOCHROME P-450 ISOFORMS

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Cytochrome P-450 is a key enzyme of the hepatic microsomal chain for oxidation of hydrophobic substances [1]. This group includes many xenobiotics which may enter the body, including some widely used drugs, such as aminopyrine, codeine, morphine, and so on, and also substances of endogenous nature, such as steroids and fatty acids. Introduction of certain xenobiotics into the body causes induction of cytochrome P-450, with an increase in its concentration in the liver and the appearance of new isoforms of the enzyme [10]. Phenobarbital is one of the most extensively studied and widely used inducers of cytochrome P-450. We know that a two-threefold increase in the cytochrome P-450 concentration in the liver is due mainly to more rapid biosynthesis of this enzyme [4, 5]. Until recently, it was held that the rate of degradation of microsomal proteins, including components of the microsomal oxidation chain, on induction by phenobarbital is delayed or remains unchanged [7, 11]. However, evidence has recently been obtained [12] to show that more rapid degradation of cytochrome P-450 and of other enzymes of the microsomal oxidation chain may take place under the influence of phenobarbital.

The aim of this investigation was to study the effect of aminopyrine, a substrate of the mono-oxygenase system of the liver, on the degradation rate of cytochrome P-450 isoforms. Da-

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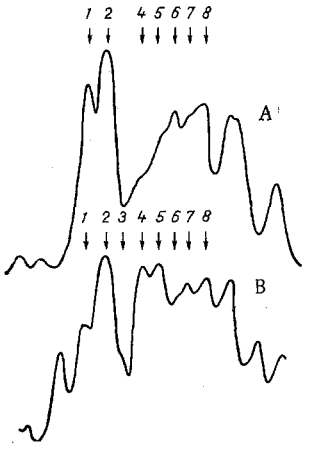

Fig. 1

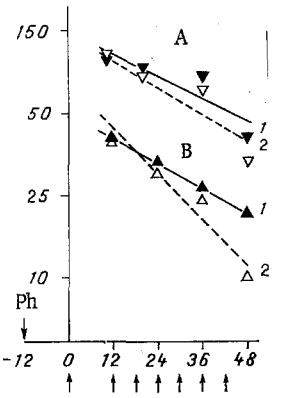

Fig. 2

Fig. 1. Densitogram of electrophoretic separation of liver microsomal proteins of control mice (A) and mice receiving phenobarbital (36 h after injection) (B). Arrows indicate protein strips whose molecular weights are given in Table 1.

Fig. 2. Decrease in specific radioactivity of cytochrome P-450 isoforms after injection of $NaH^{14}CO_3$. Abscissa, time (in h); ordinate, radioactivity (in cpm/liter·10^2). A) Protein with mol. wt. of 54 kilodaltons, B) protein with mol. wt. of 56 kilodaltons. 1) Phenobarbital, 2) phenobarbital + aminopyrine. First arrow on left indicates injection of $NaH^{14}CO_3$, remaining arrows — injection of aminopyrine. At each point the livers of three animals were pooled. Results of one typical experiment are shown. Ph) Phenobarbital.

TABLE 1. Content of Microsomal Proteins Separated by Electrophoresis (in % of total area of densitogram)

Experimental conditions	No. of protein strip							
	1	2	3	4	5	6	7	8
	58 kilodaltons	57 kilodaltons	56 kilodaltons	54 kilodaltons	52,5 kilodaltons	51 kilodaltons	50 kilodaltons	48,5 kilodaltons
Control	5,7	5,6	—	2,1	2,4	1,9	2,3	3,6
Induction by phenobarbital (36 h after injection)	2,0	4,4	1,2	4,0	4,1	1,6	2,7	4,4

Legend. Results of one typical experiment are given.

ta were obtained on acceleration of cytochrome P-450 degradation under substrate loading conditions *in vivo*.

EXPERIMENTAL METHOD

Experiments were carried out on C57BL/6 mice weighing 17-19 g. The animals were kept at a temperature of 24°C on a balanced protein diet and received food and water *ad libitum*. In each experiment data for control animals, animals receiving a single dose of phenobarbital, and animals receiving aminopyrine after previous induction with phenobarbital were compared. All mice were given an injection of 0.5 mCi $NaH^{14}CO_3$ at point "0" as radioactive precursor for protein biosynthesis, and a solution (pH 7.3) of arginine (2.2 g/kg) and glutamic acid (0.55 g/kg) every 6 h to inhibit reutilization. The animals were given sodium phenobarbital in a dose of 100 mg/kg, 12 h before administration of the label. Animals of the other group also received aminopyrine in a dose of 28 mg/kg every 6 h, starting from 12 h after administration of the label. Control mice received an equal volume of 0.9% NaCl. All solutions were injected intraperitoneally. Microsomes were isolated as described previously [3], using repre-

TABLE 2. Half-Life of Microsomal Proteins Separated by Electrophoresis (M ± m)

No. of protein strip	Half-life, h		
	I	II	III
1	23,7±0,8	22,6±3,5	95
2	23,4±0,3	26,6±2,9	114
3	27,8±4,1	15,7±1,0	57*
4	35,1±1,9	29,9±2,6	85
5	19,0±5,8	15,5±2,4	82
6	18,4±1,7	19,6±1,4	107
7	22,0±1,5	21,3±1,0	97
8	20,0±2,3	22,7±2,1	114

Legend. I) Half-life of liver microsomal proteins of mice receiving phenobarbital, II) half-life of liver microsomal proteins of mice receiving phenobarbital and aminopyrine, III) ratio II/I (in %). *P < 0.05.

cipitation in a solution of 100 mM sodium pyrophosphate and 100 mM sodium citrate to remove any adsorbed protein. Microsomal proteins were fractionated by electrophoresis in the presence of Na dodecylsulfate [8] and radioactivity was determined in the separate protein strips after treatment with tissue solubilizer TS-1 (from Koch-Light, England). Radioactivity was calculated at all points for the whole liver. The half-life of the proteins was determined by the equation $t_{1/2} = \ln 2/k$, where k is the velocity constant of degradation, numerically equal to the tangent of the angle of slope of the straight line between coordinates of time and the logarithm of specific radioactivity. The tangent of the angle of slope was determined by the method of least squares.

EXPERIMENTAL RESULTS

The main task of the investigation was to determine the rate of degradation of microsomal proteins, particularly cytochrome P-450, in control animals and after substrate loading. For this purpose the effect of injection of large doses of aminopyrine, a mono-oxygenase substrate, on the degradation rate of cytochrome P-450 isoforms was studied, on the assumption that more intensive operation of the microsomal mono-oxygenase system may accelerate degradation of its components. This hypothesis was based on the results of experiments *in vitro*, which showed that the oxygenase reactions in microsomes are accompanied by inactivation of cytochrome P-450 [2]. Aminopyrine was demethylated in microsomes of the control mice at a high velocity (4.2 nmoles/nmole cytochrome P-450/min). Injection of phenobarbital induced demethylating activity of the microsomes relative to aminopyrine by 1.7 times.

It will be clear from Fig. 1 that electrophoresis of proteins in a concentration gradient of polyacrylamide gel led to separation of microsomal proteins with mol. wt. of between 48 and 58 kilodaltons, i.e., the region which contains isoforms of cytochrome P-450, into eight protein strips. In this way the set of cytochrome P-450 isoforms could be investigated. However, cytochrome P-450 isoforms differing from one another by less than 1 kilodalton could not be separated in this way [9]. Those protein strips whose specific content increased significantly after induction by phenobarbital are of the greatest interest. Comparison of the results of electrophoresis of control and phenobarbital-induced microsomes (Table 1) showed that on induction the area of the peaks corresponding to strips with mol. wt. of 52.5, 54, and 56 kilodaltons was considerably increased. The strip with mol. wt. of 56 kilodaltons, accounting for 1-1.5% of the total area of the gel obtained by electrophoresis of liver microsomal proteins from mice induced with phenobarbital, could not be found in the liver microsomes of the control animals. The cytochrome P-450 isoform induced by phenobarbital, with mol. wt. of 56 kilodaltons, was evidently not present in the microsomes of the control animals. Induction of the cytochrome P-450 isoform with mol. wt. of 56 kilodaltons, which was isolated in a pure form, by phenobarbital in mice was also demonstrated in [6]. The relatively low protein content in this strip and the good linearization of the curve of the fall of specific radioactivity between semilogarithmic coordinates (Fig. 2B) suggest that only one cytochrome P-450 isoform was located in the strip with mol. wt. of 56 kilodaltons. Meanwhile the poor linearization of the curve showing the decrease in specific radioactivity in the strip with mol. wt. of 54 kilodaltons (Fig. 2A) can be explained by the presence of more than one cytochrome P-450 isoform in this strip. The results in Table 2 indicate that loading mice, induced by phenobarbital be-

forehand, with aminopyrine leads to a significant, twofold acceleration of degradation of the cytochrome P-450 isoform with mol. wt. of 56 kilodaltons. It is quite probable that this isoform is more specific for aminopyrine and, for that reason, this is the one which oxidized aminopyrine in the liver of phenobarbital-induced mice. The tendency toward acceleration of degradation on loading with aminopyrine also was found for two protein strips with mol. wt. of 52.5 and 54 kilodaltons (Table 2), the content of which on the densitogram also was sharply increased after induction by phenobarbital. These strips evidently include several isoforms of cytochrome P-450, which prevents acceleration of degradation of one of them from being recorded. This possibility is confirmed by the relatively high (4-5%) specific protein content in these strips on the densitogram, and also by the fact that the curves showing the decrease in specific radioactivity in these strips are not linearized between semilogarithmic coordinates.

The results of the present investigation thus provide experimental proof of an increase in the degradation rate of one isoform of cytochrome P-450 on administration of aminopyrine, a substrate oxidized in the mono-oxygenase system of the liver microsomes, to animals.

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