It can be concluded from these results that acute alcoholic hepatitis and active alcohol-induced cirrhosis of the liver are accompanied by intensification of LPO. This is manifested as an increase in the concentration of fluorescent end products in the plasma. A higher level of LPO is associated with active cirrhosis with an edema-ascites syndrome and acute alcoholic hepatitis; this is reflected both in the actual concentrations and the time course of normalization of the level of fluorsecent products in these forms of alcohol-induced liver disorder.

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DETECTION OF *DE NOVO* SYNTHESIZED FORM OF MICROSOMAL CYTOCHROME P-448 BY AUTOFLUOROGRAPHY

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UDC 612.351.11.015.36:577.152.112

KEY WORDS: monooxygenases; *de novo* synthesis; autofluorography

The monooxygenase enzyme system, consisting of cytochrome P-450 and NADPH electron transport chain, catalyzes oxidation of hydrophobic foreign compounds (xenobiotics) and thus promotes their elimination from the body. Monooxygenases hydroxylate a wide range of substrates, some of which are genetic inducers of components of the system. More than 300 substances, differing in chemical structure and biological effects, significantly increasing the rate of biotransformation of xenobiotics, and in the substrate specificity of their enzyme system, due to a selective increase in the concentration of certain forms of cytochrome P-450, are now known. For example, during induction by the polycyclic aromatic hydrocarbon (PAH) β-naphthoflavone, five forms of cytochromes possessing different spectral, catalytic, and immunologic properties, and also differing in molecular weight and primary structure, have been isolated from the microsomal fraction of rat liver [7]. After injection of another PAH, namely 3-methylcholanthrene (MCh), only one basic, *de novo* synthesized form of cytochrome (P-448) was isolated from liver microsomes and its characteristics determined sufficiently well. As regards other forms of cytochrome induced by MCh, no data on this problem exist. An increase in the content of certain forms of microsomal cytochromes, which lies at the basis of induction by xenobiotics, may be the result of an increase in the rate of *de novo* protein synthesis or the rate of recursor activation, or a decrease in the rate of degradation. Direct proof of *de novo* syn-

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 101, No. 1, pp. 29-30, January, 1986. Original article submitted October 25, 1984.

0007-4888/86/0001-0035$12.50

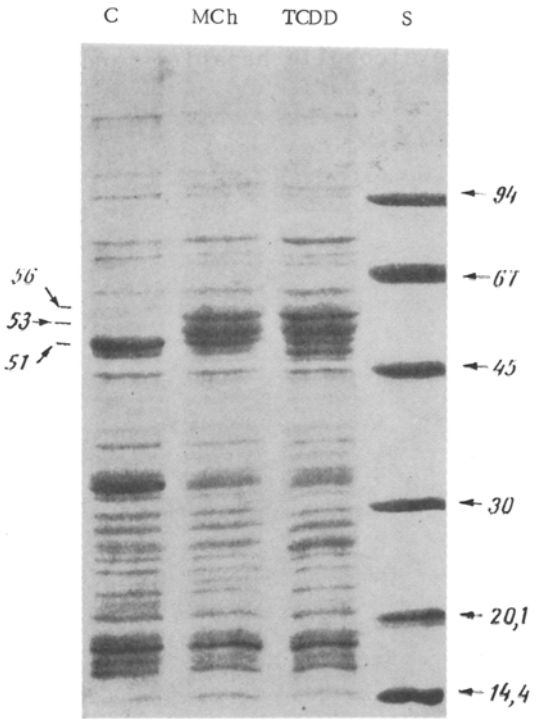

Fig. 1.

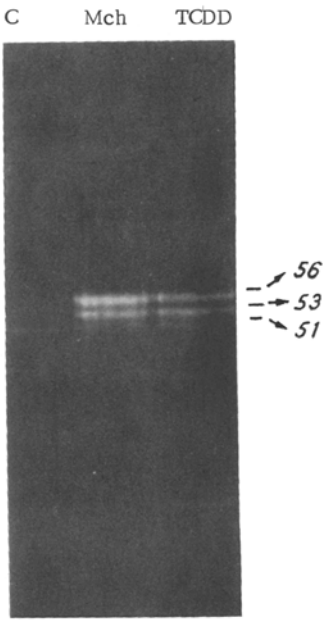

Fig. 2.

Fig. 1. Electrophoresis of microsomes in polyacrylamide gel. MCh, TCDD) Microsomes obtained from liver of rats induced with MCh and TCDD; C) control microsomes, S) standard proteins. Numbers denote molecular weight (in kilodaltons).

Fig. 2. Autoflurography of gels after electrophoresis of microsomes labeled with ^{14}C-leucine. Exposure 15 days. Legend as to Fig. 1.

thesis of forms of cytochrome P-450, induced by xenobiotics, has been obtained by investigation of incorporation of radioactive amino acids into microsomal protein [4] in the form of sums of different forms of cytochromes in the total microsomal fraction of liver [3, 5]. It has been observed that maxima of radioactivity cannot be accurately correlated with the arrangement of bands of cytochrome P-450 apoenzymes of microsomal preparations from the liver of animals injected *in vivo* with inducers and labeled amino acids, in widely used methods of electrophoretic analysis.

A simple and informative method of detection of *de novo* synthesized forms of microsomal cytochrome P-450 is suggested in this paper. The method is based on autofluorography of the gel after electrophoretic fractionation of liver microsomal proteins of rats previously injected with inducers of components of the monooxygenase system, followed by ^{14}C-leucine.

EXPERIMENTAL METHOD

Male Wistar rats weighing 50 g were used. The animals were kept on a standard laboratory diet, but deprived of food on the night before sacrifice. MCh (40 mg/kg) and 2, 3, 7, 8-tetrachlorodibenzodioxine (TCDD, 5 μg/kg), dissolved in the minimal volume of vegetable oil, were injected intraperitoneally 16 h before sacrifice, and L-1-^{14}C-leucine (250 μCi, from Amersham Corporation, England) was injected intraperitoneally twice, 8 and 4 h before sacrifice. Liver microsomes were obtained by differential centrifugation. The cytochrome P-450 concentrations were determined on a Hitachi 556 spectrophotometer (Japan) [10]. Activity of benzpyrene hydroxylase was measured as described previously [1] on a Hitachi MPF-4 spectrofluorometer (Japan). Protein was determined by Lowry's method [8]. Electrophoresis of the microsomal fraction was carried out in a 7.5-15% polyacrylamide gel gradient [6] on an apparatus from Pharmacia Fine Chemicals (Sweden). The molecular weight of the microsomal proteins was determined with the aid of standard proteins from Serva (West Germany): phosphorylase B (mol. wt. 94 kilodaltons (kd)), albumin (67 kd), ovalbumin (43 kd), carbonic anhydrase (30 kd), trypsin inhibitor (20.1 kd), and α-lactalbumin (144 kd). Autofluorography was carried out by the method in [2]. Radioactivity was determined on a Mark II liquid scintillation counter (USA).

EXPERIMENTAL RESULTS

After intraperitoneal injection of MCh and TCDD no appreciable increase in the cytochrome concentration could be found in the microsomal fraction of rat liver 16 h of induction (MCh microsomes 0.6; TCDD microsomes 0.5, control microsomes 0.5 nmole/kg). However, a shift of the maximum of the CO peak toward the region of shorter wavelength (448 nm) could already be observed. Investigation of the hydroxylating activity of cytochromes P-448 during induction for 16 h revealed an increase in the rate of hydroxylation of benzpyrene up to 350% compared with the control in the case of MCh induction and 300% after injection of TCDD into the animals. The results indicate that after 16 h new forms of cytochromes, characteristic of each induction, appear in the microsomes.

During electrophoretic fractionation under dissociating conditions (Fig. 1) of liver microsomal fractions of control and MCh- and TCDD-induced rats polypeptides with mol. wt. of 51, 53, and 56 kd were recorded in the region of migration of chemoproteins (48-58 kd) in the experimental microsomes, whereas a protein with mol. wt. of 51 kd was recorded in the control microsomes. The appearance of new protein bands (53 and 56 kd), together with the results of spectral and kinetic investigations, afford proof of *de novo* synthesis of microsomal proteins, namely specific forms of cytochromes characterisitic for the particular inducers.

After injection of MCh and TCDD into the animals a significant increase was observed in incorporation of the labeled amino acid in the experimental microsomes compared with the control (up to 340 and 260% respectively), evidence of active *de novo* synthesis of microsomal proteins in response to injection of these xenobiotics into the animals.

To detect *de novo* synthesized apoenzymes of different forms of cytochrome in the total liver fraction, the method of autofluorography of the gel after electrophoretic fractionation of proteins of the experimental microsomes, isolated from the liver of rats treated with TCDD and MCh 16 h before sacrifice, and receiving 250 μCi of 1-^{14}C-leucine 8 and 4 h before sacrifice, was suggested. An autofluorograph of gels of experimental and control microsomes after electrophoresis is shown in Fig. 2. In the region of migration of proteins with mol. wt. 48-58 kd two bands of intensive fluorescence appeared in the experimental microsomes, and there were two other bands located in the region of migration of proteins with lower molecular weight, which also were recorded in the control microsomes. The results of autofluorography afford proof of the fact of *de novo* synthesis of microsomal proteins migrating on electrophoresis in the 48-58 kd region. Complete agreement between bands of cytochrome P-448 apoenzymes and bands of light on the x-ray film was demonstrated by superposition of densitograms of the gel plates after electrophoretic fractionation of microsomal proteins with respect both to the intensity of staining with Coomassie blue and the intensity of illumination of the x-ray film as a result of radiation from ^{14}C-leucine incorporated into the cytochrome proteins during the period of *de novo* synthesis.

It can be concluded from these results obtained by autofluorography, which is used for the first time to analyze constitutive and *de novo* synthesized mono-oxygenases, that during induction of the mono-oxygenase system of rat liver with MCh and TCDD at least two forms of cytochromes (of the P-448 type) with mol. wt. of 53 and 56 kd were synthesized *de novo*.

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