ENZYMIC IMPRINTING IN ADULT ANIMALS

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Activation of the microsomal monooxygenase system in the liver by various inducers leads to changes in xenobiotic biotransformation [6]. The specific effect of the inducers acting on adult animals is short in duration, unlike that in fetuses and newborn animals, in which increased enzyme activity is maintained for a long time or even throughout life. This phenomenon, which has been called enzymic imprinting [4, 5], has been observed following administration of steroid hormones [11], phenobarbital [9, 15], 16-α-isothiocyanopregnenolone-3-acetate [4], tetrachlorodibenzo-*p*-dioxine [12], and Aroclor 1254 [3]. The existence of this phenomenon is due both to increased sensitivity of fetuses and newborn animals to endogenous or exogenous inducers and to the fact that they contain specific mechanisms of fixation of altered enzyme activity induced by these factors. Thus enzymic imprinting is an essential component of the adaptive strategy of the developing organism, forming, along with genetic factors, its individual sensitivity to unfavorable environmental influences. The mechanism of fixation of the level of enzyme activity, modified by the influence of inducers, may be closely connected with intensive proliferation processes taking place in fetal and neonatal liver tissues. It can accordingly be postulated that artificial enhancement of proliferation in the adult liver as a result of partial hepatectomy will modify the conditions that enable fetal and neonatal tissues to fix a level of enzyme activity modified through the action of inducers. The aim of this investigation was to test this hypothesis experimentally.

EXPERIMENTAL METHOD

Experiments were carried out on 97 noninbred female albino rats weighing 200-250 g, bred at the Research Institute of Oncology, and kept on a standard diet. At the age of 3 months and under ether anesthesia the animals underwent partial hepatectomy with removal of two-thirds of the liver, which was followed after 30 min (group 1, $n = 9$), 48 h (group 2, $n = 8$), 6 days (group 3, $n = 8$), or 3 weeks (group 4, $n = 8$) the animals were given the Aroclor analog Sovol (a single dose of 500 mg/kg body weight, in sunflower oil, intraperitoneally). In the corresponding controls the following animals were used: 39 intact female rats of the same age (group 5), nine rats receiving Sovol (group 6), eight hepatectomized rats receiving an injection of the solvent 48 h after the operation (group 7), and eight animals undergoing a mock operation and receiving Sovol (group 8). The experimental animals 7, 14, 30, 60, and 90 days after administration of Sovol (or the solvent), and also the intact animals were killed by cervical dislocation and the liver removed with aseptic precautions and homogenized in 0.15 M KCl solution in the ratio (w/v) of 1:3. The S_9-fractions were prepared from the resulting homogenate by the method described previously [13] and used to determine activity of the key enzymes of xenobiotic metabolism and of metabolic activation in the *Salmonella*-microsomal test. Activity of aryl-hydrocarbon hydroxylase (AHH) was determined by the method in [8] on a "Hitachi" fluorometer with activation at $\lambda_{min} = 396$ nm and fluorescence at $\lambda_{max} = 522$ nm, and expressed in fluorescence units/30 min, relative to microsomal protein concentration. Aminopyrine-N-demethylase (APD) activity was measured spectrophotometrically by the method in [14] and expressed in nanomoles formaldehyde formed in 30 min/mg protein. Activity of 7-ethoxycoumarin-de-ethylase (ECD) was measured by the method in [7] on a "Hitachi" fluorometer with activation at $\lambda_{min} = 390$ nm and fluorescence at $\lambda_{max} = 440$ nm and expressed in μg hydroxycoumarin formed in 30 mg/mg microsomal protein. In all cases the protein concentration in the liver microsomes was determined by the method in [10], using bovine serum albumin as the standard. In the modern version of the Ames-E test [13], on cells of *Salmonella typhimurium* of strains TA98 and TA100, the ability of the isolated S_9-fractions of

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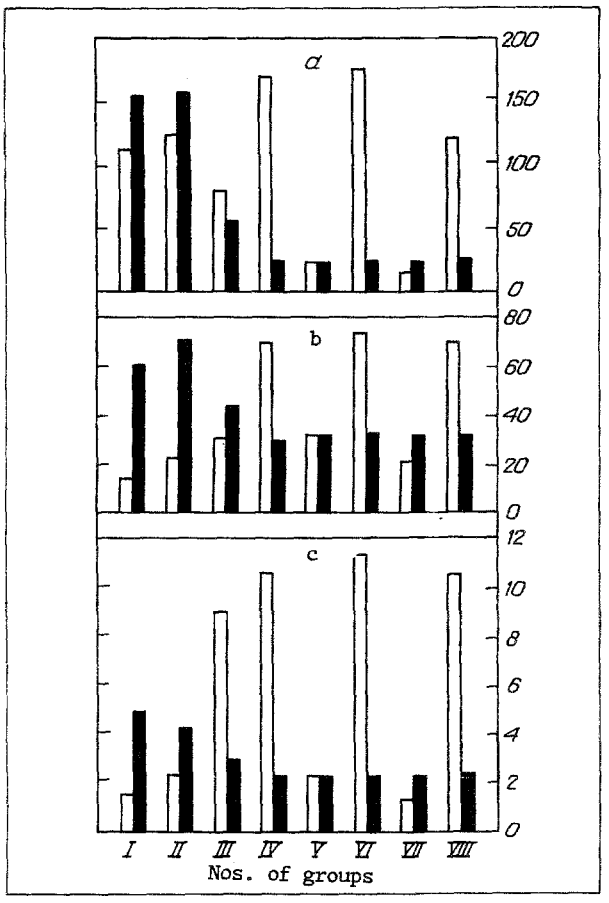

Fig. 1. Activity of key enzymes of xenobiotic metabolism in different experimental groups 1 week (□) or 3 months (■) after injection of Sovol.

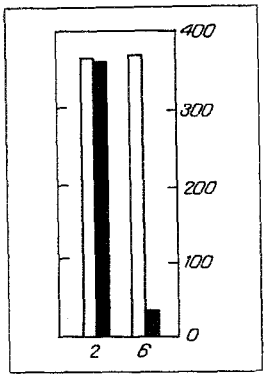

Fig. 2. Metabolic activation of benz(a)pyrene by liver tissue of rats of groups II and IV 2 weeks (□) and 3 months (■) after injection of Sovol in *Salmonella*-microsomal test on bacteria of strain TA98.

the liver to induce metabolic activation of benz(a)pyrene was demonstrated and expressed as the number of his^+-revertants per dish.

The experiments in each group were repeated at least twice and gave similar results, which were pooled.

EXPERIMENTAL RESULTS

The investigations showed that the level of activity of the enzymes studied in intact animals (group 5) did not change significantly in the course of the experiments and amounted for AHH to 16.6 fluorescence units/30 min/mg protein, for ECD to 2.45 μg hydroxycoumarin/30 min/mg protein, and for APD to 34.4 nmoles HCHO/30 min/mg protein. Under the influence of Sovol alone (group 6) the greatest increase in activity of the enzymes studied was observed after 1 week: AHH up to 160-200, ECD up to 12, and APD up to 80-100 units, after which the values of these parameters fell, to regain the spontaneous level after 1 month. In animals receiving an injection of the solvent after hepatectomy (group 7) activity of ECD and APD remained virtually unchanged during the observations, but AHH activity after 1 week was below the spontaneous level, although after 1 month it had regained the control level. After the mock operation, followed by injection of Sovol (group 8), the results were qualitatively similar to those for enzyme activity in group 6. Different results were obtained, however, in group 1. In this case the effect of the inducer was similar in animals receiving Sovol 30 min and 48 h after hepatectomy (groups 1 and 2). AHH activity in these groups after 1 week was 110-130 units, and this was followed by an increase to 150 units, at which level it remained for at least 3 months. In group 3 (injection of Sovol 6 days after hepatectomy) AHH activity was increased to 70 units after 1 week and 160 units after 1 month, after which it fell to regain the control level in 3 months. In group 4 (injection of Sovol 3 weeks after hepatectomy) the results were virtually identical with those of group 6, namely a sharp increase in enzyme activity after 1 week followed by a decrease to the control level after 1 month. The dynamics of the change in activity of the other enzymes in this group was qualitatively similar to the changes in AHH (Fig. 1). For instance, APD and ECD activity in group 4 was 10, 23, 33, and 65, and 1.2, 2.4, 9, and 10.5 units respectively, after 3 months it was 60, 65, 47, and 32 and 7, 4.4, 3, and 2.4 units respectively. These results correlated closely with those of the investigation of metabolic activation of benz(a)pyrene in Ames' test (Fig. 2). For instance, the number of his^+-revertants per dish 2 weeks after injection of Sovol averaged 370-380 in groups 2 and 6, whereas after 3 months the numbers were 365 and 36 revertants respectively.

Thus Sovol, which is a mixture of polychlorinated biphenyls, has a broad spectrum of inducing action, leading to increased activity of several key enzymes of xenobiotic metabolism [1]. In the present experiments, after injection of Sovol into intact rats (group 6) and rats undergoing a mock operation (group 8), maximal activity of the enzymes studied was observed after 1 week. Similar results also were obtained in group 4, in which Sovol was injected 3 weeks after the operation. In groups 1-3 AHH activity 1 month after hepatectomy was 8 times higher than the spontaneous level, and activity of ECD and APD was twice as high; in groups 1 and 2, moreover, this increased level of enzymic activity continued for at least 3 months (which was confirmed by integral evaluation of metabolic activity of benz(a)pyrene in the *Salmonella*-microsomal test), and in group 3, it fell gradually, while remaining a little above the spontaneous level (by 1.25-1.5 times) by the end of the experiment. This "delay" of the response to induction in groups 1-3 compared with groups 6 and 8 can be explained by competitive relations between the tissue-specific and proliferative functions of the cells [2]. Meanwhile, compared with the response of hepatocytes functioning under ordinary conditions, processes taking place in the proliferating cell, by interfering with the mechanisms responsible for its tissue-specific response to the inducer, lead to the appearance of a qualitatively new response, namely a stable and long-lasting increase in activity of the induced enzymes. The mechanism lying at the basis of this phenomenon may perhaps be that the inducer—receptor complex, binding with regulatory regions of the genome of the proliferating cells, acquires the ability to modify the genetic program of the hepatocytes, and thus to act as a trigger. On the other hand, the possibility cannot be ruled out that exposure to the inducer in the period of intensive hepatocyte proliferation may lead to proliferation mainly of those cells whose basic tissue-specific function is the detoxication of xenobiotics. Thus enzymic imprinting is not necessarily connected only with the early stage of ontogeny, but it may also be observed in adult animals, and consequently this phenomenon can be regarded as a manifestation of the universal response of proliferating cells to the action of inducers.

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IS XANTHINE OXIDASE A UNIVERSAL SOURCE OF SUPEROXIDE RADICALS IN LIVER DAMAGE INDUCED BY ISCHEMIA AND REPERFUSION?

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It has now been shown that an essential role in the development of ischemic damage of organs is played by oxygen radicals [5, 8], and the most widely held hypothesis is that a key position in the development of ischemic damage is occupied by superoxide radicals, arising under the influence of xanthine oxidase. The basis for this hypothesis consists of two groups of facts obtained chiefly in experiments on a model of ischemia in vitro. First, ischemia leads to rapid breakdown of high-energy phosphates [3]. This may result in the accumulation of substrates of the xanthine-oxidizing enzyme, namely hypoxanthine and xanthine. Second, during ischemia is various organs (intestine, heart, liver, etc.) the change was observed from xanthine dehydrogenase (the D-form of the enzyme) into xanthine oxidase (the O-form of the enzyme), which can generate superoxide radicals [5]. On this basis it was concluded that the conditions are created during ischemia which may lead during subsequent reperfusion to an increase in the rate of formation of superoxide radicals.

Since the main results relative to the role of the xanthine oxidase system have been obtained on a model of ischemia of the liver in vitro and are not entirely consistent, the aim of the present investigation was to study on a model of total ischemia of the liver in vivo whether substrates and products of the xanthine oxidase reaction accumulate whether the D-form of the enzyme changes into the O-form.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats weighing 180-220 g, under hexobarbital anesthesia. Total ischemia of the liver was induced by applying microforceps to the vascular pedicle of the central and left lateral lobes of the liver for a period of 2 h. Reperfusion was carried out by removing the forceps, and after ischemia the nonischemic lobes, amounting to about 30% by volume, were resected.

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