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Inhibition of a Hepatic Microsomal Enzyme System After Head X-Irradiation of Rats.* (32587)

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Studies in the recent past from various laboratories have shown that the activity of hepatic drug metabolising enzymes can be influenced by a variety of factors: for example, species, sex, age, nutritional status, drugs, chemicals and alterations in hormonal level (1,2). Exposure to ionising radiation may also be added to this long list of modifiers of enzyme activity. Hietbrink and DuBois(3) have demonstrated the inhibitory effect of x-irradiation on the development of hepatic microsomal enzymes that metabolise organophosphates. Using weanling rats irradiated to the head alone they also found evidence for an abscopal inhibitory effect. Recent investigations in our laboratory have revealed that *in utero* exposure of rats to low doses of x-irradiation (25 or 50 R) produced in the male offspring a significant suppression of the development of the hepatic microsomal enzyme system that metabolises hexobarbital (4). Irradiation at 21 days of age (total body or head alone) also suppressed the developmental increase of enzyme activity normally seen in the males, but to a lesser extent than in the prenatal series. Inhibition of the enzyme system, both direct and abscopal, was observed in the adult males also, but only at radiation doses above 500 R (total body or liver) or above 1,000 R (head alone).

Methods. Sprague-Dawley rats were used in these studies. *In the prenatal series,* rats received 25 or 50 R of x-irradiation (at 11-12 R/min) to the pelvic region on the 14th day of gestation. The offspring were examined at 10, 20, 40, and 80 days of age. *In the postnatal series,* 21 day old male rats received a single dose of 200 R (49-50 R/min) to the

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total body or head alone. The irradiated animals were examined at 30, 40, 45, and 50 days of age. *In the adult series,* the animals were exposed to various doses of radiation as follows: a) 500 R, 1,000 R, 2,000 R, or 10,000 R—body alone with head shielded. b) the same doses to head alone with body shielded. The physical factors of radiation were: 250 Kv, 30 mA, filter—0.5 m cu and 1 mm Al, half value layer—1.45 mm cu, dose rate—280 R/min. During irradiation, the unanesthetised animals were held in lucite tubes provided with a large number of air holes. For shielding, a 3.5 mm thick sheet of lead was employed.

For determination of the hepatic enzyme activity, the irradiated animals were decapitated at the selected intervals, a piece of tissue (~200 mg) from the periphery of the right lobe of liver excised and weighed rapidly after removing the contaminating blood and fluid. When the samples were to be stored, as was often the case when doing a large series, the whole operation was performed in a cold room, the tissue frozen on a bed of dry ice and stored frozen in plastic tubes in a refrigerator until ready for use. The enzyme activity was measured by determining the rate of substrate disappearance in a whole liver homogenate system according to the method of Yam and DuBois(5). This method employing whole liver homogenates has certain advantages over previous methods utilising microsomal fractions. It is a simpler procedure, and provides for *in vivo* quantitative comparisons of the enzyme activities of different animals. The reaction system consisted of the following: potassium phosphate buffer, pH 7.4, 20 μM; magnesium chloride, 30 μM; sodium glucose-6-phosphate, 3 μM; NADP, 1.3 μM; and a 20% homogenate of liver in cold isotonic KCl containing 0.25% nicotinamide, 0.15 ml (= 30 mg tissue) with water to make a final volume of 0.8 ml. The reaction mixture was incubated in 15 ml centrifuge tubes in a metabolic shaker in an atmosphere of air at 37.5°C. In addition to the sample tubes, each of the runs included a) a tube containing all the above constitutents but kept in ice for 30 minutes (for recovery) and b) a tube with all the constitutents except hexobarbital and kept at 37.5°C (for tissue blank). At the end of

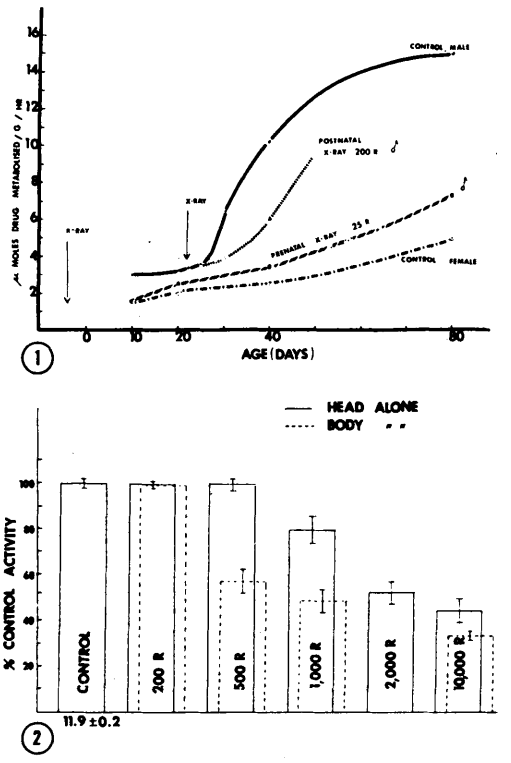

FIG. 1. The effects of prenatal and early postnatal x-irradiation and the development of hexobarbital metabolizing enzyme system in rat liver. The development of the enzyme system in control male and female rats is also shown for comparison.

FIG. 2. The effects of varying doses of head x-irradiation on the hexobarbital metabolising enzyme system in adult male rat liver are compared with the effects of radiation to the body alone. The results are expressed as per cent of enzyme activity in the controls.

the incubation period, the unmetabolised hexobarbital in the reaction mixture was determined by the method of Cooper and Brodie (6). The amount of hexobarbital metabolised was calculated from the difference between the amounts of hexobarbital recovered in the absence and presence of enzyme action. The mean recovery of hexobarbital from the tube kept in ice was 91-92% of the added amount. The enzyme activity is expressed as μ moles of hexobarbital metabolised per g of liver (wet weight) per hour.

Results and discussion. The marked sex difference in the activity of the hexobarbital metabolising enzyme system is illustrated in Fig. 1. Other investigators have also observed this sex difference in the activity of

hexobarbital metabolising enzyme system in rat liver(7). Prenatal irradiation suppressed the development of the enzyme system in the male offspring such that the males now resembled the females with respect to the enzyme activity (Fig. 1). No effect of radiation was detected in the female offspring. In view of the low enzyme activity even in nonirradiated control females, further tests were not done in females.

It may be seen that the enzyme development was suppressed in rats receiving 200 R early postnatally, but the magnitude of this suppression, in spite of the higher radiation dose, is less than that noted in the prenatally irradiated animals. There were 3 groups in the early postnatal series: a) those receiving total body irradiation b) those receiving radiation to the body alone with head shielded and c) those receiving radiation to the head alone with the remainder of the body shielded. Enzyme development was suppressed in all the 3 groups and the results were not statistically different from each other.

The effect of radiation in adult rats is shown in Table I.† Enzyme activity was measured at 3 days post-irradiation. Exposure of the head alone to 500 R of x-irradiation did not affect the hepatic enzyme activity. Even though the liver was shielded from radiation in these experiments, it was of interest to determine the extent of scattering to liver and its contribution to the suppression of enzyme activity noted in the head irradiated animals.

The following experiments were done for this purpose: a) The radiation dose under the shielded area, at various distances from the periphery of the shield (1/2″, 1″, 1 1/2″), was measured by placing a Victoreen dosimeter inside a phantom (lincolnshire bolus, a specially prepared material containing light magnesium carbonate 13% w/w in a sugar base, made by Boots Pure Drug). The readings were 18% (at 1/2″), 5.4% (at 1″) and 3.7% (at 1 1/2″) of the total dose delivered to the head.

TABLE I. Effect of X-Irradiation on Hexobarbital Metabolising Enzyme System in Adult Male Rat Liver. (Enzyme activity is expressed as μmoles of drug metabolised per g wet weight liver per hour ± S.E.)

	X-irradiation (head only)		
Control	1,000 R	2,000 R	10,000 R
11.9 ± 0.2	9.5 ± 1.0	6.3 ± 0.6	5.4 ± 0.8

b) The radiation dose to the liver was also determined directly by placing a Victoreen dosimeter, wrapped in plastic, between the hepatic lobes of an anesthetised rat and repeating the experiment under conditions identical to the head irradiation of unanesthetised rats. It was found that the liver received 5-6% of the total dose delivered to the head.

c) Varying doses of radiation from 100 R and up were delivered to the body alone (with head shielded) or liver alone (with the remainder of the body shielded). Figure 2 shows the results of these studies. Here the effects of radiation of body alone are shown in relation to the effect of head irradiation.

It can be seen that doses of 100 or 200 R body or liver radiation produced no changes in the enzyme activity in adult liver, while doses of 500 R and above produced marked suppression of the hepatic enzyme system. The maximal amount of radiation dose received by the liver when 1,000 R was given to the head alone, was 50-60 R. Since there was no inhibition of enzyme activity even up to 200 R of body irradiation, it is evident that the inhibition of the hexobarbital metabolising enzyme system noted in the adult rats after 1,000 or 2,000 R head irradiation is an abscopal effect and is not a result of direct action of radiation on liver. The effect seen at the higher radiation doses may be more complex and may include both direct and abscopal type of effects.

Hypophysectomy in early postnatal life

† No inhibition was noted in an earlier experiment when the enzyme activity was measured employing the reaction mixture of Conney *et al* [J. Pharmacol., v130, 1 (1960)] but using whole homogenates in place of the microsomal fraction. Even in the control rat, enzyme activity was very low (1 μ M/g/hr) by this method, which may explain the failure to detect any inhibition in the irradiated sample. In contrast, it should be noted that the reaction mixture employed in the present studies yielded a value of 12-14 μ M/g/hr for adult male control rats. It appears that the low enzyme activity seen in the earlier experiment may be attributed to the differences in the amount of tissue (500 mg) and cofactors in the reaction mixture.

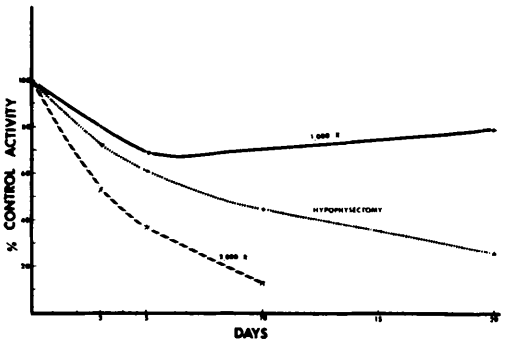

FIG. 3. The response of the hexobarbital metabolising enzyme system in adult male rat liver to a) 1,000 R head x-irradiation, b) 2,000 R head x-irradiation and d) hypophysectomy are compared.

has been reported to suppress the normal developmental increase of enzyme activity seen in male rats(8). It is shown in the present studies (Fig. 3) that hypophysectomy in adult rats (65 days old) also results in an inhibition of the hepatic enzyme system. Although the mechanisms underlying the abscopal effects of head irradiation on liver enzyme activity cannot be outlined yet, collateral evidence suggests that radiation impairment of the hypophyseal regulation may be responsible for the distant effects observed. It is also apparent from our results that the central nervous system exerts a regulatory role not only in the development but also in the maintenance of an optimal level of enzyme activity in liver.

Summary. Exposure to x-irradiation *in utero* or during the early postnatal life (total body or head alone) produced a suppression of the development of hexobarbital metabolising enzyme system in liver. Abscopal inhibition of the hepatic enzyme system after x-irradiation was also noted in adult male rats, but with higher radiation doses, suggesting that the central nervous system plays a regulatory role not only in the development but also in the maintenance of optimal level of enzyme activity in liver.

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Serological Specificity of Types A and B Botulinal Toxins and Antitoxins. (32588)

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Types A and B botulinal toxins and antitoxins appear to be quite specific as determined by animal neutralization tests(1). Multiple precipitin bands and cross-reactions have been observed, however, in gel-diffusion tests. Lamanna and Lowenthal(2) showed that type A crystalline botulinal toxin formed two precipitin bands with type A antitoxin in the Oudin gel-diffusion test and one band with type B antitoxin. They demonstrated that one of the homologous bands and the cross-reacting band were attributable to the hemagglutinin in type A toxin and its antibody present in the homologous and heterologous antitoxins. Gendon(3) demonstrated numerous bands between crude type A toxin