Microsomal Epoxidation of Aldrin to Dieldrin in Rats

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Liver microsomes contain mixed-function oxidases (mfo) which are responsible for the oxidation of various drugs and insecticides. The exact chemical nature of this system has not been determined, although it is known to contain, in addition to several enzymes, cytochrome P-450 which requires oxygen and NADPH for its action. It has also been shown that many compounds are able to stimulate or induce the mfo activity of liver microsomes, ranging from barbiturates to tranquilizers, insecticides, and polycyclic hydrocarbons (CONNEY and GILMAN, 1963; CONNEY, 1965; REMMER, 1959). The increase in oxidative activity of liver microsomes is associated with an increase of cytochrome P-450, shown independently by REMMER and MERKER (1965) and ORRENIUS (1965). GELBOIN and BLACKBURN (1963) and CONNEY (1957) have shown that this increase in enzyme activity is due to the synthesis of new enzyme protein in the microsomes. It is also known that the mfo activity of the microsomes varies with the compounds used for induction.

During the past few years considerable work has been carried out on the epoxidation of aldrin to dieldrin. An increase in the epoxidation of aldrin and heptachlor as a result of induction with DDT analogs in rats and quail is reported by GILLETT et al. (1966), and with other inducing agents in different animal species, both in vivo as well as in microsomal systems, by several other workers (GILLETT, 1968; GILLETT and CHAN, 1968; PLAPP and CASIDA, 1970; HODGSON and PLAPP, 1970; and YU and TERRIERE, 1971, 1972). However, studies on the epoxidation of aldrin to dieldrin as a result of induction of hepatic microsomal systems with phenobarbital in rats appear to be lacking. In the present investigation, therefore, comparative studies on the conversion of aldrin to dieldrin were carried out in vitro using liver microsomes obtained from rats, pre-treated and not pre-treated with sodium phenobarbital.

MATERIALS AND METHODS

Male rats, Sprague-Dawley derived strain (140-150 g), obtained from Flow Laboratories, Dublin, Va., were employed in the present study. For induction prior to preparation of microsomes, each rat was given 3 daily intraperitoneal injections of sodium phenobarbital (75 mg/kg) prepared fresh daily in de-ionized

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distilled water shortly before use. Twelve hrs after the last injection, rats were killed by a blow on the head, livers were removed immediately, weighed, and rinsed free of blood in chilled sucrose solution. The chilled livers were minced and homogenized in a Potter-Elvehjem homogenizer in 0.25 M sucrose at 0°C to make a 10% homogenate. The microsomes were prepared according to the procedure described by REMMER et al. (1967) by subjecting the liver homogenate to fractional centrifugation at 4°C in a Beckman Spinco Model L-2 Ultracentrifuge. At least two rats were used for each preparation of liver microsomes. The microsomal pellet thus obtained was washed with 0.15 M KCl by rehomogenizing and centrifuging, and suspending the final pellet in 0.1 M phosphate buffer, pH 7.4.

For incubation of aldrin as substrate with rat liver microsomes, the procedure as described by WONG and TERRIERE (1965) was adopted with modifications, the major change being in the use of phosphate buffer instead of Tris-buffer. A typical incubation flask contained NADP (1.8 μmoles) and glucose-6-phosphate (18 μmoles) in 1.0 ml and glucose-6-phosphate dehydrogenase (0.4 Kornberg units) in 0.4 ml of buffer, a known amount of microsomes suspended in buffer, plus a sufficient amount of phosphate buffer to make a total volume of 6 ml. The microsomal protein concentration was estimated by the method of LOWRY et al. (1951), and cytochrome P-450 content by carbon monoxide difference spectra (OMURA and SATO, 1964) using a recording spectrophotometer (Spectronic 505, Bausch and Lomb). The amount of cytochrome P-450 was calculated as nmoles/mg microsomal protein, using an extinction co-efficient of 0.091.

A constant amount of aldrin (10 μg) in 0.02 ml methyl cellosolve was added to the incubation flask which contained microsomes and co-factors and incubated at 37°C in a Dubnoff metabolic shaking incubator for different time intervals. After the incubation period, the reaction was stopped by adding 5 ml hexane to the flask and extracting with 10 ml portions of hexane, using a total volume of 30 ml. The hexane extract was dried over anhydrous sodium sulfate. One μl of this extract was injected into a gas chromatograph (Packard Instruments) equipped with an electron capture detector. The chromatographic conditions were: 18" x 1/4" glass column containing 10% DC 200 on Anakrom AB 50/60 mesh; column temperature 195°C; detector 220°C; and gas flow (nitrogen) 60 ml/min.

Aldrin (HHDN, purity 99.7%) and dieldrin (HEOD, purity 99.9%) from Shell Chemical Co. were used as reference standards; the percentages of each were calculated by measuring peak heights on the chromatograms.

RESULTS AND DISCUSSION

Injection into the gas chromatograph of hexane extracts of microsomes from rats not pre-treated with phenobarbital incubated with aldrin gave two peaks with the same retention times as aldrin

and dieldrin standards, approximately 4.5 min for aldrin and 8.2 min for dieldrin, with a relative difference of 1.82. However, the conversion of aldrin to dieldrin by liver microsomes from uninduced rats was limited even when the substrate (aldrin) was incubated for increased intervals of time or with increased amounts of microsomal protein (Table 1). This suggests that there is a maximum conversion of aldrin to dieldrin by liver microsomes from rats not pre-treated with phenobarbital, regardless of the microsomal protein concentration or incubation time.

Conversion of aldrin to dieldrin by microsomes from rats not pre-treated with phenobarbital increased linearly with increasing amounts of microsomal protein from 2 to 12 mg, but a further increase in the microsomal protein concentration resulted in inhibition of the epoxidation of aldrin. GILLETT et al. (1966) also reported that at shorter incubations and lower amounts of microsomal protein the epoxidation of aldrin to dieldrin increased linearly, but at longer incubation times and higher protein concentrations, the rate of aldrin disappearance usually exceeded the rate of appearance of dieldrin.

When microsomes obtained from rats pre-treated with phenobarbital were incubated with aldrin conversion to dieldrin was complete, as shown by chromatograms which showed only one peak, whose retention time was the same as standard dieldrin. This suggests that induction with phenobarbital increases the oxidative activity of rat liver microsomes. This is apparent from the fact that induction increases mfo activity, as estimated directly from the alteration of epoxidation of aldrin to dieldrin, and indirectly from an increase of the cytochrome P-450 level of the liver microsomes. The amount of cytochrome P-450 of the microsomes from induced rats was 2.512 ± 0.137 (SEM) nmoles/mg microsomal protein, as compared to uninduced rats which gave only 0.871 ± 0.022 (SEM) nmoles/mg microsomal protein, an increase of approximately 190%. OPPELT et al. (1970) have also shown that in rats treated with phenobarbital for 4 days, cytochrome P-450 increased by approximately 205%. The increase in mfo activity of liver microsomes from rats pre-treated with phenobarbital appears to be so significant that even when only 4 mg microsomal protein were incubated for a period of just 15 min, epoxidation of aldrin to dieldrin appeared to be almost quantitative (98.2%) (Table 1). It should be emphasized that the maximum amount of aldrin converted to dieldrin was only 60.9% even when 12 mg microsomal protein were used from rats not pre-treated with phenobarbital.

NAKATSUGAWA et al. (1965) also studied the epoxidation of aldrin to dieldrin and found that when 2.5×10^{-5} M aldrin was incubated with rabbit liver microsomes only 57% was converted to dieldrin, while 43% remained unchanged. WONG and TERRIERE (1965) studied the effect of substrate concentration by incubating 10 to 40 µg of aldrin with the rat hepatic microsomes equivalent to 750 mg of fresh liver weight; they found that 10 µg was the optimum amount of substrate, which gave relatively greater conversion of aldrin to dieldrin than higher quantities.

TABLE 1

Percent conversion of aldrin to dieldrin when 10 µg of aldrin was incubated at 37°C with various concentrations of hepatic microsomal protein from induced and uninduced rats for various incubation times

	Percent conversion of aldrin to dieldrin	
Microsomal protein concentration (Incubation time 4 hr)	Microsomes prepared from rats not pre-treated[a/]	Microsomes prepared from rats pre-treated with phenobarbital[b/]
2 mg	12.2	96.3
4 mg	28.6	98.5
8 mg	36.0	100.0
12 mg	60.9	100.0
16 mg	40.2	100.0
20 mg	29.7	100.0
Incubation time (4 mg microsomal protein)		
15 min	39.4	98.2
30 min	45.0	97.8
60 min	40.0	97.3
120 min	49.0	97.3
180 min	39.0	97.3
280 min	40.0	97.5

a/ Cytochrome P-450 = 0.871 ± 0.022 (SEM) nmoles/mg microsomal protein

b/ Cytochrome P-450 = 2.512 ± 0.137 (SEM) nmoles/mg microsomal protein

This study has demonstrated that the mfo system of rat hepatic microsomes is dramatically enhanced by induction with phenobarbital. Regardless of the protein concentration or the reaction time there appears to be a maximum limit for the oxidation of aldrin to dieldrin in microsomes from uninduced rats. After induction, however, conversion is nearly quantitative. Furthermore, substrate inhibition has been demonstrated when the microsomal protein concentration is increased beyond the optimum value. Workers using the conversion of aldrin to dieldrin as a measure of the activity of a microsomal system would do well to establish these relationships in advance of drawing conclusions about their system on this basis.

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