Aldehyde Oxidase Activity during the Perinatal Period

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Abstract □ Aldehyde oxidase activity was studied during the perinatal period. Data indicated that hepatic tissue from nonpregnant, pregnant, fetal, and newborn rabbits did not differ significantly in its ability to reduce 2,6-dichloroindophenol in the presence of acetaldehyde substrate. In contrast, the placental tissue demonstrated only minimal aldehyde oxidase activity.

Keyphrases □ Aldehyde oxidase activity—perinatal period □ 2,6-Dichloroindophenol, acetaldehyde presence—analysis method □ Colorimetric analysis—spectrophotometer

This study concerns the activity of aldehyde oxidase during the perinatal period. Johns *et al.* (1) have demonstrated that the folic acid antagonist methotrexate is a substrate of liver aldehyde oxidase in various lower animals. The level of enzyme activity correlates well with differences in toxicity of various species to the antifolate. Rabbit liver aldehyde oxidase also has the ability to oxidize a wide variety of substrates, many of which are commonly used drugs (2, 3). Aldehyde oxidase appears to be the same as quinine oxidase (2), but distinct from xanthine oxidase (4).

This investigation was undertaken to gain further insight into factors altering drug toxicity during the perinatal period. Metabolism of a drug usually, but not always, results in drug detoxification. These metabolic changes may alter not only drug potency, but may also change drug distribution. Oxidative processes (Phase I) have been found uniformly to be relatively deficient in activity in newborn and fetal animals, while conjugation and synthetic reactions (Phase II) are more varied (5). Pregnancy and the hormones associated with the condition have been shown to alter the metabolism of certain drugs by both animals and humans (6, 7). It is also of interest that placental tissue shares many of the metabolic functions of the liver during gestation (8). Therefore, the authors examined the activity of rabbit liver aldehyde oxidase during gestation and in the newborn and determined aldehyde oxidase activity in the placenta.

Data to be presented indicate that the activity of aldehyde oxidase is very similar when comparison is made between liver from pregnant, nonpregnant, fetal, or newborn rabbits. However, the placentas from pregnant rabbits at term had very low aldehyde oxidase activity.

EXPERIMENTAL

Aldehyde oxidase was prepared from livers of nonpregnant, pregnant, fetal, and newborn rabbits and from placentas by the ammoniacal ammonium sulfate fraction method described by Johns *et al.* (1). This procedure has been estimated to result in a 10- to 15-fold concentration of enzyme activity. In a few cases the enzyme preparations were frozen for subsequent assay. In these instances, all the tissues (fetal, adult, and placental) were handled in a similar manner. There appeared to be little difference between enzyme activity in fresh tissue preparations and those frozen for 24–48 hr. Enzyme activity was determined by the method of Mahler (9) which is based on the fact that 2,6-dichloroindophenol is reduced by acetaldehyde in the presence of the enzyme. The reduction of 2,6-dichloroindophenol was followed spectrophotometrically at 600 mμ. Protein nitrogen was determined by a modified Kjeldahl method (10) and the enzyme activity was expressed in terms of absorbance change per milligram of protein.

Each experiment consisted of placentas taken from pregnant animals, and the livers from nonpregnant, pregnant, and fetal New Zealand white rabbits. In most experiments, six to eight placentas and fetal livers were collected. All pregnant animals were within five days of term. The aldehyde oxidase activity present in livers of newborn New Zealand rabbits (1 day old) was also determined.

RESULTS AND DISCUSSION

The results obtained from five different sets of experiments are seen in Fig. 1. The ordinate is expressed as change in absorbance per milligram of protein nitrogen while the abscissa is time in seconds. The standard errors indicate that the results were rather variable. It can be seen that although the hepatic tissue from nonpregnant rabbits usually had a higher enzyme activity, it was not significantly different from the activity seen in other hepatic tissues. An experiment utilizing livers from newborn rabbits demonstrated aldehyde oxidase activity comparable to that seen for fetal and adult livers. There was no significant difference in activity of the enzyme prepared from livers of nonpregnant, pregnant, fetal, or newborn rabbits. In contrast to these data from hepatic tissues, the placenta showed very little enzyme activity in all experiments.

It is reasonable to conclude that other substrates of aldehyde oxidase would be handled in a manner similar to acetaldehyde by these same tissues, but such data are not yet available. Studies of the hydroxylation of methotrexate by perinatal tissues are now in progress.

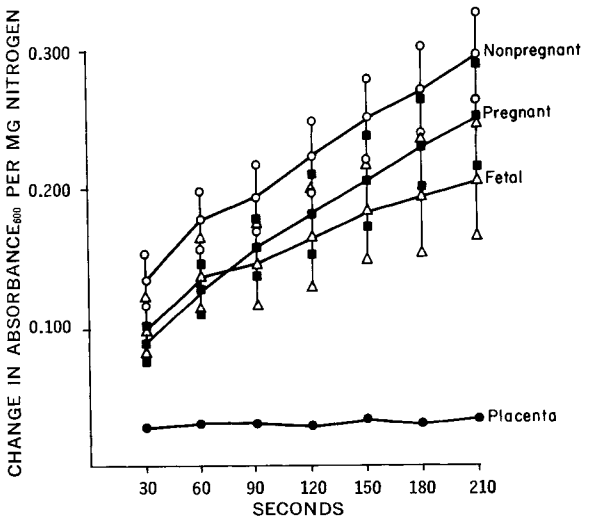

Figure 1—*Changes in the absorbance$_{600}$ per milligram nitrogen are plotted along the ordinate and time in seconds is plotted along the abscissa. There are no statistically significant differences between enzyme activity of nonpregnant* (○), *pregnant* (■), *or fetal hepatic tissue* (△). *The placental tissue* (●) *was essentially devoid of activity. Data presented represent the mean (middle symbol) of five determinations (animals) plus and minus the standard error (same symbol above and below mean).*

The tissue and subcellular distribution of mammalian aldehyde oxidizing enzyme has recently been studied by Deitrich (11). Activity was found in liver, adrenal, intestine, kidney, ovary, testis, adipose tissue, uterus, heart, lung, brain, spleen, skeletal muscle, seminal vesicles, and bladder. Liver exhibited the greatest degree of activity. Thus, the placenta appears to be unique in not having the capacity to oxidize aldehydes.

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Identification of Low Molecular Weight Aliphatic Esters from Rates of Alkaline Hydrolysis

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Abstract □ The second-order rate constants for the alkaline hydrolysis of 27 low molecular weight aliphatic esters have been determined at 25° in 37.27% acetone–water solution. These rate constants are characteristic of the entire ester molecule, and therefore provide more information for identification purposes than do other chemical methods for the characterization of esters.

Keyphrases □ Aliphatic esters, low molecular weight—identification □ Alkaline hydrolysis rates—aliphatic ester identification □ Rate constants, second-order—ester hydrolysis

Kinetic measurements provide a potentially powerful approach to the characterization of organic compounds because rate constants can be very sensitive to minor structural alterations. Moreover, the technique is simple, inexpensive, and it seldom requires large samples. Earlier papers in this series have described the identification of alcohols from rates of alkaline hydrolysis of their 3,5-dinitrobenzoate esters (1), of sugars from their rates of oxime formation (2), and of aliphatic amines from rates of acylation by cinnamic anhydride (3).[1] For this method to be practicable, first-order or pseudo-first-order kinetics must be observed, reaction conditions and the method of analysis must be common to all members of a class of compounds, and a large number of rate constants must be determined under these common conditions. This note reports rate constants for the alkaline hydrolysis of 27 aliphatic carboxylic acid esters of low molecular weight.

[1] This paper is part IV in the series "Precise Kinetic Measurements Applied to the Identification of Organic Compounds." For Part III, see *Reference 3*.

The reactions were followed by the pH-stat method, using a pH meter as a manually operated pH-stat (4). The solvent was an acetone–water mixture (see *Experimental*), and the esters studied include all of those that are soluble to the required extent in this solvent. First-order rate constants were obtained from Guggenheim plots (5), and were converted to second-order constants by dividing by the essentially constant concentration of hydroxide ion; the pH meter was used merely as an indicator to keep the pH constant, rather than as a source of information about hydroxide-ion activity (which would be questionable in this solvent). It is extremely important to bear in mind the difference between second-order rate constants for ester hydrolysis as calculated on the concentration and activity bases (6).

Table I gives the esters, their observed atmospheric boiling points, the mean second-order rate constants, and the standard deviations (of a single observation). The means are based on three to five determinations. It is seen that the reproducibility is about 1–2% for most esters.

These 27 esters represent only seven different values of saponification equivalent, and many pairs of them (especially isomers) have similar boiling points. But despite these similarities, most of the esters can be differentiated by a combination of boiling point and rate constant. Of course, one should submit authentic samples to measurement to check these values under one's own conditions, especially when a comparison of very similar constants is to be made. The method described here is unusual in that it is probably only the second general chemical method for characterizing