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The Influence of Cage Bedding on the Metabolism of Sulphobromophthalein Sodium by an Hepatic Cytosol-located Enzyme System

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Abstract

The use of cage bedding prepared from pinewood shavings has been shown to be associated with an increase in the activity of sulphobromophthalein sodium (BSP) *S*-aryltransferase in the hepatic cytosol in rats housed on this substance. This increase was associated with enhanced secretion rates of dye into the bile due to an elevation in the biliary excretion rate of conjugated BSP. Analysis of the hepatic dye content at the time of maximal excretion of BSP into the bile indicated that this phenomenon was due to increased intrahepatic conjugation of BSP. This observation emphasizes the importance of considering environmental factors that may influence results when designing experiments on hepatic metabolism.

Introduction

The hepatic microsomal enzyme systems are responsible for the metabolism of a wide variety of exogenously administered compounds. For some time it has been observed that these compounds are often metabolized at widely varying rates by individual humans and by different animal species and strains (Davies *et al.* 1969). It is probable that the varying rates of metabolism are related to a variation in activity of the microsomal enzymes. The administration of various drugs and other foreign compounds has been found to alter the activity of these enzyme systems (Conney 1967). More recently it has been appreciated that environmental factors such as food additives, cigarette smoke (Conney *et al.* 1967; Welch *et al.* 1968) and cage bedding (Ferguson 1966; Vesell 1967) can also influence enzyme activity in humans and animals.

By contrast, less is known about factors that influence the functioning of enzymes in the cytosol (the soluble fraction of the liver cell). This study, which arose out of an investigation of the transport system for sulphobromophthalein sodium (BSP), illustrates the influence of one certain exogenous factor on the BSP *S*-aryltransferase system (the enzyme that is responsible for the conjugation of BSP with glutathione).

Materials and Methods

Albino Wistar female rats weighing 170–250 g were used in these studies. They were housed for a period of 1 week prior to experimentation on either a standard pine sawdust bedding (prepared from *Pinus radiata*) used in the animal facility or on bedding consisting of small low-density polyethylene granules (made from ethylene polymerized with organic peroxides). Unconjugated BSP was purchased commercially and dissolved in sterile isotonic saline prior to intravenous administration.

Hepatic Enzyme Studies

Animals were anaesthetized with diethyl ether and through a midline abdominal incision were exsanguinated via the aorta. The liver was rapidly removed, blotted and weighed before being transferred to chilled beakers. Using the spectrophotometric assay described by Goldstein and Combes (1966), the conjugating activity of the BSP *S*-aryltransferase enzyme was measured on aliquots of cytosol prepared by ultracentrifugation of homogenates of these livers. Briefly, livers were homogenized with a volume of 0·1M pyrophosphate buffer, pH 8·2, equal to four times the weight of the liver. These homogenates were first spun at 12800 *g* for 10 min at 0°C in a MSE Mistral 6L centrifuge. The supernatant so obtained was then centrifuged at 144000 *g* for 30 min at 0°C in an Hitachi preparative ultracentrifuge model 55PA.

Results are expressed as micromoles of BSP conjugated per milligram of cytosol protein per 5 min as well as per gram of liver wet weight. The hepatic cytosol protein concentration was measured by the method of Lowry *et al.* (1951).

Biliary Excretion Studies

For these studies each animal was prepared with an external femoral vein cannula for the administration of dye. An abdominal incision was made and then a polyethylene cannula (PE-10) was placed in the common bile duct. Next an indwelling arterial cannula (PE-50) was inserted in the abdominal aorta by way of the iliac artery for the collection of blood samples. The abdominal incision was then sutured. Diethyl ether was used for surgical preparation and maintenance anaesthesia throughout infusion studies. Body temperature was monitored by means of a rectal probe attached to a Yellow-Spring Tele-thermometer and maintained at 37–38°C by means of a heating pad beneath the animal.

Dye was administered as a priming dose of 1·8 μmol/100 g followed by a constant infusion of 0·22 μmol per 100 g per minute for a period of 60 min. At the end of the experiment animals were exsanguinated from the abdominal aorta. The liver was removed, rapidly blotted, weighed and immediately frozen in liquid nitrogen. The concentration of BSP in plasma samples was measured by the method of Gaebler (1945).

Bile was collected into previously tared bottles every 10 min throughout the constant infusions. The bile volume was considered equivalent to the weight of bile. Dye concentration was measured in a Unicam spectrophotometer set at 580 nm on appropriately diluted bile samples to which alkali had been added. The total BSP excretion in any sample was calculated as the product of BSP concentration and bile volume. The rate of dye excretion recorded for each animal was calculated by averaging the values from each collection period after a steady excretion rate had been achieved (defined as a variation in consecutive collection periods of 10% or less). This usually occurred within 20–30 min of the commencement of the infusion. For chromatography, aliquots of bile were applied to Whatman No. 1 filter paper. These chromatograms were developed by descending chromatography and the subsequent determinations of the distribution of BSP compounds in bile were carried out by previously described methods (Combes 1959). BSP metabolites identified by this technique are referred to collectively in this paper as conjugated BSP.

Hepatic Uptake Studies

The rate of initial hepatic uptake of 12 μmol/100 g of unconjugated BSP given intravenously was measured in anaesthetized temperature-controlled animals housed on either pine sawdust or polyethylene granules. Blood was obtained from the aorta from 1·5 to 2 min and the livers were removed at 2 min, blotted and weighed. BSP content was measured as outlined below and results were expressed as μmoles per gram of liver wet weight.

Determination of Hepatic BSP Content

Triplicate samples of liver were extracted using a method (Whelan and Combes 1971) involving extraction with methanol. Total BSP content was measured on the methanol extracts and correction was made for the plasma content of BSP. For chromatography this was followed by heptane partition, concentration by flash evaporation and application to Whatman No. 1 filter paper (Whelan and Combes 1971). BSP compounds in plasma were similarly analysed chromatographically as previously described (Whelan and Combes 1971).

Statistical Analysis

The Mann–Whitney *U*-test for non-parametric statistical analysis (Siegel 1956) was used for evaluation of the results of the above experiments.

Results

Studies of Enzyme Activity

Since agents that are known to induce microsomal enzyme activity have at times been noted to increase hepatic size (Klaassen 1969), total protein content (Argyris 1968), DNA and RNA concentrations (Juchau and Fouts 1966; Argyris 1968) and hepatic cytoplasm (Staubli *et al.* 1969), the results in these studies are expressed as enzyme activity per milligram of cytosol protein as well as per gram of liver. The results (Table 1) show that animals bedded on pine sawdust had a significantly higher ($P < 0{\cdot}05$) BSP *S*-aryltransferase activity than their counterparts bedded on plastic granules. The functional significance of this observation was assessed by comparison with the results of studies on BSP transport across the liver.

Table 1. Effect of cage bedding on BSP *S*-aryltransferase activity

Values given are means ± standard deviation

Type of cage bedding	No. of animals	Mean body weight (g)	Mean liver wt (% of body wt)	Hepatic cytosol protein concn (mg/g liver)	Enzyme activity (μmol BSP conjugated/5 min): Per gram of liver	Per milligram of protein
Pine sawdust	5	196±35	3·3±0·3	25·0±2·2	16·96±4·40*	0·68±0·18*
Polyethylene granules	5	207±22	3·2±0·2	24·4±2·6	11·22±1·46	0·46±0·06

* Significantly higher ($P < 0{\cdot}05$) than the comparable value for animals housed on polyethylene granules.

Biliary Excretion Studies

Animals housed on pine sawdust attained a higher concentration of total dye in bile, and a higher maximal rate of biliary excretion of dye, when compared with the group of animals housed on plastic granules (Table 2). Animal weights, liver weights, bile flow, total hepatic content of BSP and terminal plasma levels of the dye were similar in both groups.

Table 2. Maximal biliary excretion rates for BSP

Values given are means ± standard deviation

Type of cage bedding	No. of animals	Mean body weight (g)	Mean liver wt (% of body wt)	Bile volume (ml/100 g/ 10 min)	Concn of dye in bile (μmol/ 100 ml)	Maximal excretion (μmol/100 g/ 10 min)	Concn of dye in plasma (μmol/100 ml)	Concn of dye in liver (μmol/g)
Pine sawdust	5	187±14	3·6±0·4	0·10±0·01	1645±212*	1·56±0·11*	18·2±5·7	0·55±0·02
Polyethylene granules	5	205± 4	3·7±0·3	0·10±0·01	1324±185	1·27±0·24	21·6±6·5	0·51±0·07

* Significantly higher ($P < 0{\cdot}05$) than the comparable value for animals housed on polyethylene granules

Composition of BSP Compounds in Liver and Bile

A similar percentage of the dye excreted into bile was in the conjugated form in both groups of animals (Table 3). However, since the total excretion of dye into bile was

greater in the group housed on pine sawdust (see Table 2), the amount of conjugated dye excreted was also higher in the group on pine sawdust (Table 3).

Although the total concentration of dye in the liver at the end of 1 h of intravenous perfusion was similar in both groups (see Table 2), a significantly greater amount was in the conjugated form in animals bedded on pine sawdust.

Table 3. Distribution of BSP compounds in liver and bile

Values given are means ± standard deviation

Type of cage bedding	No. of animals	Amount of dye in liver				Amount of dye excreted into bile per 10 min			
		In conjugated form		In unconjugated form		In conjugated form		In unconjugated form	
		%	μmol/g	%	μmol/g	%	μmol/100 g	%	μmol/100 g
Pine sawdust	5	71·5* ±4·5	0·39* ±0·03	28·5* ±5·4	0·16 ±0·03	77·8 ±3·6	1·21* ±0·10	22·2 ±3·6	0·35 ±0·07
Polyethylene granules	5	59·7 ±3·2	0·31 ±0·03	40·3 ±3·2	0·20 ±0·04	79·2 ±2·2	1·00 ±0·20	20·8 ±2·2	0·26 ±0·05

* Significantly different ($P < 0·05$) from the comparable value for animals housed on polyethylene granules.

Hepatic Uptake Studies

Although livers from animals housed on pine sawdust tended to contain a higher amount of dye 2 min after intravenous injections of single doses of unconjugated BSP ($0·44 \pm 0·06$ *v.* $0·36 \pm 0·06$ μmol/g liver), there was no statistically significant difference between mean values in each group ($P = 0·06$).

Discussion

These studies demonstrate that the metabolism of BSP, a dye which is conjugated by an hepatic cytosol-located enzyme system, is influenced by an environmental factor, namely the type of product used for cage bedding. In these experiments the activity of the BSP *S*-aryltransferase system, as assessed by *in vitro* techniques, was stimulated by pine sawdust to levels higher than those obtained with plastic bedding. This enhanced enzyme activity appears to be functionally important in that it was associated with significant increases in maximal rates of dye transfer into bile in rats perfused with unconjugated BSP, due predominantly to an increased secretion of conjugated forms of the dye.

Since conjugated and unconjugated BSP share a common transport step in the movement of BSP across the liver cell into bile (Whelan and Combes 1971), it could be argued that enhanced secretion of conjugated BSP might result from an effect of the pine sawdust on the excretory apparatus rather than being the result of increased metabolism. However, examination of the intrahepatic disposition of dye at a time when maximal biliary excretion rates of BSP were being achieved revealed that most of the dye in the liver at that time was in the conjugated form in animals bedded on pine sawdust. This observation indicates that enhanced secretion of conjugated BSP is a result of increased intrahepatic conjugation of BSP.

These findings have a practical application in that they provide additional evidence for the need to ensure that the design of studies to assess hepatic metabolism should allow for environmental factors such as cage bedding material which may influence the results of such experiments.

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