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INSUFFICIENT CONJUGATE GLUCURONIDATION ACTIVITY: A POSSIBLE FACTOR IN POLYCHLORINATED BIPHENYL (PCB) TOXICITY

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SUMMARY

Considerable evidence indicates that phenolic and biphenolic compounds are detoxified and excreted primarily via conjugate glucuronidation in man. Since fetuses, neonates and certain enzyme deficient adults have significant functional deficiencies in their capacities to excrete toxic compounds via conjugate glucuronidation, it is predicted that these groups of individuals are biochemically predisposed to accumulate polychlorinated biphenyls (PCB's), a widely distributed and highly toxic environmental contaminant.

INTRODUCTION

Since the late 1960's when polychlorinated biphenyls (PCB's) were first recognized as a potentially widespread environmental contaminant, hundreds of research papers have been published concerning their structure, industrial uses, presence in the environment, toxicity to numerous animals from insects to man, tissue storage, metabolism and excretion (1,2). As a result of such data accumulation, regulatory agencies are now trying to approach the problem of standard setting with respect to PCB's. Standards for air, water and food are necessary if the total human exposure is to be regulated and controlled. One important component in standard setting is a consideration of the individuals within the population who may be at high risk to the pollutant because of various genetic, physiological, psychological and behavioral traits. Individuals lacking the ability to detoxify and excrete PCB's represent such a high risk group since they will be unable to prevent undue accumulation of toxic levels of PCB's within the body.

It is the intention of this paper to: (1) establish the theoretical basis for why individuals lacking a sufficiently functional conjugate glucuronidation process may tend to accumulate unusually high levels of PCB's; (2) identify and quantify (where possible) those groups of individuals composing this high risk group; and (3) stimulate clinical trial of the proposed hypothesis.

GLUCURONIDATION IN THE EXCRETION OF TOXIC SUBSTANCES

Mammals, including rabbits, dogs, and humans, have been reported to excrete PCB's, in part, by conjugate glucuronidation and/or sulfonation (1,3). The urinary excretion of biphenyl and 4 chlorobiphenyl has been studied in rabbits. Biphenyl glucosiduronic acid and 4-hydroxybiphenyl were isolated from urine. Rabbits fed 4-chlorobiphenyl excreted 4-(p-chlorophenyl)-phenol and 4-chlorobiphenyl glucosiduronide. Twice as much 4-chlorobiphenyl as biphenyl was excreted as the glucosiduronic acid derivative. It was suggested that other low chlorinated biphenyls are excreted in a similar manner (3).

In dogs injected with 2,4,4'-trichloro-2'hydroxydiphenyl ether, nearly 100% of the material excreted in their urine and feces over a 5-day period appeared as the glucuronide or sulfate conjugate. Human adults similarly exposed excrete 65% in urine and 20% in feces after intravenous injection. It is excreted as a free compound or as a glucuronide (1). Other evidence suggests that some chlorinated biphenyls are hydroxylated by species such as the rat and pigeon. No evidence of reductive dechlorination was observed in either the trout, rat or pigeon (4).

During the excretory process foreign substances (such as PCB's) usually first undergo several metabolic transformations including oxidations, reductions, and hydrolysis. It often happens that following an oxidative, reductive or hydrolytic reaction, conjugation of the foreign substance with either glucuronic acid, sulfate, glycine, cysteine, methyl or acetyl occurs. Of these reactions, glucuronic acid conjugation is probably the most important in the animal kingdom since it occurs extensively in man and all laboratory animals with the exception of the cat. In the cat, glucuronic acid conjugation is not entirely absent, but occurs at a low level compared with other mammalian species. Conjugations are generally regarded as detoxification mechanisms, for compounds which undergo these reactions are converted into products which are usually less toxic and more rapidly excreted than their precursors (5,8).

The widespread occurrence of glucuronic acid conjugation may be due to the facility with which glucuronic acid can be produced in the body from carbohydrate sources and the variety of chemical groups to which glucuronic acid can be transferred enzymatically. The other conjugation mechanisms are more restricted in their occurrence and this is probably because of the limited availability of the conjugating agents such as glycine and cysteine (via glutathione) and to the small number of chemical groupings to which conjugating agents such as sulfate, glycine, cysteine, methyl, and acetyl can be transferred. The activity and amount of the transferring enzyme concerned in these conjugations are probably also limiting factors. Glycine conjugation is confined mainly to aromatic carboxyl groups, sulfate to phenolic hydroxyl groups an-

conjugation to some aromatic hydrocarbons and halogenated hydrocarbons, and methylation to certain hydroxyl and amino groups, and acetylation to some amino and hydrazine groups. However, glucuronic acid conjugation can occur with several types of hydroxyl, amino, and carboxyl groups and with sulfhydryl groups (5-8).

The effect of conjugation of a substance with glucuronic acid is to produce a strongly acidic compound which is more water-soluble at physiological pH values than the precursor. The majority of foreign substances are ultimately cleared from the body via their excretion in the urine and bile, most often in the form of polar conjugates such as glucuronides with the avenue of excretion of the glucuronide probably varying with the species of animal considered (5, 6).

GROUPS AT HIGH RISK TO PCB's

A. Fetuses and Neonates: Immature Enzyme Detoxification Systems

It is apparent that individuals lacking the ability to form either conjugate glucuronides or sulfates or both will be at high risk with respect to toxic compounds of a phenolic nature. The occurrence of low levels of glucuronide formation in the cat has been suggested as an explanation of the widely observed phenomenon in veterinary literature (9-12) that phenolic compounds should not be used on or around cats because of increased incidence of toxicity in this species. Furthermore, intravenous injections of phenol in cats, pigs, dogs and goats revealed that cats were at least 2 times more sensitive with respect to fatality caused by the phenol (13). Toxicity in this study was related to the partial deficiency in the cat to conjugate phenol with glucuronic acid. The author concluded that the cat is more likely to be poisoned by acute doses of phenol and is more likely to become chronically affected with phenol toxicity due to the inability to rapidly form and excrete glucuronides. Biochemical studies with the cat have also indicated that conjugate glucuronidation is the major pathway for the detoxification and excretion of phenol (e.g., more than twice as effective as sulfonation) (13). Because cats are hypersusceptible to phenolic-like compounds as a result of their diminished capacity to form glucuronides, it is strongly suspected that human embryos, fetuses, and neonates (2 to 3 months old) which also are deficient in a functional conjugate glucuronidation system will also be predisposed to the toxic effects of phenolic compounds including PCB's (8,14,15).

Usually by the age of 2 to 3 months, adult levels of most enzyme systems are achieved (8,14,15). Unfortunately, this "developmental immaturity" in the unborn and the very young may predispose them to the toxic effects of certain substances since they may be unable to detoxify and excrete them as quickly as necessary (16). It has already been pointed out that human adults do excrete, in part, PCB's via conjugate glucuronidation. Clinical experience has shown that infants may respond differently to doses of drugs which are easily tolerated by older children and adults. Presumably this is because older children and adults have fully functioning enzyme detoxification systems while the mechanisms in the very young have broad significance since the absence of such mechanisms prevents the prompt elimination of toxic substances from the body. A further problem for the very young with regard to PCB's is that in addition to having a difficult time excreting PCB's they may also consume more PCB's per unit of body weight than at any other time during their life span. For example, samples of human milk from two cities in California contained average PCB levels of 60 ppb and 100 ppb, respectively. Based on a daily milk intake of 150 ml/kg, breast fed infants in California would ingest about 9 μg/kg/day of PCB's. A range of 1-3 μg/kg/day has been suggested as a "reasonable" level for an Acceptable Daily Intake (2,16). One μg/kg/day has been reported as being 100 times less than the lowest "no effect" level in animal studies (17). Thus, in children the margin is reduced to about a factor of 10 if excretory activity is equivalent with adults. However, as a result of the immature enzyme systems, the safety factor of 10 may be considerably reduced. How much is presently unknown.

A tragic example of toxicity arising from the inability to form glucuronides has been reported. The drug chloramphenicol, which is known to be metabolized in humans by the action of the glucuronic pathway, caused the death of more than 30 premature babies who had been treated with the antibiotic for infectious diseases. Theoretically, the premature babies were not able to conjugate the drug with glucuronic acid and thus the drug could only be slowly excreted and so tended to accumulate in the body to toxic proportions eventually leading to death (15).

An additional problem encountered by many neonates is that approximately five percent of the mothers of normal infants secrete milk which inhibits the activity of glucuronyl transferase (and thus the glucuronidation process) by more than 20 percent via the action of a steroid present in the breast milk. Inhibition of this glucuronyl transferase has been reported for up to 49 days after birth. Clinically these children have been reported to develop unusually severe neonatal jaundice. This condition develops because glucuronide formation which assists in the elimination of bilirubin (breakdown product of hemoglobin) is partially inhibited. Cow's milk does not contain sufficient amounts of this steroid to affect a noticeable inhibition of the glucuronidation process. Consequently about 5 per cent of the neonates breast fed would be expected to have their ability to excrete PCB's impaired (18). Administration of the antibiotic novobiocin has also been associated with unconjugated hyperbilirubinemia in infants (19). Novobiocin is a noncompetitive inhibitor of glycuronyl transferase activity *in vitro* (20). Thus, children receiving concomitant exposure to novobiocin and PCB's would be expected to have their ability to detoxify and excrete PCB's impaired.

B. Individuals with Genetic Deficiencies and Modified Liver Function

After the neonatal period, a broad range of conditions is associated with the improper or incomplete development of the glucuronide conjugation system. The usual physiological problem associated with these conditions is the inadequate detoxification and excretion of bilirubin. This

spectrum extends from the frequently occurring "mild" condition known as Gilbert's syndrome to the very rare, but severe and often fatal Crigler-Najjar syndrome (21).

Since the clinical effects associated with these syndromes are considered to be caused by metabolic disturbances of the glucuronide conjugation scheme, it is expected that PCB elimination in these individuals would be impeded. The population incidence of Gilbert's syndrome has been variously reported as 1 in 200 males (23), 7 percent based on examinations of 100 medical students (24) and 6 percent of 297 healthy individuals [252 healthy National Blood Transfer Service donors, and 47 healthy medical students (197 males and 102 females)] with no difference in frequency between males and females (25).

Concomitant exposure to PCB's and other drug-like chemicals may potentiate the toxic effects of PCB's. For example, rats and monkeys given SKF525A (β-diethylaminoethyl 2, 2 diphenylpentanoate, a non-specific inhibitor for many of the microsomal enzymes especially hepatic microsomal enzymes) during the initial 24 hours of exposure to PCB succumbed rapidly as compared to the control group (22). Of possible significance is the fact that SKF525A inhibits the proper functioning of the glucuronic pathway (15).

Individuals with liver infections may also be at high risk with respect to PCB's. For example, depression of glucuronide synthesis has been observed in humans with infectious hepatitis (15).

Even though PCB-like pollutants require a functional conjugate glucuronidation system for their excretion, this does not imply that such substances are merely passive molecules in this process. For example, synthesis of microsomal enzymes such as glucuronyl transferase can be stimulated by drugs such as phenobarbital. Phenobarbital treatments have been successfully employed with patients with a partial defect in bilirubin conjunction in order to reduce the levels of bilirubin in the blood (26,27,28,29). Thompson et al (30) demonstrated similar results with oral administration of dichlorodiphenyl trichloroethane (pp'DDT). Although it has been suggested that the beneficial effects of phenobarbital or DDT are caused by their induction of glucuronyl transferase in the liver (31), conclusive evidence still remains to be demonstrated (28,32,33). Finally, supportive evidence by Allan et al (22) has recently showed that phenobarbital treatment prior to PCB exposure may reduce PCB toxicity by stimulating liver enzyme induction in rats.

With regard to public health implications, it is still not resolved whether or not people with a partial glucuronyl transferase deficiency may accumulate significant quantities of PCB-like substances. These substances seem capable of inducing microsomal enzymes which lead to their (PCB's) metabolism and excretion. This constitutes a built-in safety feature which should help to prevent these individuals from accumulating PCB-like chemicals in their bodies. In contrast, individuals with the Crigler-Najjar syndrome seem to have an absolute deficiency of glucuronyl transferase, and thus do not have such a built-in compensatory safety system. However, it is not known how effective such a system is in those with the partial enzyme deficiency. It should also be noted that substances which require the activity of glucuronyl transferase for metabolism and excretion and which do not affect microsomal enzyme induction would be expected to accumulate within the body of individuals who have any type of deficiency of glucuronyl transferase.

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