Inositol and the Toxicity of Four Isomers of Benzenehexachloride for the Rat. (17993)

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The widespread distribution of i-inositol and its unknown roles in the maintenance of life processes have stimulated search for an antimetabolite which might elucidate these problems. Microbiological(1,2), toxicological(3-6), and physical data(7,8) indicate that γ-benzenehexachloride (γ-BHC) is not the structural analogue of i-inositol, as was originally proposed by Slade(9). Consequently, examination of the other available isomers of benzenehexachloride for toxicity and anti-inositol properties was undertaken.

Technics and the basal diet* were identical with those employed previously(6). Each isomer† was incorporated in the diet at the level of 0.6 mg/g food by solution in lard, while i-inositol replaced an equivalent weight of sucrose. Twenty weanling 20-25 day-old male rats weighing 44-50 g each were placed on each diet, fed *ad libitum,* and weighed twice weekly for 4 weeks. The variables in the diets, growth rates and mortality are presented in Table I.

Comparison of growth rates of animals on the basal diet with and without added inositol indicates that this strain of rat requires no dietary inositol under these experimental conditions. α- and δ-BHC produced only minimal growth depression without fatality at the level fed. However, the β and γ isomers were relatively toxic. Significant growth retardation occurred only in animals receiving the γ isomer. At the level fed, this isomer produced an initial weight loss, growth retardation, characteristic irritability, hyperactivity, violent convulsions, and death in over 50% of the animals. This syndrome appeared in some animals within the first 24 hours of the experiment; of the 40 rats receiving γ-BHC, 20 were dead by the eleventh day. Tolerance to the drug then developed and near normal growth of survivors resulted. Neither the convulsive disorder nor the growth retardation was alleviated by i-inositol, fed at a ratio of 1:0.75 moles of γ-BHC. Other ratios up to 5.3:1.0 have produced similar results in the rat(6) and the mouse (unpublished data from this laboratory).

The β-isomer, on the other hand, produced no growth retardation during the first 2 weeks; the first signs of its toxicity, appearing about the seventh day, were increased docility, sluggish movements and torpor, followed infrequently by paralysis of the hind quarters. A progressive increase of lethargy to coma in all animals, interrupted occasionally by localized or generalized convulsions, with incontinence of urine and rare opisthotonus, was observed. After the development of narcosis, it was difficult to determine if the animals were dead or in antemortem coma, due to a progressive hypopnea and falling pulse rate for 48 hours preceding death. At this stage, a rapid loss of weight ensued. Of the 40 rats receiving the β-isomer, half were

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* Basal diet: Labco casein, 15.0; l-cystine, 0.3; salts 3A, 2.0; salts 3B, 2.0; celluration, 2.0; B-complex mixture, 1.0; choline chloride, 0.4; fat soluble vitamin mixture, 2.0; lard, 8.0; sucrose, 67.3%.

† The authors with to express their thanks to Mr. Jerome Martin of Commercial Solvents Corporation for his generous gift of the 4 isomers, the uncorrected melting points were: α = 156.5-157.1; β = 311.5-312.0; γ = 112.0-113.0; δ = 136.5-137.0.

TABLE I.
Growth Rates and Mortality of Rats Receiving Isomers of BHC. (20 rats on each diet at start of experiment).

Basal diet supplement				Growth increments (g/wk)				
Inositol mg/g food	Isomer 0.6 mg/g food	Initial wt (avg) g	Final (28 d) wt, g	1	2	3	4	Avg g gain per wk
—	—*	45.7	177.3	27.7	32.3	36.4	35.2	32.9
.5	—	46.0	177.4	30.4	29.5	35.4	36.1	32.9
—	α*	46.8	168.7	24.5	31.2	32.9	33.3	30.5
.5	α*	46.6	171.5	25.8	34.7	34.0	30.4	30.5
—	β†	46.0	—	27.3	31.3	0		29.3‡
				(2)	(7)	(20)		
.5	β	46.7	—	28.3	33.1	0		30.7‡
				(1)	(8)	(20)		
—	γ	45.6	138.6	3.8	26.2	34.7	28.3	23.3
				(7)	(10)	(11)	(11)	
.5	γ	45.9	131.7	1.6	21.6	33.2	29.4	21.5
				(7)	(11)	(12)	(12)	
—	δ	45.7	165.5	19.8	32.4	34.6	33.0	30.0
.5	δ	45.5	167.4	22.1	33.5	33.3	33.0	30.5

* One rat died of an apparently pre-existing disease.
† Numbers in parentheses indicate total number of deaths.
‡ Avg gain over the 2 wk preceding death.

dead by the seventeenth day, the remainder dying before the twenty-third day. On post-mortem examination, the disagreeable odor accompanying benzenehexachloride was emitted, particularly from the abdominal organs. Aside from an empty gastrointestinal tract, absence of depot fat, minimal color changes in the kidneys and liver, and adrenal enlargement (possibly subsequent to coma-induced inanition), the only gross finding of interest was marked distension of the urinary bladder. The latter finding with the paresis noted previously resembled "cord bladder," suggesting a peripheral as well as central action of the β-isomer.

Microscopic examination of the tissues failed to reveal any significant findings. Tissues were immediately fixed in 4% formalin and stained with haematoxylin-eosin. The brain and spinal cord were examined in addition after Weigert's stain, osmic acid preparation, phosphotungstic acid, and Mallory's connective tissue stains. No gliosis, demyelinization or fat granulation were observed. In the β-BHC animals, a patchy non-specific pyknotic shrinkage of a few of the cortical ganglion cells, interpreted as a late phenomenon of profound coma was occasionally observed. No other changes in the neurons or neurocytes were evident. These observations suggest a biochemical rather than an anatomic lesion.

Food consumption was similar for the controls and the α, β, and δ-BHC groups, while the animals receiving γ isomer ate less. The most striking differences in food consumption are found in the first week, during which the γ-BHC groups consumed about half as much food as the other groups. Intakes were slightly depressed in the β and δ-BHC animals in the first week, returning to normal in the second week. The onset of fatal coma in the remaining β-BHC animals in the third week reduced their intake to zero in the fourth week.

The food intake of the animals determines the dosage of the isomer. The intakes of the

TABLE II.
Food Intake of Rats Receiving 0.6 mg BHC/g Food (g food/rat/wk).

Supplement	Wk of exp. 1	2	3	4
—	52.0	66.8	86.4	96.2
I*	52.9	68.1	87.7	98.1
α–BHC	50.4	62.5	85.1	99.4
α + I	52.6	70.1	93.7	101.4
β–BHC	48.0	66.3	71.8	—
β + I	46.1	65.2	63.7	—
γ–BHC	26.4	50.3	69.9	85.7
γ + I	23.9	45.3	68.6	80.9
δ–BHC	42.1	66.0	83.4	96.0
δ + I	41.0	65.9	81.6	92.2

* i–Inositol at a level of 0.5 mg/g food.

α, β, and δ isomers were all similar—about 28 mg per rat for the first week, while consumption of the γ isomer was limited to 15 mg. These dosages are in the range of 60 mg/kg/d for the former 3 isomers and 45 mg/kg/d for γ-BHC. The apparent discrepancy between intake and dosage level (28 and 15 mg/rat/wk compared with 60 and 45 mg/kg/d) is explained by the gain in body weight permitted by the α, β, and δ isomers, while the rats consuming γ-BHC lost about 8% of their body weight over the first 4 days of the experiment (from 45.7 g to 42.4 g body weight on the fourth day). In the presence of hyperactivity, a decreased food intake is unexpected. Thus the γ isomer may depress appetite due to a disagreeable odor or flavor, and increase the metabolic needs of the organism without compensatory increase in food intake.

These results clearly indicate that under the conditions of this experiment, this strain of rat does not require dietary inositol; that α- and δ-BHC are relatively inert toxicologically; that the β- and γ isomers are toxic; and that these toxicities are not alleviated by dietary inositol.

The mechanism by which the β and γ isomers exert their toxic effects is poorly understood. The simple alkyl halides such as chloroform and carbon tetrachloride produce well-defined postmortem findings completely absent in the animals receiving these cycloalkyl chlorides. Dallemagne(10) has presented suggestive data which illustrate relative toxicity of the 4 isomers administered by mouth to the rat; an inverse relationship between melting point and toxicity is described. Using Taylor's data(9) he notes the toxicity was greatest with the lowest melting isomer, γ-BHC, and decreased to no toxicity with the highest melting isomer, β-BHC, over a test period of one week. However, our results, obtained over longer periods of time, indicate that β-BHC is more toxic than γ-BHC, a finding which corroborates the work of Fitzhugh *et al.*(11).

Chaix(2) attributes difference in toxicity to variation in solubility of the isomers in different cellular media. The early onset of γ toxicity in our experiment, compared to the longer induction period of the β toxicity, might be due to recognized differences in solubility(9). The lower solubility of the β isomer might retard its transfer across the intestinal membrane, thereby delaying the appearance of toxic signs. Once the critical level for narcosis is reached, further oral intake ceases; however, absorption probably continues, since the pronounced odor of BHC observed at postmortem in the abdominal cavity possibly indicates a residuum of the β isomer yet to be absorbed. Further light has been shed on the mechanism of action by Dallemagne *et al.*(12) who demonstrated in acute toxicity experiments in dogs that there was a synergistic effect in the production of convulsions between the γ isomer and eserine. They interpreted their results as indicating that the γ isomer exerts its convulsive effects by inhibition of cholinesterase. Interestingly enough, counteraction among the isomers has been noted; McNamara and Krop(4) demonstrated that preparation of the animal by the intravenous administration of either the β or δ isomer negated the convulsive effects of the γ isomer on later administration by the same route. Thus, when the role of absorption is eliminated, the convulsive and narcotic actions of these drugs counteract each other, and differences in solubility are probably no longer so important a factor. In our opinion, the opposite effects of the γ and β isomers, convulsive and narcotic, respectively, are best explained not by physical properties but by stereochemical differences between the molecules. The possible isomers of BHC as listed by Bijvoet(7) and Kauer(13) contain a structure analogous to i-inositol, which has been reported to have one polar(14) and 5 equatorial hydroxyl substituents (p,e,e,e,e,e) (7,15). Of the 6 isomers of BHC already

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isolated, the following may be eliminated as analogues if their proposed structures are confirmed: α, since it is apparently a racemate, Cristol(16) having obtained the dextrorotatory enantiomorph; β (e,e,e,e,e,e)(17, 18); γ (p,p,p,e,e,e)(7,8); and zeta (p,e,e, p,e,e)(17). Our data also rule out the first 3 isomers mentioned and the δ isomer on biological evidence. The question of which isomer of BHC is analogous in structure to i-inositol must await further isolation and characterization studies.

The marked differences in physical properties (solubility, melting points, dipole moments) and in chemical reactivities between the hexahydroxy- and hexachlorocyclohexanes are not in accord with the accepted antimetabolite concept of biological competition. Therefore, true specific competitive antagonism will probably not be demonstrated between such physically dissimilar classes of molecules. However, the antithetical physiological properties of the BHC stereoisomers are unique and may therefore become of great interest in elucidating the mechanisms of narcosis and idiopathic convulsive disorders, such as epilepsy.

Summary. The toxicities of the α, β, γ, and δ isomers of hexachlorocyclohexane were not affected by inositol in the rat. The convulsive and narcotic effects of the γ and β isomers are discussed in detail.

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Some Effects of Testosterone Propionate on Mice Irradiated with X-rays.* (17994)

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We have recently demonstrated that administration of desoxycorticosterone acetate after exposure of mice to lethal doses of X-rays reduces the mortality produced by irradition(1,2). It has also been demonstrated that the administration of this hormone protects the liver of mice against radiation induced fatty changes(3) and prevents the radiation induced depletion of sudanophile substances(4) of the adrenal cortex. In order to obtain more complete information on the significance of these findings for the mechanism of total body irradiation, our studies on the pharmacological analysis of this process have been extended to include testosterone propionate. The use of this substance suggested itself because testosterone is not only a chemical relative of desoxycorticosterone but also exercises some similar metabolic effects.

Material and methods. Swiss male white mice (Rockland Farms Stock) 22 g $\pm$ 15% of body weight were used in these experiments. Irradiation took place in the previously described manner(5). The X-ray dose was in all instances 500 r/air (HVL 0.75 mm Cu).

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