INHIBITION OF GROWTH BY BENZENE HEXACHLORIDE ISOMERS AND PROTECTIVE EFFECT OF GLUCOSE AS MEASURED BY CELL COUNTING TECHNIQUE

H. T. HOPKINS

(WITH FOUR FIGURES)

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Introduction

The purpose of this study is to evaluate the effects of benzene hexachloride isomers on root growth and the antagonism of these effects by selected chemical compounds. Interest has developed in gamma benzene hexachloride because it is highly toxic to insects (**40**). Five isomers of benzene hexachloride have been isolated (**19**) and the structural properties of most of them are well understood (**40**). The gamma and delta isomers cause mitotic arrest at metaphase (**8, 9, 13, 28, 30**). The toxicity of the gamma isomer, known commercially as Lindane, has been studied extensively (**8, 14, 18, 23, 28, 34, 38**), but little work has been done on the delta isomer (**8, 38**). Benzene hexachlorides possess certain properties characteristic of typical narcotic compounds, two of which are the lack of polar groups in the molecule and the limited solubility in water. Owing to the lack of polar groups, it would be predicted that benzene hexachloride (BHC) molecules would accumulate preferentially in the lipid phase boundaries of the protoplast (**2**). Following such accumulation, changes in cell structure and organization may occur which greatly influence the direction and rate of chemical reactions (**13, 33**).

One of the first reported antagonists of gamma benzene hexachloride was *meso*-inositol (**9, 12, 22, 27**). Earlier, this finding was of interest because of the supposed structural analogy between gamma benzene hexachloride and the biologically important inositols. It now appears, however, that the delta isomer, and not the gamma isomer, is isomorphous with *meso*-inositol (**17**). D'AMATO (**12**), on the other hand, reported a delaying action of *meso*-inositol on the C-mitotic and C-tumor action of gamma benzene hexachloride, which occurred at 15 to 19° C, but not at 26 to 27° C. D'Amato also observed a delaying action of sucrose and other sugars and ascribed these effects to a change in permeability to the BHC. Antagonisms of this type are suggestive of those observed against x-radiation by sodium cyanide (**3**), thiourea (**31**) and vitamin P (**41**).

LEVAN and ÖSTERGREN (**30**) have discussed the mechanism of C-mitotic action at some length. These workers describe C-mitotic action as a narcosis of those factors which govern growth by cell division, and C-tumor action as a narcosis of those factors which govern growth by cell tension. C-tumor formation has a threshold separate from C-mitosis (**37**). Mc-

ELROY (**33**) has presented a unifying interpretation of the mechanism of inhibition of cellular activity by narcotics, *in vivo*. He stressed that narcosis consists fundamentally of the inhibition of the Pasteur effect. McElroy's interpretation is quite useful in that it encompasses most theories heretofore advanced on the mechanism of narcosis. Furthermore, it aids materially in explaining why diverse inhibitors of cellular function appear to affect a variety of processes in somewhat the same way, even though the actual site of combination of the inhibitors in cells may be different.

Materials and methods

The rooting medium adopted after preliminary study was the special crepe paper called Kimpack, often used by seed technologists. Pieces of Kimpack eight inches square and 0.3 inch thick were placed in waxed cardboard boxes eight and one half inches square and one and one half inches deep. The required amount of BHC was pipetted from the diluted stock solution into a dispenser fitted with an aluminum sprinkler head and acetone containing 0.08% Tween 20 was added to give a volume of 130 ml. which was adequate for thorough impregnation. The treated papers were dried in a forced draft oven at 30° C for eight hours. Since BHC will volatilize under these conditions, it is imperative to maintain a constant drying time if comparable data are to be obtained between experiments. The initial BHC concentration is calculated from the volume of water used to moisten each piece of dried Kimpack and the weight of BHC added. Purified alpha, beta, gamma and delta isomers of BHC were used.

Plant material consisted of *Cucurbita pepo* (L.), variety Early Yellow Prolific squash, and *Carthamus tinctorius* (L.), Nebraska Hybrid, number 852. Previous work indicated squash roots to be sensitive to a large number of chlorinated hydrocarbon insecticides and related compounds (**25**).

All experiments were carried out in a dark room at a temperature of $27 \pm 1°$ C and a relative humidity of about 50%. Seedlings with primary roots 1 to 3 mm. long were transplanted to treated and untreated papers previously moistened with 130 ml. of aerated tap water, then covered with moist paper toweling. A uniform planting and harvesting schedule was adhered to so as to avoid the variability associated with mitotic cycles (**21**). All experiments were terminated 48 hours after transplanting. Twenty-five seedlings were removed from the treated papers and root tips 10 mm. long were excised under water. The root tips were preserved in glacial acetic acid because standard killing solutions did not permit good maceration following transfer to chromic acid. The over-all length of primary roots was then recorded, allowing for the portion removed.

A composite sample of five root tips was placed in one ml. of 5% chromic acid and macerated after standing six hours, according to the method of BROWN and RICKLESS (**7**). Ten to fifteen root tips were studied in each treatment. Using a special pipette, a small aliquot of the macerated root suspension was transferred to the counting chamber of a Spencer

Bright-Line haemacytometer for counting of the cells. The volume of the chamber was 0.9 cubic millimeter. The microscope used for counting had a 35 mm. Leitz objective, 20 × compensating oculars and no condenser. The slide was illuminated with a Spencer microscope lamp fitted with a blue Corning glass filter, number 429. Counts were completed within 24 hours after transfer of root tips to the chromic acid in order to avoid difficulties with disintegration of the cells. The total number of cells and the number of non-vacuolated cells were determined in treated and untreated root tips. By means of this technique BROWN and RICKLESS (**7**) determined the effect of mineral nutrients, sugar and yeast extract on the rate of cell division in excised roots of *Cucurbita pepo* (L.) during a period of 12 hours. They determined the rate of cell division by dividing the average increment in number of cells by the average number of non-vacuolated cells in the root during the interval. A modification of this procedure has been used in this work. The number of non-vacuolated or meristematic cells in squash seedling root tips was found to remain constant during the four 12-hour intervals of a 48-hour experiment. The total number of cells, however, decreased progressively during this time. The number of vacuolated cells, obtained by taking the difference between total and non-vacuolated cells, likewise decreased. The assumption is made that during the first 48 hours, the dominant process in squash roots is cell division.

The ratio between non-vacuolated and vacuolated cells in untreated root tips during a period of 48 hours is plotted in figure 2. This ratio is termed the index of growth. It is of interest that the shape of the curve is similar to that of the classical growth curve. The cell counting technique is quantitative; and measures change in the combined processes of cell division, differentiation, and organization. MASCRE' and DEYSSON (**32**) used a mitotic index for comparing the effects of various chemicals on mitosis in the onion root. The average number of cells and the number of non-vacuolated cells between duplicate samples from the same bottle could be counted with an error of 5% as claimed originally for this method (**7**). A coefficient of variability of 15% was observed among 25 random samples for counts of cells. For counts of non-vacuolated cells the coefficient of variability was 20% among 25 random samples.

The extent of protection exhibited by various chemical compounds against concentrations of benzene hexachloride isomers inhibiting cell division was studied. The following compounds were dissolved in water and applied in concentrations ranging from 10^{-6} to 10^{-2} M to Kimpack paper treated with BHC: acetylcholine chloride, adenylic acid, beryllium sulphate, cysteine chloride, desoxyribose nucleic acid and ribose nucleic acid, dimethylamine, D-glucose, D,L-glyceraldehyde, ethanol and calcium chloride mixtures, *i*-inositol, iodoacetic acid, magnesium chloride, octanoic acid, phloridzin, sodium arsenate, sodium arsenite, sodium diethyldithiocarbamate, sodium fluoride, sodium pyrophosphate, thiamine hydrochloride and 2,4-dinitrophenol (**10, 15, 24, 27, 29, 42, 44**). All solutions were adjusted to

a pH of 6.0 with the exception of iodoacetic acid and sodium diethyldithiocarbamate which were used at a pH of 5.0 and 7.5, respectively. The effect of these compounds on the growth of squash roots was determined in preliminary experiments.

Inorganic and labile phosphate analyses were made on root tips essentially according to the method of KALCKAR (**26**). The method permits the determination of inorganic phosphorus in the presence of labile phosphate esters. Excised root tips weighing 500 mg. were cooled quickly to $-5°$ C, then transferred in 10 ml. of ice cold trichloroacetic acid to a Potter-Elvehjem homogenizer (**43**) made of stainless steel. The final concentration of the trichloroacetic acid was 5%. During homogenization the stainless steel tube was kept in ice. The cold homogenized suspension was centrifuged for one to two minutes at 12,000 RPM in an angle-head centrifuge. The tube was placed in ice and a 0.5 ml. aliquot of the supernatant liquid was pipetted into 1.0 ml. of 0.1 *N* sodium acetate, and 3.0 ml. of sodium acetate–acetic acid buffer, pH 4, was added. This sample was analyzed for inorganic phosphate. Labile phosphate was determined on another aliquot which was incubated at 30° C for 30 minutes before neutralizing with the acetate–acetic acid buffer. The phosphate color was developed in both samples by adding 1.0 ml. of ammonium molybdate reagent at a concentration of 1% in 0.05 *N* sulphuric acid, and 1.0 ml. of ascorbic acid at a concentration of 1% in water. Absorption of light was measured with a Beck-

TABLE I

ENZYME INHIBITORS WHICH AFFECT THE GROWTH OF ROOTS OF *Cucurbita pepo.*

Name of compound	Concentration (molarity)	Mean total cells per root ($\times 10^3$)	Mean non-vacuolated cells per root ($\times 10^3$)	Index of growth	Mean root length
					mm.
Sodium arsenite	2.3×10^{-6}	87.4	44.0	1.01	64
	2.3×10^{-5}	80.4	38.9	0.94	61
	4.5×10^{-5}	87.2	44.4	1.04	57
2,4-Dinitrophenol (DNP)	4.2×10^{-6}	59.0	27.1	0.85	71
	4.2×10^{-5}	56.7	17.3	0.44	25
	4.2×10^{-4}	61.4	17.5	0.40	12
Sodium arsenate	2.5×10^{-6}	84.6	36.2	0.75	58
	1.2×10^{-5}	69.3	24.4	0.54	44
	2.5×10^{-4}	52.4	14.6	0.39	25
Iodoacetate	4.1×10^{-6}	86.1	39.8	0.86	75
	4.1×10^{-5}	87.0	43.6	1.00	66
	4.1×10^{-4}	73.5	32.9	0.81	51
	1.0×10^{-3}	64.7	12.8	0.25	26
Beryllium sulphate	7.3×10^{-6}	83.2	38.1	0.84	46
	7.3×10^{-5}	74.4	35.3	0.90	39
	7.3×10^{-4}	65.0	35.0	1.17	48
	7.3×10^{-3}	62.8	22.0	0.54	38
Untreated		82.0	38.7	0.90	70

mann spectrophotometer at 700 millimicrons, after the color had developed for 10 minutes.

The phosphate analyses were made to campare the effect of 2,4-dinitrophenol (DNP), which causes the release of inorganic P, with that of benzene hexachloride. Determination of labile phosphate, although specifically a measure of adenosine triphosphate (ATP), may be interpreted as such if a marked decrease in labile P occurs in the presence of the stimulant (**33**).

Results

It seemed desirable to characterize the capacity of squash roots to respond to selected chemical compounds, many of which are considered rather specific inhibitors of enzyme systems. Indices of growth for five compounds having inhibitory effects on root growth are shown in table I. Sodium arsenite and iodoacetate reduce sulfhydryl groups, and sodium arsenate and DNP act to uncouple oxidative phosphorylation (**4**). Sodium arsenate may also inhibit adenosine triphosphatase. Beryllium sulphate is considered to be an inhibitor of the prosthetic group of adenosine triphosphate (**15**). Sodium arsenite at a concentration of 2.3×10^{-4} M stopped root

TABLE II

EFFECT OF BENZENE HEXACHLORIDE ISOMERS ON TWO PLANT SPECIES.

Species	Isomer and dosage	Mean total cells per root ($\times 10^3$)	Mean non-vacuolated cells per root ($\times 10^3$)	Mean root length	C-tumor*
				mm.	
Cucurbita pepo	alpha BHC 1.1×10^{-3} M	62 ± 7	9.3 ± 4	32 ± 12	++
	beta BHC 1.1×10^{-3} M	78 ± 19	36.0 ± 6	82 ± 37	None
	gamma BHC 1.1×10^{-3} M	66 ± 38	1.6 ± 0.8	14 ± 1.9	+++
	delta BHC 1.1×10^{-3} M	44 ± 0.5	1.8 ± 0.04	10 ± 1.9	+
	None	67 ± 6	34.0 ± 0.3	75 ± 12	None
Carthamus tinctorius	alpha BHC 2.2×10^{-3} M	96 ± 9	45.0 ± 14	44 ± 12	None
	beta BHC 2.2×10^{-3} M	68 ± 7	33.0 ± 8	44 ± 18	None
	gamma BHC 2.2×10^{-3} M	84 ± 30	16.0 ± 5	26 ± 11	+
	delta BHC 2.2×10^{-3} M	46 ± 4	3.3 ± 3	17 ± 8	+
	None	84 ± 10	44.0 ± 6	67 ± 12	None

*+ = slight C-tumor, ++ = intermediate C-tumor, +++ = pronounced C-tumor.

growth in the first 12 hours of the experiment. Lower concentrations of arsenite were without effect. Sodium arsenate, iodoacetate and DNP lowered the growth index and over-all root lengths at concentrations of 10^{-3} M or lower. Beryllium sulphate inhibited root growth at all concentrations used, but the rather high concentration of 7.3×10^{-3} M was required to lower the growth index. The most striking effect on cell numbers is produced by DNP and sodium arsenate. Glyceraldehyde, phloridzin, sodium fluoride, acetylcholine, sodium diethyldithiocarbamate, and sodium pyrophosphate were without effect on root growth. These results indicate that the cell counting technique is useful in judging the growth inhibiting effects of water soluble chemicals.

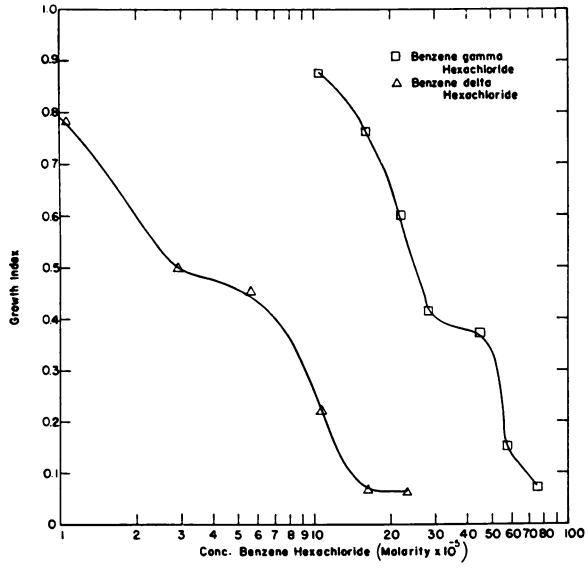

FIG. 1. Dosage response curves for the effect of gamma and delta benzene hexachloride on the growth of *Cucurbita pepo* roots.

Detailed screening experiments using four isomers of benzene hexachloride were carried out on squash, *Cucurbita pepo* (L.), and a safflower hybrid, *Carthamus tinctorius* (L.). Results are shown in table II. The C-tumor which appears in response to the gamma isomer is quite pronounced. The condition known as C-tumor arises from an unimpeded tension in all directions tending to make the root cells isodiametric (**30**).

The effect of varying concentrations of the gamma and delta isomers on the growth index is shown in figure 1 in which the logarithm of the concentration of benzene hexachloride is plotted against the growth index. Comparison of the curves shows that the concentration at which the gamma isomer produced a 50% lowering of the growth index was about fivefold higher than that for the delta isomer.

Results from an experiment in which roots of squash were maintained

in contact with various concentrations of gamma benzene hexachloride for periods of 14 to 48 hours are shown in figure 2. The sharply rising index of growth between 24 and 36 hours in untreated roots reflects the large number of non-vacuolated cells in proportion to vacuolated cells. High concentrations of BHC abolish this condition. It is apparent from the curve for the untreated roots that the growth index approaches unity at 48 hours.

Results from an experiment in which squash roots were placed in contact with gamma benzene hexachloride for 2 to 36 hours and then transferred to untreated media for recovery in a 48 hour experiment are shown in table III. These data reveal an interaction of time and concentration. It is also evi-

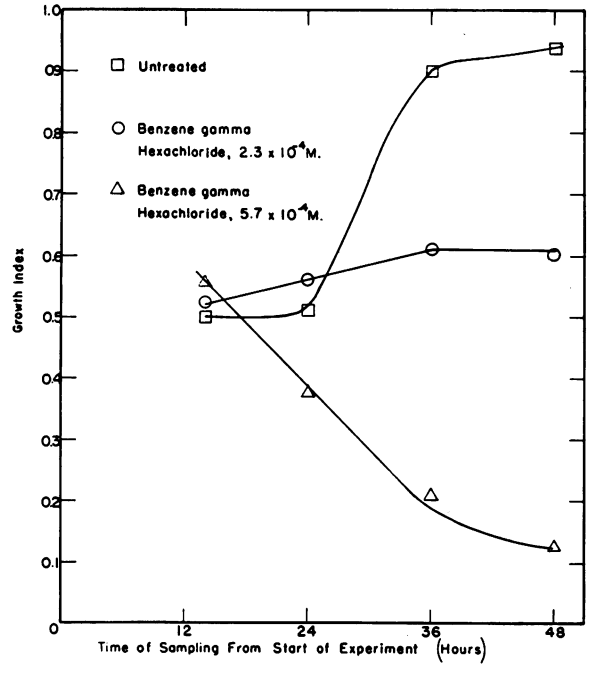

FIG. 2. Time course of the growth index for untreated and treated roots of *Cucurbita pepo* seedlings.

dent that the growth index decreases rather uniformly with increasing time of contact with the exception of the contact periods of 14 hours at the higher dosages.

Among the 22 compounds studied, D-glucose was the most effective antagonist of both the gamma and delta isomers of benzene hexachloride as shown in table IV. The lengths of untreated roots, however, exceeded those of the roots grown in glucose and BHC. Glucose gave no protection against a BHC concentration of 5.7×10^{-4} M. Dinitrophenol at 10^{-4} M with a concentration of BHC producing a C-tumor, 5.7×10^{-4} M, abolished the reaction(s) responsible for the production of the tumor, but the over-all growth was suppressed. The inhibitory effect of the combination of BHC with DNP was the same as if either compound had been used singly.

TABLE III

CELL NUMBERS, ROOT LENGTHS, AND GROWTH INDEX, AS AFFECTED BY VARIOUS CONTACT INTERVALS AND CONCENTRATIONS OF GAMMA BENZENE HEXACHLORIDE.

BHC dosage	Contact time	Recovery time	Mean total cells per root ($\times 10^3$)	Mean non-vacuolated cells per root ($\times 10^3$)	Mean root length	Growth index
	hrs.	*hrs.*			*mm.*	
2.3×10^{-4} M	2	46	90 ± 3	42 ± 4	90 ± 12	0.88
	6	42	81 ± 9	37 ± 2	79 ± 11	0.84
	14	34	62 ± 22	24 ± 15	64 ± 10	0.63
	24	24	82 ± 32	31 ± 16	47 ± 7	0.61
	36	12	61 ± 20	22 ± 14	40 ± 10	0.56
3.4×10^{-4} M	2	46	89 ± 14	36 ± 13	83 ± 11	0.68
	6	42	88 ± 10	36 ± 16	75 ± 12	0.69
	14	34	83 ± 20	40 ± 16	68 ± 9	0.93
	24	24	82 ± 10	32 ± 2	46 ± 8	0.64
	36	12	64 ± 10*	23 ± 12	39 ± 8	0.56
5.7×10^{-4} M	2	46	76 ± 20	33 ± 4	88 ± 18	0.77
	6	42	67 ± 1	23 ± 5	79 ± 10	0.52
	14	34	73 ± 19	43 ± 11	62 ± 8	1.43
	24	24	59 ± 28*	20 ± 16	40 ± 8	0.51
	36	12	46 ± 6*	8 ± 2	25 ± 6	0.21
None	....	....	68 ± 5	33 ± 1	57 ± 10	0.94

*Evidence of C-tumor.

TABLE IV

PROTECTION EXHIBITED BY GLUCOSE AGAINST INHIBITORY EFFECTS OF GAMMA AND DELTA BENZENE HEXACHLORIDE.

Concentration BHC isomer	Concentration of glucose	Mean root length	Growth index	Percentage protection*
		mm.		%
gamma BHC:				
2.8×10^{-4} M	2.1×10^{-5} M	50 ± 13	0.87	132
2.8×10^{-4} M	2.1×10^{-4} M	45 ± 9	0.55	54
2.8×10^{-4} M	4.2×10^{-4} M	52 ± 15	0.79	112
2.8×10^{-4} M	None	44 ± 13	0.33	....
None	4.0×10^{-4} M	75 ± 10	0.93	....
None	None	73 ± 13	0.74	....
delta BHC:				
2×10^{-5} M	4.3×10^{-6} M	37 ± 18	0.42	31
2×10^{-5} M	4.3×10^{-5} M	26 ± 9	0.42	31
2×10^{-5} M	4.3×10^{-4} M	54 ± 15	0.86	88
2×10^{-5} M	None	25 ± 12	0.18	....
None	4.0×10^{-4} M	73 ± 10	0.90	....
None	None	70 ± 10	0.95	....

*$\frac{\text{Growth index for BHC and Glucose Combination} - \text{Growth index for BHC}}{\text{Growth index for Untreated} - \text{Index for BHC}} \times 100.$

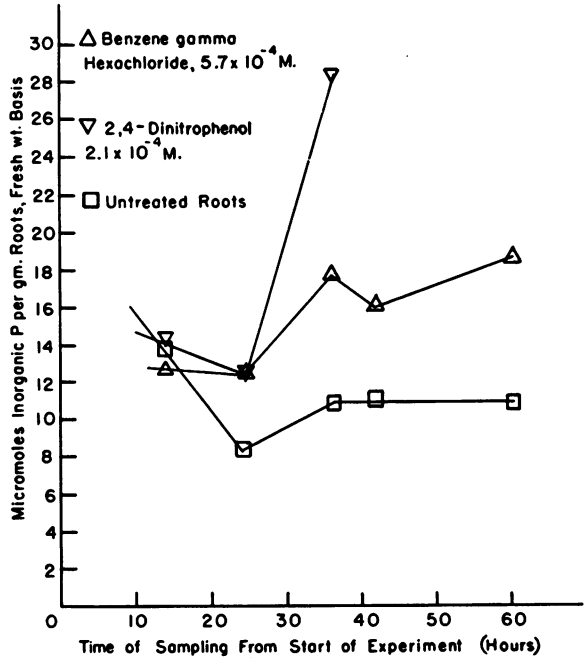

FIG. 3. Time course of the release of inorganic phosphate in untreated and treated roots of *Cucurbita pepo* seedlings.

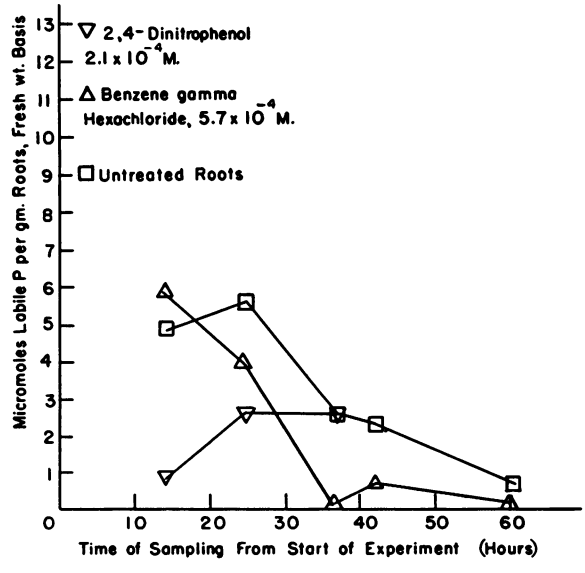

FIG. 4. Time course of the decrease in acid-labile phosphate in untreated and treated roots of *Cucurbita pepo* seedlings.

Analyses for inorganic and labile phosphate in trichloroacetic acid extracts of squash roots grown in contact with DNP and BHC for varying periods of time are shown in figures 3 and 4. Analyses of untreated roots for the same time periods are also shown. Presence of DNP causes an accelerated release of inorganic P between 24 and 36 hours. A parallel release of inorganic P is caused by BHC. The amount of labile phosphate in the roots treated with DNP for 14 hours was much less than the amount in the untreated roots. During further treatment the labile phosphate increased and at 36 hours was equal in amount to that in the untreated roots. On the other hand, treatment with BHC caused a sharp decrease in the amount of labile phosphate and at 36 hours there was little or no labile phosphate present. These results are in agreement with what would be predicted for the effect of stimulants in the concept advanced by McELROY (**33**).

Discussion

The cell counting technique adapted from BROWN and RICKLESS (**7**) is useful in judging the inhibitory effects of both water-soluble and water-insoluble compounds on growth. The striking effects of DNP, arsenate, arsenite, and iodoacetate on the growth of squash roots shown in table I reveal the dependence of the processes of cell division and differentiation upon enzyme systems concerned in the mobilization of energy (**13**). Both DNP and arsenate are considered as stimulants whose action ultimately may abolish the Pasteur effect (**33**). The inhibitory effect of sodium arsenite and iodoacetate reveals the presence in squash roots of sensitive enzyme systems containing sulfhydryl groups. It is not clear, however, whether the —SH groups which arsenite specifically reduces are part of the proteins in the mitotic spindle, or whether these groups are parts of the enzyme systems concerned in mobilization of energy for the mitotic process (**13**). DUSTIN (**16**) believes that cell division is dependent upon a concentration of —SH groups in the cell which must be maintained within narrow limits. NICKERSON and VANRIJ (**35**) suggest that two groups of chemicals can poison the mitotic process. One group includes the water-soluble chemicals which are specific for sulfhydryl groups and directly affect cell division. The second group is the water-insoluble narcotics which affect the formation of new, discontinuous phase boundaries at the end of cell division.

Rather specific effects of isomers of benzene hexachloride on root growth are suggested by the data in figure 1, by virtue of the narrow range of concentrations over which they act. The nature of these effects cannot be predicted on the basis of the experiments carried out here. It is suggested, however, that the presence of benzene hexachloride molecules in phase boundaries of the protoplast may initially disorganize the lipid component and cause the disorganization of the chain of respiratory processes (**2, 35**). The results in table II reveal a variation between species in the growth response to some of the BHC isomers. In squash roots the alpha isomer

lowers the number of non-vacuolated cells and causes C-tumor formation, but in safflower roots neither response was observed. The assumption that lipid soluble compounds denature proteins by changing the organization of lipid residues in the polypeptide chains making up the proteins allows for variation among species (**13, 37**). Within a given species, a difference in the spatial configuration of the molecule is associated with a difference in response. The molecular shape of a given isomer may determine the amount of the compound which can penetrate the root (**1, 33**). Reactions may occur at the surface of the cell, however, which obviate the necessity of penetration (**24, 35, 44**).

Data in table III indicate that development of the C-tumor in the highest concentration of BHC is accompanied by a large decrease in the total number of cells and the number of non-vacuolated cells. This observation is at variance with the observations of LEVAN and ÖSTERGREN (**30**) who found that in the tumor induced by colchicine the total number of cells remains unchanged. Possibly the BHC tumor is structurally unlike that induced by colchicine. The increase in the growth index at 14 hours for the highest concentration of BHC in table III is in sharp contrast to the rather low index for the treatment with 2.3×10^{-4} M BHC. This condition arises from the increased number of non-vacuolated or meristematic cells at 14 hours induced by 5.7×10^{-4} M BHC. BLUMENTHAL (**6**) has reported an increase in mitotic activity in some tissues during phases of an imposed starvation period.

The C-tumor appears 12 hours earlier in the treatment with 5.7×10^{-4} M BHC than in the 3.4×10^{-4} M treatment. This situation seems analogous to that reported by NORTHEN for colchicine (**36**), in which the concentration and time of contact were of importance in causing the dissociation of labile spindle proteins. In general, the data in table III indicate irreversible effects of BHC at all concentrations after 14 hours of contact.

The effect of glucose at 4×10^{-4} M in protecting against growth inhibiting concentrations of the gamma and delta isomers of BHC, table IV, is essentially a delaying action similar to that reported by CORNMAN for colchicine (**11**) and D'AMATO for gamma benzene hexachloride (**12**). It is suggested that glucose may act for a time principally as a phosphate acceptor coincident with the stimulated fermentative reactions induced by the BHC (**4, 20**). High concentrations of BHC may quickly disorganize the system through which the glucose protection operates (**33**).

The effect of DNP at 10^{-4} M in subduing the C-tumor reaction induced by BHC at 5×10^{-4} M is a type of antagonism frequently reported (**5, 16**). SANTARATO (**39**) found that streptomycin in association with sulphapyridine in molar ratio of 1:2 decreased both the respiration and glycolysis of the system with which he worked. Further study is required to establish whether the association of DNP with BHC alters the course of tumor formation by some modification of respiration and fermentation.

A close relationship in point of time is noted between the altered phos-

phate metabolism in the roots shown in figure 4, and the lowered growth index in figure 2 for the treatment with BHC at 5.7×10^{-4} M. The accelerated release of inorganic P and the decrease in labile P occurred after 14 hours. Similarly, the irreversible effect of BHC as measured by the growth index in the transfer experiments and the altered phosphate metabolism after 14 hours are believed to be causally related. These results support the belief that BHC affects cellular function by inhibition of enzyme systems involved principally in reactions of synthesis. At the same time, the similarity between the effect of DNP and BHC in causing an accelerated release of inorganic P suggests initial activation of hydrolytic enzymes (**33**). The low values for labile P, though not a measure of ATP *per se*, are suggestive of accelerated hydrolysis of labile phosphate esters in the roots after treatment with DNP or BHC. McElroy (**33**) cites numerous examples in which inhibitors of cell division may cause the rapid breakdown, or prevent the formation, of certain energy-rich phosphate esters.

Summary

The effect of four purified isomers of benzene hexachloride and 22 additional compounds on the growth of seedling roots was determined by the use of a cell counting technique. The delta isomer is equal to or greater in phytotoxicity than the gamma isomer. The alpha isomer in high concentration inhibits growth and causes formation of a C-tumor in squash roots, but has neither effect on safflower roots. The implication of these data is discussed.

The inhibition of growth of squash roots by low concentrations of benzene hexachloride, sodium arsenate, DNP and iodoacetate is causally related to the inhibition of the number of non-vacuolated or meristematic cells between 24 and 48 hours.

Irreversible effects of the gamma isomer on root growth after 14 hours contact are indicated by data from transfer experiments.

A relationship in point of time was noted between growth inhibition and the phosphate metabolism in squash roots induced by high concentrations of gamma benzene hexachloride. This finding is discussed.

Among 22 compounds studied for their protective effects against concentrations of gamma and delta benzene hexachloride inhibiting growth, D-glucose was the most effective. In addition, 2,4-dinitrophenol antagonized the C-tumor reaction induced by gamma benzene hexachloride.

The alpha, beta, and gamma isomers of BHC were purified by the Department of Chemistry, University of Maryland, from crude materials furnished by the Penn Salt Company. The purified delta isomer was furnished by the California Spray Chemical Company. The writer gratefully acknowledges the valuable contributions of all cooperating individuals.

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U. S. DEPARTMENT OF AGRICULTURE
PLANT INDUSTRY STATION
BELTSVILLE, MARYLAND

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