THE EFFECT OF VARIOUS HERBICIDES ON GLUCOSE METABOLISM IN GARDEN PEAS *PISUM SATIVUM* L.

by

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INTRODUCTION

For many years man has sought to control nature's green carpet, the plant world. From the time primitive man sank his first crude hoe into the rich earth and discovered the art of farming, man has been plagued with the problem of the removal of unwanted plants. He has improved his techniques for tilling the soil, improved his crops to yield greater quantities of better quality, and has even found methods for controlling pests and disease. With the advent of the industrial revolution he climbed on giant tractors pulling great steel plows, but was still compelled to laboriously pull weeds by hand. It was not until the advent of the scientific age that chemical means were developed for the control of weeds.

In 1934 Koyl (45, p. 363) and his co-workers discovered a compound in human urine that was markedly active in promoting cell elongation in plants. When purified the compound was found to be indole-3-acetic acid. Later in the same year Koyl found this same substance in yeast. It was sometime, however, before this substance was found in higher plants.

Koyl's discovery lead others to look for different

active substances, and in 1935 Zimmerman found a-naphthalene acetic acid to be active. Irvine's discovery in 1938 of the growth regulating properties of 2-napthoxy acetic acid stimulated many workers to develop phenoxy acid herbicides such as 2,4-D which was first reported by Zimmerman in 1942 (45, p. 263-266).

Today there are many chemicals in commercial use as weed killers and many more in the experimental stages. A great deal of work has been done on the field usage of these herbicides such as selectivity, methods of application, and dosage rates; however, very little is known of the way in which they work, or the biochemical mechanism which is affected.

The first phase in the action of herbicides is their absorption into the tissue after application to the plant. Application may be either to the above ground portion of the plant such as the leaves or to the roots. The method of absorption is still unknown, however, several suggestions have been made. Van Overbeek (44, p. 300) has suggested that there is a correlation between the lipophilic properties and leaf absorption. Ross and Ludwig (35, p. 65-95) found not only a relationship between leaf absorption and an oil water coefficient but also a correlation with water solubility. Other suggestions may be found in the literature but as yet no one

theory has been found which will explain all the experimental results.

Uptake by the roots has been less thoroughly studied than uptake of the leaves; the only informative work being that of Blackman (48, p. 318). Performing time course experiments in the uptake of C^{14} labeled 2,4-D in intact seedlings, Blackman found a steady uptake with resistant species. In susceptible plants there was a rapid uptake followed by a transfer of the herbicide back into the solution. Intermediate plants acted about the same as susceptible plants except that after the loss of 2,4-D there was a gradual uptake as the absorption mechanism recovered. Blackman suggests that this phenomenon is relevant to the basic difference between susceptible and non-susceptible plants. It would appear from the foregoing discussion that absorption, though different in susceptible and non-susceptible plants, is not the cause of the plant's failure to survive, although it may be an effect of the failure of some other mechanism.

The next phase in the scheme of herbicidal action is the translocation of the herbicide to the site of activity within the plant. In order for translocation to proceed at least four factors must be satisfied. First, the herbicide must be absorbed as has been discussed. Second, there must be sufficient photosynthetic activity to supply

the necessary assimilates for movement through the phloem. Jaworski (27, p. 272-275) has shown that photosynthetic products are necessary for the movement of herbicide in the phloem. When photosynthesis stops, the movement of herbicide also stops, but movement can again be restarted by application of any of a number of carbohydrates. The third factor that must be satisfied is that translocation from actively photosynthesising leaves toward the roots must be occuring. The last factor to be satisfied is that the herbicide reach actively growing tissue and not dormant tissue. Yamaguchi (48, p. xlii) has shown that 2,4-D is readily absorbed in parenchyma tissue but, if the plant is rapidly growing, the translocation rate is fast enough to move the herbicide to the active growing tips. It would appear then that the mechanism of action for the herbicide is not absorption or translocation.

Aside from absorption and translocation and the easily detectable physiological effects of wilting, stomatal closing, protophasmic streaming and the like, many biochemical effects have been studied. Butts and Fang (9, p. 212-213) found that there was an inhibition of CO_2 assimilation caused by 2,4-D in photosynthesis, the inhibition being proportional to the amount of herbicide used. The previous confirms the work of Freeland (16, p. 319-324).

Mineral uptake is inhibited by auxin herbicide (48, p. 327-328) (8, p. 5) as is the uptake and metabolism of indole-3-acetic acid (IAA). In phosphate metabolism there appears to be a decrease in the upward movement of phosphate to the leaves, the decrease being proportional to the amount of 2,4-D used. 2,4-D treatment causes an increase in hexose diphosphates in the stem while there is a slight decrease in the leaves (13, p. 365-368). Remmert (34) has found that there is an inhibition to phosphorylation caused by 2,4-D, which French and Beevers (17, p. 660-666) suggest is not due to an uncoupling but to a drawing off of the ATP for use in the herbicidally induced growth process. Cyclic photosynthetic phosphorylation in insolated chloroplasts, however, does not appear to be affected by herbicidal treatment (4, p. 10-21) (26, p. 974-984).

It has been known for many years that auxin causes an increase in growth accompanied by an increase in respiration (17, p. 666). Axelrod and Beevers (5, p. 267-298) have described the mechanisms of carbohydrate breakdown quite satisfactorily in normal tissue. The question which now arises is, how is the respiration of normal plants affected by herbicidal treatment.

Humphreys and Dugger (21, p. xxii) working with 2,4-D found that treatment did not alter the RQ which

remained near 1.0 in pea seedlings. This would indicate that carbohydrate was the main substrate being oxidized in both the treated and untreated plants. They also found that the QO_2 values for the treated plants were greater than those for the untreated, signifying a stimulation of the respiratory rate. In a later paper (23, p. 530-536) they found the reducing sugars, starch and sucrose content of the untreated seedling and 2,4-D treated seedling to be the same. Thimann (41, p. 777-780), however, found IAA decreased the sugar content by one-third in peas. Working with pea root Humphreys and Drugger (22, p. 136-140) found that application of 2,4-D caused an increased metabolism of glucose -C^{14} via the pentose pathway. This increased metabolism is not due to a greater amount of respiratory substrate being present. In their most recent work utilyzing 2,4-dinitrophenol DNP they suggest that 2,4-D and DNP which have the same properties of increasing respiration via the pentose cycle do so by blocking synthetic metabolic pathways (24, p. 112-116).

This thesis will be concerned with studies, not only of 2,4-D and IAA, but also other herbicides of varying characteristics. Studies will be made of the respiratory rate, catalytic pathways, absorption, and synthetic and catabolic products of metabolism.

MATERIALS AND METHODS

MATERIALS

The work done for this thesis was carried out exclusively with the common garden variety of peas, Pisum sativum Alaskan. The seeds were purchased from the Ferry-Morse Seed Company of Mountain View, California. They were from the 1957 crops with a germination rate of 97% and were purchased in 25 pound lots.

The labeled glucose used was purchased as follows: glucose 1-C^{14} (G-1-C^{14}) Volk Radio Chemical Company and Nuclear Chicago Corporation; glucose 6-C^{14} (G-6-C^{14}) Volk Radio Chemical Company and Nuclear Chicago Corporation; and the glucose U-C^{14} (G-U-C^{14}) from Volk Radio Chemical Company. These were purchased in either 0.05 or 0.10 millicurrie quantities in the dried form. All glucose solutions were made up in this laboratory to an activity of 1 uc per ml and to 1 mg/ml with inactive reagent grade glucose. Under no exception was a different concentration used in the experiments reported in this thesis.

The herbicide solutions were made up to the concentrations given in the tables. A list of herbicides, the symbols to be used and supplier is given in Table I. All herbicides used were of the highest obtainable purity.

TABLE I

HERBICIDES AND PLANT GROWTH REGULATORS USED IN THESE EXPERIMENTS

Chemical Name	Abbreviations*	Supplier
2,4-dichlorophenoxyacetic acid	2,4-D	Monsanto Chemical Company
2,6-dichlorophenoxyacetic acid	2,6-D	American Chemical Paint Company
2,4,5-trichlorophenoxyacetic acid	2,4,5-T	Pittsburgh Coke and Chemical Co.
2,4,6-trichlorophenoxyacetic acid	2,4,6-T	American Chemical Paint Company
Indole-3-acetic acid	IAA	Nutritional Biochemicals Corp.
Gibberelic acid, Potassium salt	GA	Merck and Company, Inc.
a-naphthaleneacetic acid	a-NAA	Westville Laboratories
b-naphthaleneacetic acid	-NAA	University of Chicago
3-(p-chlorophenyl) 1,1-dimethylurea	CMU	E. I. Du Pont De Nemours and Co.
3-amino-1,2,4-triazole	ATA	American Cyanamid Company
N-phenyl isopropylcarbamate	IPC	Locally Recrystallized
Maleic Hydrazide, sodium salt	MH	Naugatuck Chemical Company
Phenylmercuric acetate	ØMA	Eastman Organic Chemicals
2-chloro-4,6-bis(ethylamino-s-triazine)	Simazin	Geigy Chemical Corporation
Ethyl N,N-di-n-propylthiocarbamate	EPTC	Stauffer Chemical Company
a.a-dichloropropionic acid	Dalapon	Dow Chemical Company
2,3,6-trichlorobenzoic acid	2,3,6-TBA	Heyden Chemical Corporation
2,3,5-triiodobenzoic acid	TIBA	Eastman Organic Chemicals

*Abbreviations used here are those used by Hubert Martin, "Guide to the Chemicals used in Crop Protection", Canada Department of Agriculture, (1957)

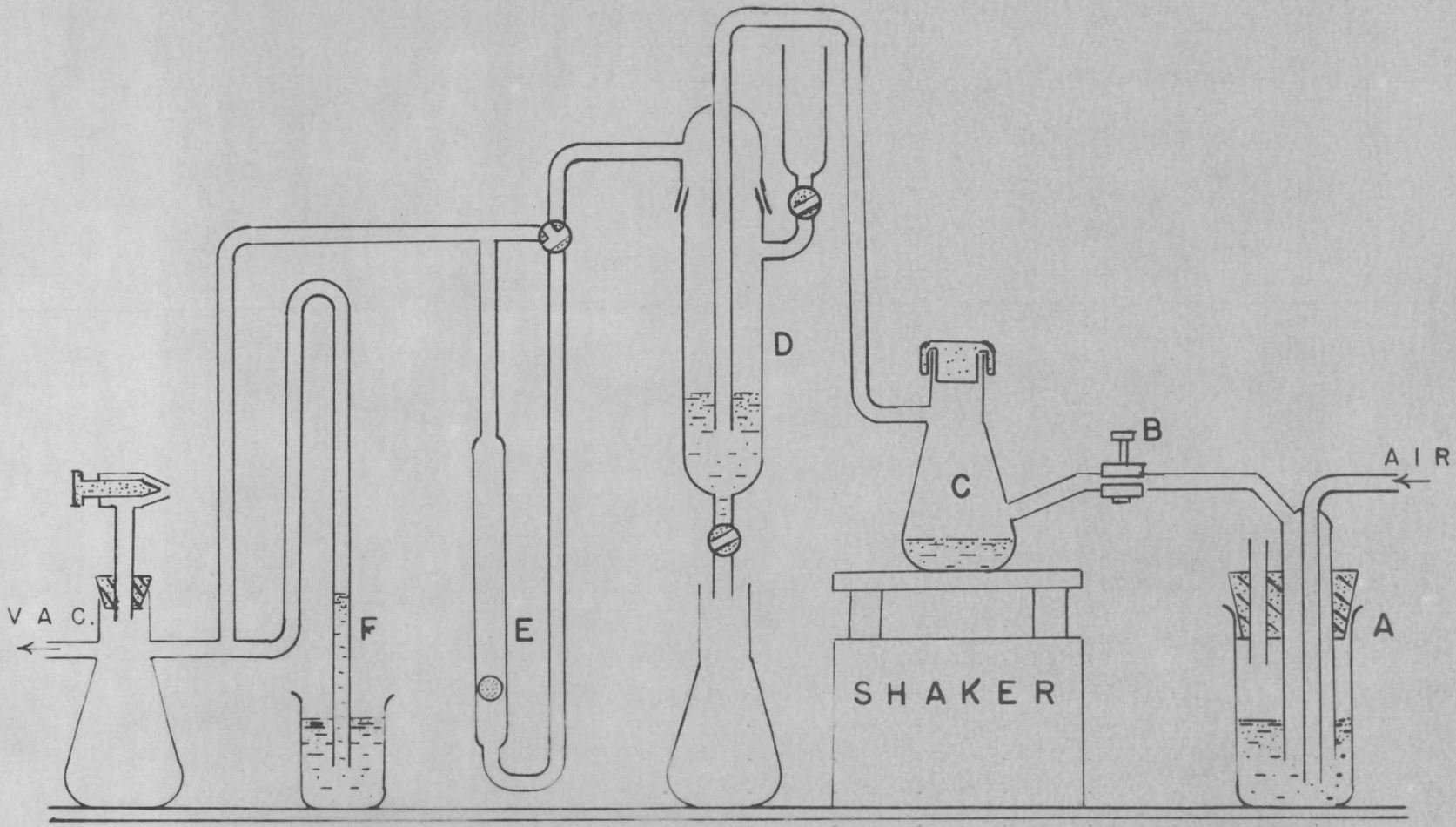


FIGURE I.

RESPIRATOR

The sodium hydroxide solution used for absorption was made up from a technical grade saturated solution available within the department. The phosphate buffer solution was made up in stock solution at 2×10^{-2} molar and diluted for use as a buffer.

The respirometer used in these experiments is one of our own design. It is diagramed in cross section in Figure 1 with the various components lettered. CO_2 absorber (A) with pressure regulator is necessary for the cleaning of the air which is drawn from the building's compressed air lines. The CO_2 absorber is filled with 100 ml of 5 N NaOH and is vented to the atmosphere for removal of excess air. The intake manifold serves to distribute the air under pressure to each of the eight reaction flasks (C). Screw clamps (B) are used to regulate the flow of air through the respirometer. The reaction flask was made by adding an entrance tube near the bottom and an exit tube near the top of a 50 ml pyrex erlenmeyer flask. This arrangement was designed to permit complete air circulation throughout the entire flask. The tops of the flasks were turned down to permit the use of serum bottle caps as seals and also to allow access to the flask for the possible withdrawal of samples and the addition of H_2SO_4 for stopping the reaction.

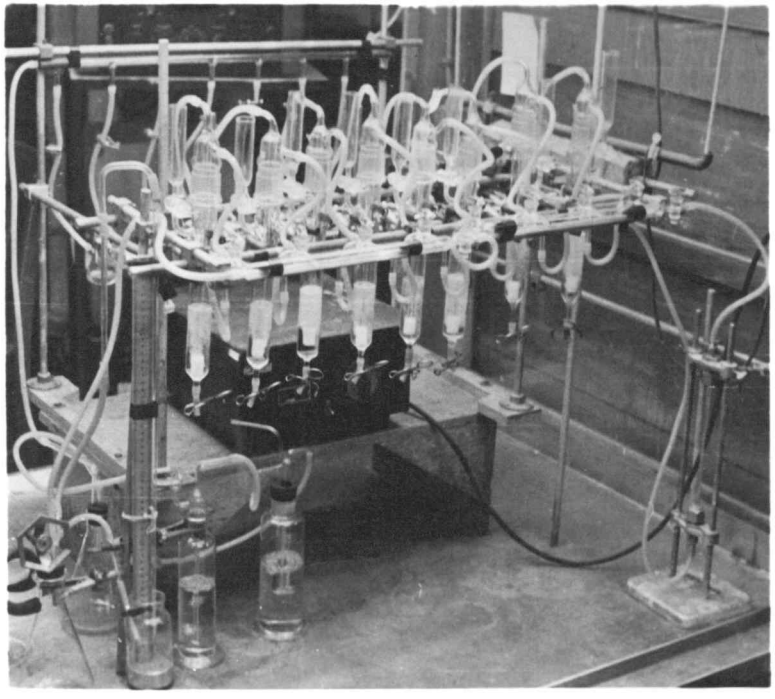
The flasks are fixed in a row of eight to a vibrating

shaker which supplies the necessary agitation for mixing the solution and tissue (Figure 2).

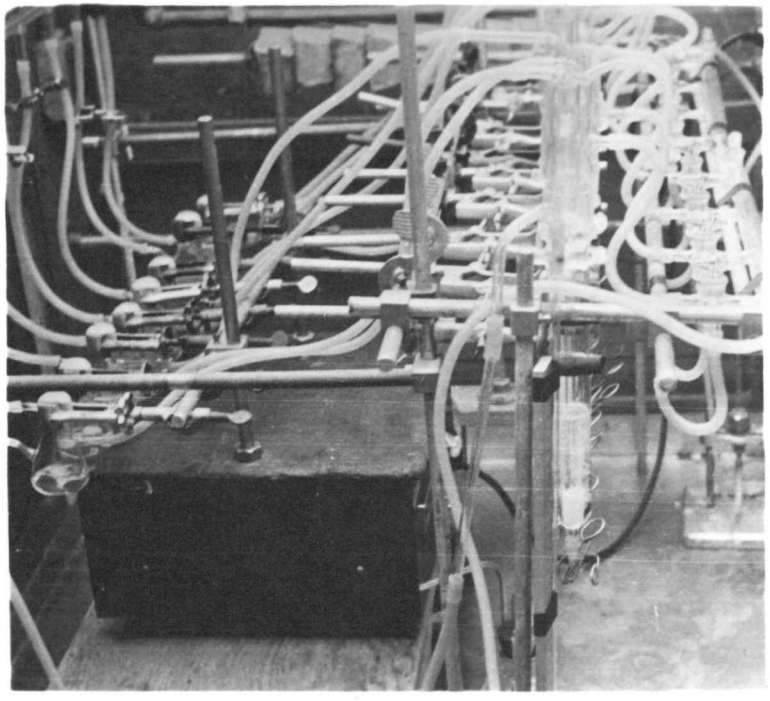
The exhaust from the flask is connected with soft rubber surgical tubing to the CO_2 absorbers (D). These absorbers are made of two parts. The outer part has a drain at the bottom which is closed off by a pinch clamp, and has a capacity of about 80 cc. The inner part serves as a cap for the outer portion consisting of a standard taper glass joint to fit the outer portion, a vent tube for removal of the air after the CO_2 has been extracted, an intake tube with a sintered glass end which extends down to the bottom of the outer portion, and a valve and reservoir arrangement for adding fresh NAOH.

The vent from the absorber is connected directly to a three-way stopcock. One exit from the stopcock is connected to the exhaust manifold, the other to a second manifold or flow manifold. The two manifolds serve as collection mechanisms for returning the air to a single stream. The flow manifold serves to divert the air through the flow meter (E) for the measurement of air velocity. By use of the three-way stopcocks any one or combination of absorbers can be diverted and measured. The flow meter then returns the air to the exhaust manifold. The exhaust reservoir serves as a vacuum reservoir and water trap. The needle valve allows the vacuum to be

FIGURE 2



a, Respirometer Front View



b, Respirometer Side View

finely adjusted and the manometer (F) registers the vacuum pressure. The vacuum reservoir is in turn connected to a water aspirator.

The entire respirometer is contained in a large hood with access to two sides and placed under twelve 40 watt fluorescent lights. The temperature here remained between 27° and 29° C.

Counting and sample preparation equipment will be considered later in the section on preparation of samples for counting.

Plant Material Preparation

The seeds to be used in the experiment were rolled in moist paper towels, 40 seeds to a roll, placed in 400 ml beakers with one inch of water in the bottom, and the beaker containing four rolls placed in a germination chamber. The germination chambers were constructed of two aluminum pots 11 inches in diameter, placed one on top of the other. An inch of water was left on the bottom to maintain a saturated atmosphere. Germination was allowed to proceed in the dark and at room temperature for 72 hours. At that time the seedlings had developed a primary root about 30 to 40 mm long, but the epicotyl, though emerged to a maximum of 5 mm, had not yet straightened. The seedlings were removed from the germination chamber

and those to be used in the experiment were then selected for their uniformity.

Treatment and Preparation of Tissue

Treatment was carried out in specially prepared trays. The treatment trays were constructed of plastic sandwich boxes 11.5 cm by 10.5 cm by 3.4 cm. Into the top were drilled 64 evenly spaced holes about 3 mm in diameter. The trays had a capacity of 350 ml. One seedling was placed in each hole and 300 ml of both the treatment and control solutions added at the same time from previously prepared solutions. The filled trays were then covered with moist paper towels and allowed to stand for two hours. The control solution was a 10^{-2} M KH_2PO_4 solution made up from the previously prepared stock solution. The herbicide solutions were made up by mixing equal portions of the stock potassium phosphate solution and stock 2×10^{-4} M herbicide solution. All herbicide stock solutions were made up to identical concentrations with the exception of Simizan (2.5×10^{-5} M) and IAA (5.35×10^{-5} M). All solutions were stored in tightly stoppered bottles until used.

At the end of the two hour absorption period the moist paper towels were removed and the seedlings placed in 200 ml beakers. They were washed three times with tap

water and stored under paper towels until used. The apial 20 mm of the root tip of each seedling was immediately removed with a single edged razor blade. The root tips were then blotted dry with a paper towel and weighed, all weights being adjusted to within 20 mg by cutting off sections from the previously cut end. Root tips were then placed in the reaction flasks containing 10 ml of 1×10^{-2} M phosphate buffer and one ml of substrate solution added. The flasks were attached to the respirometer and shaken gently by means of a mechanical shaker. The CO_2 absorbers were filled with 50 ml of fresh 0.5 N NaOH solution which had a low CO_2 content. CO_2 free air was passed through by means of a vacuum on the exhaust manifold and pressure on the intake manifold at a rate of 55 cc per minute. The flow of air was regulated by screw clamps on the intake tubing leading from the intake manifold to the flask. The reaction was allowed to progress at room temperature and under constant light conditions for six hours. Samples were removed at intervals during the six hour incubation period, at one, two, four and six hours, and stored in 125 ml erlenmeyer flasks stoppered with rubber stoppers. In experiments with G-U-C^{14}, all flasks contained G-U-C^{14} as a substrate, but in the experiments using G-1-C^{14} and G-6-C^{14} four flasks contained the former and four the latter.

At the end of the six hour period 0.5 ml of 10 N H_2SO_4 was added to each of the flasks with a syringe to stop the reaction and drive off any dissolved CO_2. Shaking and CO_2 free air flushing were continued for fifteen minutes at which time the flasks and final CO_2 sample were removed. The tissue was removed, washed in tap water three times, placed in 10 ml beakers with about 8 ml of 95% ethanol and the beakers stored in covered sandwich boxes.

Preparation of Samples for Counting

The carbonate was counted as $BaCO_3$ by being prepared in the following manner. One ml of 10% $BaCl_2$ solution was added to each of the carbonate samples, the samples were shaken and then allowed to stand overnight. The $BaCO_3$ was filtered out on previously weighed one inch glass fiber filter disks (#934-AH, Huribut Paper Co.) especially prepared for use in one inch planchets. Filtration was accomplished by placing the filter disk in a Tracer Lab E-8B precipitation apparatus mounted on top of a standard one liter filter flask. The entire filtration process was carried out at a maximum vacuum of five inches of Hg. The alkaline solution containing the precipitated $BaCO_2$ was filtered, the erlenmeyer flask was rinsed with distilled water and the last particles of

$BaCO_2$ removed with the aid of a rubber policeman. The flask was then rinsed twice and the walls of the precipitation apparatus washed down with 95% ethanol. The $BaCO_3$ was then dried with ethyl ether. The dried sample was placed on a Tracer Lab E-1A sample tray until all the $BaCO_3$ samples were prepared. These were then weighed, the weight of the $BaCO_3$ being the difference between the filled and empty filter disk. The prepared filter disks were then placed in marked one-inch stainless steel planchets and counted in a Tracer Lab SC-33 "1000" scaler using a SC59 standard manual sample changer and UTGC-2 geiger tube with a window thickness of 1.8 mg/cm^2. Background was measured with a new clean one inch stainless steel planchet.

Preparation of the ethanol extract and residue was accomplished by grinding the tissue in ethanol using a porcelain mortar and pestle, filtering the residue on glass fiber filter disks as was described for the preparation of the $BaCO_3$ samples and collecting the ethanol extract in a 16 x 150 mm culture tube. Before drying the residue with ethyl ether the culture tube was removed from the filter flask and the volume made up with 95% ethanol. The dried residue was then weighed on the filter disk, placed in a one-inch stainless steel planchet, and counted on the equipment described above.

A 1.0 ml aloquot of the ethanol extract was pipetted directly into a one-inch stainless steel planchet and dried under an infra red heat lamp. The remaining 9 ml of extract was stoppered and stored in the refrigerator. When dry, 1.0 ml of 95% ethanol was added and the planchet again dried. This second drying assuring a uniform plate over the entire surface of the planchet. The ethanol extract was then counted as previously described.

Counting Corrections and Calculations

All the data reported in this thesis were corrected to counts as $BaCO_3$ in the following manner.

A correction for $BaCO_3$ self-absorption was obtained from a self-absorption curve kindly supplied by Dr. Sheng Chung Fang of this department.

Counts as residue were corrected to counts as $BaCO_3$ by multiplying the actual counts per minute by a factor of 2.2 per 25 mg of residue. This factor was arrived at by combustion experiments carried out in the following manner. Various weights of residue were burned in a CuO combustion chain, the CO_2 absorbed in CO_2 free 0.5 N sodium hydroxide, precipitated as $BaCO_3$ by $BaCl_2$, plated and weighed. This $BaCO_3$ was then counted, corrected for self-absorption and the correction factor calculated by

dividing the counts per minute as $BaCO_3$ by the counts per minute as residue. Although this value of 2.2 was used through this work, it appears from more recent work, that the value may be slightly more or less, for greater or lesser amounts of residue. The difference is small, however, and does not affect the final calculations significantly.

The counts per minute obtained from the alcohol extract were corrected to counts per minute as $BaCO_3$ by multiplying by a factor of 1.25. This value was also obtained by combustion experiments.

No scaler or geiger tube correction was necessary since the same scaler and tube were used in each experiment.

Methods for Calculation of Results

Absorption was calculated by the summation of the counts recovered as respiratory CO_2, in the residue and in the alcoholic extract. The percent absorption was calculated on the basis of 100 percent for the control by dividing the absorption of the treated seedlings by that of the control. The absorption of the controls differed very little from one run to another.

The percent absorbed C^{14} recovered as respiratory CO_2 was calculated by dividing the counts per minute as

respiratory CO_2 by the total counts per minute absorbed. In a similar manner, values were calculated for the residue and the alcoholic extract.

The C6/C1 values were calculated by dividing the counts per minute recovered as respiratory CO_2 from glucose-6-C^{14}, by the counts per minute recovered as respiratory CO_2 from glucose-1C^{14}.

The F values are simply a ratio of the C6/C1 value for the treated plant, divided by the C6/C1 value for a control, run at the same time. The result is a value either greater than, equal to, or less than unity. If the value is equal to unity, there is no difference in the C6/C1 values which indicates no difference in the metabolism of the treated and untreated seedlings. Any deviation in the F value from unity indicates a shift in the C6/C1 ratio of the treated plant. In other words, a deviation from unity indicates some effect caused by the herbicide. Analysis of both the F values and the recovery data must be undertaken in order to determine whether the shift is in the pentose or glycolytic pathways and whether inhibition or stimulation is responsible for the shift.

EXPERIMENTAL

The Phenoxy Acids; 2,4-dichlorophenoxy acetic acid Concentration Studies

Preliminary work was carried out with 2,4-D in order to determine the proper herbicide concentration to be used. Humphreys and Dugger (22, p. 136-140) in their work used 1 x 10^{-3} molar 2,4-D; however, this was found to cause physiological damage to the seedlings when used in this laboratory. The root tissue became quite dehydrated and its absorption of substrate was reduced to about one-fourth that of tissue treated with 1 x 10^{-4} molar 2,4-D. Treatment with 1 x 10^{-5} molar 2,4-D caused only slight inhibition to substrate absorption while showing no noticeable physiological damage. The same is found for 1 x 10^{-4} molar solutions, but the substrate absorption is slightly less.

Experiments carried out using G-1-C^{14} and G-6-C^{14} showed that 1 x 10^{-4} molar 2,4-D solutions had little effect on the C6/C1 value for CO_2, while 1 x 10^{-5} M solutions increased this value indicating a stimulation in the glycolytic oxidation of glucose. A 1 x 10^{-3} molar solution, however, caused a decisive reduction in the C6/C1 ratio, indicating either an inhibition of glycolytic oxidation or a stimulation of the pentose pathway. The

data indicates that the former is the case, since the respiratory CO_2 production is greatly decreased. Because of the difference in inhibition between the C-6 carbon (73%) and the C-1 carbon (47%), it would appear that glycolysis is more strongly inhibited although both are affected. Since both carbons are not affected equally; the effect of 2,4-D is not simply one of absorption inhibition.

Solutions of both 1×10^{-3} molar and 1×10^{-4} molar 2,4-D show a stimulation of CO_2 output from both the first and sixth carbon atoms, the former solution showing equal stimulation of the first and sixth carbon atoms, the later showing a greater stimulation of the sixth carbon atom.

It appears that low concentrations of 2,4-D owe their stimulatory powers to a stimulation of glycolytic oxidation while higher concentrations owe their inhibiting powers to inhibition of both the glycolytic and the pentose pathways.

All concentrations of 2,4-D used caused a reduction in the utilization of C1 and C6 carbon atoms for cellular synthesis. In both the treated and untreated tissue the C6 carbon is more readily utilized than the C1 as is shown by the C6/C1 ratio for the residue. Low concentrations of 2,4-D tend to inhibit the utilization of both carbons; however, the C6 carbon is more readily inhibited. A 1×10^{-4} M solution of 2,4-D appears to inhibit

utilization of both equally well as does the 1 x 10^{-3} M solution. Examination of the CO_2/residue values show an increase with increasing concentration of 2,4-D in the case of C1 utilization; however, a decrease when C6 is the substrate carbon. High herbicide concentrations inhibit the utilization of the C6 carbon atom and stimulate the C1 carbon atom, while low herbicide concentrations reverse the utilization to a C6 carbon atom stimulation and an inhibition of C1 carbon atom utilization.

Time course studies of the respiratory CO_2 show virtually no effect on the C1 carbon except during the later part of the period when the lower concentrations appear to exhibit a more pronounced stimulation. There appears to be a direct relationship, on the other hand, between 2,4-D concentration and CO_2 elimination from the C6 carbon. A concentration of 1 x 10^{-5} molar causes a stimulation in respiratory CO_2 throughout the time studied. The 1 x 10^{-4} molar concentration appears to show an inhibition during the early period from which it recovers showing a stimulation during the last part of the period. Again the 1 x 10^{-3} molar concentration shows a decisive inhibition. Thus after treatment of the root tissue with 2,4-D it appears that as the plant utilizes the glucose, the C1 carbon is stimulated by decreased concentrations of 2,4-D while the C6 carbon atom is

unhibited under these same conditions.

This is further emphasized by Figure 3a which plots a decreasing C6/C1 ratio with increasing concentration, again explained by an increased C1 catabolism and a decreased C6 catabolism.

The C6/C1 ratio appears after a short time lapse to return to near normal for untreated tissue with the exception of the most concentrated 2,4-D solutions. Figure 3b follows the C6/C1 ratios for CO_2 over a six-hour time period.

From analysis of the data just described it was decided to use a herbicide concentration of 1×10^{-4} M in further studies since it did not cause any physiological damage to the plant, nor did it display any of the stimulatory effects common to the plant growth regulators. At this concentration and treatment period the tissue seemed readily able to overcome the toxicity of the herbicide and return to normal, thus permitting the study of treated tissue without placing it under excessive stress.

Faludi (12, p. 273-282) found similar results in potato tissue while studying amino acid content. He found 10^{-5} M to stimulate growth and 10^{-3} M to inhibit growth. In every case he found a decrease in the amino acid content, the decrease being dependent on 2,4-D concentration.

TABLE IIa

2,4-D CONCENTRATION STUDIES: RECOVERY DATA

Concentration M	Substrate	Treatment	% Absorbed C^{14} Recovered as CO_2 per hour 1hr.	2hr.	4hr.	6hr.	No. Runs	% Absorbed C^{14} Recovered as CO_2	Residue	EtOH Sol.	No. Runs	CO_2 Residue
10^{-5}	G-1-C^{14}	2,4-D + B	5.5	7.2	15.6	28.1	3	49.1+3.1	38.6+2.2	12.3+1.7	3	1.27
		Buffer only	6.7	9.2	14.6	27.4	3	54.0+5.8	35.5+4.3	10.3+2.2	20	1.52
	G-6-C^{14}	2,4-D + B	5.2	10.1	15.2	27.2	3	42.9+5.9	45.9+6.5	11.2+3.0	3	.94
		Buffer only	6.7	11.5	15.9	25.0	3	40.1+2.7	46.3+2.4	13.4+2.1	20	.87
	G-U-C^{14}	2,4-D + B	3.9	7.4	17.1	27.3	3	36.8+2.3	49.5+2.3	13.7+4.5	3	.75
		Buffer only	3.8	7.9	17.4	26.8	3	47.4+5.8	41.0+4.6	11.5+2.7	3	1.15
10^{-4}	G-1-C^{14}	2,4-D + B	5.2	6.6	12.4	31.6	3	52.6+4.2	34.5+2.2	12.9+2.0	3	1.52
		Buffer only	6.7	9.2	14.6	27.4	3	54.0+5.8	35.5+4.3	10.3+2.2	20	1.52
	G-6-C^{14}	2,4-D + B	4.5	8.3	13.8	29.8	3	39.3+3.3	49.5+3.2	14.8+2.6	3	.79
		Buffer only	6.7	11.5	15.9	25.0	3	40.1+2.7	46.3+2.4	13.4+2.1	20	.87
	G-U-C^{14}	2,4-D + B	2.5	5.5	13.3	32.6	6	54.5+6.0	36.0+5.0	9.5+2.0	6	1.51
		Buffer only	3.8	7.9	17.4	26.8	6	47.4+5.8	41.0+4.6	11.5+2.7	27	1.15
10^{-3}	G-1-C^{14}	2,4-D + B	7.1	4.2	12.5	31.8	3	25.8+10	11.5+4.5	63.0+11	3	2.24
		Buffer only	6.7	9.2	14.6	27.4	3	54.0+5.8	35.5+4.3	10.3+2.2	20	1.52
	G-6-C^{14}	2,4-D + B	6.3	3.7	14.1	30.8	3	9.9+6.0	14.4+6.7	78.1+11	3	.69
		Buffer only	6.7	11.5	15.9	25.0	3	40.1+2.7	46.3+2.4	13.4+2.1	20	.87

Note: + represents ± deviation.

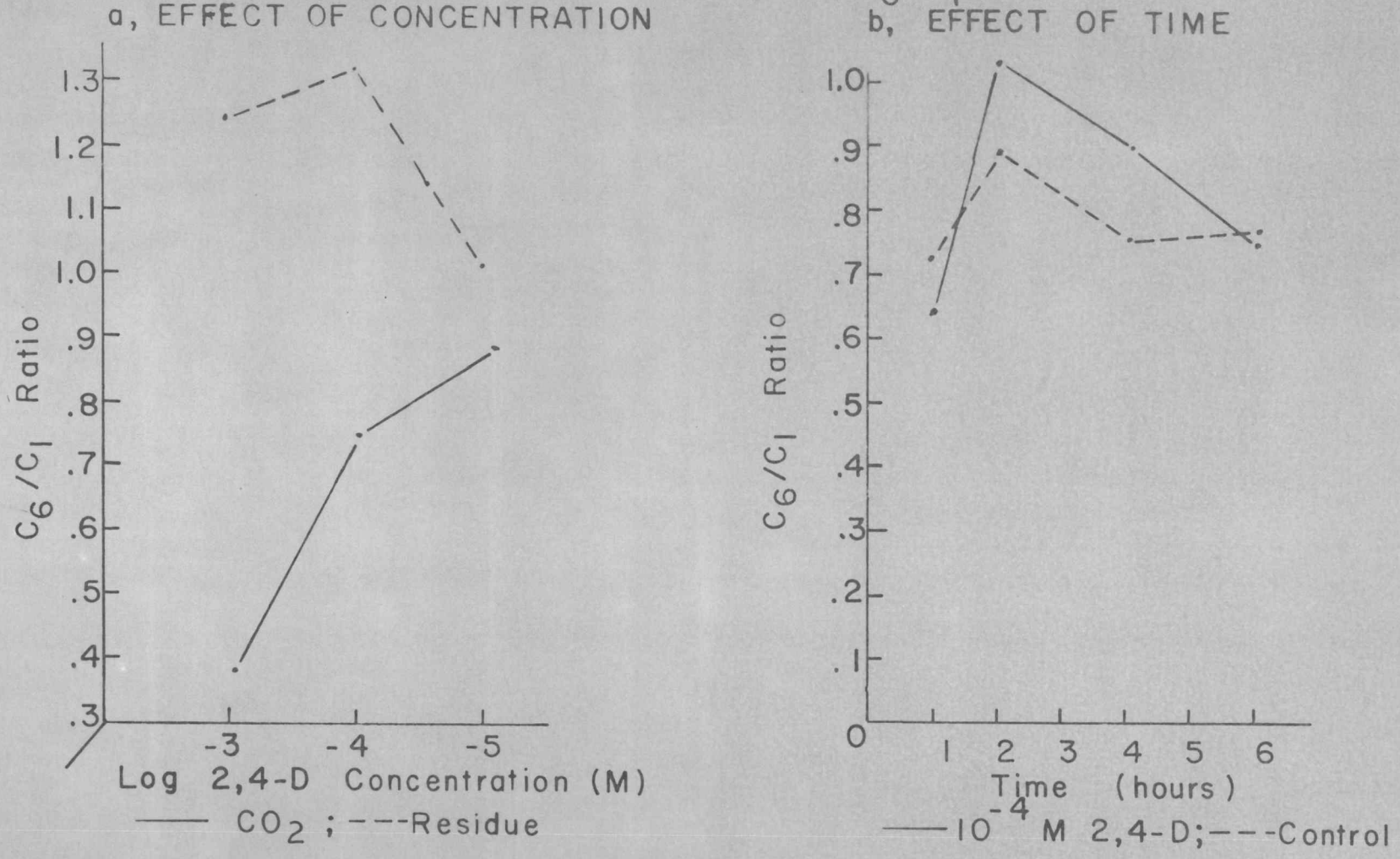
TABLE IIb

2,4-D CONCENTRATION STUDIES: METABOLISM AND ABSORPTION DATA

Compound	Concentration M	% Abs.	F. Values			C6/Cl Values for Respiratory CO_2/hr.				C6/Cl Values for six hour period		
			CO_2	Residue	EtOH Sol.	1hr.	2hr.	4hr.	6hr.	CO_2	Res	CO_2/Res
2,4-D	10^{-5}	85.5 ± 6.8	1.14 ± 0.11	.946 ± .130	1.03 ± 0.02	.790	1.12	.810	.790	.877 ± .080	1.19 ± .023	.738
2,4-D	10^{-4}	79.7 ± 6.5	.978 ± 0.05	1.12 ± 0.13	1.03 ± 0.12	.640	1.04	.910	.750	.750 ± .053	1.33 ± .03	.564
2,4-D	10^{-3}	12.3 ± 3.0	.476 ± .081	.788 ± .220	1.03 ± 0.03	.355	.295	.290	.290	.366 ± .070	1.25 ± .061	.292
Buffer	-	100	1.00	1.00	1.00	.761	.956	.843	.699	.754 ± .064	1.34 ± 0.15	.575

FIGURE 3

THE EFFECT OF 2,4-D ON THE C_6/C_1 RATIO



The Phenoxyacetic Acids

The pheonxy acids used in these experiments were comprised of two which are herbicidally active, 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and two which are herbicidally inactive, 2,4,6-trichlorophenoxyacetic acid (2,4,6-T) and 2,6-dichlorophenoxyacetic acid (2,6-D). Of the two active herbicides, 2,4,5-T is the more active, while both 2,6-D and 2,4,6-T show absolutely no herbicidal activity.

In studying the absorption (Table IIIb) no correlations can be drawn between herbicidal activity and inhibition to absorption. It does appear, however, that the number of substituted chlorines on the ring does affect the absorption. Those with two chlorines on the ring, 2,4-D and 2,6-D, have nearly similar absorptions, 79% and 73% respectively, while those having three chlorines, 2,4,5-T and 2,4,6-T, have identical absorptions somewhat lower, 58%.

Glucose U-C^{14} shows no effect as a result of treatment by any of the phenoxy compounds used with the exception of the last hourly period. During this period 2,4,6-T, a non-toxic compound, shows the greatest stimulation although no inhibition is found using any of the

compounds.

In respiratory CO_2 studies, as in absorption studies, no correlation can be found between C6/C1 values and herbicidal activity. A toxic compound 2,4-D, and 2,4,6-T, a non-toxic compound, produce ratios very near that of the untreated seedlings. A toxic compound, 2,4,5-T, causes a definite decrease in the ratio and 2,6-T, a non-toxic compound, causes an increase.

The time course studies of the C6/C1 ratios for respiratory CO_2 show no correlation between herbicidally active and inactive compounds except during the first hourly period when the C6/C1 values for the active compounds increase slightly more rapidly. The 2,6-D causes a slightly different time course effect, which by noting the control, can be seen to be due to some external influence and not to the compound since its control differs.

Residue studies of C6/C1 ratios again show no correlation with toxicity. Only 2,4-5-T causes a large change due to a decrease in C1 utilization as seen in Table IIIb. The 2,4,6-T causes a slightly greater inhibition to C1 than C6 while 2,4-D produces an equal decrease in C1 and C6 utilization. The 2,6-D is the only compound which causes a decrease in the C6/C1 ratio which is due to a decrease in C6 utilization, while the C1 utilization remains about the same as the control. Again

the number of substituted chlorines seems to have more effect on the plant than the herbicidal activity. The tri-substituted compounds caused a greater decrease in Cl utilization and the di-substituted compounds caused either no effect or a greater inhibition to the C6 utilization.

Examination of the CO_2/residue values indicates that all the herbicides, both those herbicidally active and those inactive, caused either no change or an increase in this ratio for Cl utilization. The C6 utilization, however, does have a correlation with herbicide activity. The 2,4-D and 2,4,5-T either caused no change or a slight decrease in the CO_2/residue ratio while the inactive 2,6-D and 2,4,6-T caused an increase. Since the change is found in the inactive compounds and not in the active ones, this cannot be an effect of herbicidal activity.

In every case, with the exception of 2,4,6-T, there was an equal increase in the percent of C/1 and C/6 carbon atoms found in the alcoholic extract. However, the overall incorporation of all six carbon atoms decreased; the herbicidally inactive 2,4,6-T caused a greater decrease. This again signals a decrease in glycolytic oxidation since glycolysis and Krebs cycle are responsible for the bulk of the soluble compounds.

In summary, consideration will be given to the

relative values for the C6/C1 as was described earlier. These F values like the previously described work do not show any correlation with the herbicidal activity of the compound. All but 2,6-D show a decrease in F value indicating the plant is placing more reliance on the pentose pathway. This was as previously stated due to a decrease of C6 utilization caused by inhibition to the glycolytic pathway. The 2,6-D, on the other hand, shows an increased F value indicating more reliance on the glycolytic pathway for the oxidation of glucose. This appears to be due to increased activity in C6 utilization.

A similar situation exists in the case of the residue F values. All the compounds, with the exception of 2,6-D, increase the F value indicating relatively more C6 carbon atoms incorporated into cellular residue than C1. This is due to a greater inhibition of C1 than C6 utilization. The 2,6-D again showed inhibition to the C6 carbon utilization.

The F values for the alcoholic extract, with the exception of 2,4,5-T treated plants, remain unchanged. The 2,4,5-T, however, shows a greater increase in alcohol soluble compounds formed from the C6 carbon.

From the data presented here it is postulated that the herbicidal activity of 2,4-D and 2,4,5-T is not due to their effect on glucose metabolism, since no significant

correlation can be found between the effect on metabolism and its activity.

The Natural and Synthetic Hormones

The natural and synthetic hormones used in this work were a-naphthalene acetic acid (a-NAA), b-naphthalene acetic acid (b-NAA), gibberellic acid (GA), and indole-3-acetic acid (IAA).

A mixture of a-naphthalene acetic acid and b-naphthalene acetic acid is sold commercially under the trade name of "Rootone", which is widely used in the rooting of cuttings. Galston has shown that there is an adaptive increase in IAA oxidase activity following application of NAA (18, p. 373-380).

A great deal of work concerning the function and metabolism of the natural growth hormone in the plant has been carried out. Fang, et al., have described the effect of light and 2,4-D on IAA distribution (14, p. 253-259) and metabolism (15, p. 26-32). Turian has described an increase in glycerophosphatase (43, p. 368-370) while others have shown various responses in ascorbic acid oxidase, catalase, peroxidase, pectin methyl esterase, polyphenol oxidase and glucose-6-phosphate dehydrogenase (48, p. 329-332). In most cases the critical concentration between stimulation and inhibition is 10^{-4} M to 10^{-6} M,

TABLE IIIa

THE PHENOXYACETIC ACIDS AND THEIR ANALOGS RECOVERY DATA

Concentration M	Substrate	Treatment	% Absorbed C^{14} Recovered as Respiratory CO_2 1hr.	2hr.	4hr.	6hr.	No. Runs	% Absorbed C^{14} Recovered as CO_2	Residue	EtOH Sol.	No. Runs	CO_2 Residue
	G-1-C^{14}	2,4-D + B	5.2	6.6	12.4	31.6	3	52.6±4.2	34.5±2.2	12.9±2.0	3	1.52
		Buffer only	6.7	9.2	14.6	27.4	3	54.0±5.8	35.5±4.3	10.3±2.2	20	1.52
10^{-4}	G-6-C^{14}	2,4-D + B	4.5	8.3	15.8	29.8	3	39.3±3.3	49.5±3.2	14.8±2.6	3	.79
		Buffer only	6.7	11.5	15.9	25.0	3	40.1±2.7	46.3±2.4	13.4±2.1	20	.87
	G-U-C^{14}	2,4-D + B	2.5	5.5	13.3	32.6	6	54.5±6.0	36.0±5.0	9.5±2.0	6	1.51
		Buffer only	3.8	7.9	17.4	26.8	6	47.4±5.8	41.0±4.6	11.5±2.7	27	1.15
	G-1-C^{14}	2,4,5-T + B	6.6	5.8	10.9	33.8	3	56.7±5.7	25.0±1.0	18.3±2.0	3	2.26
		Buffer only	7.2	8.7	16.7	25.3	3	54.0±5.8	35.5±4.3	10.3±2.2	20	1.52
10^{-4}	G-6-C^{14}	2,4,5-T + B	5.8	9.2	13.5	29.0	3	34.9±3.0	40.9±1.0	24.3±2.0	3	.86
		Buffer only	6.4	10.4	14.4	27.1	3	40.1±2.7	46.3±2.4	13.4±2.1	20	.88
	G-U-C^{14}	2,4,5-T + B	6.9	6.8	16.1	27.0	6	50.3±8.6	32.4±5.8	17.0±2.1	6	1.55
		Buffer only	6.5	14.3	17.4	22.1	6	47.4±5.8	41.0±4.6	11.5±2.7	27	1.15
	G-1-C^{14}	2,4,6-T + B	4.6	5.8	12.3	32.5	3	59.5±3.0	24.4±0.5	16.1±1.0	3	2.44
		Buffer only	5.9	8.7	17.3	25.4	3	54.0±5.8	35.5±4.3	10.3±2.2	20	1.51
10^{-4}	G-6-C^{14}	2,4,6-T + B	3.9	7.3	12.0	32.5	3	42.3±4.0	35.6±2.0	21.1±2.0	3	1.19
		Buffer only	5.7	10.4	15.0	26.9	3	40.1±2.7	46.3±2.4	13.4±2.2	20	.87
	G-U-C^{14}	2,4,6-T + B	3.1	5.7	10.0	35.6	3	60.0±7.2	23.2±4.3	15.8±4.0	8	2.58
		Buffer only	4.9	9.4	14.8	28.1	3	47.4±5.8	41.0±4.6	11.5±2.7	27	1.15
	G-1-C^{14}	2,6-D + B	3.7	9.1	13.7	29.9	3	53.8±2.4	29.5±1.4	16.4±1.1	3	1.82
		Buffer only	6.0	11.3	15.0	26.3	3	54.0±5.8	35.5±4.3	10.3±2.2	20	1.52
10^{-4}	G-6-C^{14}	2,6-D + B	3.7	8.8	13.9	29.8	3	44.7±1.0	37.3±1.0	17.9±1.0	3	1.20
		Buffer only	5.5	11.8	16.0	25.4	3	40.1±2.7	46.3±2.4	13.4±2.2	20	.87

TABLE IIIb

THE PHENOXY ACIDS AND THEIR ANALOGS: METABOLISM AND ABSORPTION DATA

Compound	Concentration M	% Abs.	F. Values			C6/Cl Values for Respiratory CO_2/hr.				C6/Cl Values for six hour period		
			CO_2	Residue	EtOH Sol.	1hr.	2hr.	4hr.	6hr.	CO_2	Res.	CO_2/Res
2,4-D	10^{-4}	79.7 ± 6.5	.978 ± .50	1.12 ± 0.13	1.03 ± 0.12	.640	1.04	.911	.753	.750 ± 0.53	1.33 ± 0.03	.564
2,4,5-T	10^{-4}	57.9 ± 13.0	.692 ± 0.01	1.30 ± 0.02	1.22 ± 0.15	.530	.951	.743	.529	.610 ± .027	1.66 ± 0.16	.368
2,4,6-T	10^{-4}	58.3 ± 6.3	.838 ± 0.124	1.57 ± 0.16	.998 ± 0.121	.611	.900	.702	.710	.711 ± 0.66	1.45 ± 0.06	.491
2,6-D	10^{-4}	73.1 ± 4.5	1.16 ± 0.07	.881 ± .063	.918 ± .061	.819	.802	.847	.830	.833 ± .049	1.25 ± 0.08	.666
Buffer	-	100	1.00	1.00	1.00	.722	.905	.760	.777	.754 ± .064	1.34 ± .011	.575

although no single concentration can be picked out which will cause the same direction of response to all the enzymes studied.

Absorption studies indicate both a-NAA and b-NAA cause a large decrease in glucose absorption, b-NAA causing the greatest. IAA caused a small decrease and GA caused only a slight decrease. The synthetic plant hormones caused a much larger decrease in absorption than did the natural hormones.

Studies of respiratory CO_2 using glucose U-C^{14} showed no overall effect on glucose metabolism caused by the natural hormones; however, the synthetic hormones caused a stimulation in CO_2 elimination. The greater stimulation was caused by b-NAA, the most active of these hormones. A time course evaluation (Figure 4) shows no difference in G-1-C^{14} or G-6-C^{14} oxidation between any of the natural hormones; however, during the last two hour period the synthetic hormones appear to increase CO_2 evolution faster than the natural hormones. Again b-NAA increases the fastest.

Studies using glucose-1-C^{14} and glucose-6-C^{14} and the synthetic hormones have much effect on the C6/C1 ratio with the exception of b-NAA. The b-NAA is the more active hormone and also causes the greatest stimulation of C1 utilization or pentose oxidation. There is no reason, however,

to believe that the physiological response of these hormones is caused by glucose catabolism alone.

Analysis of Table 4b shows that the natural hormones have a slightly different effect on the C6/C1 ratio during the course of the first two hours than do the synthetic hormones. The latter seem to stimulate C1 oxidation at first, then they return to a normal rate of oxidation, while the natural hormones follow more closely the control.

In analyzing the data gathered concerning incorporation of glucose carbon atoms into cellular residue there is no overall effect caused by the natural hormones. There is, however, a slight preference for C1 atoms caused by GA and a slight decrease in C1 and C6 atoms caused by IAA. Analysis of the C6/C1 ratios further illustrates this fact. The synthetic hormones decrease greatly the utilization of all glucose atoms with a greater decrease in C1 and C6 utilization. Since there is only a small change in the ratio of glycolytic and pentose oxidation, it would appear that the synthetic hormones inhibit anabolism of glucose to cellular residues. The greater inhibition results in C1 anabolism. This is further substantiated by examination of the C6/C1 ratios for cellular residues. In Table 4b it can be seen that there is a large increase in the ratios caused by a greater inhibition to C1 utilization than to C6 utilization.

The ratio of CO_2/residue values for respiratory CO_2 and

cellular residue permit an easy inspection to the differences between the two classes of hormones. The synthetic hormones falling into one class which is much above the control and the natural hormones which have values around that of the control. The synthetic hormones appear to decrease the amount of cellular formation, while the natural hormones have no effect on cellular formation.

Analysis of Table IVa shows that the percent of C^{14} recovered from G-U-C^{14} as alcoholic soluble compounds decreases with treatment of the plants by synthetic hormones, again b-NAA being the most effective. The natural hormones again cause no change over the controls. The recovery from G-1-C^{14} and G-6-C^{14} was erratic with no correlation possible.

Other Herbicides Affecting Physiological Activity

Of the herbicides to be considered in this section two affect meristematic activity and the other causes considerable dehydration of the plant tissue.

N-phenylisopropylcarbamate (IPC) was first suggested by Templeman and Secton (40, p. 630) for use as a growth substance. It is produced by the Pittsburg Plate Glass Company and marketed under the trade name of "Propham". The most active of a series of arylurethanes as a growth regulating substance, it has the properties which allow it to interfere with nuclear division. IPC is reported to cause

TABLE IVa

THE NATURAL AND SYNTHETIC HORMONES: RECOVERY DATA

Concentration M	Substrate	Treatment	% Absorbed C^{14} Recovered as Respiratory CO_2 1hr.	2hr.	4hr.	6hr.	No. Runs	% Absorbed C^{14} Recovered as CO_2	Residue	EtOH Sol.	No. Runs	CO_2 Residue
	G-1-C^{14}	a-NAA + B	2.7	4.4	9.7	36.8	3	64.5±6.1	23.0±3.3	12.5±2.8	3	2.80
		Buffer only	6.2	8.5	14.5	29.1	3	54.0±5.8	35.5±4.3	10.3±2.2	20	1.52
10^{-4}	G-6-C^{14}	a-NAA + B	2.8	6.7	11.5	33.8	3	46.4±6.6	40.9±1.8	12.5±4.8	3	1.13
		Buffer only	5.0	10.9	16.4	25.6	3	40.1±2.7	46.3±2.4	13.4±2.1	20	.87
	G-U-C^{14}	a-NAA + B	3.7	4.6	12.5	33.3	6	46.6±4.7	31.5±2.3	21.1±5.2	6	1.48
		Buffer only	6.1	9.8	9.8	32.3	6	47.4±5.8	41.0±4.6	11.5±2.7	27	1.15
	G-1-C^{14}	b-NAA + B	2.5	3.7	9.0	37.9	3	69.9±6.3	18.2±2.7	11.9±3.0	3	3.84
		Buffer only	4.4	8.3	13.9	29.2	3	54.0±5.8	35.5±4.3	10.3±2.2	20	1.52
10^{-4}	G-6-C^{14}	b-NAA + B	2.5	6.1	10.7	35.0	3	45.2±5.3	33.1±2.7	21.7±3.9	3	1.37
		Buffer only	5.0	11.0	16.4	25.7	3	40.1±2.7	46.3±2.4	13.4±2.1	20	.87
	G-U-C^{14}	b-NAA + B	2.6	6.5	12.4	33.0	6	50.5±4.9	25.7±1.7	24.5±5.6	6	1.96
		Buffer only	5.0	10.3	15.1	27.3	6	47.4±5.8	51.0±4.6	11.5±2.7	27	1.15
	G-1-C^{14}	GA + Buffer	3.6	7.1	16.6	28.1	3	56.2±3.5	35.4±2.4	8.3±0.8	3	1.58
		Buffer only	3.8	7.8	17.4	26.8	3	54.0±5.8	35.5±4.3	10.3±2.2	20	1.52
5 ppm	G-6-C^{14}	GA + Buffer	5.2	10.2	15.7	26.4	3	39.2±1.3	46.3±1.5	10.3±2.2	3	.85
		Buffer only	5.9	10.7	17.3	24.4	3	40.1±2.7	46.3±2.4	13.4±2.1	20	.87
	G-U-C^{14}	GA + Buffer	5.2	8.9	13.4	29.6	5	48.0±6.6	41.3±6.1	10.3±1.6	5	1.16
		Buffer only	4.8	9.3	16.2	26.7	5	47.4±5.8	41.0±4.6	11.5±2.7	27	1.15
	G-1-C^{14}	IAA + Buffer	3.0	6.7	11.9	33.2	3	56.1±7.9	34.4±4.9	9.5±3.7	3	1.63
		Buffer only	3.9	8.4	15.7	28.1	3	54.0±5.8	35.5±4.3	10.3±2.2	20	1.52
10^{-4}	G-6-C^{14}	IAA + B	4.4	9.2	13.0	30.2	3	40.9±3.7	44.8±2.7	14.2±2.7	3	.91
		Buffer only	6.8	11.1	13.6	27.4	3	40.1±2.7	46.3±2.4	13.4±2.1	20	.87
	G-U-C^{14}	IAA + B	5.3	6.9	12.7	31.2	6	51.4±5.2	37.4±6.8	11.1±5.1	6	1.38
		Buffer only	5.6	8.5	15.4	27.6	6	47.4±5.8	41.0±4.6	11.5±2.7	27	1.15

TABLE IVb

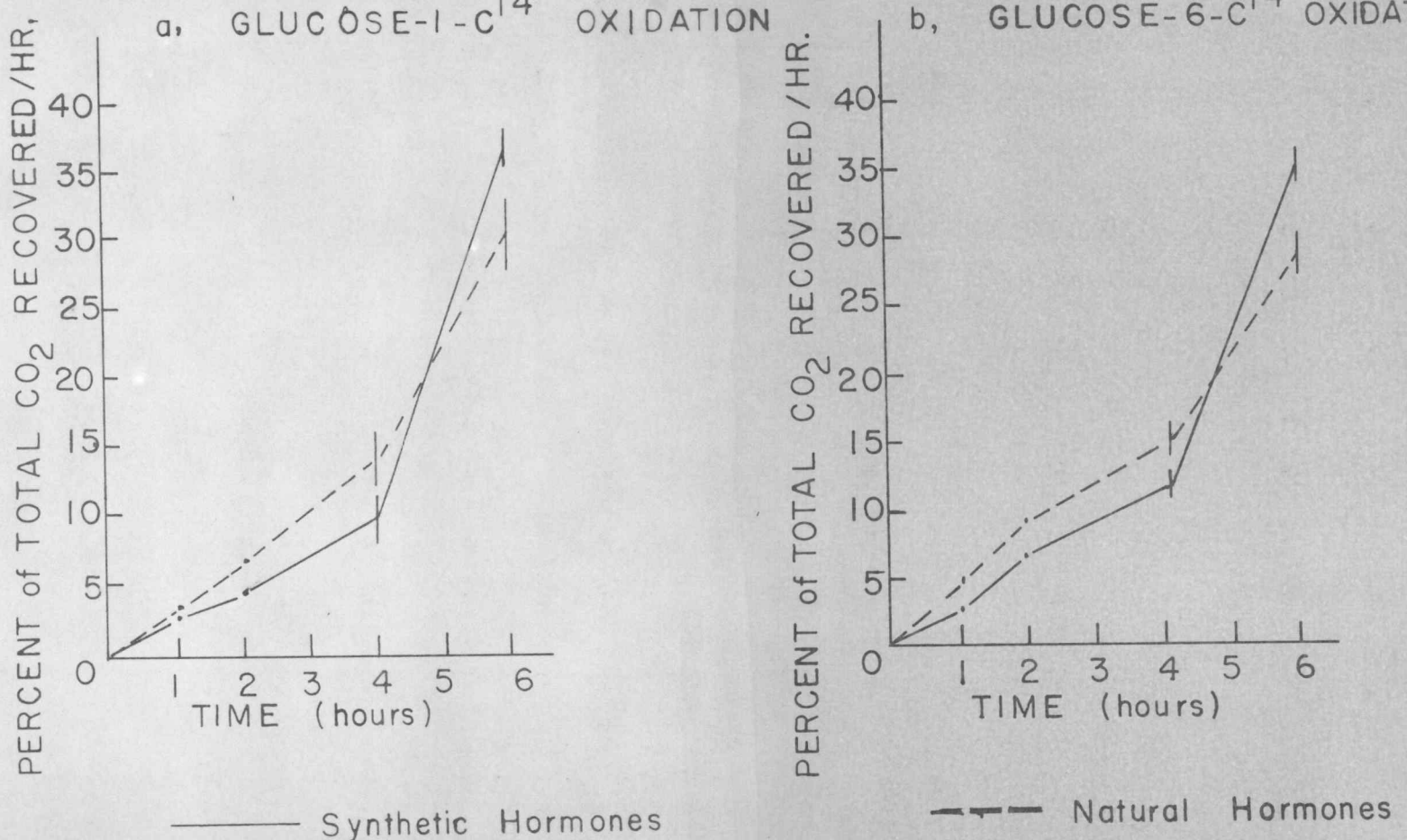
THE NATURAL AND SYNTHETIC HORMONES: METABOLISM AND ABSORPTION DATA

Compound	Concentration M	% Abs.	F. Values CO_2	Residue	EtOH Sol.	C6/C1 Values for Respiratory CO_2/hr. 1hr.	2hr.	4hr.	6hr.	C6/C1 Values for six hour period CO_2	Res.	CO_2/Res.
a-NAA	10^{-4}	63.4 ± 16.2	.940 ± .091	1.14 ± 0.12	.781 ± 0.12	1.03	1.17	.820	.578	.715 ± .037	1.81 ± 0.16	.396
b-NAA	10^{-4}	49.9 ± 10.9	.900 ± .120	1.31 ± 0.05	1.21 ± 0.06	1.14	1.25	.792	.694	.645 ± .059	1.71 ± 0.13	.378
GA*	5ppm	96.6 ± 3.6	1.02 ± 0.03	.920 ± 0.04	1.21 ± 0.07	1.02	1.00	.660	.660	.699 ± .045	1.31 ± 0.10	.534
IAA	10^{-4}	86.6 ± 5.9	1.01 ± 0.04	1.03 ± 0.08	1.22 ± 0.01	1.09	1.04	.830	.690	.776 ± .076	1.33 ± 0.17	.584
Buffer	-	100	1.00	1.00	1.00	1.06	.930	.750	.640	.754 ± .064	1.35 ± 0.15	.560

* This data was taken from earlier work now in press. "The Influences of Gibberellic Acid on the Metabolism of Indoleacetic acid, Acetate and Glucose in Roots of Higher Plants" S. C. Fang, John B. Bourke, V. L. Stevens and J. S. Butts. Department of Agricultural Chemistry, Oregon State College, Corvallis, Oregon. Technical Paper Number 1256. Accepted for publication in Plant Physiology.

FIGURE 4

THE EFFECT OF THE NATURAL AND SYNTHETIC HORMONES ON GLUCOSE OXIDATION



a decrease in root respiration (39, p. 178-189) and an increase in sugars (29, p. 43-49). It is specifically more active on graminaceous plants than on dicotyledons and is used almost exclusively as a selective grass killer. It is particularly effective for control of grasses in peas, potatoes and clover. It is not absorbed through the leaf to any extent, but is rapidly taken up through the roots. For this reason it is applied to the soil, usually before planting of annual crops, at a rate of three to five pounds per acre. IPC is believed to be completely non-toxic to man.

The second herbicide having meristematic activity is 1,2-dihydropyridazine-3,6-dione or Maleic Hydrazide (MH). Its plant growth regulation properties were first described by Schoene and Hoffman in 1949 (36, p. 588-590).

It is used selectively against grasses, being toxic to meristematic tissue, inhibiting mitosis and causing loss of apical dominance (20, p. 205-212). It is readily translocatable in plants and is commonly used to prevent sucker development in tobacco. It is also useful for the prevention of sprouting in root crops, such as onions and beets. That MH inhances IAA oxidase activity (2, p. 439-459) combines with free thiol groups (32, p. 232)(20, p. 212), inhibits growth by blocking dehydrogenase activity (25, p. 465) and increases the synthesis of "gibberellic like hormones" (7, p. 489-497) have all been reported in the

literature; however, the work is as yet unconfirmed. MH also increased oxygen consumption although it does not affect the C6/C1 ratio in leaf disks (37, p. 233-237).

MH is a non-irritant having an oral LD in rats of about 2 g/kg. Like ATA, however, MH may be cancer producing (11, p. 407-408).

The last herbicide in this group is commonly used as a fungicide. Phenylmercuric acetate (PMA) is one of the many mercury compounds used in crop protection; however, it has little power unless it is converted to the chloride. It is used as a selective herbicide for the control of crabgrass on lawns but has the disadvantage of being highly toxic to mammals.

Root tissue treated with PMA reacted violently, becoming highly dehydrated and exhibiting a highly reduced substrate absorption. The C6/C1 value for respiratory CO_2 was decreased while the same value for cellular residue was increased. The overall formation of CO_2 was greatly reduced as was the incorporation of glucose atoms into cellular residue. The ability of PMA treated tissue to oxidize glucose to CO_2 was greatly stimulated during the first hour, but fell off rapidly with time (Figure 5). Both the first and sixth carbon atoms behaved similarly. IPC and MH did not show this, nor did they differ from the control. The amount of soluble compounds was vastly increased, probably

in an effort to retain moisture. It would appear that the primary effect of PMA on the tissue was to dehydrate the cells and that the dehydration may be responsible for the changes in metabolism.

MH, on the other hand, showed only slight alterations in the metabolic scheme. Absorption was only slightly affected with no effect on the oxidation of the C-6 and C-1 carbon atoms of glucose, except an overall slight decrease. No substantial effect is noted on the utilization of glucose carbon atoms for cellular construction. There is, however, a slight inhibition to anabolism which is the same for all glucose solutions. Time course studies of respiratory CO_2 and the corresponding C6/C1 values show no effect caused by the herbicide with the exception of a slightly higher pentose activity during the last few hours.

IPC shows a definite decrease in absorption and overall carbon oxidation. Conversion of the C-6 carbon atom to CO_2 remains unchanged, while that of the C-1 increases. This would be due to an increase in pentose cycle activity as is illustrated by the decreased C6/C1 value for respiratory CO_2. As with MH, there is no effect on utilization of glucose carbon atoms for insoluble cellular material with the exception of a slight shift favoring C-1 anabolism. The alcoholic extract is likewise unaffected. Time course studies of the respiratory CO_2 show an early inhibition to

oxidation followed by a late stimulation, glycolysis being the less active through the entire period.

In general, HM and IPC cause only a slightly higher pentose cycle activity while PMA causes a great deal of physiological damage resulting in metabolic changes.

The Pre-emergent Herbicides

Four herbicides will be considered in this group which find their principal function as pre-emergent herbicides. The first, 2-chloro-4,6-bis (ethylamino)-5-triazine (Simazin) is a promising pre-emergent herbicide for use in corn, tomatoes, grapes and asparagus at 2 to 20 lbs./acre. At higher concentrations Simazin can be used as a soil sterilant, although it does not exhibit any fungicidal properties. Simazin is only active when applied to the soil, by affecting some mechanism in photosynthesis, probably the Hill reaction. It is not believed that this is the site of herbicidal activity since both susceptable and tolerant plants behave similarly to Simizan (31, p. 432-435). Glucose appears to partially overcome the toxicity of Simizan.

Ethyl N,N-di-n-propylthiocarbamate (EPTC) is a liquid herbicide introduced by Stauffer Chemical Company in 1956. It is a promising pre-emergent herbicide; however, most crops are resistant to post-emergent applications. It does not inhibit germination but is toxic to the young seedlings.

TABLE Va

OTHER HERBICIDES AFFECTING PHYSIOLOGICAL ACTIVITY: RECOVERY DATA

Concentration M	Substrate	Treatment	% Absorbed C^{14} Recovered as Respiratory CO_2 1hr.	2hr.	4hr.	6hr.	No. Runs	% Absorbed C^{14} Recovered as CO_2	Residue	EtOH Sol.	No. Runs	CO_2 Residue
	G-1-C^{14}	IPC + B	2.9	6.88	14.1	31.1	3	60.5±3.9	31.4±2.7	8.1±2.2	3	1.93
		Buffer only	3.6	7.72	17.0	28.3	3	54.0±5.8	35.5±4.3	10.3±2.2	20	1.52
10^{-4}	G-6-C^{14}	IPC + B	4.5	8.46	14.3	29.2	3	38.6±1.3	48.4±1.0	12.9±3.1	3	.80
		Buffer only	6.0	11.5	16.6	24.5	3	40.1±2.7	46.3±2.4	13.4±2.1	20	.87
	G-U-C^{14}	IPC + B	5.0	7.82	15.3	28.3	5	43.8±5.0	43.6±4.1	12.7±1.0	5	1.00
		Buffer only	6.4	9.80	15.0	27.0	5	47.4±5.8	41.0±4.6	11.5±2.7	27	1.15
	G-I-C^{14}	MH + B	4.7	7.71	15.5	28.3	4	45.2±7.2	38.1±4.4	16.7±3.2	4	1.19
		Buffer only	6.7	9.87	16.8	25.1	4	54.0±5.8	35.5±4.3	10.3±2.2	20	1.52
10^{-4}	G-6-C^{14}	MH + B	6.4	9.85	15.1	26.8	4	33.9±4.8	48.3±1.6	17.8±3.6	4	.70
		Buffer only	6.8	10.7	17.2	24.0	4	40.1±2.7	46.3±2.4	13.3±2.1	20	.87
	G-U-C^{14}	MH + B	5.1	9.40	16.2	26.6	4	40.4±2.9	45.8±3.8	13.8±1.0	4	.88
		Buffer only	5.7	11.1	16.7	24.9	4	47.4±5.8	41.0±4.6	11.5±2.7	27	1.15
	G-1-C^{14}	PMA + B	44.2	19.9	10.7	7.40	3	6.90±2.3	4.7±1.0	88.3±1.9	3	1.48
		Buffer only	4.5	7.48	16.4	27.6	3	54.0±5.8	35.5±4.3	10.3±2.2	20	1.52
10^{-4}	G-6-C^{14}	PMA + B	69.5	5.98	7.23	5.04	3	2.2 ±0.7	5.7 ±0.7	93.0±1.3	3	.39
		Buffer only	5.5	10.1	15.3	26.9	3	40.1±2.7	46.3±2.4	13.4±2.1	20	.87
	G-U-C^{14}	PMA + B	30.8	10.4	19.2	10.3	5	13.1±3.6	11.7±2.0	75.3±7.3	5	1.12
		Buffer only	4.6	9.40	17.4	26.6	5	47.4±5.8	41.0±4.6	11.5±2.7	27	1.15

Note: ± represents ± deviation.

TABLE Vb

OTHER HERBICIDES AFFECTING PHYSIOLOGICAL ACTIVITY: METABOLISM AND ABSORPTION DATA

Compound	Concentration M	% Abs.	F. Values			C6/C1 Values for Respiratory CO_2/hr.				C6/C1 Values for six hour period		
			CO_2	Residue	EtOH Sol.	1hr.	2hr.	4hr.	6hr.	CO_2	Res.	CO_2/Res.
IPC	10^{-4}	86.5 ± 3.4	.937 ± .029	1.02 ± .08	1.06 ± .08	1.00	.929	.629	.575	.641 ± .026	1.55 ± 0.13	.414
PMA	10^{-4}	11.1 ± 3.7	.681 ± .286	2.94 ± 2.45	.818 ± .218	.729	.139	.311	.314	.447 ± .304	1.43 ± 0.13	.312
MH	10^{-4}	95.0 ± 6.5	.859 ± .038	1.13 ± .04	1.02 ± .12	.935	.882	.678	.652	.757 ± .051	1.28 ± 0.11	.590
Buffer	-	100	1.00	1.00	1.00	.880	.828	.693	.698	.754 ± .064	1.35 ± 0.15	.560

The effect is a darkening of the leaves followed by necrosis. Leaves never emerge from their sheath. It is selectively toxic to many weed seeds, especially the grasses, and may find broad use in corn, alfalfa, soybeans and the like. It is somewhat more toxic to mammals than most herbicides having an LD 50 of 1.63 g/kg.

Like EPTC, a,a-dichloropropionic acid (Dalapon) is a liquid herbicide. It was first introduced by the Dow Chemical Company in 1953 and exhibits great promise as a grass herbicide. It causes physiological responses to the plant when absorbed through either the roots or leaves. Some trouble is encountered in handling because of irritation to the eyes, but toxicity is extremely low.

The fourth, 2,3,6-trichlorobenzoic acid (2,3,6-TBA) is a pre-emergent herbicide used in weed control which was introduced in 1954 by Heyden Chemical Company. The 2,3,6-TBA exhibits growth regulating properties which may be used in the control of dicotyledonous weeds, particularly in corn. It has also found use in brush control. Its toxicity has an LD 50 of about 1 g/kg.

Metabolic studies utilizing glucose-1-C^{14}, glucose-6-C^{14} and glucose-U-C^{14} as substrates indicate that all these herbicides affect substrate absorption to about the same extent (Table VIb). The 2,3,6-TBA inhibits substrate absorption slightly more than the other three.

Consideration of the C6/C1 values for respiratory CO_2 reveals that all the herbicides tend to reduce this value indicating more reliance on C-1 or pentose oxidation. Time course studies of these values show no difference in the normal trend toward the pentose cycle over a six-hour period. There is, however, a general lowering of the values over the entire period following treatment with Dalapon, 2,3,6-TBA and EPTC. Simazin, however, only shows a lowering during the first hour, the remaining time its value appears very near that of the control. In general the trend appears to be a lowering of the respiratory CO_2 C6/C1 values following treatment with the pre-emergent herbicides followed by a return to the normal.

Analysis of the percent recovery of absorbed C^{14} as CO_2 reveals no correlation among the four herbicides used here. EPTC causes no change in recovered CO_2 when using G-1-C^{14} or G-U-C^{14}; however, there is a small inhibition to G-6-C^{14} oxidation. Since there is no effect on incorporation of any of the carbon atoms into the cellular residue this would indicate an inhibition to glycolysis.

Simazin, on the other hand, causes an increase in G-1-C^{14} oxidation and a decrease in glucose-U-C^{14} oxidation. Because of the decrease in C-1 anabolism into cellular residue this would tend to indicate a general decrease in pentose cycle oxidation with an increase in C-1

elimination causes by the decrease in the assimilation of the C-1 into tissue.

The 2,3,6-TBA shows an overall increase in CO_2 elimination with the C-1 elimination showing the greatest increase. Carbon incorporation into cellular residue remains unaffected except for C-1 incorporation which decreases. This would tend to indicate an overall increase in metabolism, but like Simazin, with increased C-1 CO_2 production owing to the decrease in C-1 incorporation into cellular residue.

The C-1 carbon following treatment with Dalapon shows increased CO_2 production and decreased incorporation into cellular residue. C-2 CO_2 elimination is decreased while no effect is noted on its incorporation into residue. This decrease may be due to an accumulation of glycolytic intermediates in the cell as indicated by the increase in activity in the soluble fraction.

Treatment with Dalapon in G-U-C^{14} experiments results in no change in metabolism. In general it appears that Dalapon does not cause any substantial change in pathway but rather an inhibition to cellular incorporation from pentose cycle intermediates and a possible accumulation of glycolytic intermediates. The overall incorporation of glucose carbons into the residue appears to be only slightly affected because of the increase in pentose cycle

operation cancelling out the decrease in residue formation from glucose-1-C^{14}. The accumulation of glycolytic intermediates, however, indicates some inhibition to the pyruvic enzyme.

Examination of the anabolic function or CO_2/residue values shows that EPTC and Dalapon have little effect on the function while Simazin and TBA increase the function. This increase is due to a decrease in the incorporation of glucose into cellular material.

This group of pre-emergent herbicides appears to have no correlation between one herbicide or another. EPTC and Dalapon appear to inhibit glycolysis but only slightly. The 2,3,6-TBA appears to show an overall increase in pentose cycle operation which may be due only to the elimination of CO_2 which would ordinarily be used in tissue construction. EPTC did not affect cellular residue formation while 2,3,6-TBA and Simizan caused a decrease in C-1 cellular incorporation.

The Non-selective Herbicides

The two herbicides described in this section are generally used non-selectively; however, they may be used specifically. The first, 3-(p-chlorophenyl)-1, 1-dimethyl urea (CMU) is more commonly called Monuron and is sold under the trade names of "Telvor" and "Karmex" which have

TABLE VIa

THE PRE-EMERGENT HERBICIDES: RECOVERY DATA

Concentration M	Substrate	Treatment	% Absorbed C^{14} Recovered as Respiratory CO_2 1hr.	2hr.	4hr.	6hr.	No. Runs	% Absorbed C^{14} Recovered as CO_2	Residue	EtOH Sol.	No. Runs	CO_2 Residue
	G-1-C^{14}	EPTC + B	3.7	9.00	15.0	28.6	3	52.6±9.1	33.5±4.5	13.8±5.7	3	1.57
		Buffer only	4.5	9.61	14.3	28.7	3	54.0±5.8	35.5±4.3	10.3±2.2	20	1.52
10^{-4}	G-6-C^{14}	EPTC + B	4.3	10.8	16.2	27.5	3	35.1±3.3	45.8±1.0	19.1±5.7	3	.77
		Buffer only	5.4	12.3	17.1	25.0	3	40.1±2.7	46.3±2.4	13.4±2.1	20	.86
	G-U-C^{14}	EPTC + B	3.6	6.43	14.7	31.8	6	47.1±7.9	42.1±5.4	10.5±2.9	6	1.12
		Buffer only	4.3	7.16	16.0	28.2	6	47.4±5.8	41.0±4.6	11.5±2.7	27	1.15
	G-1-C^{14}	Simazin + B	4.5	7.74	14.3	29.5	3	60.6±3.1	30.0±3.2	8.4±1.5	3	2.02
		Buffer only	4.7	8.30	16.4	27.0	3	54.0±5.8	35.5±4.3	10.3±2.2	20	1.52
10^{-5}	G-6-C^{14}	Simazin + B	6.1	10.8	16.3	25.2	3	41.1±1.9	44.5±2.4	14.4±3.4	2	.92
		Buffer only	7.4	12.9	17.4	22.4	3	40.1±2.7	46.3±2.4	13.4±2.1	20	.86
	G-U-C^{14}	Simazin + B	7.7	12.6	12.1	28.4	6	41.3±4.0	47.1±4.3	11.6±1.0	6	.88
		Buffer only	8.1	9.60	13.0	28.1	6	47.4±5.8	41.0±4.6	11.5±2.7	27	1.15
	G-1-C^{14}	TBA + B	4.2	7.00	14.4	32.0	3	62.7±4.7	27.9±2.9	9.40±1.9	3	2.24
		Buffer only	4.5	8.85	16.2	27.2	3	50.4±5.8	35.5±4.3	10.3±2.2	20	1.52
10^{-4}	G-6-C^{14}	TBA + B	5.4	8.60	14.0	30.8	3	48.2±8.3	43.3±1.8	16.8±3.1	3	1.11
		Buffer only	6.8	11.5	14.9	26.0	3	40.1±2.7	46.3±2.4	13.4±2.1	20	.86
	G-U-C^{14}	TBA + B	4.2	7.95	12.2	31.8	5	49.3±2.4	38.9±2.4	11.9±1.2	5	1.27
		Buffer only	6.9	9.55	15.2	26.6	5	47.4±5.8	41.0±4.6	11.5±2.7	27	1.15
	G-1-C^{14}	Dalapon + B	3.4	6.75	13.0	32.0	3	57.9±7.3	31.5±5.2	10.5±2.1	3	1.83
		Buffer only	4.8	8.50	12.6	30.0	3	54.0±5.8	35.5±4.3	10.3±2.2	20	1.52
10^{-4}	G-6-C^{14}	Dalapon + B	5.2	10.0	14.1	28.2	3	32.5±6.1	45.7±1.0	18.8±1.4	3	.71
		Buffer only	6.4	10.4	14.8	26.8	3	40.1±2.7	46.3±2.4	13.4±2.1	20	.86
	G-U-C^{14}	Dalapon + B	3.2	7.06	14.7	30.2	5	47.6±7.6	40.1±5.7	12.2±2.2	5	1.18
		Buffer only	3.9	7.70	16.8	27.4	5	47.4±5.8	41.0±4.6	11.5±2.7	27	1.15

TABLE VIb

THE PRE-EMERGENT HERBICIDES: METABOLISM AND ABSORPTION DATA

Compound	Concentration M	% Abs.	F. Values			C6/C1 Values for Respiratory CO_2/hr.				C6/C1 Values for six hour period		
			CO_2	Residue	EtOH Sol.	1hr.	2hr.	4hr.	6hr.	CO_2	Res.	CO_2/Res.
EPTC	10^{-4}	91.6 ± 5.7	.812 ± .090	1.17 ± .03	1.23 ± .12	.795	.820	.735	.658	.667 ± .015	1.17 ± .03	.570
Simazin	10^{-5}	94.0 ± 6.6	.986 ± .088	1.03 ± 0.13	1.07 ± 0.16	.935	.967	.788	.592	.682 ± .039	1.03 ± 0.13	.651
2,3,6-TBA	10^{-4}	82.7 ± 6.1	947 ± .108	1.13 ± .06	1.19 ± .07	.906	.748	.581	.549	.637 ± .007	1.13 ± .06	.563
Dalapon	10^{-4}	89.3 ± 8.3	.858 ± .032	1.07 ± 0.04	1.41 ± 0.13	.939	.923	.679	.549	.622 ± .063	1.31 ± 0.10	.475
Buffer	-	100	1.00	1.00	1.00	.993	.969	.757	.595	.745 ± .064	1.35 ± 0.15	.560

FIGURE 5

THE EFFECT OF THE PHYSIOLOGICALLY ACTIVE HERBICIDES ON GLUCOSE OXIDATION

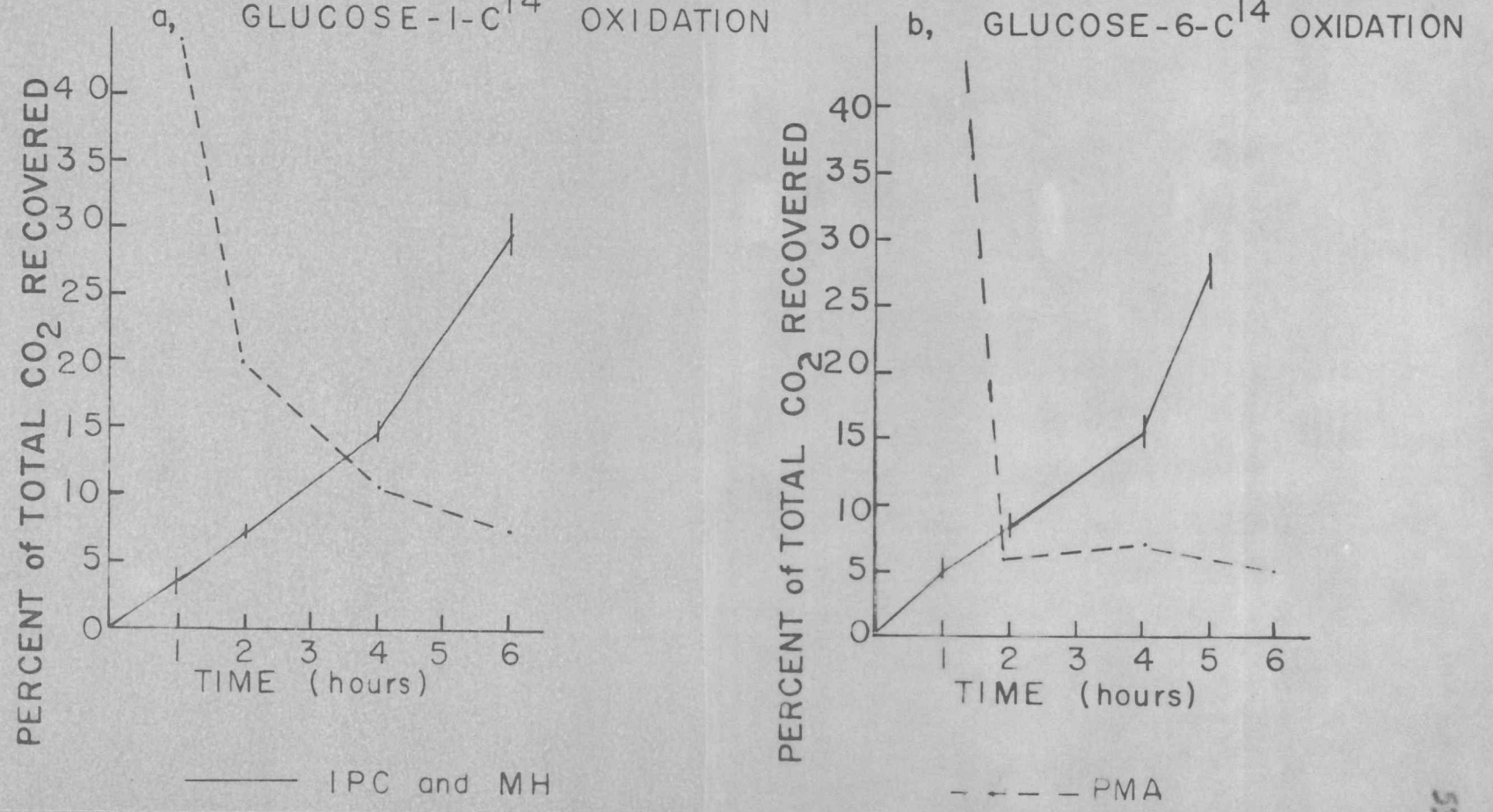
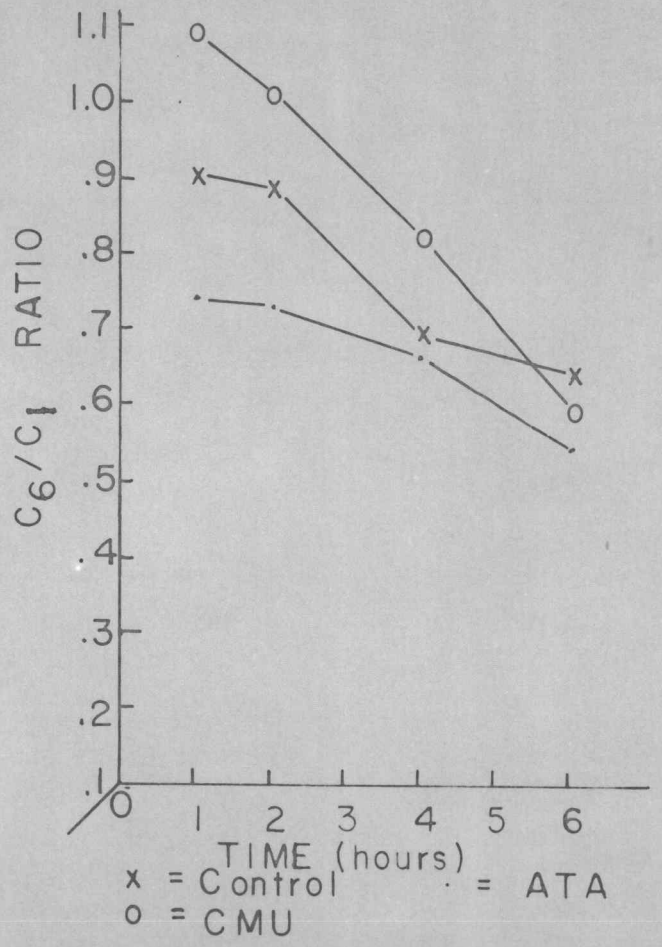
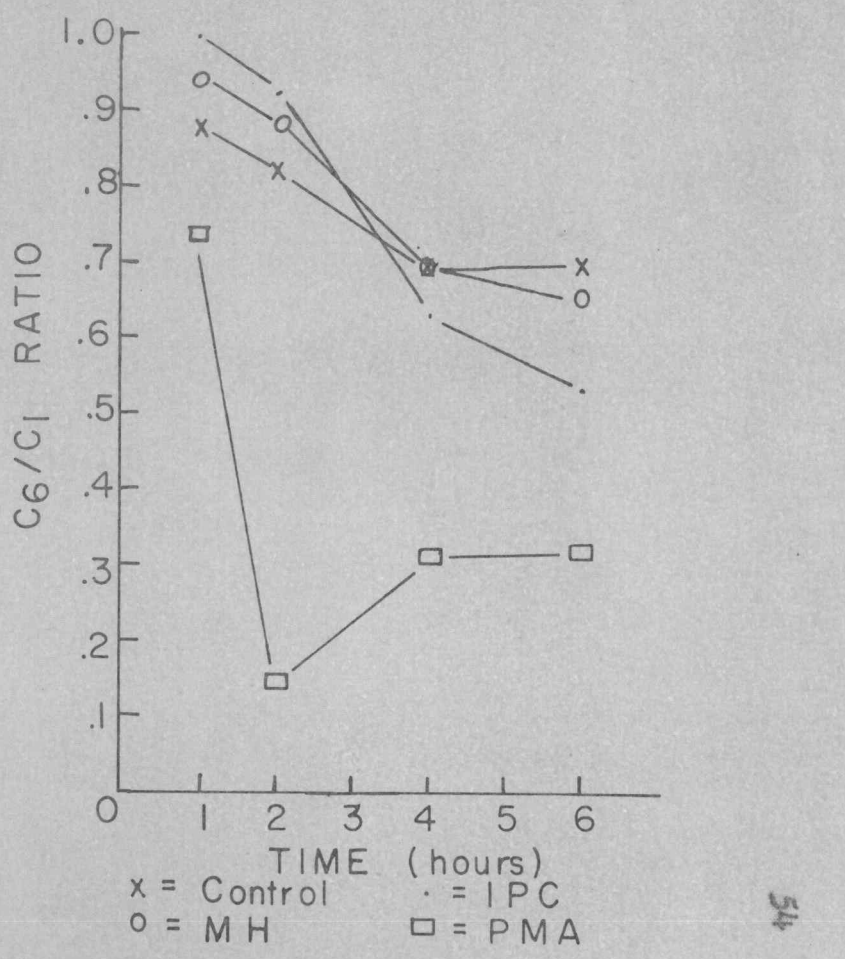


FIGURE 6

THE EFFECT OF SEVERAL HERBICIDES ON THE C_6/C_1 RATIOS





about 80 percent active components. CMU was the first of a series of substitutal urea compounds to be suggested as soil sterilants and herbicides. It has a low mammalian toxicity, the acute oral LD to rats being 3.7 g/kg. CMU is a general herbicide for application to either the soil or foliage; however, it is somewhat less effective when applied to the foliage. It has a known inhibition to the Hill reaction of photosynthesis (26, p. 980), uncoupling phosphorylation, has little effect on germinating seeds until the shoots have emerged above the ground and causes chlorosis. Its absorption through the leaves is poor, while root absorption is high (10, p. 1-14). It is used non-selectively at 20 to 80 lbs./acre or less effectively selectively in certain crops such as pineapple, sugar cane, citrus and grapes at 1 to 6 lbs./acre (28, p. 40-42).

The second herbicide to be considered in this section is 3-amino-1,2,4-triazole (ATA) which is sold under the trade name of "Weedazol" which contains 50 percent active compound.

It is relatively non-toxic to mammals, the acute LD to rats being 14.7 g/kg. In long term feedings, however, it can produce cancer.

It is a general herbicide used to kill plants, brush and hardwood. ATA finds only limited use selectively. It is rapidly absorbed by aerial plant parts and roots

inducing chlorosis, which is not corrected by iron treatment, followed by death of the plant (30, p. 218-226).

ATA is reported to stimulate respiration in leaf sections (30, p. 218-226), inhibit the formation of plastids (33, p. 674-678) and lower the catalase activity. Herbert also found stimulation of respiration generally increased in the leaves and rhizomes following treatment with ATA (19, p. vi).

Treatment of pea roots with 1×10^{-4} M CMU and ATA produced results shown in Table VIIa and VIIb. There was no apparent physiological damage to the tissue. CMU only slightly reduced the substrate absorption while ATA caused no change.

In studies utilizing respiratory CO_2 production, no difference between CMU treated seedlings and control plants could be found. ATA, however, caused a decrease in the C6/C1 ratio which appears to be due to an increase in C-1 oxidation during the last two-hour period. Time course studies show an early inhibition to CO_2 evolution from both the C-1 and C-6 carbon atoms of glucose followed by a slight stimulation in C-1 evolution. The C-6 oxidation approaches the control near the end of the 6-hour period. A study of the C6/C1 ratios for respiratory CO_2 over the 6-hour period show an early decrease in the ATA and an increase in the CMU C6/C1 ratios which approach the control after six hours.

It would appear then, that the overall effect of CMU

on glucose oxidation is slight while ATA shows an increase in C-1 oxidation. Oxidation of uniformly labeled glucose shows no effect caused by either CMU or ATA.

The incorporation of glucose carbons is unaffected by CMU treatment; however, a slight decrease in the C6/C1 ratio can be induced by treatment with ATA. This is caused by a slight increase in C-1 carbon incorporation. No effect is induced on the C-6 carbon atom or when G-U-C^{14} is used.

No effect is induced upon the breakdown of glucose to alcoholic soluble compounds with treatment by CMU; however, once again ATA causes a slight increase in the incorporation of C-6 carbon atoms into alcohol soluble compounds.

In summary there appears to be no affect on glucose metabolism when pea roots are treated with CMU; however, ATA appears to cause a slight increase in pentose phosphate oxidation. This increase is probably found only during the latter part of the experimental period.

TABLE VIIa

THE NON-SELECTIVE HERBICIDES: RECOVERY DATA

Concentration M	Substrate	Treatment	% Absorbed C^{14} Recovered as Respiratory CO_2 1hr.	2hr.	4hr.	6hr.	No. Runs	% Absorbed C^{14} Recovered as CO_2	Residue	EtOH Sol.	No. Runs	CO_2 Residue
10^{-4}	G-1-C^{14}	ATA + B	4.5	8.01	15.0	29.7	3	48.3±8.2	39.0±3.4	12.7±5.5	3	1.24
		Buffer only	5.0	9.41	15.0	26.9	3	54.0±5.8	35.5±4.3	10.3±2.2	20	1.52
	G-6-C^{14}	ATA + B	5.5	9.62	16.5	25.9	3	38.3±6.8	47.7±1.0	17.1±6.4	3	.81
		Buffer only	6.2	11.2	16.8	24.5	3	40.1±2.7	46.3±2.4	13.1±2.1	20	.87
	G-U-C^{14}	ATA + B	6.4	9.12	15.5	26.8	4	42.1±5.9	44.1±1.5	13.8±2.7	4	.95
		Buffer only	4.8	11.5	14.7	27.2	4	47.4±5.8	41.0±4.6	11.5±2.7	27	1.15
10^{-4}	G-1-C^{14}	CMU + B	3.3	6.97	14.1	31.8	3	52.6±6.0	32.6±1.4	11.1±1.3	3	1.61
		Buffer only	3.9	7.73	16.4	27.8	3	54.0±5.8	35.5±4.3	10.3±2.2	20	1.52
	G-6-C^{14}	CMU + B	5.2	10.1	16.5	25.9	3	44.9±5.6	43.8±2.3	15.2±1.3	3	1.02
		Buffer only	5.4	10.9	15.7	26.2	3	40.1±2.7	46.3±2.4	13.4±2.1	20	.87
	G-U-C^{14}	CMU + B	5.5	11.2	15.7	26.0	4	43.4±7.1	43.3±2.1	13.3±5.0	4	1.00
		Buffer only	7.3	10.1	14.6	26.6	4	47.4±5.8	41.0±4.6	11.5±2.7	27	1.15

Note: ± represents ± deviation.

TABLE VIIb

THE NON-SELECTIVE HERBICIDES: METABOLISM AND ABSORPTION DATA

Compound	Concentration M	% Abs.	F. Values			C6/C1 Values for Respiratory CO_2/hr.				C6/C1 Values for six hour period		
			CO_2	Residue	EtOH Sol.	1hr.	2hr.	4hr.	6hr.	CO_2	Res.	CO_2/Res.
CMU	10^{-4}	92.6 ± 3.4	1.07 ± .06	.913 ± .039	.963 ± .090	1.09	.101	.821	.591	.701 ± .019	1.39 ± 0.10	.504
ATA	10^{-4}	97.7 ± 7.0	.814 ± .041	1.11 ± .05	1.30 ± .13	.745	.73	.665	.545	.633 ± .081	1.23 ± 0.10	.514
Buffer	-	100	1.00	1.00	1.00	.900	.892	.697	.643	.754 ± .064	1.35 ± 0.15	.560

SUMMARY

In every case with the exception of PMA there was no appreciable herbicidal effect on glucose metabolism that can explain the toxicity of the various herbicides used. Herbicides which have a high toxic effect on peas cause very similar effects to those for which peas show complete tolerance. As examples, 2,4-D and 2,4,5-T are highly toxic to peas yet cause almost the same effect as the inactive compounds 2,4,6-T and 2,6-D. Likewise IPC which is used as a grass killer in pea crops shows a greater effect on peas than 2,4-D.

All the compounds with the exception of 2,6-D cause either no change or a decrease in the C6/C1 ratio regardless of activity. There is, however, a slight shift toward the pentose pathway.

All the compounds caused a decrease in substrate absorption, the amount of inhibition depending on some other property of the compound than its herbicidal activity. No correlation can be found between either C6/C1 ratios or residue incorporation and absorption.

2,4-D, 2,4,6-T, 2,6-D, a-NAA, GA, IAA, Simazin and CMU appear to cause only a slight change in glucose oxidation. 2,3,6-TBA, 2,4,5-T and IPC cause the highest increase in pentose pathway activity while EPTC, Dalapon, and 2,4,5-T

cause the greatest inhibition to glycolysis.

The general conclusion reached in this thesis is that glucose metabolism is not the site of herbicidal activity in the pea seedling. This combined with the work of Stevens (49, p. 1-50) who found that acetate metabolism was not affected by herbicidal treatment removes from the list of possible herbicidally active sites two very important phases of plant metabolism.

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