ON THE ACTION OF PLANT GROWTH REGULATORS

by

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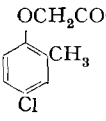
I. INTRODUCTION

The mode of action of "plant growth regulators" or "auxins" has been the subject of controversy almost from 1928 when WENT[1] concluded that "there is no growth without growth substance". In this paper we are concerned only with those compounds related to phenoxyacetic acid, in particular 2-methyl-4-chloro phenoxyacetic acid — "MCPA". It is proposed to refer to these compounds as "growth regulators", reserving the term "auxin" for those regulators which are natural to the plant.



BONNER[2] demonstrated that, under conditions in which aerobic respiration was inhibited by iodoacetates, the growth of *Avena* coleoptiles was also inhibited and that auxin (β-indole acetic acid) increased the oxygen uptake of the coleoptile, thus linking the activity of this compound with its stimulating effect on respiration. BERGER, SMITH AND AVERY[3] confirmed that auxin increases the oxygen uptake of *Avena* coleoptiles. Respiration and salt uptake being inter-related, this would result in an enhanced uptake of salts and hence also, of water. Thus, increased growth by cell extension was attributed to an increased water uptake under the influence of auxin.

Polysaccharide transformations have also been observed. REINDERS[4] reported that starch grains disappeared when potato slices were treated with β-indoleacetic acid, an effect which she ascribed to an increased rate of respiration. Other workers, notably MITCHELL AND WHITEHEAD[5], have confirmed these findings. EYSTER[6] interpreted the action of a growth regulator in terms of a release of the enzyme diastase from a bound to an active state.

HEYN[7] advanced an alternative view; he considered that auxin within a plant produced an increase of cell wall plasticity, thus allowing the cell to enlarge more readily by reducing the mechanical resistance of the cell wall. COMMONER AND MAZIA[8], however, found that potato slices with their cells in a flaccid condition still exhibited an increase in the rate of salt and water uptake under the influence of growth regulators, an indication that cell wall resistance in that experiment was not a limiting factor.

SKOOG, SCHNEIDER AND MALAN[9] postulated that a growth regulator acted as a coenzyme and had to be capable of combining with the apoenzyme and with the substrate. They suggested this from observations that pretreatment of split pea stems with phenyl butyric acid, (itself inactive in the pea test), resulted in a loss of activity by subsequent applications of indoleacetic acid. They regarded phenyl butyric acid, therefore, as being capable of only one of the above required interactions.

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Favouring a more general approach, VELDSTRA[10,11] regarded growth regulation as resulting from an association between the growth regulator molecule and the lipoid portion of the protoplasmic membrane, producing a change in its permeability to water and ions. Recently, his views have broadened to include also physicochemical interaction with an enzyme[12].

Here the problem is considered in relation to three stages that are involved when the growth regulator travels from a medium external to the plant to its ultimate site of action:

a. Entry into the plant system by the leaves or through the roots.

b. Transit from the point of entry to its ultimate site.

c. Possible interaction with an enzyme system.

We are mainly concerned with an adsorption process that may occur during stage (b) and its influence upon the physiological response of various plant species.

The monolayer technique, employing a Langmuir trough, was used. This method has been employed in a study of biological problems by several workers, notably RIDEAL AND SCHULMAN[13,14] and by CLOWES, DAVIS AND KRAHL[15]. The authors have attempted to establish

1. The form (dissociated or otherwise) in which the organic acid exerts its effect.

2. The nature of the polar groups within the plant with which "MCPA" can interact.

3. Principles involved when "MCPA" interacts with molecules containing two or more polar groups.

4. A study of monolayers produced directly from the exudates of crushed plant tissue and their interaction with "MCPA". Wheat coleoptiles, cress seedlings and tomato shoots were used. This choice of plants was made in view of the resistance of wheat to growth regulators and the high susceptibility of cress seedlings and tomato plants.

2. METHODS OF EXPERIMENT

a. *Apparatus*

A Langmuir trough and surface balance accurate to 0.2 dynes/cm were used to follow the "Force-Area" (F-A) characteristics of the monolayers and their surface potentials (ΔV-A) were determined by means of a radioactive electrode in circuit with a valve electrometer. The trough was housed in a metal-lined box which was earthed and thermostatically maintained at 20° C. Known weights of material in a suitable solvent were applied to the air/water interface with an Agla micrometer syringe.

b. *Experimental*

To study the interaction of "MCPA" with the monolayer of a compound, (F-A) and (ΔV-A) btrves were produced for the monolayer on a substrate containing no "MCPA" and compared with chose from a similar monolayer in equilibrium with a substrate containing "MCPA" of known concentration. The latter stage was performed either by spreading the film on a substrate containing u known weight of "MCPA" or by injecting an aqueous solution of "MCPA" with a bent pipette, ceneath a monolayer already on the surface.

c. *Computation of results*

To obtain an adsorption isotherm, several differing concentrations of "MCPA" were injected beneath a given monolayer, and the changes in surface potential ($\delta\Delta V$) observed.

In Fig. 1, $\delta\Delta V$ and $\frac{c}{\delta\Delta V}$ are plotted against the concentration of "MCPA" injected under a monolayer of gliadin at pH 4.0 and A = 0.52 metres^2/mg.

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It will be noted that the data give a linear relationship between $\frac{c}{\delta\Delta V}$ and c. If we may regard $\delta\Delta V$ as a direct measure of the amount of "MCPA" adsorbed on the monolayer, then the fraction of the monolayer covered is given by

$$\Theta = \frac{KC_1}{a + KC_1} = \frac{\delta\Delta V_1}{\delta\Delta V_s}$$

where $\delta\Delta V_s$ = surface potential change corresponding to complete saturation of the surface by "MCPA".

Thus the adsorption is of the Langmuirian type, a form also given by the application of the law of mass action.

The extent of interaction in any conditions may therefore be assessed if $\delta\Delta V_s$ is known. This was determined graphically from the inverse slope of the straight line Fig. 1.

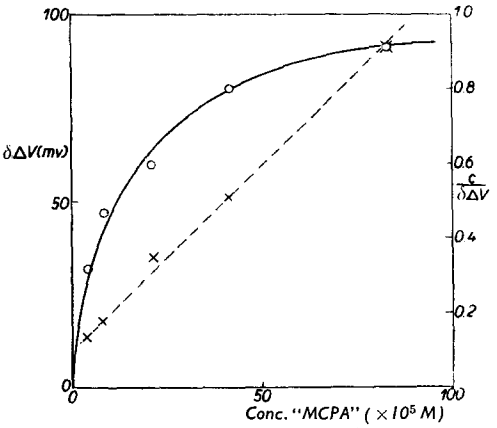

Fig. 1. Gliadin injected with "MCPA" at pH 4.0, 20° C.
——O—— $\delta\Delta V$ (A = 0.52 m²/g) *versus* concentration
——×—— $\frac{c}{\delta\Delta V}$ *versus* concentration

3. EXPERIMENTAL RESULTS

a. *Factors involved*

Three factors likely to influence these interactions were first investigated:

1. Substrate pH.
2. Ionic strength of the substrate.
3. Type of buffer solution.

1. *Substrate pH*

"MCPA" is an organic acid of dissociation constant $3.2 \cdot 10^{-4}$. Thus, over an extended pH range in this region, ions and undissociated molecules coexist, and it is important to assess their contributions to the interaction over a range of pH.

Octadecyltrimethylammonium chloride was selected for this investigation because, as a strong base, its (F-A) and (δV-A) characteristics do not vary significantly over a large range of pH (1.0–8.0). Changes in its interaction with "MCPA" on varying the pH may be interpreted in terms of a change in the "MCPA" molecule.

Two methods were employed to detect this change:

i. The extent of interaction by a fixed concentration of injected "MCPA" was computed at a range of pH values. It was anticipated that the organic acid ion would interact to a different extent from that of the molecule.

ii. The saturation value $\delta\Delta V_s$ was assessed at each pH. Assuming that a 1.1 complex (*i.e.* quaternary ammonium salt: "MCPA" = 1:1) is formed between the monolayer and the injected molecule, then $\delta\Delta V_s$ for a complex involving the ion would differ from that involving the undissociated molecule.

The quaternary salt was spread from 60°/80° petrol ether/alcohol solution on hydrochloric acid, acetate and phosphate buffers of pH 1.0–8.0, the ionic strengths of which were I = 0.001–0.01. "MCPA" was injected at several concentrations, from which

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adsorption isotherms were computed at a number of pH values. The extent of interaction $\left(\frac{\delta\Delta V_{3.3}}{\delta\Delta V_s}\right)$ was calculated in each case for C = $3.3 \cdot 10^{-5}$ *M* "MCPA" and in Tables I and II these values are presented together with the corresponding values of $\delta\Delta V_s$.

TABLE I

OCTADECYLTRIMETHYLAMMONIUM CHLORIDE INJECTED WITH "MCPA"

Values of $\frac{\delta\Delta V_{3.3}}{\delta\Delta V_s}$ and $\delta\Delta V_s$ with changing pH

C = $3.3 \cdot 10^{-5}$ *M* Substrate HCl

pH	*1.0*	*2.0*	*2.3*	*2.5*	*3.0*	*4.0*	*5.4*
$\frac{\delta\Delta V_{3.3}}{\delta\Delta V_s}$ (90 sq. A)	0.29	0.28	0.32	0.41	0.39		
$\delta\Delta V_s$		255		253	273	205	177

TABLE II

SUBSTRATE ACETATE BUFFER

pH	*3.6*	*4.0*	*4.3*	*4.7*	*4.9*	*5.4*	*8.0 (Phosphate)*
$\frac{\delta\Delta V_{3.3}}{\delta\Delta V_s}$ (90 sq. A)	0.42	0.43		0.48		0.51	0.33
$\delta\Delta Vs$	252	207	184	184	160	176	163

In Fig. 2 are given the (*F-A*) curves for the quaternary salt injected with $83 \cdot 10^{-5}$ *M* "MCPA" on substrates of various pH values.

Tables I and II indicate that the extent of adsorption falls off below pH 2.5. As the dissociation constant of "MCPA" is $3.2 \cdot 10^{-4}$, it appears that the undissociated form of "MCPA" predominates in the region of reduced adsorption, whereas above pH 2.5 the ionic form mainly is adsorbed. Below pH 3.6, penetration of the monolayer ensues at all film areas (Fig. 2), whereas above this pH a measure of contraction occurs at high surface areas. The quaternary ammonium salt gives rise to a gaseous monolayer, the

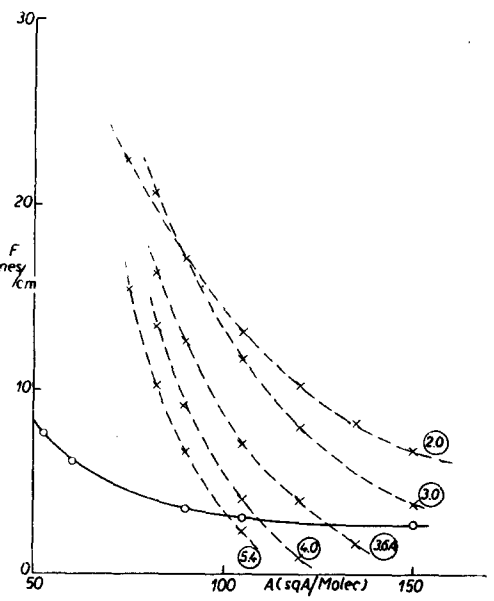

Fig. 2. *F-A* curves for $C_{18}H_{37}\overset{+}{N}(CH_3)_3\}Cl^-$ injected with $83 \cdot 10^{-5}$ *M* "MCPA" at various pH values (– – – × – – –); No "MCPA" (——○——) 20° C. Substrates HCl except pH 3.6, acetate buffer (3.6 *A*)

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cohesion between individual long chains being counterbalanced by repulsion between the positive charges on neighbouring molecules, thus, the contraction above pH 3.6 implies that, by adsorption of a negative "MCPA" ion, the repelling force has been decreased.

We note also that the lower value of $\delta\Delta V_s$ (180 mV) above pH 4.0 (Tables I and II) corresponds to the adsorption of the "MCPA" ion. The value 250 mV below pH 4.0 would then result from the adsorption of undissociated "MCPA".

Although there is some discrepancy between the values on the two substrates, and also the surface pH may vary from the bulk pH by at least one unit[16], we conclude that "MCPA" exists predominantly in the ionic state above pH 4.0.

2. *Ionic strength of the medium*

Monolayers of the quaternary salt were spread on substrates of pH 3.0 HCl (I = 0.001) whose ionic strengths were adjusted from I = 0.001 to I = 0.01 by suitable addition of sodium chloride. "MCPA" was then injected. The extent of interaction was identical on all three substrates, and we may therefore disregard any effect on the interactions from changing ionic strength.

The ionic strength of acetate buffers employed in the investigation lay between I = 0.0015 (pH 3.6) and I = 0.01 (pH 5.4), these low concentrations being selected to reduce the concentration of carboxyl groups to a minimum whilst maintaining a constant pH in the presence of injected "MCPA".

3. *Influence of buffer solution*

It is important to establish that the interactions involving "MCPA" are independent of the nature of the substrate ions.

Table III presents $\delta\Delta V_{83}$ and $\delta\Delta V_{3.3}$ (C = $3.3 \cdot 10^{-5}$ *M*) on injecting "MCPA" beneath octadecyltrimethylammonium chloride on three substrates at various pH values.

TABLE III

OCTADECYLTRIMETHYLAMMONIUM CHLORIDE INJECTED WITH "MCPA"
Values of $\delta\Delta V_{83}$ and $\delta\Delta V_{3.3}$ on three substrates at various pH values

Substrate pH	*Conc. "MCPA" $\cdot 10^5$ M*	*Surface Potential Change (mV)*		
		HCl	*Acetate Buffer*	*Phosphate Buffer*
3.0	83	273	—	—
(+ NaCl to I = 0.002)	3.3	107		
3.6	83	—	252	—
	3.3		105	
5.4	83	176	176	—
(+ NaCl to I = 0.002)	3.3	85	90	
8.0	83	—	—	163
	3.3			56

It is seen that the extent of interaction $\frac{\delta\Delta V_{3.3}}{\delta\Delta V_s} \approx \left(\frac{\delta\Delta V_{3.3}}{\delta\Delta V_{83}}\right)$ is similar on hydrochloric acid and acetate buffer substrates, whereas on a phosphate buffer there is a small

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reduction in adsorption. It appears, however, that the nature of the substrate solutions used in this investigation plays an insignificant role in these interactions.

b. *Long-chain compounds*

A large variety of polar groups exist within a plant as constituents of proteins, lipoids, polysaccharides and in simpler molecules—purines, amino acids, organic acids alcohols, aldehydes and sugars.

Long-chain compounds having the following polar groups were therefore interacted with "MCPA": amine hydrochloride, ester, ketone, phenol, quaternary ammonium salt, amide, peptide, mercaptan and primary alcohol.

1. *Eicosylamine hydrochloride* ($C_{20}H_{41}\overset{+}{N}H_3\}$ Cl^-)

Significance was attached to the interaction of "MCPA" with this monolayer since positively charged nitrogen atoms are of common occurrence in proteins, *e.g.* the amino acid arginine containing the guanidinium group $H_2\overset{+}{N}$=C(NH$_2$)–NH–.

The C_{20}-amine hydrochloride was spread from petrol ether/alcohol solution on HCl substrates of pH 2.0, 2.7, 3.8, 5.6 and on $N/100$ NaOH. The ionic strength of all solutions was adjusted to I = 0.01 by the addition of NaCl. Suitable mixtures of the free "MCPA" acid and its sodium salt were employed to avoid pH changes which would result from injecting the free acid alone.

In Fig. 3 are presented the (F-A) curves for the above monolayer injected with $3.3 \cdot 10^{-5} M$ "MCPA" at several pH values and with $41 \cdot 10^{-5} M$ "MCPA" at pH 3.8.

Table IV presents the surface potential of these monolayers on injecting "MCPA" at pH 3.8.

The C_{20}-amine hydrochloride forms a monolayer that is more condensed than either the C_{16} or C_{18} compound on HCl[17].

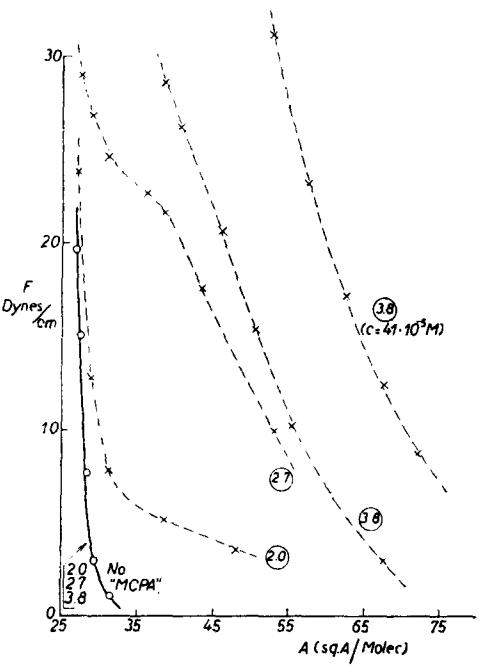

Fig. 3. F-A curves for $C_{20}H_{41}\overset{+}{N}H_3\}Cl^-$ on pH 2.0, 2.7 and 3.8 HCl (——○——). Injected with $3.3 \cdot 10^{-5}$ M "MCPA" (– – –×– – –). 20° C.

There is disagreement between workers regarding the surface potentials of these films. Thus, PORTER[18], using acetate-barbital buffers of pH 3.0–6.0, obtained values considerably less than those determined in this work, due no doubt to an interaction of the buffer solution itself with the monolayer. In general, however, there is agreement with MARSDEN AND SCHULMAN[19] who employed the C_{18} compound, a value of about $\Delta V = 700$ mV being obtained at $A = 27$ sq. A/molec. which decreased considerably on substrates of high pH.

There is a stronger interaction with the amine than with the quaternary ammonium

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salt (§ 3. a.I.). This difference may be due to hydrogen bonding between the amine and the organic acid but not by the quaternary ammonium salt. Moreover, the negative "MCPA" ion can approach nearer to the positive charge of the amine hydrochloride.

TABLE IV

THE SURFACE POTENTIALS OF EICOSYLAMINE HYDROCHLORIDE MONOLAYERS INJECTED WITH VARYING CONCENTRATIONS OF "MCPA" AT pH 3.8

Control ($c = 0$)		$C = 3.3 \cdot 10^{-5}$ *M*		$C = 41 \cdot 10^{-5}$ *M*	
A sq. A/molec.	ΔV *(mV)*	*A sq. A/molec.*	ΔV *(mV)*	*A sq. A/molec.*	ΔV *(mV)*
31.2	620	55.2	114	67.2	16
28.8	670	45.6	138	57.6	27
27.8	695	38.4	188	52.8	40
27.4	705	33.6	236	50.4	51

The influence of pH on the interaction agrees well with the observations on the quaternary ammonium salt (§ 3. a.I.) Thus at pH 2.0, little or no adsorption or penetration takes place by $3.3 \cdot 10^{-5}M$ "MCPA", at pH 2.7, penetration occurs with squeezing out of the injected molecule at 23 dynes/cm, whereas at pH 3.8 to 5.6 penetration occurs forming a complex which is stable to pressures over 30 dynes/cm.

At pH 12.0, where undissociated amine is present with the organic acid ion, no interaction occurs.

These data suggest that "MCPA" may be adsorbed strongly to proteins.

2. *Octadecyl trimethyl ammonium chloride*

As described in § 3. a.I, interaction occurred at all pH values studied (1.0–8.0) (Fig. 2). The extent of interaction, however, was somewhat less than that of the long chain amine hydrochloride. Adsorption to lecithin which is believed to be present in plant protoplasmic membranes, may therefore take place.

3. *Methyl pentadecyl ketone*

Dissolved in 60°/80° petrol ether, the ketone was spread on pH 3.6 acetate buffer and on pH 8.0 phosphate buffer.

At pH 3.6, the injection of "MCPA" resulted in a penetration of the monolayer and a small lowering of ΔV. The association was weak, for at 4 dynes/cm, the injected molecule was squeezed out rapidly. At pH 8.0. where "MCPA" ions are involved, no interaction was detected.

4. *Miscellaneous*

"MCPA" was found to give no significant interaction with either hexadecyl phenol at pH 3.6 and pH 5.5 or with monolayers of *n*-octadecyl acetamide ($C_{18}H_{37}NHCOCH_3$), stearamide, phytosterol, cetyl alcohol and eicosyl acetate at pH 8.0.

5. *The sulphur-hydryl group –SH*

It was not possible to spread a long-chain mercaptan on the liquid surface. To in-

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vestigate the carboxyl-sulphur-hydryl group interaction, therefore, stearic acid was spread on pH 3.6 acetate buffer, the dimercaptan B.A.L. $\begin{matrix}CH_2SH\\|\\CHSH\\|\\CH_2OH\end{matrix}$, being injected. No penetration or surface potential change occurred.

Summarising, "MCPA" interacts strongly with positively charged nitrogen atoms and feebly with the keto group. In order of decreasing reactivity with "MCPA" at pH 3.6,

$$-\overset{+}{N}H_3 \gg -\overset{+}{N}(CH_3)_3 \gg C=O \gg -CONH_2 > \text{C}_6\text{H}_4(OH)$$

This suggests that attention should be focussed on the interaction of "MCPA" with proteins and the lipoid lecithin, both of which are widely distributed in plants.

c. *Gliadin, lecithin and lipoprotein*

1. *Gliadin*

The wheat protein, gliadin, was selected for its ease of spreading at the air/water interface. According to CHIBNALL's analyses[20] the molecule at pH 5.0 contains polar groups in the following ratio:

$$50 - \begin{cases} COOH \\ COO^- \end{cases}, \quad 25 - CONH_2, \quad 3 - CH_2OH, \quad 1 \quad \begin{matrix} H_2\overset{+}{N} \\ \diagdown \\ C\,NH - \\ \diagup \\ H_2N \end{matrix} \quad 1 \quad \begin{matrix} -C-\overset{+}{N}H \\ \| \quad\quad \| \\ CH \quad CH \\ \diagdown \quad \diagup \\ NH \end{matrix}$$

A small number of $-\overset{+}{N}H_3$ groups are also present. The adsorption of "MCPA" to gliadin is essentially that of the interaction of a carboxyl group with a positively charged nitrogen atom, the latter being surrounded by protein carboxyl groups, a fraction of which are ionised at a given pH. It was desirable, therefore, to study the interaction of a gliadin monolayer with "MCPA" at a range of pH values.

Monolayers were produced between pH 3.6–5.4 (acetate buffers) and at pH 8.0 (phosphate buffer). The acid was injected at each pH value and an adsorption isotherm computed as described in § 2.c.

Table V presents the lowering of surface potential $\delta\Delta V$ which results from the injection of varying concentrations of "MCPA" beneath monolayers of gliadin at a series of pH values.

TABLE V

$\delta\Delta V$ VERSUS CONCENTRATION FOR GLIADIN MONOLAYERS INJECTED WITH "MCPA" AT VARIOUS pH VALUES

pH	$\delta\Delta V_{4.1}$	$\delta\Delta V_{8.3}$	$\delta\Delta V_{21}$	$\delta\Delta V_{41}$	$\delta\Delta V_{83}$	$\delta\Delta V_s$
3.6	42	66	94	119	138	162
4.0	32	47	60	80	91	96
4.9			38	62	72	90
5.4			25	35	50	83

The extent of interaction $\frac{\delta\Delta V_{21}}{\delta\Delta V_s}$ ($C = 21 \cdot 10^{-5} M$) was calculated at each pH and in Fig. 4, this function is plotted against pH; the variation in ΔV with pH of the uninjected gliadin monolayer is also included.

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The most rapid change of ΔV with pH takes place (Fig. 4) between pH 3.6–6.0, where the dominant reaction is $-COOH \rightarrow -COO^-$, all other groups remaining positively charged. Thus the fall in adsorbability with increasing pH (Fig. 4) is to be attributed to the appearance of $-COO^-$ groups on the protein. No adsorption occurs at pH 8.0. "MCPA", itself negatively charged above pH 4.0, is evidently unable to approach the positively charged centres with which it would normally interact owing to the repulsion between like negative charges. Thus it is necessary to regard not only the centres with which "MCPA" interacts positively, but also the arrangement and number of those by which the organic acid is repelled.

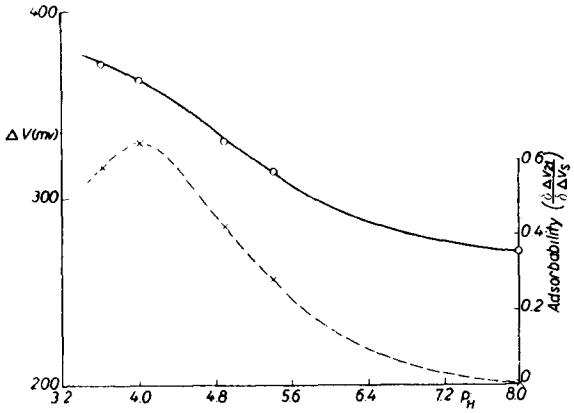

Fig. 4. Gliadin, 20° C.
——O—— Variation of surface potential at $A = 0.52$ m²/mg with change of pH
--- × --- Effect of pH on adsorbability $\frac{\delta \Delta V_{21}}{\delta \Delta V_s}$ of "MCPA"

Below pH 4.0, the extent of adsorption decreases. This we ascribe to the loss of negative charge on the "MCPA' molecule in this pH range.

2. Lecithin

This lipoid is widely distributed in plants, and it is believed to occur associated with proteins in the protoplasm. The molecule contains two ionisable polar groups, separated by a distance of 7A. A hypothesis by VELDSTRA[10] was based upon the interaction of lecithin with the growth regulator, thereby altering the permeability of the membrane in which the lipoid occurred, to ions and nutrients.

Ground-nut lecithin was freshly prepared from its cadmium chloride complex by the method of LEVENE AND ROLF[21]. It was spread from a chloroform/alcohol mixture on to acetate buffers of pH 3.6–5.4. "MCPA" was injected at these pH values and adsorption isotherms produced. Fig. 5 illustrates an injection experiment at pH 4.0. The extent of interaction was plotted against pH (Fig. 6) and as for gliadin, the plot of ΔV for uninjected lecithin against pH is included.

The (ΔV-pH) curve agrees reasonably with that of HUGHES[22], a rapid change in ΔV for the uninjected film occurring around pH 3.6. This results from ionisation of the phosphate group in a molecule of lecithin.

Close correspondence between the (adsorbability-pH) and (ΔV-pH) curves is evident in this system, suggesting that the fall in adsorbability with increasing pH results from the appearance of negative phosphate ions in the lecithin molecule.

When pH $>$ 4.0, adsorption of "MCPA" to the lipoid is insignificant. This leads us to question the importance of this lipoid in plant growth regulation activity and hence to doubt the validity of VELDSTRA's permeability regulation hypothesis. To test their suggestions, VELDSTRA AND HAVINGA[23] spread lecithin on pH 4.0 acetate buffer and injected growth regulators of varying structure beneath. On the basis of (F-A) curves only, they concluded that the interaction "came up to expectation". We cannot agree with this conclusion since in the work described herein, the degree of penetration is low and surface potential measurements indicate little or no polar interaction at pH $>$ 4.0.

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In view, however, of the limiting effect of the negative phosphate ion on the adsorption of "MCPA" to lecithin, it was desirable to investigate the interaction of a lipoprotein with "MCPA", proteins in plants frequently being associated with lecithin in this way.

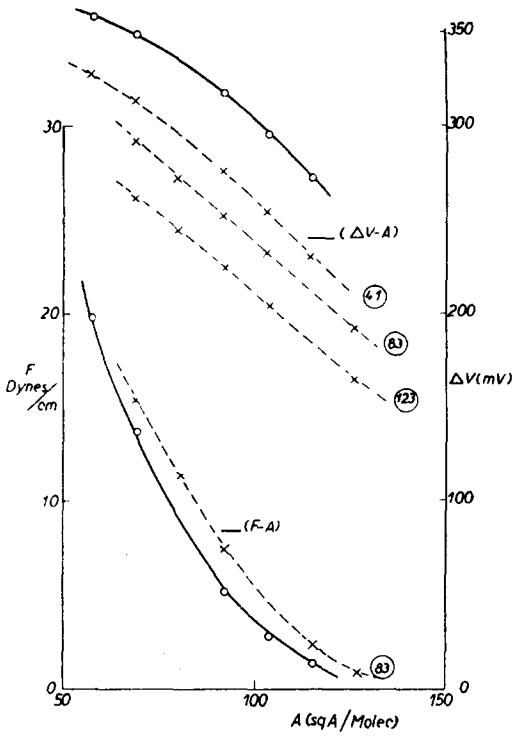

Fig. 5. *F-A* and *ΔV-A* curves for lecithin, 20° C.
—— O —— on pH 4.0 acetate buffer
--- × --- injected with "MCPA"
Ringed numbers = conc. "MCPA" $\cdot 10^5$ *M*

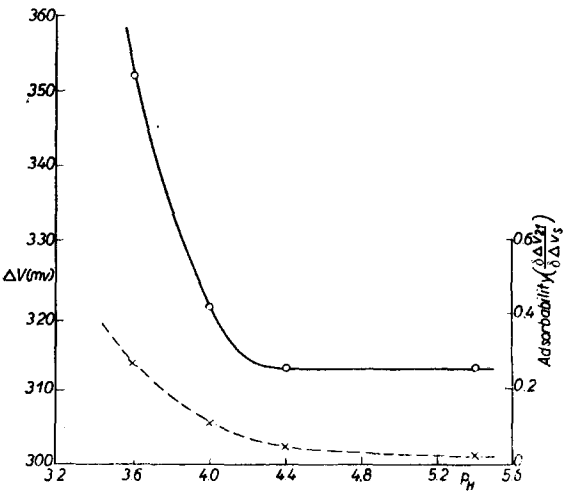

Fig. 6. Lecithin, 20° C.
—— O —— Variation of surface potential at 90 sq. A with change of pH
--- × --- Effect of pH on adsorbability $\frac{\delta \Delta V_{21}}{\delta \Delta V_s}$ of "MCPA"

3. *Lipoprotein*

The low extent of anion adsorption to lecithin has been shown to result from its negative phosphate ion. Thus in a lipoprotein complex, its influence may extend to the protein which is in association with the lecithin molecule. Gliadin, which interacts strongly with "MCPA", may be unable to do so when in combination with lecithin by reason of the negative ion and steric factors introduced by the bulky quaternary ammonium group.

The lipoprotein was formed by the admixture of equal weights of gliadin and lecithin. It was interacted at similar pH values to those of gliadin and lecithin and by injecting "MCPA" at a number of concentrations, adsorption isotherms could be plotted using the data in Table VI.

The extent of interaction $\frac{\delta \Delta V_{21}}{\delta \Delta V_s}$ ($C = 21 \cdot 10^{-5} M$) was then determined at each pH, the plot of $\frac{\delta \Delta V_{21}}{\delta \Delta V_s}$ versus pH and ΔV ($A = 1.36$ m²/mg) versus pH being presented in Fig. 7.

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TABLE VI

$\delta\Delta V$ VERSUS CONCENTRATION FOR LIPOPROTEIN INJECTED WITH "MCPA" AT VARIOUS pH VALUES

pH	$\delta\Delta V_{21}$	$\delta\Delta V_{11}$	$\delta\Delta V_{83}$	$\delta\Delta V_{123}$	$\delta\Delta V_{166}$	$\delta\Delta V_{s}$
3.6	53	77	126		186	320
4.0	30	70	100	105		155
4.4		36	58	79	85	170
4.9		33	54		70	110
5.4			37	50	60	156

In the pH range at which "MCPA" becomes undissociated (< pH 4·0), the extent of interaction decreases, as noted for gliadin. The correlation between the two curves of Fig. 7 is here less striking. The rapid rise of ΔV with decreasing pH (5.0–4.2) — presumably due to the conversion $COO^- \rightarrow COOH$ — is not accompanied by a rapidly increasing adsorption of "MCPA" to the lipoprotein monolayer. It would appear that the negative phosphate ion is so situated in the complex that interaction of negatively charged "MCPA" with the positively charged protein centres is prevented. Not until the phosphate ion becomes undissociated (pH < 3.6) will conditions be suitable for high adsorption. Lecithin, therefore, plays an important role in this system, namely that of "shielding" the protein with which it is associated from interaction with the organic acid.

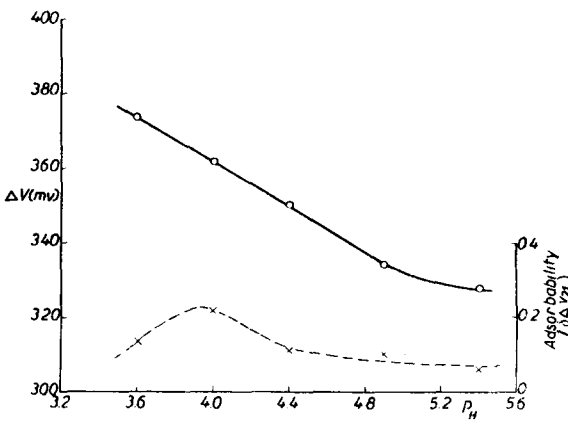

Fig. 7. Lipoprotein, 20° C.
—— O —— Variation of surface potential (A = 1.36 m²/mg) with change of pH
--- × --- Effect of pH on adsorbability $\frac{\delta\Delta V_{21}}{\delta\Delta V_{s}}$ of "MCPA"

Two considerations are here worthy of note.

a. In transit through the plant, "MCPA" will encounter both proteins and lipoids (lecithin is widely distributed in plants). Interaction with the proteins may occur extensively, thus progressively adsorbing it to sites not concerned in the physiological response. In this way its final concentration may be insufficient to perform its function at the ultimate site of action. However, if lecithin accompanies the protein, then "wasteful" adsorption is considerably reduced, permitting more "MCPA" to fulfil its true role. Thus the existence of lecithin and similar molecules within the plant may play an important part in growth regulation by "MCPA".

b. Resistant species may differ from the susceptible species, in the proportion of lecithin-like molecules that they contain. The former, containing less of the above molecules, would permit of greater adsorption to sites not concerned in the physiological response. This consideration is investigated experimentally in the following section.

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d. *Monolayers produced directly from plant tissue*

To attach biological significance to the interaction of "MCPA" with a given monolayer, the monolayer should contain a representative sample of molecules from the biological system under investigation. Thus we have injected "MCPA" beneath gliadin and the purified proteins isolated from clover (not recorded) and on similar lines, VELDSTRA used proteins from the stems of peas. The results in neither case have serious implications from a biological standpoint.

In this section, an attempt is described to overcome partially these limitations. A simple technique is developed whereby proteins, lipoids, etc. are spread as monolayers at the air/water interface, directly from plant tissue. There remains the limitation that proteins become extended when spread, thus altering their configuration.

Nevertheless, the possibility now exists of elucidating differences in the interaction of "MCPA" with representative molecules from resistant and susceptible species. Of the former, wheat coleoptiles were studied and of the latter, cress seedlings and tomato shoots.

Preliminary work established that a monolayer could be produced by an appropriate method from these species, which was stable up to 20 dynes/cm. Moreover, it was reproducible from member to member of the same variety of plant.

1. *Tomato*

Young tomato shoots were selected and a section about ¼" long which included the growing point, was used to produce a monolayer. Held by forceps, the cut end of the section was "touched" on to the surface lightly and allowed to remain there for a few seconds. Further cuts were made, the cut surface being well crushed each time. This technique was continued until a monolayer was built up on the surface. The film was then "homogenised" by expanding it to a large area for a short period to allow freer movement of the individual molecules on the surface, after which the film was compressed. Fifteen minutes was then allowed for equilibrium to be established. In this period, the pressure rose continuously for ten minutes, with an accompanying change of ΔV. Steady conditions prevailed thereafter.

As it was impossible to ascertain the weight of material spread, standard conditions were maintained in different experiments by spreading material until a pressure of 2 dynes/cm was built up. The above procedure was adopted for all the films. Moreover, as neither the weight nor the molecular weight of the substances spread are known, areas cannot be expressed in the usual units. Instead, for simplicity, the distance "l" between the mica float and the movable barrier, being proportional to the total film area, was used.

Preliminary work

i. To establish that the film was produced by molecules in the stem interior and not from waxy material on the external surface, a well-washed tomato shoot was immersed. An insoluble film was produced, but on compression no condensation of the film occurred in contrast to those described above, which were solid at low pressures.

ii. A surface containing a plant monolayer was scanned by the radioactive electrode. It was thereby shown that these monolayers were homogeneous over the entire surface.

iii. Finally, the (ΔV-l) characteristics of monolayers from tomatoes of different variety and grown in various localities were studied in a similar way and were found to be identical.

It was therefore concluded that these monolayers were reproducible, constant from plant to plant and characteristic of molecules within the portion of the plant taken.

The interaction with "MCPA"

Monolayers from tomato shoots were produced on substrates of varying pH by the

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procedure already described. Fig. 8 presents their (F-l) and (ΔV-l) curves at two pH values. The films were all solid at pressures of 2 dynes/cm.

"MCPA" was injected by a different method from that employed hitherto. A solution of the acid was injected below the monolayer by means of a bent pipette, whereas in previous work the monolayer was spread on the acid-containing substrate. Varying amounts of "MCPA" were injected and adsorption isotherms constructed. The (adsorbability–pH) curves were also plotted (Fig. 9) and the (ΔV-pH) curves (Fig. 10).

The injection of "MCPA" resulted in a penetration of the monolayer with an accompanying change of surface potential. The extent of interaction decreased with increasing pH.

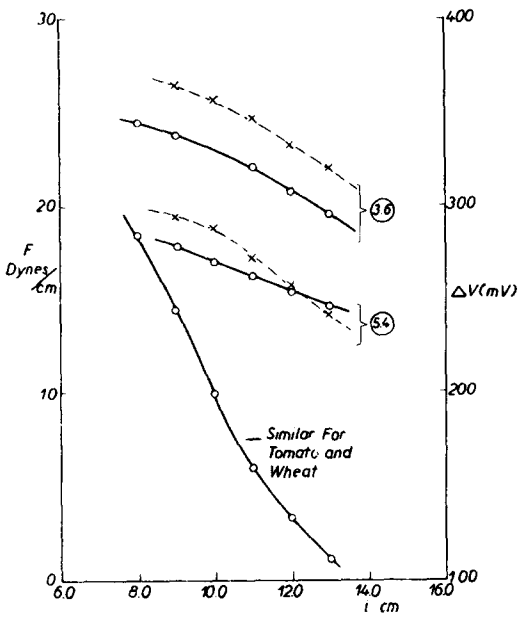

Fig. 8 (F-l) and (ΔV-l) curves for tomato shoot monolayers (—— o ——) and wheat coleoptile monolayers (– – – × – – –) on pH 3.6 and 5.4 acetate buffers. 20 °C.

2. *Cress*

Like tomato plants, cress seedlings are highly susceptible to applications of "MCPA". 7-day old cress seedlings used in this investigation were grown from seeds of variety "Sutton's Plain". These were placed on wet filter papers in a petri dish, a second dish being inverted over the first to reduce evaporation. The growing temperature was 23° C.

After 7 days, 1" seedlings had emerged, of which three were used for a single experiment. The procedure and experimental conditions were identical to those described for tomato shoots, monolayers being spread on substrates of similar pH.

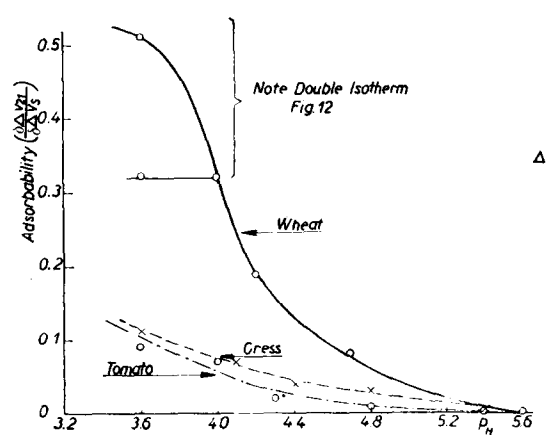

Fig. 9. (Adsorbability — pH) curves for tomato, cress and wheat monolayers. 20° C.

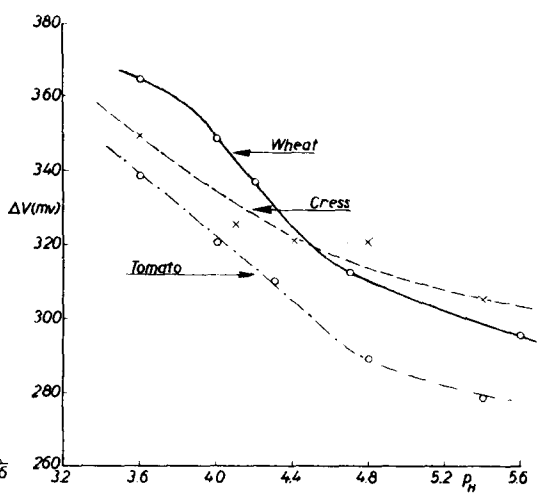

Fig. 10. (ΔV — pH) curves for tomato, cress and wheat monolayers. 20° C.

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The (F-l) and (ΔV-l) characteristics of these films were similar to those from tomato shoots, and they were solid at low pressures. The injection of "MCPA" resulted in a penetration of the monolayer, and in a change of surface potential similar in magnitude to that for tomato monolayers. (Fig. 11) presents the adsorption isotherms for this system.

(Fig. 9) illustrates the (adsorbability-pH) curves and finally (Fig. 10) the (ΔV-pH) relation for monolayers from cress seedlings.

Again, it is important to note the low degree of adsorption of "MCPA" to these monolayers in the pH range studied.

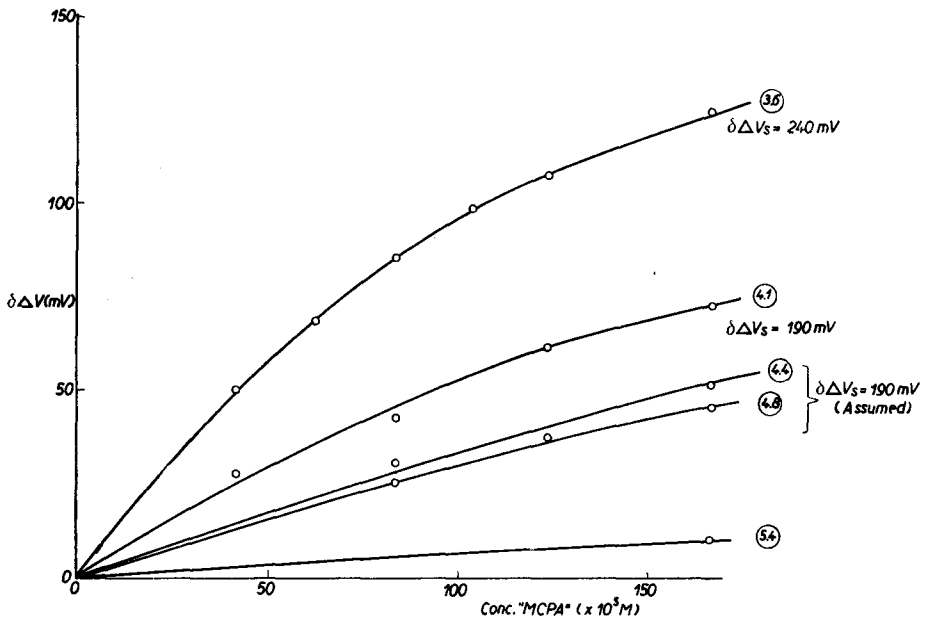

Fig. 11. Cress seedling monolayers, 20° C. Change in surface potential ($\delta\Delta V$) at l = 12.0 cm, *versus* concentration of injected "MCPA" at various pH values

3. *Wheat*

This was selected as representing a resistant species. 7-day old coleoptiles of variety "Toogood's Holdfast" were produced by a method similar in principle to that of cress seedlings.

Two coleoptiles of length about 1½" were employed for each monolayer, the enclosed leaves having previously been removed. The technique was identical to that applied to cress and tomato monolayers and the (F-l) and (ΔV-l) curves for these monolayers spread on acetate buffers of pH 3.6–5.4 are given in Fig. 8.

Adsorption isotherms were produced at each pH (Fig. 12) and the (adsorbability-pH) and (ΔV-pH) curves are presented in Figs. 9 and 10 respectively.

In general characteristics, the monolayers resemble those from cress seedlings and tomato shoots, and they are solid at low pressures.

At pH 3.6, however, an interesting and reproducible feature not found with the preceding monolayers is apparent in the adsorption isotherms of Fig. 12. A normal

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isotherm is at first produced, followed rapidly by a second. The plot of $c/\delta\Delta V$ against c consists of two straight lines whose inverse slopes give rise to saturation values $\delta\Delta V_{s1} = 86$ mV and $\delta\Delta V_{s2} = 143$ mV. At no other pH, in the range studied, was this behaviour repeated.

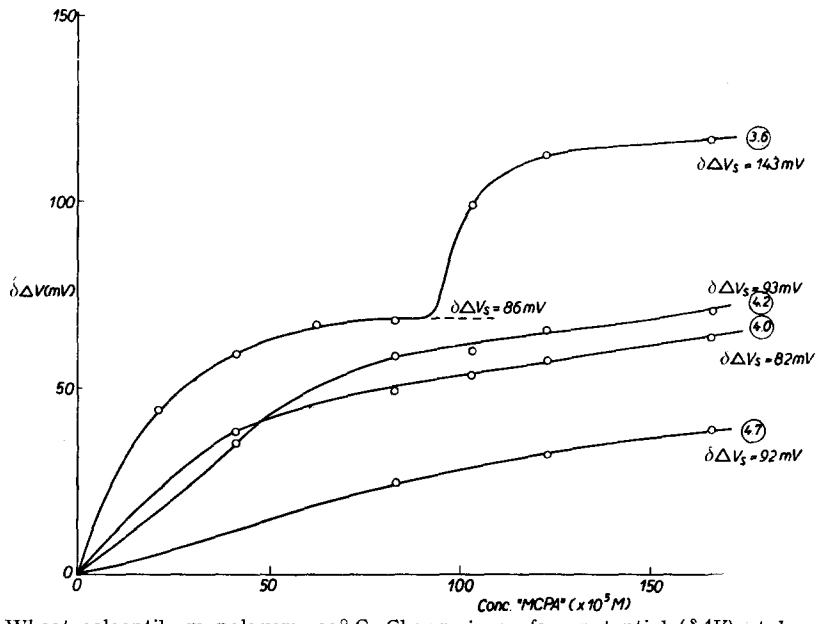

Fig. 12. Wheat coleoptile monolayers, 20° C. Change in surface potential ($\delta\Delta V$) at $l = 12.0$ cm, *versus* concentration of injected "MCPA" at various pH values

It would appear that when a certain concentration of "MCPA" has been injected, a reorientation takes place either in the monolayer or at the surface of the supporting phase.

The adsorption of "MCPA" to wheat monolayers is clearly stronger than that to those of cress and tomato. The adsorption isotherm flattens out at concentrations far below that of the two susceptible species. For comparative purposes, Table VII presents the extent of adsorption of "MCPA" ($c = 21 \cdot 10^{-5} M$) to the three species, in the pH range studied.

TABLE VII

WHEAT, CRESS AND TOMATO INJECTED WITH "MCPA"

Extent of adsorption $\left(\frac{\delta\Delta V_{21}}{\delta\Delta V_s}\right)$ at various pH values

pH	*Adsorbability* $\frac{\delta\Delta V_{21}}{\delta\Delta V_s}$		
	Wheat	*Cress*	*Tomato*
3.6	0.51 0.32	0.11	0.09
4.0	0.32	0.07	0.07
4.3	0.16	0.04	0.02
4.8	0.05	0.01	0.01
5.4	0.01	—	—

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The adsorption by wheat is approximately five times greater than that of cress and tomato.

4. DISCUSSION

As outlined in the Introduction (a, b, c), three stages must be passed through by a growth regulator before it can effect a physiological response.

It was suggested Section 3.c.3 that the susceptibility of a plant to growth hormones may depend upon the extent to which the hormone is adsorbed to sites, not concerned in the physiological response during Stage b above, high adsorption during this stage being associated with low susceptibility.

If, however, growth regulation depends upon the adsorption of a growth regulator to plant cell membranes, thus altering their permeability (VELDSTRA's original theory), then exactly the reverse would be anticipated, high adsorption giving rise to high physiological activity.

The results of Section 3.d clearly support the former suggestion. In Fig. 9 the (adsorbability — pH) curves for cress, tomato and wheat are plotted together and it is interesting to note that the adsorption of "MCPA" to cress and tomato monolayers is similar and of a different order from that to wheat. The adsorption of "MCPA" by the latter at the lower pH values is 4–5 times greater than that of the other two. It is premature to formulate an hypothesis on the basis of only three experiments; further resistant and susceptible species must be studied. Preliminary evidence, however, strongly suggests that adsorption to non-enzyme systems is a powerful controlling factor in growth regulation. But the surface pH at which this differentiation occurs *in vitro* must be reconciled to that within the plant.

Unfortunately, little is known even of bulk pH values at specific points within a plant, but pH 5.5 may be taken as an overall value for many species. However, the surface pH will differ from that in the bulk by an amount depending on the isoelectric point of the molecules (proteins, lipoids) in that surface. Assigning pH 5.0 to this value, the above surfaces may differ at pH 5.5 by approximately —(0.2–0.4) pH units from that in the bulk, whereas at pH 3.6–4.4 as used experimentally in Section 3.d, the surface pH may differ by approximately + (0.2–0.4) from that in the bulk. In this way, the disparity between *in vivo* and *in vitro* bulk pH values may be narrowed somewhat, assuming that the ionic strengths are equal in both systems[16].

In the main, however, VELDSTRA's suggestions lead to conclusions very similar to those herein described. Moreover, in a recent paper, he hints that selectivity in plant species may result from differences of composition in the respective plasma membranes of the two species, leading in one case to stronger adsorption of the growth regulator. This suggestion has been realized experimentally in Section 3.d.

But "stage b" adsorption cannot alone account for experimental findings, for the growth regulating activity of "MCPA" decreases with increasing pH. The reverse would be anticipated on the grounds of "stage b" adsorption only. A second factor must be considered which, by decreasing in intensity with increasing pH, reduces the growth regulating properties of the organic acid.

We revert to the interaction of "MCPA" with gliadin, which as a protein will be considered as an example of the enzyme with which the regulator may ultimately interact. It has been seen (Fig. 4) that the interaction with "MCPA" falls off rapidly between

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pH 4.0–6.0—much more rapidly than the corresponding fall for cress and tomato monolayers. Thus in the above pH range (it may differ for the true enzyme), "stage b" adsorption is decreasing slowly, but the "MCPA"—"enzyme" reaction decreases rapidly, the nett effect of an increase in pH being that of a decreasing physiological response.

In addition, the permeability of the lipoprotein membrane to organic acid anions will decrease as it becomes progressively negatively charged. Increasing pH, therefore, should reduce the permeability of the protoplasmic membranes to the "MCPA" anions, and this effect may contribute to the decreased physiological activity at high pH values. It is not proposed to pursue this aspect here.

Finally, for many years physiological activity has been attributed to the undissociated form of a growth regulator. The authors, however, attach importance to the ionic form for the following two reasons:

a. As found experimentally in section 3.a.1, "MCPA" is predominantly ionic above pH 4.0, the change to an ionic form commencing possibly at pH 2.0. At physiological pH values, therefore, the acid cannot contain a significant amount of the undissociated molecule.

b. Ionisation changes of a similar nature to the above in proteins and hence in cell protoplasmic membranes have been largely overlooked. But it is evident from work described in Section 3.c that ionisation of carboxyl groups on these molecules takes place rapidly at plant physiological pH values and exerts profound effects on their interaction with ionised "MCPA".

It is felt, therefore, that, in seeking an explanation of the increased physiological activity of growth regulators with decreasing pH, attention should be directed to changes which involve the proteins and protein complexes of the plant (and hence their protoplasmic membranes) rather than to the production of undissociated growth regulator molecules.

Concluding, the experimental work has drawn attention to a factor in growth regulation that hitherto has received little consideration. Estimations of the adsorption of "MCPA" to monolayers of a protein and lipoprotein and finally to monolayers produced directly from plant tissue lend support to the suggestion that the foundations of species susceptibility may be based upon the extent of its adsorption to sites not concerned in the physiological response.

SUMMARY

A plant growth regulator 2-methyl 4-chlorophenoxy acetic acid "MCPA" is interacted with a range of surface-active compounds, spread as monolayers on a Langmuir trough, to investigate factors involved in its mode of action within the plant.

By using quaternary ammonium salt $C_{18}H_{37}\overset{+}{N}(CH_3)_3\}Cl^-$, it is deduced that "MCPA" interacts in the ionic form above pH 4.0. It also interacts in low concentration with long-chain amines and ketones. Estimations of the adsorption of "MCPA" to monolayers of a protein, lipoid, lipoprotein and finally to monolayers produced directly from plant tissue, lend support to the suggestion that the foundation of species susceptibility may be based upon the extent of its adsorption to sites not concerned in the physiological response.

RÉSUMÉ

Dans l'intention d'étudier les facteurs qui influencent le mode d'action dans la plante d'une substance de croissance, l'acide 2-méthyl-4-chlorophénoxy-acétique ("MCPA"), nous avons fait réagir cette substance avec une série de composés à activité de surface, étendus en couche monomoléculaire sur une cuve du type Langmuir.

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L'emploi du sel d'ammonium quaternaire $C_{18}H_{37}\overset{+}{N}(CH_3)_3\}Cl^-$ nous a permis de conclure que le "MCPA" réagit sans sa forme ionique en-dessous de pH 4.0. Il réagit aussi en faible concentration avec des amines et des cétones à longue chaîne. Les résultats des déterminations de l'adsorption de "MCPA" à des couches monomoléculaires d'une protéine, d'un lipoïde, d'une lipoprotéine et finalement à des couches produites directement à partir du tissu végétal sont a l'appui de l'idée suivante: la susceptibilité des espèces pourrait dépendre de la mesure dans laquelle l'hormone est adsorbée à des parties de la plante qui ne prennent pas part à la réaction physiologique.

ZUSAMMENFASSUNG

Ein Pflanzenwuchsstoff, die 2-Methyl-4-chlorophenoxy-essigsäure "MCPA", wurde mit einer Reihe von oberflächenaktiven Verbindungen (in Form von monomolekularen Schichten auf einem Langmuirtrog verteilt), zusammengebracht, um die Faktoren, welche die Wirkungsart des Wuchsstoffes in der Pflanze beeinflussen, zu untersuchen.

Bei Verwendung des quaternären Ammoniumsalzes $C_{18}H_{37}\overset{+}{N}(CH_3)_3\}Cl^-$ wird geschlossen, dass "MCPA" oberhalb pH 4.0 in ionisierter Form reagiert. Es reagiert auch in schwacher Konzentration mit Aminen und Ketonen, welche lange Ketten enthalten. Bestimmungen der Adsorption von "MCPA" an monomolekularen Schichten eines Proteins, Lipoids, Lipoproteins und endlich an direkt aus dem Pflanzengewebe hergestellten monomolekularen Schichten stützen den folgenden Gedanken: die Empfindlichkeit der Arten könnte auf dem Umfang der Adsorption an Teilen der Pflanze, welche nicht an der physiologischen Reaktion teilnehmen, begründet sein.

REFERENCES

1 F. W. WENT, *Rec. trav. bot. néerland.*, 25 (1929) 1.
2 J. BONNER, *J. Gen. Physiol.*, 17 (1933) 63.
3 J. BERGER, P. SMITH, AND G. S. AVERY (Jnr.), *Am. J. Botany*, 33 (1946) 601.
4 D. E. REINDERS, *Proc. Acad. Sci. Amsterdam*, 41 (1938) 820.
5 J. W. MITCHELL AND M. R. WHITEHEAD, *Botan. Gaz.*, 102 (1940) 393.
6 H. C. EYSTER, *Plant Physiol.*, 21 (1946) 68.
7 A. H. J. HEYN, *Botan. Rev.*, 6 (1940) 515.
8 B. COMMONER AND D. MAZIA, *Plant. Physiol.*, 17 (1942) 682.
9 F. SKOOG, C. L. SCHNEIDER, AND P. MALAN, *Am. J. Botany*, 29 (1942) 568.
10 H. VELDSTRA, *Enzymologia*, 11 (1944) 97.
11 H. VELDSTRA, *Biochim. Biophys. Acta*, 1 (1947) 364.
12 H. VELDSTRA, *Biochim. Biophys. Acta*, 3 (1949) 278.
13 J. H. SCHULMAN AND E. K. RIDEAL, *Proc. Roy. Soc.*, B 122 (1937) 46.
14 J. H. SCHULMAN AND E. K. RIDEAL, *Nature (Lond.)*, 144 (1939) 100.
15 G. H. A. CLOWES, W. W. DAVIS, AND M. E. KRAHL, *Am. J. Cancer*, 36 (1939) 98.
16 J. F. DANIELLI, *Biochem. J.*, 35 (1941) 470.
17 N. K. ADAM, *Proc. Roy. Soc.*, A 126 (1929) 526.
18 E. F. PORTER, *J. Am. Chem. Soc.*, 59 (1937) 1883.
19 J. MARSDEN AND J. H. SCHULMAN, *Trans. Faraday Soc.*, 34 (1938) 748.
20 A. C. CHIBNALL, *The contribution of the analytical chemist to the problem of protein structure.* 2nd Procter Memorial Lecture. Leeds. (1945).
21 P. A. LEVENE AND I. P. ROLF, *J. Biol. Chem.*, 72 (1927) 587.
22 A. HUGHES, *Biochem. J.*, 29 (1935) 430.
23 H. VELDSTRA AND E. HAVINGA, *Rec. trav. chim.*, 67 (1948) 855.

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