RESEARCHES ON PLANT GROWTH REGULATORS

XVII. STRUCTURE AND ACTIVITY. ON THE MECHANISM OF THE ACTION III

by

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I. INTRODUCTION

Preceding investigations concerning structure and activity of plant growth substances and their mutual relation led to the conception that, as to the structure, a peculiar relation (also to be taken in a spatial sense) between lipophilic basal ring system and hydrophilic acid group is essential for a high growth activity (VELDSTRA, 1944). In this respect the required spatial structure reminds strongly of the relations in the group of wetting agents and penetrants (cf. page 280). Considering the actions to be expected from such types of compounds, a physico-chemical mechanism of action was deemed the more probable one, and, based on the colloid-chemical investigations of BUNGENBERG DE JONG *et al.*, the working hypothesis was developed that this action would consist of an influencing of the permeability (intrability) of the protoplasmic membranes.

As to the relation structure/activity these views were further put to the test with more extensive material, which came at our disposal from own investigations and from the literature, whereas with respect to the mechanism of the action ample investigations were carried out with the aid of model-systems (HAVINGA, VELDSTRA, 1948; BOOIJ, BUNGENBERG DE JONG, 1949; BOOIJ, VELDSTRA, 1949).

After a discussion of the results of the structual investigations, the consistencies of the latter investigations on model systems for the view concerning the action will be considered, also in connection with studies on biological systems and opinions developed elsewhere.

II. STRUCTURE AND ACTIVITY

The essential structural requirements for compounds to possess a high growth activity were given in the former investigations by

A. Basal ring system (non-polar part) with high interface activity.

B. Carboxyl group (polar part)—in general a group of acidic character—in such a spatial position with respect to the ring system, that on adsorption of the active molecule to a boundary (the non-polar part playing the most important rôle) this functional group will be situated as peripherically as possible.

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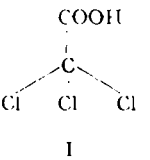
It was now considered to what extent lipophilic structures, other than those studied up till now (benzene-, naphthalene- or indolenuclei, or closely related structures) might be capable of filling the function of "attaching system" (requirement A), simultaneously carrying the COOH group in a position required according to B.

The simplest type conceivable which would meet these requirements as far as B is concerned, is without doubt trichloroacetic acid (I), though it very probably will be very much too water-soluble.

Here the "special" form of the requirements for a growth substance, as formulated by KOEPFLI, THIMANN, AND WENT (1938) is completely abandoned and attention is paid only to both demands to be made generally as expressed under A and B.



I

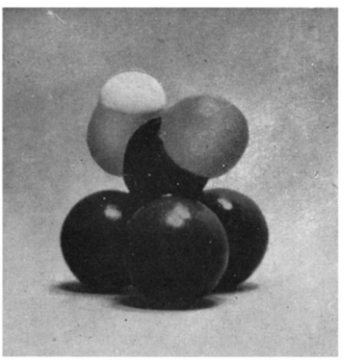
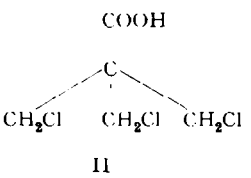
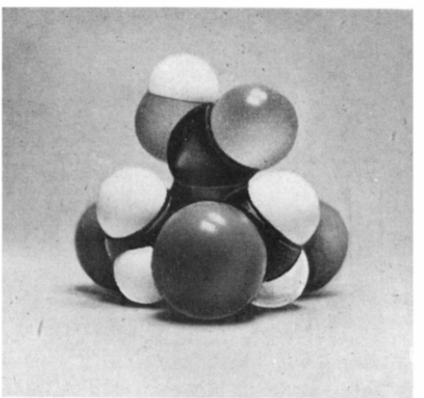


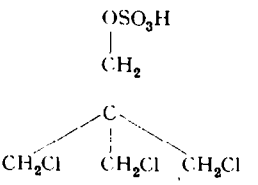
Fig. 1. STUART model of trichloroacetic acid

As already briefly communicated elsewhere (VELDSTRA, 1947) trichloroacetic acid proved to possess a very low activity in the pea test (active at $5 \cdot 10^{-3}$ mol/l)*. Though there is thus an indication of some activity, on a closer examination this evidence is not wholly conclusive because of the high concentration required. Therefore it is not absolutely sure that the effect is based on the same mechanism as that of the normal growth substances. Nevertheless the effect of this simple "ideal" type of acid in itself remains interesting, also in connection with other properties shown by it, as *e.g.*, its action on proteins, and with the behaviour of "spatial related" branched fatty acids, to be discussed hereafter (see page 280).



II





III

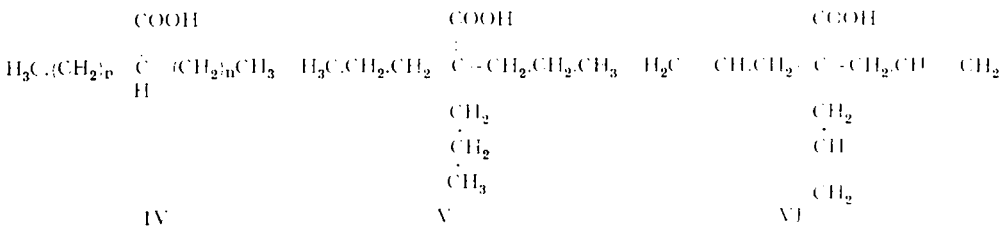
Fig. 2. STUART model of tris-(chloromethyl)-acetic acid

In order to approximate more closely the molecular dimensions of the highly active compounds, such as naphthalene acetic acid, and to obtain a higher attaching power, we investigated tris-chloromethyl acetic acid (II) and the related tris-chloromethyl ethane sulphuric acid (III).

* The acids investigated in the pea test were always used in the form of their potassium salts.

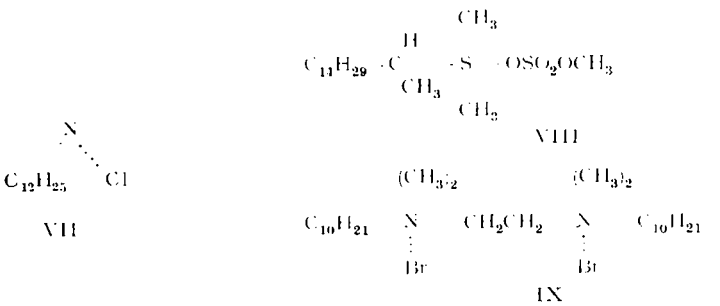
No activity in the pea test could be detected, however. As to the most interesting compound of the pair, namely tris-chloromethyl acetic acid, no definite conclusion could be arrived at because it turned out that a solution of its potassium salt rather rapidly decomposed with loss of the carboxyl group. So it proved to be impossible to determine exactly whether this compound would be active or not. The half suppression value determined polarographically (· 240) indicated however that very probably also with this compound the surface activity has not yet become sufficiently high.

As the effect of naphthalene acetic acid in the oleate coacervate is comparable with that of octanoic acid (cf. preceding paper) we reinvestigated the latter acid in the pea test over a wide range of concentrations. With the higher concentrations (≈ $5 \cdot 10^{-3}$ mol/l) the effect described before (VELDSTRA, 1947) was observed again, but in lower concentrations not the slightest inward curvatures were obtained. Further we investigated related fatty acids with branched chains, whose structures resemble more closely that of trichloroacetic acid and thus meet better the requirement B (page 278) than in the case of normal fatty acids. In fact in this way we are dealing with prototypes of the stronger wetting agents (cf. YOUNG, COONS, 1945; PRICE, 1946).



IV V VI

Of these acids: di-n-propylacetic acid (IV, n = 2), di-n-butylacetic acid (IV, n = 3), di-n-amylacetic acid (IV, n = 4), tri-n-propylacetic acid (V) and triallylacetic acid (VI), none did show any distinct physiological activity however. Only in the high concentrations (10^{-2} — 10^{-3} mol/l), near the toxic level, there appeared weak curvatures as described for comparable concentrations of the normal C_8 - C_{14} fatty acids (VELDSTRA, 1947), the objects in the pea test becoming more or less transparent. In this latter respect the branched fatty acids act more rapidly than their normal isomers. These observations induced us to study the effect of some - both mutually as with respect to the fatty acids—structurally quite unrelated wetting agents, such as the compounds VII–IX.



VII VIII IX

These compounds proved to be very toxic (already with $4 \cdot 10^{-5}$ mol/l), but quite near to these concentrations the transparency of the objects in the pea test, as mentioned before, was observed, accompanied by weak curvatures of the type obtained with the

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fatty acids in high concentrations. Particularly with compound IX, where the hydrophilic part is located in the centre of the lipophilic chain (cf. page 297) these effects were observed (see Fig. 3). In this way it becomes more probable that these curvatures do not represent a normal physiological effect, but are rather correlated with an—in this case—exaggerated form of a factor, also playing a part normally, namely with an abnormal uptake of water.

Thus with these "biped" and "tripod" compounds no significant activity could be observed and up till now this remains wholly restricted to the types derived from naphthalene, indole, substituted benzene or related ring systems.

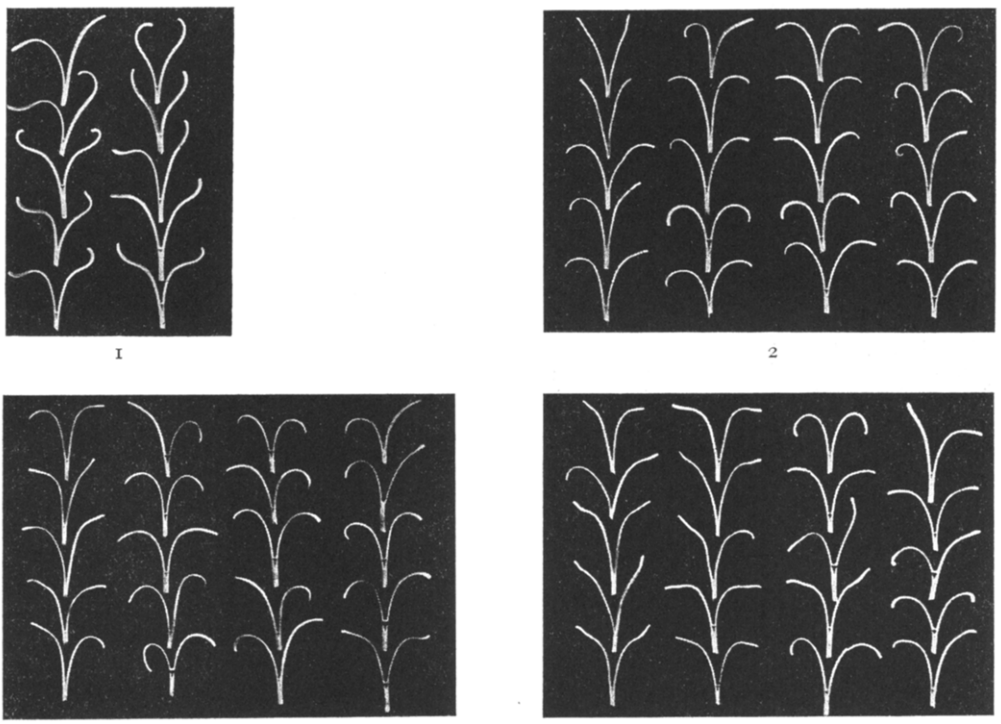

Fig. 3. Pea test

1. Naphthalene acid acetic, from l. to r.: 4; $1 \cdot 10^{-5}$ mol/l
2. Compound VII
3. Compound VIII } 4; 2; 1; $0.4 \cdot 10^{-5}$ mol/l
4. Compound IX

The bearing of these findings on the discussion of the mode of action of the growth substance will be dealt with afterwards (page 297).

As to the spatial relation of ring system and carboxyl group in the side chain some more interesting facts could be added to those already known (*cis*- and *trans*-cinnamic acid, tetrahydronaphthylidene acetic acids). As to the tetrahydro-naphthylidene acetic-acids, it was already communicated in the preceding paper that their interactions with the oleate coacervate constituted a proof for the *cis*- and *trans*-structures ascribed before to the compounds of m.p. 92° and 163°, respectively, on account of the different behaviour in the pea test (cf. VELDSTRA, 1944). Moreover a very conclusive proof now

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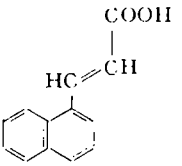
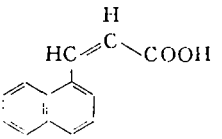
has been given by HAVINGA AND NIVARD (1948) by means of the ultraviolet absorption spectra and comparison of the difference found with that observed for comparable pairs of *cis*- and *trans*-acids. To investigate an analogy of the couple *cis*- and *trans*-cinnamic acid, *trans* β-naphthalene(1)acrylic acid (X) was synthesized and found to be totally inactive in the pea test.

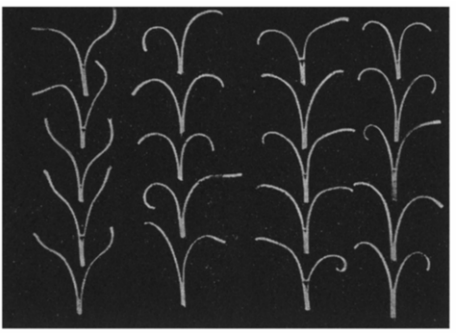


X



XI

By irradiation with ultraviolet light an isomer could be obtained (m.p. 141°, HAVINGA, NIVARD, 1948), which could be regarded as the *cis*-form (XI), and which, like *cis*-cinnamic acid, was indeed distinctly active (Fig. 4). The typical relation between spatial structure and activity as derived from the former investigations here once more comes to the fore. It is interesting that quite recently SEXTON AND TEMPLEMAN (1948), in a study of the differential effects of 2-benzoyl-benzoic acid and its derivatives, found that also with these compounds similar relations may be of importance.



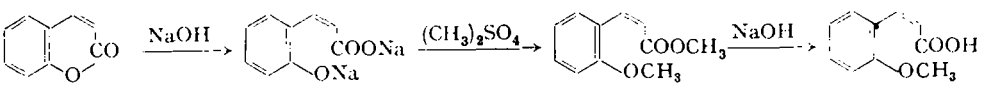
1 2 3 4

Fig. 4. Pea test

1; 3: *cis*-Naphthalene(1)acrylic acid 10 and $4 \cdot 10^{-5}$ mol/l

2; 4: *trans*-Naphthalene(1)acrylic acid 10 and $4 \cdot 10^{-5}$ mol/l

HAAGEN-SMIT AND WENT (1935) had already investigated o.methoxy *cis*-cinnamic acid (XII) and found it to be slightly active in the pea test. As this compound was highly interesting to us because of its relation to the growth inhibiting coumarin and the bearing thereof on the general problem of the structural relation between growth stimulating and inhibiting compounds (cf. VELDSTRA, HAVINGA, 1943, 1945) we prepared this cinnamic acid derivative by means of the following reactions (STOERMER, FRIEMEL, 1911):



XII

By assaying this compound in the pea test the results of HAAGEN-SMIT AND WENT could be confirmed (Fig. 5) and thus it proves possible by means of a simple series of reactions to convert a growth inhibiting substance into a stimulating one. Of course it would have been still more interesting to investigate the parent acid of the lactone coumarin, namely o.hydroxy-*cis* cinnamic acid. But as already described previously (VELDSTRA, HAVINGA, 1943) this failed, as in a solution of this acid coumarin is formed immediately.

In a comparable way *cis*-2-methoxy-β-naphthalene(1) acrylic acid (XIV) was obtained from 5.6-benzocoumarin (XIII). For the latter compound it had already been

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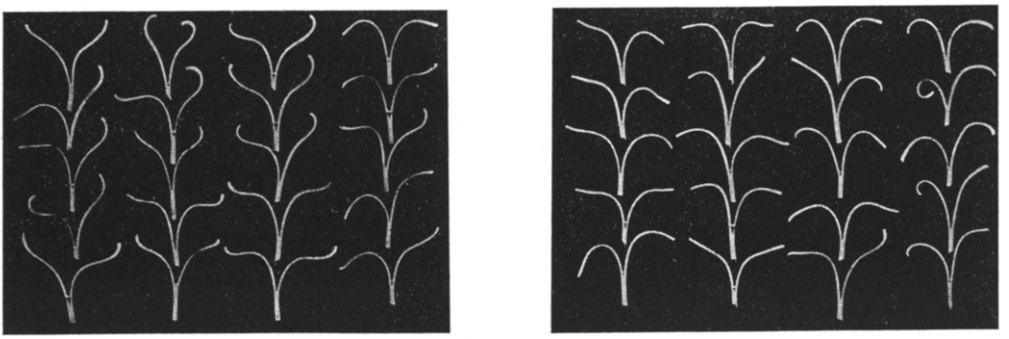
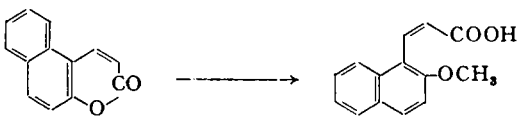


1 Fig. 5. Pea test 2

1: *cis*-o-methoxy-cinnamic acid from l. to r.
2: *cis*-2-methoxy-naphthalene(1)acrylic acid from l. to r. } 50; 25; 10; $4 \cdot 10^{-5}$ mol/l

established that its inhibiting activity was weaker than that of coumarin (VELDSTRA, HAVINGA, 1943). The methoxy-naphthalene acrylic acid, derived thereof



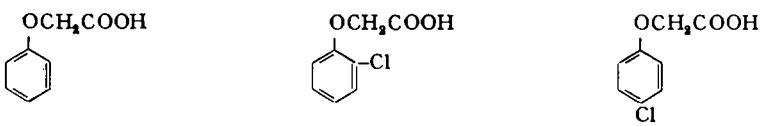
XIII XIV

was found to be active, but to a much lesser extent than the methoxycinnamic acid (cf. Fig. 5).

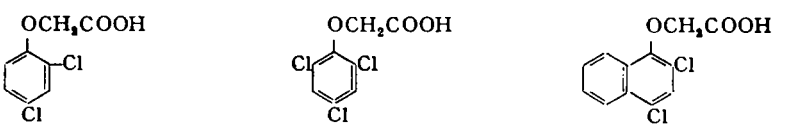
Maximal activity both in a stimulating and in an inhibiting sense thus seems to be connected with a rather well-defined molecular size, as *e.g.*, also appears from the fact that, compared with coumarin, the chloro-coumarins generally are weaker inhibitors (cf. AUDUS, QUASTEL, 1947; VELDSTRA, NAUTA, 1949).

As the growth substances have the same hydrophilic part in common, namely the COOH group, the molecular size required for maximal activity implies a definite lipophilic part in balance with the hydrophilic carboxyl group*.

One arrives at the same conclusion when comparing phenoxy-acetic acid derivatives mutually or with conformable derivatives of naphthoxy-acetic acid.



XV XVI XVII



XVIII XIX XX

Starting with phenoxy-acetic acid (XV), itself but very weakly active in the pea

* Henceforth this balance between hydrophilic and lipophilic (hydrophobic) parts of similar polar/non-polar compounds (amphipatic compounds, cf. HARTLEY, 1941) will also be denoted as HL-balance.

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test, introduction of chlorine atoms in the o- (XVI), p- (XVII) or in the o- and p- (XVIII) positions simultaneously causes in the same order an increase in activity, so that 2,4-dichlorophenoxy-acetic acid is quite as active as naphthalene acetic acid (cf. Fig. 6).

If their interface activity is measured polarographically, it proves to increase in the same sense, the activity of 2,4-D in this respect also being wholly comparable with that of naphthalene acetic acid (half suppression values (HSV): phenoxy-acetic acid 130; o.chlorophenoxy-acetic acid 17; p.chlorophenoxy-acetic acid 14; 2.4-dichlorophenoxy-acetic acid 7; naphthalene-acetic acid 5).

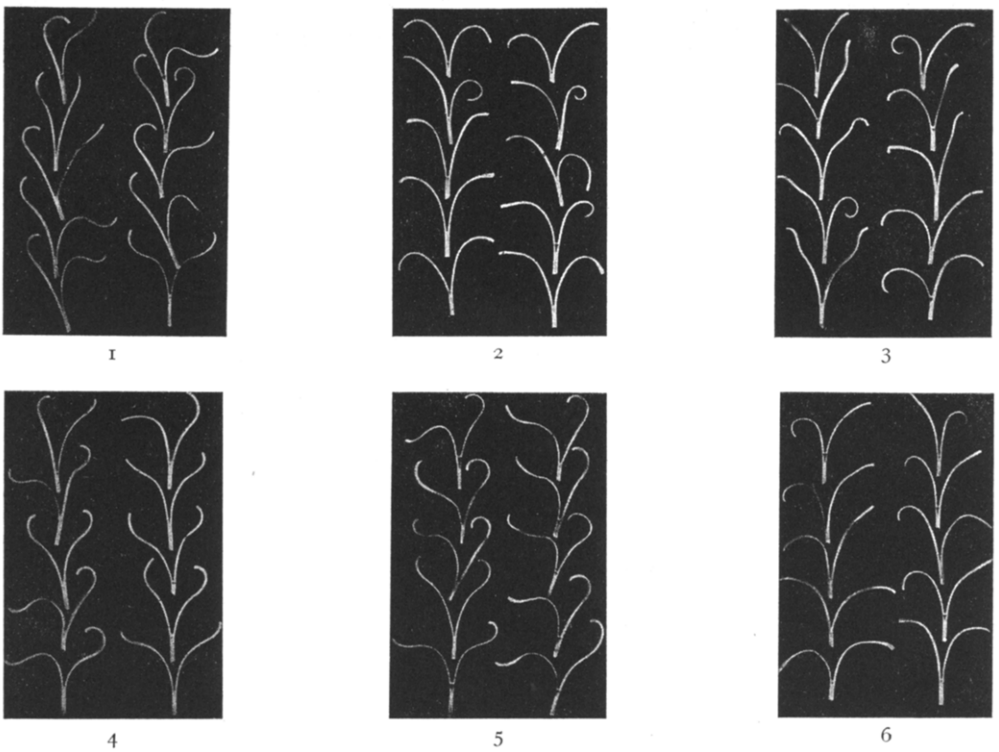

Fig. 6. Pea test

1. naphthalene-acetic acid — from l. to r.: 4; $1 \cdot 10^{-5}$ mol/l
2. phenoxy-acetic acid
3. o.chlorophenoxy-acetic acid
4. p.chlorophenoxy-acetic acid
5. 2,4-dichlorophenoxy-acetic acid
6. 2,4,6-trichlorophenoxy-acetic acid

— from l. to r.: 10; $4 \cdot 10^{-5}$ mol/l

Apparently in this series the above-mentioned balance between lipophilic (non-polar) and hydrophilic (polar) part, characteristic for maximal activity, is approximated in the best way with 2,4-D. For if one more chlorine atom is introduced, the resulting 2,4,6-trichlorophenoxy-acetic acid (XIX) proves to be practically inactive in the pea test, though its interface activity still has increased (HSV: 4,8). We believe that in the same sense in 2,4-dichloronaphthoxy-acetic acid (XX) the balance has turned too much towards the lipophilic side, this compound also possessing a very low physiological activity.

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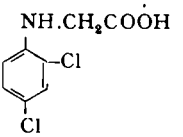
We consider it probable that with this HL-balance the real meaning of the "high interface activity of the non-polar part" (requirement A., page 287) is detailed more strictly, as this interface activity now too is brought in relation with the carboxyl group and a certain limit can be indicated. The interface activity namely not only can be too low (from which side in our former investigations this problem was mainly viewed), but also too high.

In this way the requirements for maximal growth substance activity are considered to consist of a double relation between non-polar and polar parts of the active molecule, a physico-chemical and a spatial one.

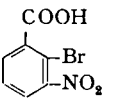
A more extensive discussion of this relation will follow after comparison of the action of growth substances and related compounds on the oleate coacervate and on biological systems (cf. page 290).

After the appearance of our first paper on the relation between chemical structure and growth substance activity, in which the active compounds known up till then were summarized, a vast amount of compounds has been investigated as to their growth activity in different centres—but mainly in the U.S.A.—(ZIMMERMAN *et al.*, 1941–1943; 1944; THOMPSON *et al.*, 1946; TEMPLEMAN, SEXTON, 1946; NEWMAN *et al.*, 1947; cf. TINCKER, 1940, 1948).

For the study of the relation structure/activity it is rather a pity that the tests used, for reasons discussed before (VELDSTRA, 1944, page 100) do not permit to draw conclusions in this respect. For this purpose the most interesting series will also have to be investigated in the pea test. Apart from the phenoxyacetic acid derivatives already mentioned, only a few of them were investigated by us up till now. For the moment we will only state that the activity of N-2,4-dichlorophenyl(1)glycine (XXI) is comparable with that of 2,4-D and naphthalene-acetic acid. Thus apparently also this type of side chain meets the requirements.



XXI



XXII

Very interesting both in a theoretical sense and to practical applications are the effects shown by substituted benzoic acids. (ZIMMERMAN, HITCHCOCK, 1942). So 2-bromo-3-nitro-benzoic acid (XXII) was reported to be mildly active for cell-elongation. We reinvestigated this compound in the pea test and found it to be active, though but very weakly.

This means that for the first time activity (let it be a very small one) is here encountered in a compound which does not fit the rule of KOEPFLI, THIMANN, AND WENT, that for the display of growth activity at least one carbon atom is required between ring system and carboxyl group or at first sight does not show the spatial relation between non-polar and polar part as we described. These requirements for *maximal activity* thus do not seem to be a "conditio sine qua non" for the appearance of growth activity in general. From a theoretical point of view this is a very interesting observation and therefore these questions were studied more in detail, also with compounds not investi-

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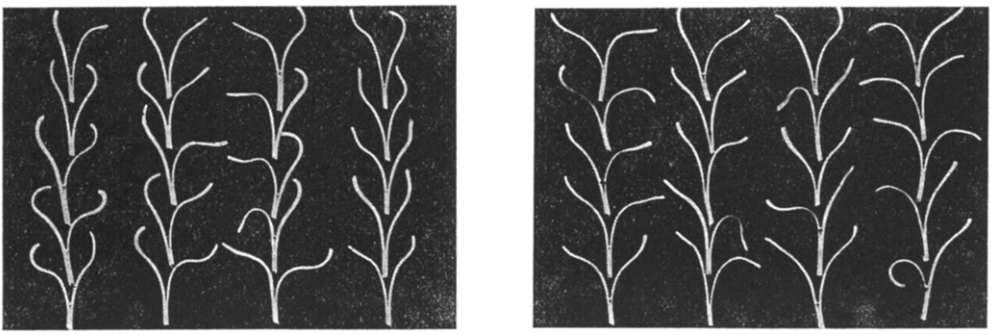

1 Fig. 7. Pea test 2

1. naphthalene acetic acid, from l. to r.: 4; 1; 0.4; $0.1 \cdot 10^{-5}$ mol/l
2. 2-bromo-3-nitro-benzoic acid from l. to r.: 50; 40; 30; $20 \cdot 10^{-5}$ mol/l

gated in this respect up till now. The results of these investigations will be discussed in a following paper.

III. ON THE MECHANISM OF THE ACTION

The investigations concerning the effect of growth substances on model systems, particularly on the oleate coacervate (BOOIJ, VELDSTRA, 1949), showed that the type of action answered expectations, but that the quantitative relations between opening effect and physiological activity in the series of naphthalene and indole derivatives were just the reverse of those one would expect on account of the view that the growth substances mainly act on the protoplasmic membranes. These divergences might be caused by the fact that the model system used does not correspond completely enough with the system physiologically reacting. On account of some arguments given in the preceding paper (BOOIJ, VELDSTRA, 1949, page 274) it was considered more probable, however, that with the view of growth substances influencing intrability/permeability the essential part of their activity had not yet been indicated and that most probably this had to be looked for within the protoplasm itself.

In order to shorten the discussion on these two possibilities and to show whether it was justified or not to base conclusions on the effects observed with the oleate coacervate, it was deemed highly important to compare the effect of some series of compounds on the coacervate with those on a biological system of such a type that one could almost certainly ascribe the effect to an interaction with protoplasmic boundaries.

One case of conformity between the effects obtained with the oleate coacervate and a biological system has already been demonstrated. The first part of the typical curve describing the opening action of normal fatty acids on the coacervate is namely reflected in the curvatures shown by these acids in the pea test (VELDSTRA, 1947). The relative figure is reproduced here once more (Fig. 8).

This parallelism, though already indicating that some biological system reacts comparably with the coacervate, does not yet furnish, however, such decisive arguments for the present discussion that it allows a choice in the above-mentioned sense. As outlined on page 280, it must moreover be taken into consideration that with these effects of the fatty acids in the pea test still other factors than those of primary importance for a normal physiological reaction may play a rôle. For this reason the relations

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found are more significant for a discussion on the *type* of action than for one on its *localization*.

For the latter purpose it seemed to us that the border tissue of the red beet (*Beta vulgaris rubra*) would offer an attractive possibility. This object has already been used previously to study the action of compounds on the plasmic boundaries. If such an action takes place, causing disturbance of the semi-permeability of endo- and ecto-plasmic membranes, the colouring matter—contained in the vacuole—can leave the

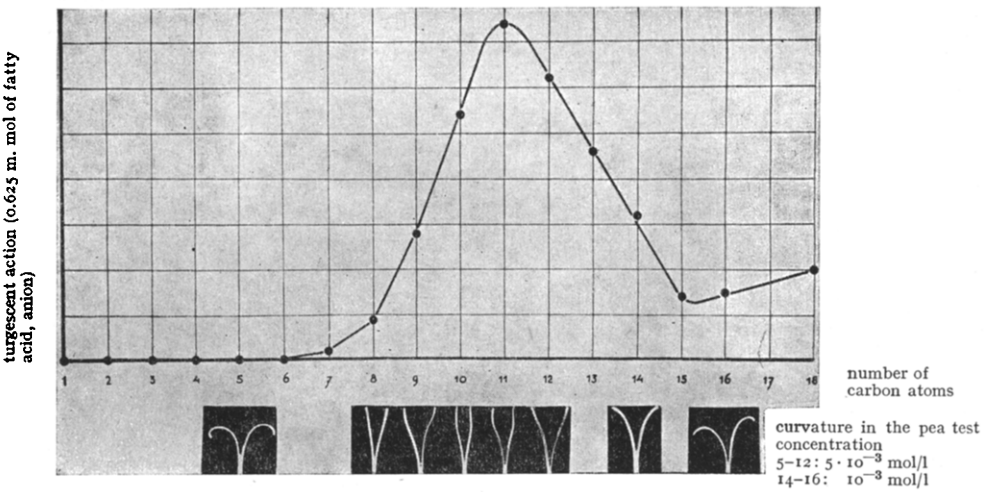

Fig. 8. Turgescent (opening) action on the oleate coacervate and growth activity in the pea test of normal fatty acids

cell and may be determined in the medium (cf. *e.g.*, GÄUMANN *et al.*, 1947). This method has been used more incidentally than for systematic investigations and it seemed attractive to study whether it could be made suitable for the comparative investigation of series of compounds.

The method adopted was as follows:

Cylinders (diameter 10 mm) were prepared from the beet by means of a suitable cork-borer and from these 10 disks (thickness 3 mm) were cut simultaneously by means of coupled razor blades. The disks were rinsed in distilled water until the colouring matter of the damaged cells had been removed, after which they remained in distilled water for one hour. After rinsing again to remove a small rest of free colouring matter, 10 disks were put into Erlenmeyer flasks containing 50 ml of a solution of the compound to be investigated. For one series the disks (100–120) from one beet were used and if some difference was observed as to the intensity of their colour, a number of comparable disks, equal to the number of flasks to be filled, were selected beforehand and of these one was put into each flask. The series consisted of different concentrations of the same compound, or of different compounds of the same (molar) concentration and were kept at 4–6° (in the refrigerator) or at room temperature.

The colouring matter leaving the cells was determined by measuring the colour intensity of the solution after suitable intervals by means of a photo-electric colorimeter (Colorimeter "Objecta", BLEEKER, Utrecht, equipped with monochromator and two photo cells) using green light (maximum 5000 Å), against blanks consisting of 10 disks immersed in the solvent only. The limit values for the absorption, reached when total discoloration occurred in a series, proved to be rather constant, indicating that the disks, selected as mentioned, always contained a fairly constant amount of colouring matter, as was shown also by running duplicates.

During the test (usually 22 hours) the blanks (in distilled water, sometimes in 12% alcohol) remained practically colourless or showed but a slight coloration.

It was observed that the p_H in the mean shifted 0.5 units towards the acid side. In comparing

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series of compounds simultaneously this effect causes no trouble, however. Buffer solutions could not be used, as their constituents influenced the test in an unfavourable way. In a further study of the beet test these observations will be analysed more in detail.

First of all the series of fatty acids, already studied with the oleate coacervate, was investigated (see Fig. 9).

Unfortunately this could not be done for the whole series, because of the fact that the solubility of the higher homologues is too small at p_H 7. From lauric acid (C_{12}) onwards this causes opalescence or a slight turbidity of the solutions, interfering with the colorimetric determination, so that measurements at the beginning of the test in this region can only be approximative. Towards the end of the test the solutions become more and more clear as the acids are adsorbed into the beet tissue, but only for lauric acid this goes so far that the measurements become absolutely reliable. Though for this reason the interpretation of the curves has to be given with some caution, as micelle formation with the higher homologues may interfere, they nevertheless indicate that there exists a maximum in the same region as found with the oleate coacervate, so that this model system seems to react comparably with a biological object. And it can be concluded almost certainly already that the absence of a parallelism between the action of growth substances (derived from indole or naphthalene) on the oleate coacervate and in the pea test (cf. the preceding paper) cannot be explained on account of the imperfection of the model system.

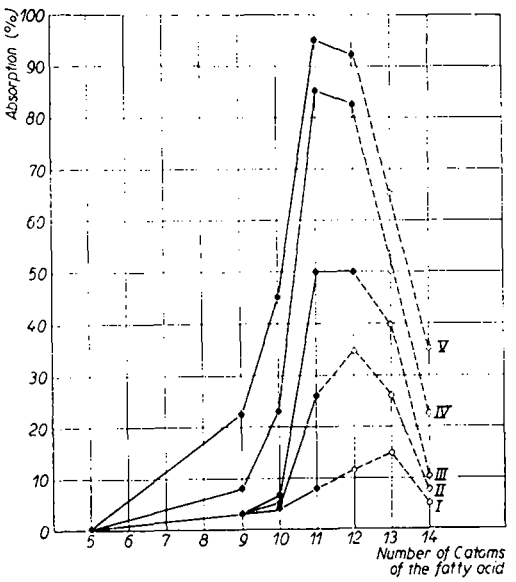

Fig. 9. Beet test. Normal fatty acids (C_5–C_{14}) I, II, III, IV, V: after 2.5; 4.5; 6.5; 12.5 and 22 hours respectively. ($4 \cdot 10^{-4}$ mol/l, water, t = 20° C). Approximative measurements are indicated by open circles and dotted lines (cf. text).

It would be of great importance if the form of the curves could be established accurately also for the higher numbers of the series. For, considered in connection with the conclusions arrived at by BOOIJ AND BUNGENBERG DE JONG (1949) in explaining the form of the curve for the action of fatty acids on the oleate coacervate, the occurrence of a maximum in the region C_{11}–C_{12}, followed by a minimum at C_{15}–C_{16} would indicate that the reacting constituent of the plasmic membrane is a system built in an orderly manner and that the length of its lipophilic part would be comparable with that of oleic acid.

It now becomes very interesting to determine the action of the growth substances in the beet test and to compare the effects with those on the oleate coacervate. The results are summarized in the Figs 10–13.

From these figures it can be deduced:

1. For naphthalene and indole derivatives the action on the membranes of the beet runs parallel to that on the oleate coacervate, as increasing hydrogenation, or

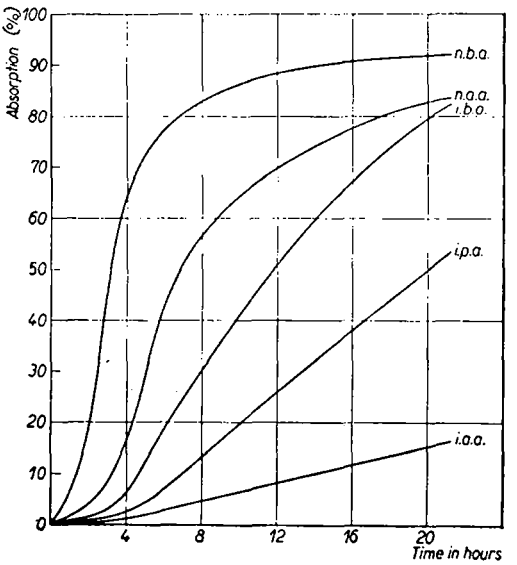

Fig. 10. Beet test: Indole acetic acid (i.a.a.); Indole propionic acid (i.p.a.); Indole butyric acid (i.b.a.); Naphthalene acetic acid (n.a.a.); Naphthalene butyric acid (n.b.a.); (10^{-2} mol/l; 12 % alcohol, t = 4° C)

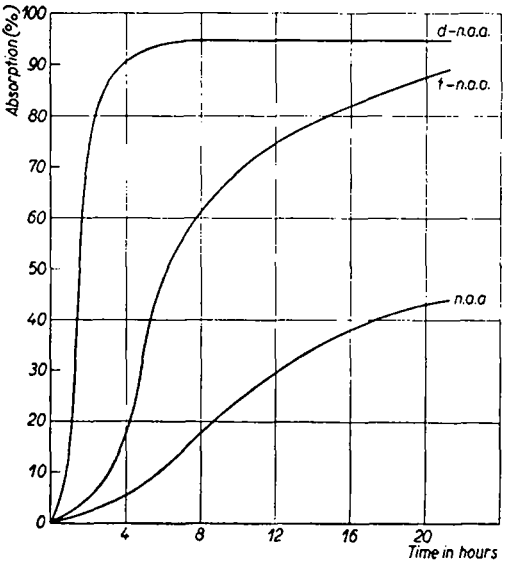

Fig. 11. Beet test: Naphthalene acetic acid (n.a.a.) and its tetrahydro- (t-n.a.a.) and decahydro- (d-n.a.a.) derivative (10^{-2} mol/l; water, t = 4° C)

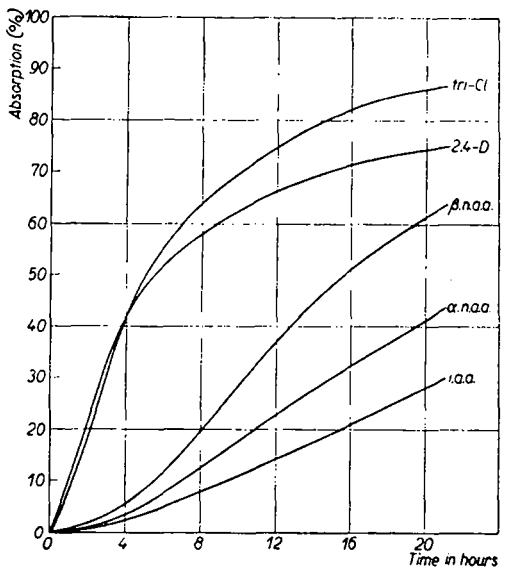

Fig. 12. Beet test: Indole acetic acid (i.a.a.); α-Naphthalene acetic acid (α-n.a.a.); β-Naphthalene acetic acid (β-n.a.a.); 2,4-Dichlorophenoxy-acetic acid (2,4-D); 2,4,6-Trichlorophenoxy-acetic acid (tri.Cl); ($2.5 \cdot 10^{-3}$ mol/l; water; t = 18° C)

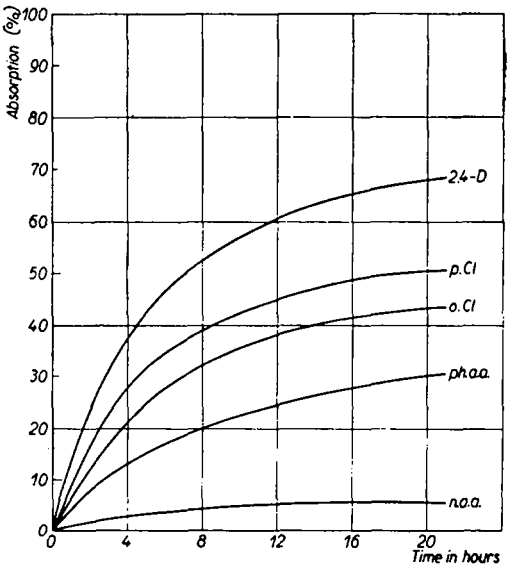

Fig. 13. Beet test: Naphthalene acetic acid (n.a.a.); Phenoxy-acetic acid (ph.a.a.); o.Chlorophenoxy-acetic acid (o.Cl); p.Chlorophenoxy-acetic acid (p.Cl); 2,4-Dichlorophenoxy-acetic acid (2,4-D); ($2.5 \cdot 10^{-3}$ mol/l; water, t = 4° C)

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lengthening of the side chain results in increasing liberation of the colouring matter.

2. The higher the activity of these compounds in the pea test (plant growth activity), the weaker their effect on the beet membranes.

3. The action of indole derivatives in the beet test is decidedly weaker than that of the comparable naphthalene compounds, on the other hand it is a well-known fact that especially with the butyric acids the indole derivative exhibits the strongest growth activity.

4. The activities of the different compounds in the beet test reflect very well their phytotoxicity as already known from the study of their effects on parts of plants or intact plants (rooting of cuttings, inducing parthenocarpy, etc.). This is very evident if one compares indoleacetic acid < naphthalene acetic acid < 2,4-dichlorophenoxy-acetic acid.

5. Phenoxy-acetic acid, and its chlorinated derivatives on the whole, affect the membranes more strongly than *e.g.*, naphthalene acetic acid.

In this series not only the actions on the coacervate (Fig. 14) and in the beet test run parallel, but—in contrast with that of naphthalene and indole derivatives—the physiological activity (exhibited at low concentrations) also increases in the same sequence. Moreover their herbicidal effects (in higher concentrations) show the same course.

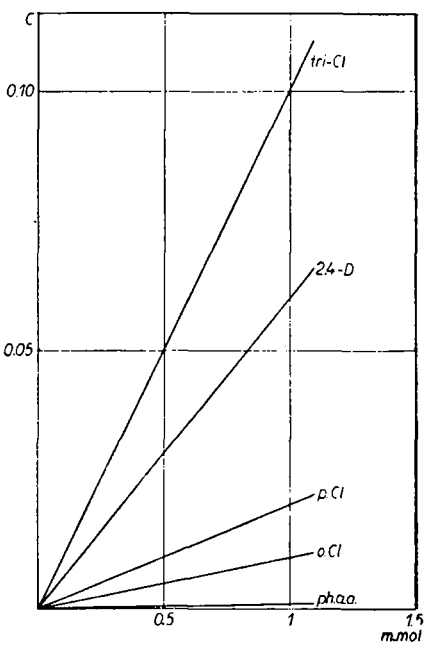

Fig. 14. Oleate coacervate. Shifting of the KCl-curve (ordinate) under influence of phenoxy-acetic acid (ph.a.a.), o.chlorophenoxy-acetic acid (o.Cl), p.chlorophenoxy-acetic acid (p.Cl), 2,4-dichlorophenoxy-acetic acid (2,4-D) and 2,4,6-trichlorophenoxy-acetic acid (tri-Cl)

These results give rise to the following considerations:

In the series of naphthalene acetic acid and its hydrogenated derivatives or naphthalene and indole acetic acid and their homologues the highest physiological (growth) activity is found at the beginning of the series, indicating that here the proper balance between lipophilic and hydrophilic parts of the molecule, required for maximal activity, has already been reached. Increase of the lipophilic character, by hydrogenation of the nucleus or lengthening of the sidechain, causes stronger interaction with (adsorption to) the protoplasmic membranes and on the whole a diminished growth activity.

Starting with the weakly active growth substance phenoxy-acetic acid with too weak a lipophilic character, increase of the lipophilic part (by introduction of chlorine atoms) is followed by increasing interaction with the membranes, but now also by an increase of the growth activity up till 2,4-dichlorophenoxy-acetic acid, where the proper balance is reached. For, if one more chlorine-atom is introduced, the activity of the resulting 2,4,6-trichlorophenoxy-acetic acid in the pea test proves to be greatly

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diminished (cf. Fig. 6) whereas the activity in the coacervate as compared to that of 2,4-dichlorophenoxy-acetic acid has still increased. See Fig. 14.

So from the dichloro acid onwards the same divergence between biological object and model system is encountered as that found with the naphthalene and indole series discussed above.

In the same sense it can be understood that chlorination of naphthoxy-acetic acid to 2,4-dichloronaphthoxy-acetic implies an "overpowering" of the lipophilic "weight" and consequently together with increasing interaction with the coacervate a diminished growth activity.

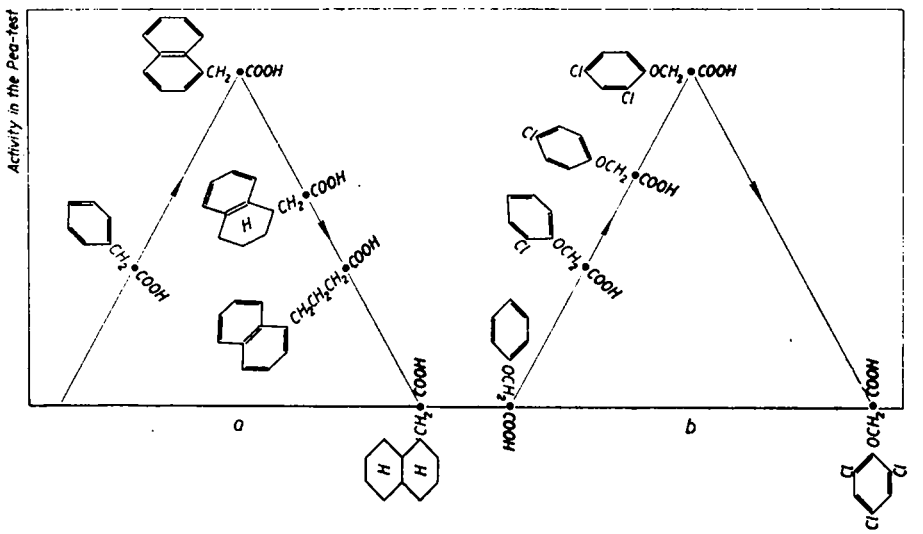

Fig. 15. Schematic representation of the balance between hydrophilic and lipophilic parts of the molecules in the series: A. Phenyl acetic acid, naphthalene acetic acid and its hydrogenated derivatives and homologues; B. Phenoxy acetic acid and its chlorinated derivatives.

The HL-balance is brought into relation with the physiological activity (in the pea test). The most favourable value in this sense (maximal activity) is indicated by a horizontal position. If the hydrophilic carboxyl group or the lipophilic part dominates, the position becomes an inclined one, with as extremes vertical positions (physiologically inactive), caused either by total dominance of the carboxyl group (compound is too water soluble) or of the lipophilic part (compound is too fat soluble).

Most likely the conclusion arrived at by TEMPLEMAN AND SEXTON (1946) in their studies on the differential effect of synthetic plant growth substances: "chlorination of the phenoxy compounds generally appears to increase their activity, whereas this is not so for chlorination of the naphthoxy compounds", can be explained on the same grounds.

In our opinion all these facts fit into the same scheme, the value of the balance between lipophilic and hydrophilic parts of the molecules being the decisive magnitude (cf. VELDSTRA, 1949). And it wholly depends on the starting points whether parallelism for activities with the biological object (pea test) and in the model system (oleate coacervate) is observed or not.

These relations are represented once more in a schematic form in Fig. 15.

We believe that from this point of view also the higher physiological activity of indole butyric acid as compared to that of naphthalene butyric acid may be understood. Of these two ring systems the indole nucleus has a considerably weaker lipophilic character than naphthalene (derived from the difference between their condensing

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actions in the oleate coacervate; compare also HAVINGA, VELDSTRA, 1948). For this reason the size of the lipophilic side chain can be somewhat longer when attached to indole than when coupled with naphthalene before the maximum balance with regard to the hydrophilic carboxyl group is reached, and thereafter the physiological activity decreases. Of course we realize that in these considerations another important factor is neglected, namely the influence of *e.g.*, introduction of chlorine atoms or of lengthening of the side chain on the position of the carboxyl group with respect to the ring system. Before *e.g.*, dipole moments of suitable derivatives will have been determined this factor cannot be analysed further, however.

The importance of the HL-balance of an amphipatic compound to its behaviour in a certain system could be clearly demonstrated in the series of di-alkyl acetic and di-alkyl malonic acids respectively, by determining their effect in the oleate coacervate (see Fig. 16).

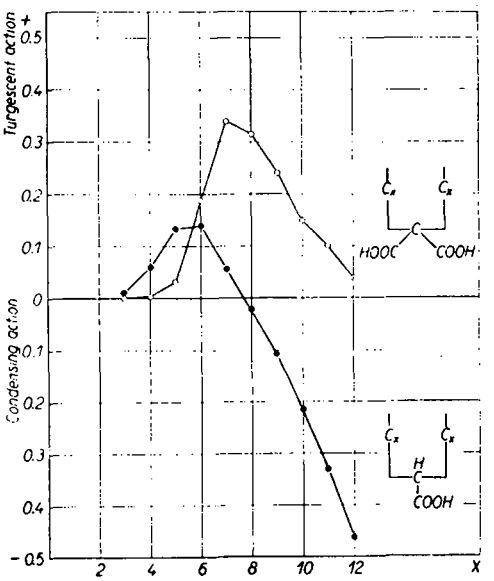

Fig. 16. Oleate coacervate. Shifting of the KCl-curve (ordinate) under the influence of di-n-alkyl acetic acids (•——•) and of di-n-alkyl malonic acids (o——o) (concentrations: $5 \cdot 10^{-4}$ mol/l)

As to the acetic acids, up to di-n-hexyl acetic acid the swelling effect increases (parallel with increasing adsorption into the micelles). Though the adsorption of the higher homologues increases still further, the swelling effect diminishes from that acid onwards as now the lipophilic "weight" of the aliphatic chains predominates more and more over the hydrophilic carboxyl group. Even so that between x = 7 and x = 8 the effect becomes a condensing one, steadily increasing by lengthening of the chains. Introduction of two $-CH_2-$ groups thus causes a reversal of the effect and possibly the often non-elucidated rôle of $-CH_3$ groups in physiologically active compounds may be understood in this sense, as contributing to the proper HL-balance.

In principle the curve for the di-alkyl malonic acids is of the same form as that of the acetic acids, but has shifted quite markedly to the swelling side and to the right. This means that at the same value of x (for $x > 6$) the swelling effect of the malonic acids is stronger than that of the acetic acids and that the influence of increasing lipophilic character becomes perceptible "later", *viz.*, at a higher value of x. Obviously this is caused by the introduction of another carboxyl group, by which the hydrophilic "weight" is doubled.

Quite clearly the action in the oleate, both in a quantitative and in a qualitative sense, is governed by the HL-balance of these acids.

If one tries to imagine why increasing the lipophilic character of a compound beyond a certain limit causes a decreasing physiological activity, in our opinion the results obtained with the beet test may offer a plausible explanation, namely that the

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interaction with (adsorption to) the protoplasmic membranes then becomes too strong. If indeed, as already suggested in the preceding paper, the primary reaction takes place in the protoplasm, too strong an adsorption to the membranes might prevent the attaining of the concentration (within the protoplasm) required for maximum activity. Furthermore too strong an interaction, with as a possible consequence, too strong an opening effect on the membranes might disturb the normal (physiological) equilibrium between the protoplasm and the outside of the cell. It is even imaginable that the latter effect is partly responsible for the herbicidal action of 2,4-dichlorophenoxy-acetic acid (for this purpose used in concentrations up till $5 \cdot 10^{-3}$ mol/l), which compound, as shown in the beet test, has a strong affinity for the membranes. In that case the well-known selectivity of this action ,as expressed by the far more greater toxicity for dicotyledonous than for monocotyledonous plants, a.o. might be connected with differences in composition and consequently in reactivity of the plasmic membranes. As to the selectivity in action of this type of compounds we truly are inclined to attribute quite generally decisive importance to the relation between HL-balance of the active compound on the one side and the composition of the system on the other side (cf. also VELDSTRA, 1944, page 154 under 3).

It now also becomes necessary to revise the view, expressed previously, concerning the inhibiting action of growth substances, if applied in excess, namely that this would be caused by a condensing action on the membranes. In the oleate coacervate only opening actions were observed, secondly they never changed to condensing ones when the concentration of the growth substances was increased. Therefore, also in connection with the experience in the beet test, it seems more likely now that in the inhibition at higher concentrations rather too strong an opening action plays a rôle. On a closer examination this is more plausible too, when considered from a colloid chemical point of view. Of course this does not exclude the possibility that in the growth inhibition by other types of compounds, such as the neutral coumarin or related lactones, such condensing actions are of importance. More probably, however, as with the growth stimulating action, the inhibiting one can neither be explained solely by these membrane effects. This appeared from the fact that the blastocholine activity in the series of indole-or naphthalene acetic acid and its hydrogenated derivatives decreases just like the growth activity by lengthening of the side chain or by increasing hydrogenation.

And so we must conclude that the view expressed before concerning the localization of the action of the growth substance has certainly been connected too one-sidedly with the membranes.

According to the results obtained with the oleate coacervate and in the beet test the interaction with the membranes may certainly occur and, moreover, is of the type that answers expectations. But it is quite certain now that in the starting hypothesis concerning growth substance action the importance of this interaction has been overestimated and that it does not constitute the "primary reaction" itself, which will more probably be found in the cytoplasm. The membrane effect, however, can influence this primary reaction in a quantitative sense and will be responsible to a large extent for the difference in activity in a series of related compounds. In this way it implies a form of selectivity.

This view leads to the following considerations:

Case A. Of the amount of a highly active growth substance (naphthalene acetic

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acid) added to a biological system part will be adsorbed to the endo- and ectoplasmic membranes (and possibly onto other interfaces outside the cytoplasm) and part will act within the cytoplasm (primary growth reaction), both parts being in dynamic equilibrium with each other and with the molecules present in the vacuole and outside the cells.

Case B. If to the same system be added a derivative of naphthalene acetic acid with increased lipophilic character, and consequently of higher interface activity (*e.g.*, naphthalene butyric acid or decahydro naphthalene acetic acid) in the same molar concentration, within a certain course of time a greater part will be adsorbed to the plasmic membranes, etc. Therefore a smaller amount of molecules will be available for the primary reaction, causing a weaker growth-response than in the case of A. The stronger the affinity for the membranes, the weaker the growth effect will be (naphthalene butyric acid: weakly active, decahydro naphthalene acetic acid: practically inactive).

If this schematic representation touches the essence of the growth substance action, it should be possible:

1. To obtain the same growth effect as in A with a lower concentration of naphthalene acetic acid, if a compound as used in B is added too. Because of its higher interface activity the B-compound will be preferentially adsorbed to the membranes and consequently of the lesser amount of naphthalene acetic acid (as compared to that in A) enough still remains available for the primary reaction. The B-compound will be the more effective in this sense, as its growth activity is lower.

Such a mixture of "underdosed" highly active growth substance and a supplementary quantity of a weaker active or nearly inactive compound should equal the maximum effect of the growth substance alone, even if the total number of molecules is smaller, as for a comparable "occupation" of the membranes, etc., a lesser amount of a higher interfacial activity compound will be sufficient.

2. The activity of a weakly active substance as mentioned in Case B (weakly active because of too strong a lipophilic character) should be enhanced in the presence of a related compound with still higher interface activity. The first-mentioned substance then will be displaced from its adsorbed state and become available to a higher degree for the primary reaction. (The proportion of the interface activity can be derived from the results obtained with the oleate coacervate or in the beet test).

Both suppositions could be substantiated by assaying mixtures of the required composition in the pea test (cf. Figs 17, 18, 19).

So it proves to be possible to obtain a maximal effect with a sub-maximal concentration of *e.g.*, naphthalene acetic acid by the addition of the less active naphthalene butyric acid or of the inactive decahydro naphthalene acetic acid in quantities which exhibit only a very weak or no activity of their own. Even if the concentration of naphthalene acetic acid is lowered to one tenth or one fourtieth of that normally used, maximum activity can be attained. Fig. 18 shows that maximal activity can be reached with half the quantity of the molecules required for naphthalene acetic acid if used alone.

The normal fatty acids of which the molecular size corresponds with that of naphthalene acetic acid [as *e.g.*, undecanoic acid (XXIII) and dodecanoic acid (XXIV)] also show this enhancing effect, though to a less extent.

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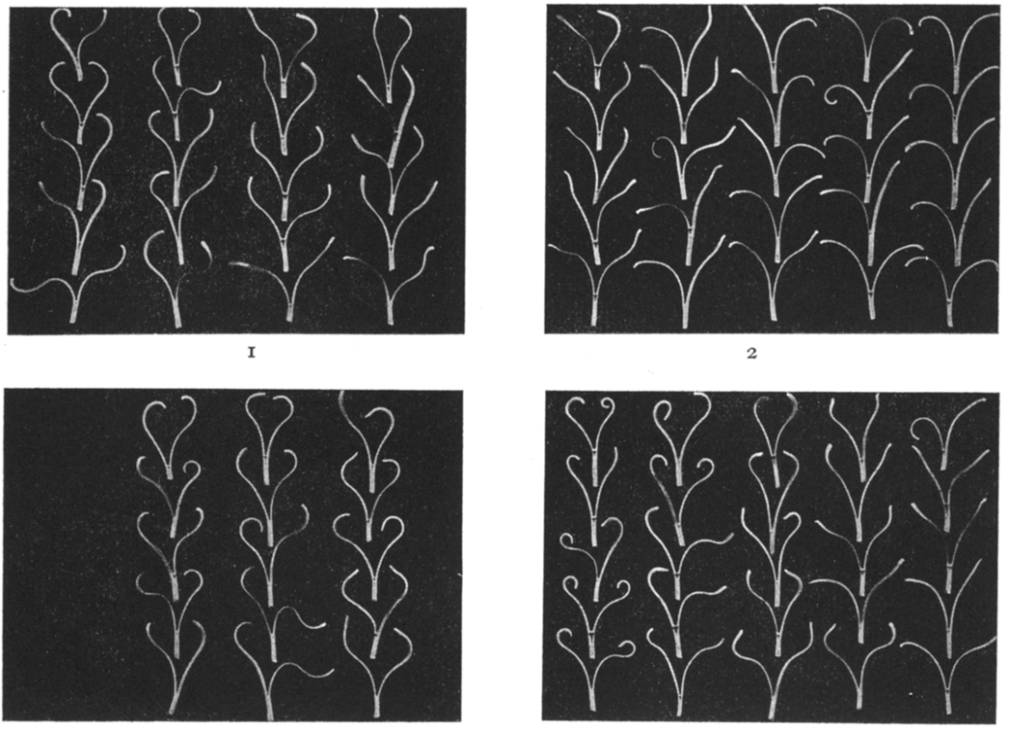

Fig. 17. Pea test. Synergistic actions.

1. Naphthalene acetic acid, from l. to r.: 4; 1; 0.4; $0.1 \cdot 10^{-5}$ mol/l
2. Naphthalene butyric acid, ,, ,, ,, ,,: 4; 2; 1; 0.4; $0.1 \cdot 10^{-5}$ mol/l
3. Naphthalene acetic acid, ,, ,, ,, ,,: $\left\{\begin{matrix}1\\2\end{matrix}\right.$ $\left\{\begin{matrix}0.4\\2\end{matrix}\right.$ $\left\{\begin{matrix}0.1\\2\end{matrix}\right.$ $\cdot 10^{-5}$ mol/l
   Naphthalene butyric acid, ,, ,, ,, ,,:
4. Naphthalene butyric acid, ,, ,, ,, ,,: $\left\{\begin{matrix}4\\4\end{matrix}\right.$ $\left\{\begin{matrix}2\\4\end{matrix}\right.$ $\left\{\begin{matrix}1\\4\end{matrix}\right.$ $\left\{\begin{matrix}0.4\\4\end{matrix}\right.$ $\left\{\begin{matrix}0.1\\4\end{matrix}\right.$ $\cdot 10^{-5}$ mol/l
   Di-n-amyl-acetic acid, ,, ,, ,, ,,:

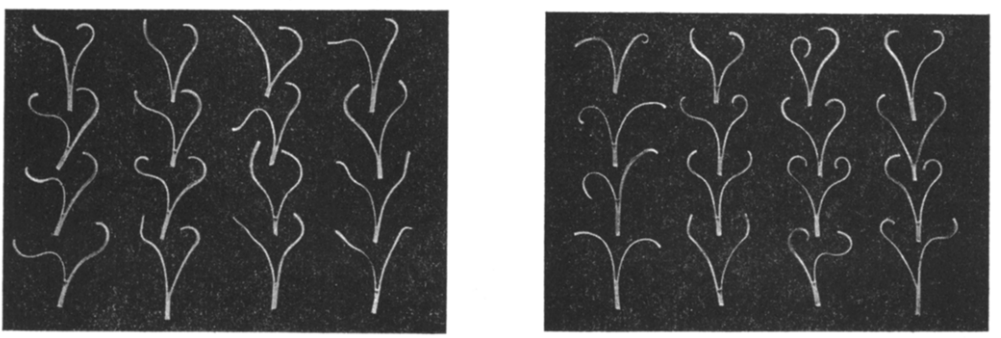

Fig. 18. Pea test. Synergistic actions.

1. Naphthalene acetic acid, from l. to r.: 4; 1; 0.4; $0.1 \cdot 10^{-5}$ mol/l
2. Naphthalene acetic acid, ,, ,, ,, ,,: $\left\{\begin{matrix}-\\2\end{matrix}\right.$ $\left\{\begin{matrix}1\\2\end{matrix}\right.$ $\left\{\begin{matrix}0.4\\2\end{matrix}\right.$ $\left\{\begin{matrix}0.1\\2\end{matrix}\right.$ $\cdot 10^{-5}$ mol/l
   Decahydro-naphthalene acetic acid, ,, ,, ,, ,,:

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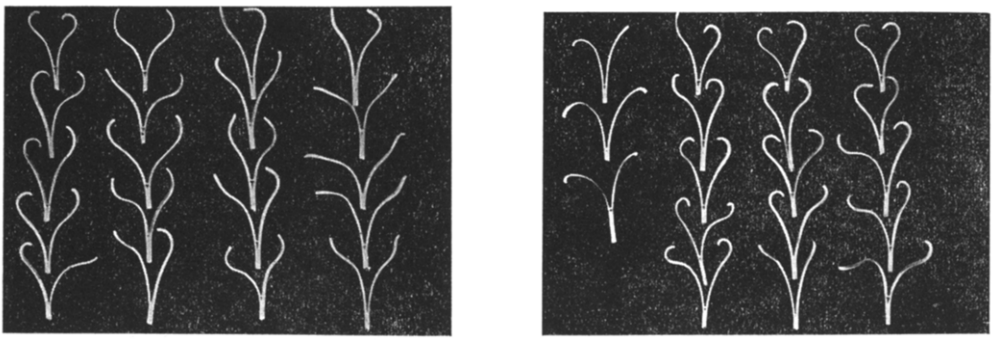

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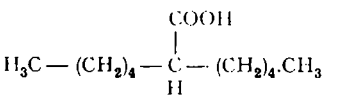
Fig. 19. Pea test. Synergistic actions.
1. Naphthalene acetic acid, from l. to r.: 4; 1; 0.4; $0.1 \cdot 10^{-5}$ mol/l
2. Naphthalene acetic acid, ,, ,, ,, ,, : $\left\{-\right.$ $\left\{\begin{matrix}1\\4\end{matrix}\right.$ $\left\{\begin{matrix}0.4\\4\end{matrix}\right.$ $\left\{\begin{matrix}0.1\\4\end{matrix}\right.$ $\cdot 10^{-5}$ mol/l
Di-n-amylacetic acid, ,, ,, ,, ,, :

$H_3C.(CH_2)_9.COOH$

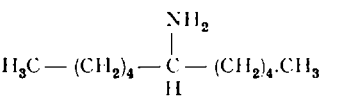
XXIII

$H_3C.(CH_2)_{10}.COOH$

XXIV



XXV



XXVI

If the carboxyl group is moved towards the centre of the carbon chain, and the molecules of the fatty acid thus assume the form known to be required for maximal activity as a wetting agent or penetrant (WILKES, WICKERT, 1937; HARTLEY, 1941; PRICE, 1946), the resulting di-n-amylacetic acid (XXV) proves to be a very potent activator, (cf. Figs 17, 19), though its activity in the beet test is even weaker than that of undecanoic acid (cf. Fig. 20) and its activity therefore cannot be explained by an unusually enhanced affinity for the membrane.

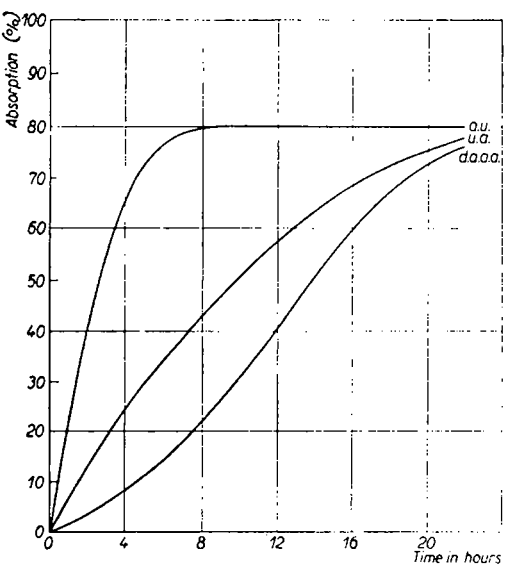


Fig. 20. Beet test. Di-n-amylacetic acid (d.a.a.a.) Undecanoic acid (u.a.); 6.Amino-undecane (a.u.) ($8 \cdot 10^{-4}$ mol/l; water; t = 20° C)

So with these fatty acids, which—in the concentrations used—are completely inactive as growth substances, the property to act as synergists seems to be connected with a certain spatial structure, in a certain sense quite comparable with that derived previously for the highly active growth substances proper.

In fact, di-n-amyl-acetic acid is a highly active "internal" wetting agent in relatively low concentrations for e.g., pea stem tissues, as these rapidly become transparent when being embedded in a

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solution of it. The uptake of water under the influence of compounds of this type is at the moment the subject of a separate investigation.

In this connection the question arose quite logically whether wetting agents in general, if showing high activity in the beet test, would be able to enhance the activity of sub-maximal concentrations of growth substances. This proved not to be the case, *e.g.*, for the types mentioned on page 280.

It was very interesting to find that 6-amino-undecane (XXVI) which is still more active in the beet test than the corresponding acid (cf. Fig. 20) does not show the enhancing effect either (cf. Fig. 21). So the carboxyl group, as with the growth substances

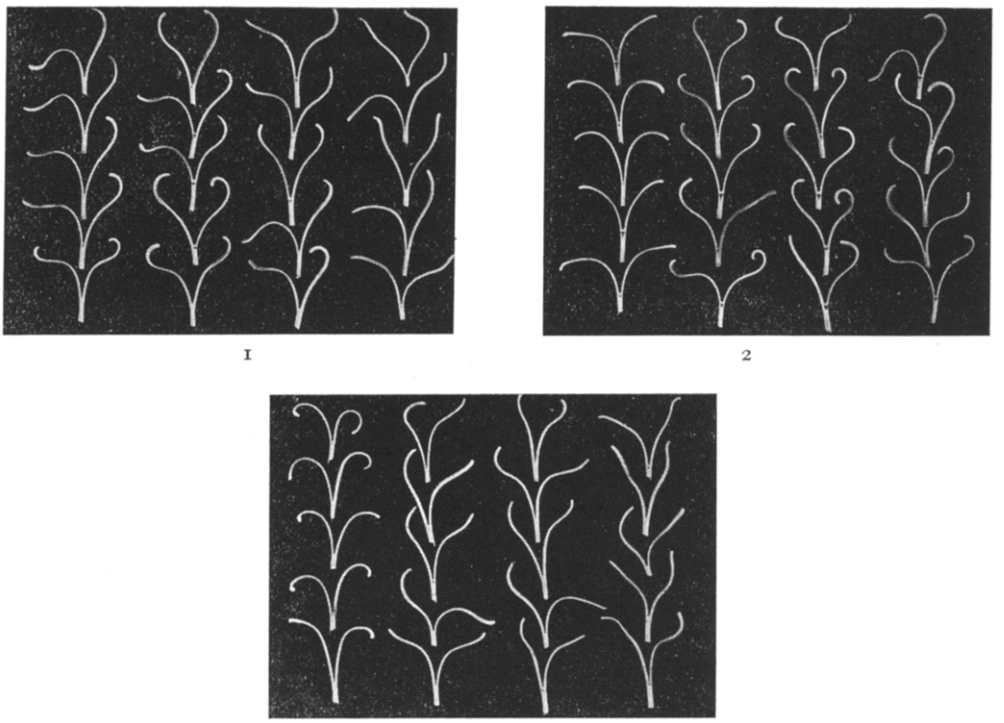

Fig. 21. Pea test. Synergistic actions.

1. Naphthalene acetic acid, from l. to r.: 4; 1; 0.4; $0.1 \cdot 10^{-5}$ mol/l
2. Naphthalene acetic acid, ,, ,, ,, ,, : $\left\{ - \left\{ 1 \left\{ 0.4 \left\{ 0.1 \right. \right. \right. \right. \right.$
   Di-n-amyl-acetic acid, ,, ,, ,, ,, : $\left. \left. \left. \right\} \right\} \right\} \cdot 10^{-5}$ mol/l
3. Naphthalene acetic acid, ,, ,, ,, ,, : $\left\{ - \left\{ 1 \left\{ 40. \left\{ 0.1 \right. \right. \right. \right. \right.$
   6.Amino-undecane.HCl, ,, ,, ,, ,, : $\left. \left. \left. \right\} \right\} \right\} \cdot 10^{-5}$ mol/l

proper, apparently has a specific function. The results of investigations with several types of carboxylic acids will be published in a separate paper. In connection with the present discussion we will only mention that it was found that acids generally show the enhancing effect if, as compared with the highly active growth substances, the lipophilic character has increased and the molecular size is of the same order*.

It is interesting in this connection that recently THIMANN and BONNER (1948) reported about the increase of growth promoting action of low concentrations of indole

* Patent application pending.

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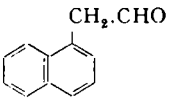
acetic acid by means of tri-iodo benzoic acid, practically inactive itself. The effect is large in the pea test, but smaller in the straight growth of *Avena* coleoptiles and in the standard *Avena* test. We will discuss these results more extensively in the above-mentioned paper.

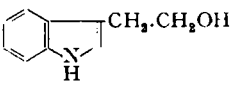
It will be attractive to study whether this type of synergistic action of structural analogues will be of importance in other cases, also because of the fact that up till now antagonistic effects have been emphasized practically exclusively in the study of the action of structural analogues (cf. VELDSTRA, 1948).

When surveying once more the data obtained in the present investigations one arrives in our opinion at the conclusion that in the action of growth substances two phases can be distinguished, namely one concerned with adsorption to protoplasmic membranes, etc., and one constituting the primary growth reaction proper, most probably proceeding within the protoplasm.

For the latter supposition further arguments were found in the behaviour of the neutral compounds naphthalene acetaldehyde (XXVII) and tryptophol (XXVIII) in the pea test.



XXVII



XXVIII

The aldehyde proved to be nearly as active as naphthalene acetic acid, whereas the alcohol only showed a small effect (cf. LARSEN, 1947, who already investigated these compounds in the *Avena* test). But in both cases the action was distinctly more rapid than that of naphthalene acetic acid. If an essential part of the growth reaction does take place inside the cell, this difference in reaction velocity may be explained in a plausible way, namely by a more rapid permeation of the neutral compounds as compared to that of the analogous acids. (Inside the cells both compounds are very probably oxidized to the acids).

As to the membrane effect, from the results with the oleate coacervate and also with the beet test it can be deduced that in a physico-chemical sense the growth substances are apt to exert an "opening" action on lipophilic membranes, but under the present conditions we cannot state definitely whether this really occurs in the physiologically active concentration region to the extent that this adsorption performs a physiological function or whether this adsorption properly speaking must be considered as a "waste" with respect to the total growth reaction. The fact that, as mentioned above, such structural details as "wetting agent type" are apparently of importance for the synergistically active acids, makes us inclined for the present to the supposition that indeed the membrane effect is of physiological importance and that the growth reaction thus proceeds in two phases. From the proportions found with the activity of mixtures of growth substances and synergistically active acids it must be deduced then that by far the greater part of the growth substance is used in the first phase and only a small fraction is actually required for the primary reaction.

In our opinion it seems very likely that the first "phase" of the growth reaction, as deduced in this way, is the same as that indicated as early as 1939 by F. W. WENT as the preparatory reaction when he proved that pre-treatment of the objects in the *Avena*

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or pea test with *e.g.*, phenyl butyric acid or cyclohexane acetic acid (not active in the elongation reaction itself) increased the response of subsequently applied indole acetic acid. The compounds active in this respect were called hemi-auxins. In comparison with the compounds investigated by us phenyl butyric acid and cyclohexane acetic acid are but very weakly active, however, as we determined by using phenyl butyric acid in our tests.

(Cf. also the concentration used by WENT for phenyl butyric acid in the pea test: $\sim 10^{-3}$ mol/l, whereas *e.g.*, di-n-amyl-acetic acid acts with 10^{-6}–$4 \cdot 10^{-5}$ mol/l).

It was stated by WENT that indole acetic acid is active both in the preparatory and in the elongation reaction, but that the former reaction requires a higher concentration than the latter. So here the proportions are comparable to those we found in the pea test with the afore-mentioned mixtures of naphthalene acetic acid and a synergist.

Furthermore according to WENT the preparatory reaction ("first phase") is insensitive to the p_H, whereas the primary reaction itself ("second phase") is dependent on it. These relations could be understood if indeed the first phase is concerned in the main with the protoplasmic membranes, *viz.*, with the outside of the cells, because for such an action one would not expect a great difference at a different p_H. As the membrane has to be passed for the primary reaction (inside the cell), the degree of dissociation of the acids plays a rôle and consequently the p_H of the medium will exert a pronounced influence.

In their important study on the interactions of growth substances in growth and inhibition, SKOOG, SCHNEIDER, AND MALAN (1942) did not agree with WENT's conclusions, as in the *Avena* test and in the cylinder test (straight growth) inhibiting effects were mainly observed if phenyl butyric acid was added to indole acetic acid; these effects were ascribed to a competitive action between the acids. Only in the cylinder test with low concentrations of indole acetic acid (0.005 mg/l) and a relatively high concentration of phenyl butyric acid a synergistic effect was observed, explained by SKOOG *et al.* as an auxin-sparing action of phenyl butyric acid.

With higher concentrations of indole acetic acid the initially occurring antagonistic effect (at low concentrations of phenyl butyric acid) is only partially compensated by increasing the concentration of phenyl butyric acid (cf. Fig. 2 of SKOOG *et al.*, reproduced in Fig. 22).

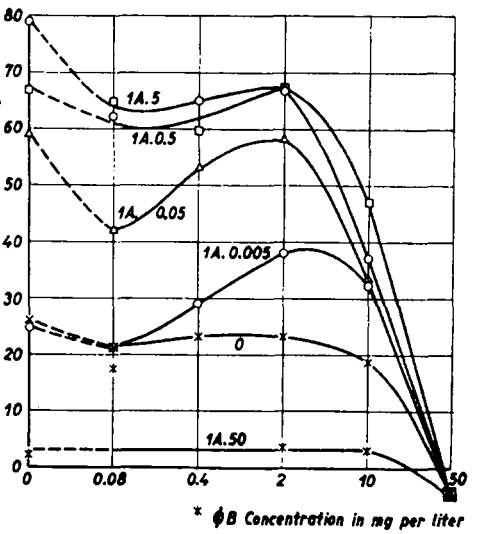

Fig. 22. Reproduction of Fig. 2 and legend according to SKOOG, SCHNEIDER, AND MALAN (1942). Per cent elongation of 3 mm long sections of *Avena* coleoptiles in the section test with 2 % sucrose solution and various concentrations of indole acetic and phenyl butyric acids. (The numbers inserted represent concentrations of indole acetic acid in mg/l).

For this reason SKOOG *et al.* concluded that in this case it is impossible to interpret the effects in terms of hemi-auxin activity of phenyl butyric acid, as then the growth response of the mixtures should equal the optimal action of indole acetic acid itself.

We deem it possible, however, that this

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optimal level is not reached because of the fact that with phenyl butyric acid—only weakly active as a synergist in our test—the toxic concentration is interfering too soon, and it remains to be investigated if under the circumstances of the test of SKOOG *et al.* the acids highly active synergistically described by us would not meet the re quirements for hemi-auxins.

For the rest it seems to us that the course of the curves does not procure arguments for a competitive action as discussed by SKOOG *et al.*, as *e.g.*, the relative inhibition caused by 0.08 mg phenyl butyric acid/litre is smaller for indole acetic acid 0.5 mg/l than for indole acetic acid 5 mg/l. For a truly competitive antagonism this would have to be the reverse and moreover it cannot be understood how by increasing the concentration of the "antagonist" phenyl/butyric acid the inhibition is overcome. In the relation metabolite/metabolite-antagonist it is only known that at very low concentrations of the antagonist, a stimulating action may occur (cf. HENRY, 1943; WOOLLEY, WHITE, 1943; WOOLLEY, 1944) and that the antagonistic effect increases with increasing concentration, which is just the reverse of what is observed in SKOOG's test.

In our opinion the apparently complex relations in the action of mixtures in SKOOG's experiments have not yet been satisfactorily unravelled. The discussion of these interesting observations can be pursued after the synergists, which we have described, have also been investigated.

For the moment we prefer to follow the "route" of a two-phase growth reaction, the more so as in one of the most important contributions to the investigations concerning the mechanism of action of plant growth substances BURSTRÖM (1941, 1942, 1945, 1947) has presented convincing evidence that the cell elongation (studied with individual wheat root cells) proceeds in two distinct phases. During the first phase the primary wall is loosened, possibly no new material being added, accompanied by increasing wall elasticity and turgor tension. This phase is highly temperature-sensitive, acceleration following increase of the temperature. Nutritional conditions, however, prove to be of little importance.

In the second phase the rate of cell elongation—slow until then—increases very much, so that two-thirds or more of the total increase in cell lengths takes place in a short time. The turgor tension now remains constant, whereas the elasticity decreases. This second phase, during which oriented cellulose strands are deposited in the wall, is strongly influenced by the nutrient supply; *e.g.*, carbohydrate supply causes an extension.

As to the influence of indole acetic acid it appeared that it accelerates the first phase, but that the second phase is more or less completely inhibited. If very low concentrations of indole acetic acid are used, the retardation of cell elongation, caused by this influence on the second phase, is followed by a stimulation of the elongation.

It now becomes most interesting to investigate whether the "phases" deduced from the more (physico-)chemical analysis of the problem have something to do with those distinguished by BURSTRÖM. In this connection one will first of all have to determine whether the acids, active synergistically in the pea test, have some typical action on the first and/or the second phase, as described by BURSTRÖM. It will depend on the outcome of these investigations* in which direction the discussions will have to be pursued.

* Prof. BURSTRÖM was so kind as to accept our proposal that these questions be studied in his laboratory at a convenient time.

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Before we terminate our considerations by giving some suggestions concerning the mode of action of the growth substances in the cytoplasm in connection with the information gathered from our experiments, it seems useful to survey briefly the views about growth substance action put forward until now by different investigators and to analyse in what respects these views converge.

As for the growth response by the natural auxins oxygen and carbohydrate are indispensable and as there is evidence that auxin action is in some way connected with respiration (see below) it is quite comprehensible that there still exists a very definite trend to deal with the auxins under the heading "co-enzymes". This means incorporation of the auxins in the ergons, mainly belonging to the vitamin B group, of which it is known with certainty that they form part of prosthetic groups of redox enzyme systems (cf. *e.g.*, the review by SKOOG, 1947, pages 542–544). SKOOG even goes so far as to suggest that, because of the aerobic nature of auxin action, together with the indispensability of the double bond in the auxins and its exact position in the ring, the reversible saturation of this double bond may simply constitute the specific reaction of the auxin molecule.

From a chemical point of view this is highly improbable, however, as the experience with biochemical hydrogenations (*e.g.*, with yeast, cf. FISCHER, 1939) has shown that hydrogenation of the double bond is difficult when connected with a tertiary C-atom (both in aliphatic chains and in hydro-aromatic ring-systems) and that α, β-unsaturated alcohols are not hydrogenated, in contrast to the primary ones. And even if the dihydro-auxin could be formed under physiological conditions, with this type of compounds it is rather certain that this hydrogenation would not be reversible (required for the supposed physiological function) as oxidation of a cyclopentane ring-system to a cyclopentenederivate is quite unknown under these conditions. The vitamins functioning in a redox-system and all compounds which can replace them to a certain extent (*e.g.*, methyleneblue, etc., with low negative redoxpotentials) are of a fundamentally different structure and from a chemical point of view there is little doubt that neither the auxins a and b, nor indole acetic acid and the synthetic compounds with growth substance action possess the redox-character required for functioning in this sense under physiological conditions. Also by studying the synthetic compounds polarographically it has already appeared that they are not easily hydrogenated (VELDSTRA, 1944). So in our opinion the "co-enzyme" conception in the chemical sense will have to be abandoned and we see more perspectives in trying to link in another sense the physico-chemical type of action—as deduced by one of us from the chemical and spatial structure of the growth substances—to the results of investigations by *e.g.*, COMMONER, THIMANN (1941), AVERY *et al.* (1943–1944), which indicate that auxins play a rôle in the relation growth/respiration. In 1941 COMMONER and THIMANN raised the question anew whether there exists some relation between growth and respiration. They were unsatisfied by the conclusions of former investigations (KÖGL *et al.*, 1936; BONNER, 1936), implying that auxin does not have an effect on the respiration of *Avena* coleoptiles, whereas to their opinion many facts point to the existence of some relation.

Considering 1st that inhibition of respiration by cyanides—which in the chain:

$$\text{oxygen} \rightarrow \text{oxidase} \rightleftarrows \text{carriers} \rightleftarrows \text{dehydrogenase} \leftarrow \text{metabolite}$$

is concerned with the oxidase—causes a proportionate inhibition of growth, 2nd that, also in non-growing tissues respiration does occur, COMMONER AND THIMANN concluded

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that the differentiation of the two processes had to be looked for in the dehydrogenase part of the chain. For this reason they studied the effect of dehydrogenase inhibitors on growth and found it to be an inhibiting one.

Particularly iodoacetate proved to inhibit growth of *Avena* coleoptiles in solutions containing sucrose and indole acetic acid. This inhibition of growth is complete with an iodoacetate concentration of $5 \cdot 10^{-5}$ mol/l whereas under these conditions the respiration is only inhibited by 10%. This part of the respiration then could be essentially linked to the growth process. The effect of iodoacetate could be removed by the four-carbon acid-ions malate and fumarate and to a lesser extent by succinate and pyruvate. These acids may function as carriers in the chain indicated above. The acids reinforce the effect of indole acetic acid on growth and also the respiration if indole acetic acid is present. After pre-treatment of the coleoptile sections with malate or fumarate the respiration of these objects is stimulated by indole acetic acid, without such a pre-treatment it has no effect and in this respect the results of the former investigations are confirmed. As under these conditions the influence of indole acetic acid on respiration and the effect on growth parallel each other, COMMONER AND THIMANN deduced that the C_4 acids are involved in a respiratory system essential for growth, which constitutes only a small fraction of the total respiration. This particular respiratory process was considered to be promoted by indole acetic acid.

ALBAUM AND COMMONER (1941) and ALBAUM AND EICHEL (1943) found similar relations for growth inhibition by iodoacetate and its reversal by C_4-acids with intact oat seedlings.

Recently THIMANN AND BONNER (1948) repeated the experiments more in detail and could confirm the previous results. Besides the C_4-acids already mentioned also citrate, malonate and maleate proved to be able to reverse the inhibition by iodoacetate. Growth of isolated sections in a solution containing indole acetic acid ($5.7 \cdot 10^{-6}$ mol/l) and sucrose ($3 \cdot 10^{-2}$ mol/l) proved to be markedly increased if the sections were only partly submerged or by oxygenating submerged sections. Obviously the aerobic condition is of great importance for the processes involved, and as shown by the experiments, primarily in the presence of indole acetic acid. The remarkable fact was established that the sensitivity, both to iodoacetate inhibition and to the growth promoting action of the organic acids, augments with increasing age of the coleoptile from which the sections are cut. The authors suggest that growth is controlled by an enzyme containing free SH-groups, essential for its activity. The concentration of iodoacetate required to effect growth inhibition is presumed to be a measure for the concentration of the enzyme. According to the authors the amount of this enzyme (per unit length of the coleoptile) would then decrease with increasing age and its effective concentration would be enhanced by oxygen. As to the latter conclusion it seems difficult to us to imagine how the effective concentration of an enzyme, for the action of which free SH-groups are essential, can be enhanced by oxygen supply. And we are inclined to ask whether it would not be more plausible to suppose that oxygen is required for the functioning of the enzyme leading to a growth response. Though of course many other questions still remain unanswered these clear-cut experiments will certainly contribute a great deal to the solution of the problem of growth substance action.

The more so if they are connected with the investigations of BERGER AND AVERY (1943, 1944) who studied the influence *in vitro* of synthetic growth substances on dehydrogenase systems of the *Avena* coleoptile. The action of cell-free extracts containing

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these enzymes was not influenced by growth substances, but if the enzyme extracts were prepared from coleoptiles, pre-treated with indole acetic acid, particularly the action of the extract containing the alcohol dehydrogenase was enhanced as compared with that of the controls. For other dehydrogenases a similar effect was not found or only to a lesser degree. This is a very interesting observation, as among the dehydrogenases the alcohol dehydrogenase is by far the most sensitive to iodoacetate. Thus the authors are led to consider auxin action as an activation of an enzyme. As activation has to be relative to a constant amount of the enzyme and it is not certain that the conditions of the experiment meet this requirement (in our opinion an enhanced extractivity of the enzyme may play a rôle as well) this conclusion, though highly alluring, cannot yet be definite on account of these experiments. It seems to us, however, that continuation of this type of experiments is of the utmost importance to the study of growth substance action. Particularly as in this way we may expect to receive an answer to the question whether growth substances are concerned with the energy consuming processes of the uptake of sugar and water by the cells. The question was put in this way by FREY-WYSSLING (1947) in a discussion on the biochemistry of cell elongation (cf. also FREY-WYSSLING, 1945), after having given as his opinion that during elongation not only the intake of sugar (opposite to the diffusion gradient and therefore requiring energy (ARISZ, 1939, 1945), supplied by the respiration) but also that of water takes place with consumption of energy. In this connection it will be of great importance to follow FREY-WYSSLING's advice not to relate respiration to coleoptiles or cells but to the quantity of protoplasm, as the cell walls and the contents of the vacuole do not consume oxygen.

This question greatly interests us as the peculiar relation between the structure of the growth substances (particularly also in a spatial sense) and their activity as deduced from our investigations made us consider them apt to perform a function related with water- and sugar-uptake. Certainly this view was at first connected too one-sidedly with membrane effects, but also for the action taking place in the cytoplasm one has to ask whether this relation structure/activity may procure a lead.

There are already many indications from other investigations that growth substance action is in some way or another related to the uptake of water and sugar by the cells. COMMONER *et al.* (1942, 1943) studied the absorption of water by potato slices and found that this was stimulated by indole acetic acid, as already stated before by REINDERS (1938). This led to the conclusion that the effect of growth substances on cell elongation largely consists of a regulation of water absorption, probably by influencing the adsorption of osmotically active salts.

VAN OVERBEEK (1944), critisizing these experiments because of the fact that they were not performed under aseptic conditions and because REINDERS (1938) had already found that increased uptake of water also occurs in distilled water, repeated them, taking these facts into consideration. Also under aseptic conditions indole acetic acid and naphthalene acetic acid proved to induce increased uptake of water, both in distilled water and in mannitol or sucrose solutions.

As the expressed sap of tissues treated with growth substances (the method used implies that the cells are crushed) possessed a lower osmotic concentration than that of non-treated tissues, VAN OVERBEEK concludes that the enhanced water uptake of the cells induced by growth substances can be due to a decreasing wall pressure only or to an increase in non-osmotic water uptake, or to both.

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SHOWACRE AND DUBUY (1947) arrived at similar conclusions in studying the relation of water availability and growth substances in the growth of *Avena* coleoptiles. One of the most interesting results is that although oxygenation does not have any considerable influence on the growth of submerged sections in the absence of growth substances, a differential effect occurs in the presence of growth substances, the sections in the aerated solutions then showing increased elongation as compared to those in solutions with inadequate oxygen supply. As the authors state: "Oxygen becomes a limiting factor only when solutions which are not aerated are used in conjunction with factors favouring maximal growth, such as the addition of growth substances".

So here once more growth substance action is linked to oxygen consumption.

KELLEY (1947), investigating relationships between respiration and the uptake of water in the oat coleoptile, also provides evidence for the highly aerobic character of the growth, using the uptake of water as a measure. Several inhibitors known to inhibit carbohydrate metabolism in animal tissues also proved to inhibit the water uptake of coleoptile segments as well as the absorption of oxygen (respiration). Both processes were inhibited simultaneously and in the same concentration range. The stimulation of respiration and of water uptake by indole acetic acid is parallel to its stimulation of growth; uptake of water proved to take place under aerobic conditions only.

In tissue cultures of carrot and topinambour in media without sugar GORIS (1947 a) observed that absorption of water by the tissues was enhanced in the presence of indole acetic acid. In the same media the decrease of the sugar reserve in carrot tissue was found to be intensified by the addition of indole acetic acid (GORIS, 1947 b), particularly in the autumn, during which, probably in contrast to the situation in spring, the auxin content of the carrot tissue itself is low.

According to analyses by SUKHORUKOV AND SEMOVSKIKH (1946) the lower parts of normal coleoptiles contain more sugar than the higher ones. By application of growth substances these differences were found to become less pronounced, indicating that growth substances facilitate the diffusion of sugar in to the tissue.

In this connection it seems interesting to quote finally two papers concerning the action of 2,4-dichlorophenoxy-acetic acid on the germination of seeds and on the growth of different micro-organisms.

HSUEH AND LOU (1947) studied the germination of barley, a typical aerobic seed, and of rice, known to be able to germinate anaerobically. The germination of barley could be completely inhibited by a treatment with solutions of 2,4-D (*e.g.*, 0.07%) whereas under the same conditions that of rice was only delayed. Similar effects were obtained by keeping the seeds under anaerobic conditions. So 2,4-D treatment seems to create a situation where oxygen is no longer available to the seeds. Also with other seeds this equivalence of anaerobic conditions and 2,4-D treatment could be established. Gas exchange analysis by means of the Warburg technique also indicated clearly that the seeds treated with 2,4-D cannot utilize very well oxygen in the air during germination and must find another source (*e.g.*, fermentation) for energy supply. Rice possessing a highly functional fermentative mechanism meets the latter requirement and is therefore rather insensitive to 2,4-D, whereas the germination of barley, the seed of which lacks such a mechanism, is inhibited.

These investigations induced WORTH AND MCCABE (1948) to determine the effect of 2,4-D on aerobic, anaerobic and facultative anaerobic micro-organisms. Their results can be summarized as follows: Those organisms which require free oxygen for respiration

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are—as the authors express it—"smothered" by 2,4-D; so they react in a manner similar to the barley seeds. If the organisms are capable of anaerobic respiration only, they are not affected by 2,4-D to any significant degree.

Though in both cases inhibitory actions mainly have been considered, the interesting results clearly have a bearing upon the problem under discussion, the more so as with very low concentrations of 2,4-D stimulating actions were observed with the seeds as well as with the micro-organisms.

All these data, to which still more could be added, indeed strongly suggest that in some way or other there exists a relation

> growth substance action / a particular fraction of respiration (oxygen absorption, cf. aerobic character of growth) / water uptake / sugar transport / changes in the properties of the cell wall.

We are not yet informed, however, about the question whether all these functions are linked up in a series (in which case their exact sequence is not known either) or if they are partially linked up in a parallel sense. And so we do not know where the relation between the functions is of a primary or only of a secondary character.

To diagrammatize the situation still more roughly once more, we may state that for growth to occur

A. nutritive matter and water has to be supplied to the cells, implying passage of membranes,
B. inside the cell (cytoplasm) chemical processes under enzymatic regulation must proceed and products of these reactions have to go the way back—passing the barriers again—to be deposited in the walls, which
C. in the meantime already must have been modified as to some of their properties.

In the beginning growth substance action has particularly been connected with C. Then one of us thought that A was the more important "site of action", but now we know that both views have certainly been too one-sided and in our opinion there is sufficient evidence that the so-called primary reaction is "covered" by B. So growth substance action would rather be many-sided, being connected with B as well as with A and C. (As to the effects of growth substances on the membranes (A) the investigations of KONINGSBERGER *et al.* concerning the influence of growth substances on isolated protoplasts are of great importance (cf. KONINGSBERGER, 1947, 1948).

The results of our investigations described in this paper (cf. page 298) induce us to suppose that a rather considerable fraction of the growth substance (including the part "wasted" by aspecific adsorption) plays a rôle in A and that, properly speaking, for this function (concerning penetration) the highly active growth substances are not the most suitable compounds, but could better be replaced in a certain sense by the synergists described earlier in this paper. As to C, we must first of all await the results of the investigations referred to on page 300 in connection with the studies of BURSTRÖM.

The relatively small fraction acting in B most probably causes the kind of response which is the most specific for the highly active growth substances and it remains to be elucidated which is the exact function in the cytoplasm and what is the meaning of the particular spatial structure—reminding of that of a wetting agent or penetrant—which we found to be characteristic for the compounds with maximal activity.

As to the function in the cytoplasm, from the investigations discussed on page 301

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it may be deduced with a high probability that enzymatic processes are involved (cf. also SWEENEY, THIMANN, 1942; WILDMAN, BONNER, 1946; BONNER, WILDMAN, 1946). As we had to reject the point of view that the auxins are part (prostethic group) of an enzyme, or to express it in other words are acting *in* an enzyme, the remaining possibility is that they are acting *on* an enzyme system, *viz.*, are regulating its activity. This view (connected with the enzyme "activation" as expressed by AVERY *et al.*, and also considered by VAN OVERBEEK, 1947) is the more attractive as for some other hormones similar relationships toward particular enzymes have already been established. The work of CORI *et al.* (PRICE, CORI, COLOWICK, 1945; COLOWICK, CORI, STEIN, 1947) provided evidence that on the one side the adrenal cortical hormone and the diabetogenic hormone of the anterior pituitary gland and on the other side insulin are functioning (in opposite sense) as regulators of the activity of hexokinase, the enzyme which catalyses the phosphorylation of hexoses by ATP.

Some authors (cf. HARROW, 1947; MEYERHOF, 1948) are for this reason already inclined to generalize that hormones enhance or lower the activity of enzymes (containing *e.g.*, vitamins as prostethic groups), in this way regulating the speed of turnover of the metabolites.

EYSTER (1943, 1946) already has put forward a view concerning the action of auxins, which in fact implies such a regulation of an enzyme system. The mechanism was understood as a release of certain enzymes, particularly of diastase, from a protein colloidal base, to which they were considered to be attached normally. This opinion was deduced from experiments of diastase on charcoal as a carrier and the influence of synthetic growth substances thereupon. For such important conclusions in a physiological sense this very simplified model system hardly seems to offer a sufficient base however. Moreover SMITH, LANGELAND, AND STOTZ (1947), repeating EYSTER's experiments under more critically controlled conditions, did not obtain convincing evidence for a direct inhibition of free diastase by indole acetic acid nor for a significant influence on the absorption equilibrium of diastase on charcoal by the same acid.

In our opinion more promising starting points in this connection may be found in the work of AVERY *et al.* (cf. page 302) and a further analysis both qualitatively and quantitatively of the enzyme systems of *e.g.*, the *Avena* coleoptile and their influencing by growth substances *in vitro* and *in vivo* may procure basic information.

Following our trend of thought it must of course be our first aim to find connecting links which may lead to an understanding of the way in which a regulation of enzymatic activity by growth substances could occur and to a judgment of the question in how far the specific spatial structure of the highly active compounds is also of fundamental importance in this connection.

Now it might seem possible at first sight to start with ideas like those given by LASNITZKI (1947) in a survey on the relation between cell proliferation, carbohydrate breakdown and hydration of enzyme protein (for animal tissues), which considerations suggest "that the intensity of carbohydrate breakdown and consequently the rate of cell proliferation largely depends on the degree of hydration of corresponding enzyme proteins, so that, within limits, an increase in hydration stimulates and decrease inhibits that enzymatic activity". And one would ask if the uptake of water induced by auxin could be connected with similar relations in plant cells. (Cf. for the significance of swelling and shrinking of protoplasm, SEIFRIZ, 1946). Though it cannot wholly be excluded that such considerations may play some rôle in future discussions on auxin action, on second

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thought objections arise which make it improbable that a solution of the problem will be found on this base.

First of all one has to take into consideration that a large part of the water taken up will be found back in the vacuole of the plant cells (absent in animal cells), whose volume is considerably enlarged during cell elongation and which does not contain proteins.

Moreover, recently LEVITT (1948), in a discussion on the uptake of water induced by auxin in aerated potato discs, pointed out on account of calculations that this uptake of water cannot, in a considerable measure, be due to protoplasmic protein hydration, and can only be explained by decreased wall pressure. Though it seems to us that with the latter statement the problem of water uptake has not yet been solved (if this were the only decisive factor, the osmotic pressure would have to decrease far more than it really does), it is clear that for our discussion other views are necessary than those indicated above.

Now it may be deemed possible that in the resting cells several enzymes are present in a bound (more or less inactive) form. Perhaps they are then bound to other cell constituents by complex relations (electrostatic forces). Or possibly they occur as an internal complex, this means that the "acting area" of the enzyme—not in the sense of prosthetic group, but in that of the atom grouping required for the catalytic actions—is masked as it were, *e.g.*, is located in the "interior" of the enzyme molecule. Disrupting of these complex relations then causes "liberation" of the active area and the enzyme action may start.

Now it is reasonable to suppose that anions with a special structure (like the growth substances) may influence similar complex relations in very low concentrations. This will occur if special relations exist between the structure of the acting anion and the structure of the enzyme to be activated or of the cell constituent to which the enzyme is bound.

Here we think also of the effect of detergents, particularly of those of anionic type, in relatively low concentrations on proteins, which implies a denaturation, *viz.*, liberation of –SH groups and increase in molecular asymmetry (cf. PUTNAM, 1948). Processes of this type, if occurring in a cell under influence of some agent may cause a definite physiological effect (cf. *e.g.*, the importance of a regulation of the effectiveness of –SH groups for the ratio cell division / cell elongation, NICKERSON, 1948). Taking into consideration that the (anionic) growth substances are related in principle to certain classes of detergents (wetting agents) as to their spatial structure, we are led to the question whether the auxins may be acting in a physico-chemically related sense on certain enzyme proteins, of course without thinking of a complete parallel of the denaturation caused by detergents.

We then would have to attribute to the auxins (and synthetic analogues with comparable action) a more or less specific affinity for enzyme systems which are important for cell elongation. Now indications for such a property have already been found by the investigations of AVERY *et al.* (cf. page 303). The fact that such low concentrations of growth substances are effective (of the order of 10^{-18} g mol/cell, cf. WASSINK, 1946) could then also be understood.

Surveying the development of opinions concerning the action of auxin we believe that in this way we arrive at a situation where the efforts from biological and chemical side show a promising convergence.

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The results of future investigations, to which we hope to contribute and of which the study of the interaction between growth substances and proteins (enzymes) will certainly constitute an important part, will decide in how far our suggestions have touched something essential concerning the action of growth substances.

SUMMARY

1. Continuing the investigations concerning the relation structure/activity of the plant growth substances, it is shown, that replacement of the lipophilic parts in the highly active compounds (derived from benzene, naphthalene or indole) by trichloro-acetyl-, tris-chloromethylacetyl-, di- or trialkylacetyl residues respectively, results in loss of activity. Only with high concentrations of the compounds, near the toxic level, weak curvatures are produced in the pea test, as already observed earlier for certain normal fatty acids. The acids with branched chains act more rapidly in this respect than their normal isomers. Similar relations are found with some structurally quite unrelated wetting agents, causing weak curvatures in the pea test particularly if the hydrophilic part is located in the centre of the lipophilic chain.

2. The importance of the spatial relation between the ring-system and the carboxyl group in the side chain, as derived formerly for the highly active compounds and reminding of the relations in the group of wetting agents and penetrants, is demonstrated once more for naphthalene-acrylic acid and derivatives. That the spatial structures ascribed to the compounds an account of their physiological activity are the correct ones, has in the meantime also been proved by means of their ultra violet absorption spectra (HAVINGA, NIVARD, 1948).

3. Comparison of the activities in series of compounds leads to the conclusion that for maximal activity, apart from the spatial relation between non-polar and polar parts of the molecule, a very definite balance between these lipophilic and hydrophilic parts (H.L. balance) is required. In this sense an upper limit is now also indicated for the requirement: "high interface activity of the non-polar part".

4. The effect of series of growth substances and related compounds on the tissue of the red beet (*Beta vulgaris rubra*)—by affecting the endo- and ectoplasmic membranes, the colouring matter leaves the vacuole and can be quantitatively measured—completely parallels that on the oleate coacervate (cf. preceding paper). So also with this biological object the effect, in a quantitative sense, is for the greater part the reverse of that which one would expect on account of the view that the primary growth substance action is mainly concerned with the protoplasmic membranes (influence upon the permeability).

5. If the primary reaction does indeed take place in the cytoplasm—an additional argument to those given in the preceding paper being the more rapid action observed for some neutral "precursors" of highly active acids as compared to that of these acids themselves—the result of the beet test can be explained in a plausible way. Increase of the lipophilic character of a compound beyond a certain limit may namely cause too strong an interaction with (adsorption to) the membranes, preventing the attainment of the "cytoplasmic" concentration required for maximal activity (apart from a direct effect of changed membrane properties).

6. It is deduced that a properly composed mixture of "underdosed" highly active growth substance and a supplementary quantity of an acid, weakly active or inactive for the reasons indicated under 5, should equal the maximal effect of the growth substance alone, even if the total number of molecules is less. Such synergistic effects of certain types of acids could indeed be established in the pea test. From the proportions found in this respect it is concluded that only a small fraction of a growth substance in a biological system is actually required for the primary reaction and that the greater part is adsorbed to the membranes (considered to perform a physiological function), has some action on the cell wall and is possibly adsorbed aspecifically at other places ("waste").

7. These findings are discussed in relation to views already put forward earlier by F.W. WENT (1939, preparatory reaction — primary reaction) and to the evidence for a two-phase growth reaction, provided by the investigations of BURSTRÖM (1941–1942–1945).

8. A short review is given of the most important biological investigations concerning growth substance action. The data suggest that a relation exists:
growth substance action/a particular fraction of respiration (oxygen absorption, cf. aerobic character of growth)/water uptake/sugar transport/changes in the properties of the cell wall.

As to the function of the growth substances in the cytoplasm it is highly probable that enzymatic processes are involved.

9. Arguments are given which lead to rejection of the view that the auxins are part (prosthetic group) of an enzyme, in other words, in our opinion the auxins do not act catalytically as co-enzymes. The remaining possibility then would be that they act *on* an enzyme system, *viz.*, that they function

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as regulators of enzymatic activity. Suggestions are given concerning the way in which this may occur, taking into consideration the results of the chemical investigations. This implies that in future investigations the interaction between growth substances and proteins (enzymes) will particularly have to be studied.

RÉSUMÉ

1. En poursuivant les recherches sur la relation structure/activité des substances de croissance, on a montré que le remplacement des parties lipophiles dans les composés hautement actifs (dérivés du benzène, du naphtalène, ou de l'indol) respectivement par des résidus trichloroacétyl, tris-chloro-méthylacétyl, di- ou trialkylacétyl, entraîne une perte d'activité. C'est seulement avec de fortes concentrations de ces composés, voisines de la dose toxique, que l'on obtient de faibles courbures dans le test de *Pisum*, comme cela a déjà été montré pour certains acides gras normaux. Dans ce domaine, les acides possédant des chaînes ramifiées, agissent plus rapidement que leurs isomères normaux. Des relations semblables ont été trouvées chez certains agents mouillants de structure tout-à-fait différente, qui entraînent de faibles courbures dans le test de *Pisum*; particulièrement si la partie hydrophile est localisée au centre de la chaîne lipophile.

2. Nous démontrons à nouveau pour l'acide naphtalène-acrylique et ses dérivés l'importance de la relation spatiale entre le système cyclique et le groupe carboxyle de la chaîne latérale, trouvée d'abord pour les composés hautement actifs et rappelant les relations du groupe des agents mouillants et pénétrants. L'étude des spectres d'absorption dans l'ultraviolet a montré, en même temps, que les structures spatiales attribuées aux composés d'après leur activité physiologique, étaient correctes (HAVINGA, NIVARD, 1948).

3. La comparaison des activités d'une série de composés conduit à la conclusion suivante: pour l'activité maxima, à part les relations spatiales entre les parties non-polaires et polaires de la molécule, il est nécessaire qu'il existe un équilibre bien défini entre les parties hydrophiles et lipophiles (balance H.L.). Maintenant, dans ce sens, une limite supérieure est indiquée aussi pour la condition requise: "haute activité interfaciale de la partie non-polaire".

4. L'effet de séries de substances de croissance et de composés voisins sur le tissu de la betterave rouge (*Beta vulgaris rubra*) — en agissant sur les membranes endoplasmique et ectoplasmique, la substance colorante quitte les vacuoles et peut être mesurée quantitativement — est tout à fait parallèle à celui exercé sur un coacervat d'oléate (voir publication précédente). Ainsi, avec cet objet biologique, l'effet, dans un sens quantitatif, est en grande partie l'inverse de celui qu'on pourrait attendre d'après l'idée que l'activité primaire de la substance de croissance est nettement liée aux membranes protoplasmiques (influence sur la perméabilité).

5. Si la réaction primaire se produit dans le cytoplasme — un argument qui s'ajoute à ceux donnés dans la publication précédente étant l'action beaucoup plus rapide de certains "précurseurs" neutres des acides hautement actifs, en comparaison avec l'action de ces acides eux-mêmes — le résultat du test de la betterave peut être expliqué d'une manière plausible. L'augmentation du caractère lipophile d'un composé au délà d'une certaine limite, doit entraîner une interaction trop grande avec les membranes, ce qui empêche l'obtention de la concentration, dans le cytoplasme, nécessaire pour l'activité maxima.

6. On en déduit qu'un mélange convenable d'une substance de croissance hautement active, mais à dose trop faible, avec une quantité supplémentaire d'un acide, faiblement actif ou inactif pour les raisons indiquées au paragraphe 5, peut égaler l'effet maximum de la substance de croissance seule, même si le nombre total de molécules est inférieur. De tels effets synergiques de certains types d'acides ont été établis dans le test de *Pisum*. D'après les proportions trouvées dans cette étude, on conclut que seule une petite fraction de la substance de croissance, dans un système biologique, est nécessaire pour la réaction primaire, la plus grande partie de la substance étant adsorbée sur les membranes (fonction physiologique aussi) et exerçant quelque action sur la paroi de la cellule.

7. Ces résultats sont discutés relativement aux idées émises par F. W. WENT (1939, réaction préparatoire, réaction primaire) et aux arguments en faveur d'une réaction de croissance en 2 phases selon les recherches de BURSTRÖM (1941, 1942, 1945).

8. On donne une brève revue des recherches biologiques les plus importantes concernant l'action des substances de croissance; les résultats suggèrent qu'il existe une relation entre l'action d'une substance de croissance / une part déterminée de la respiration (absorption d'oxygène, voir le caractère aérobie de la croissance) / l'absorption d'eau / le transport de sucre / des modifications des propriétés des parois de la cellule. Quant à la fonction des substances de croissance dans le cytoplasme, il est fort probable que des processus enzymatiques s'y trouvent engagés.

9. D'après les arguments donnés, nous sommes amenés à rejeter l'idée que les auxines font partie (comme groupement prosthétique) d'une enzyme. En d'autres termes, dans notre opinion, les auxines n'agissent pas catalytiquement comme co-enzymes. La possibilité qui subsiste alors est la suivante: Elles agissent *sur* les systèmes enzymatiques, c'est à dire qu'elles fonctionnent comme régulateurs

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de l'activité enzymatique. Certaines suggestions sont faites sur le mode d'action, et ce, d'après des résultats de recherches chimiques. Ceci indique que des recherches ultérieures sur l'action mutuelle des substances de croissance et des protéines (enzymes) seraient particulièrement indiquées.

ZUSAMMENFASSUNG

1. In Fortführung der Untersuchungen über das Verhältnis zwischen der Struktur und der Aktivität der Wuchsstoffe wurde gezeigt, dass der Ersatz der lipophilen Molekülteile in den hochaktiven Substanzen (welche vom Benzol, Naphthalin oder Indol abgeleitet sind) durch Trichloracetyl-, Tris-chloromethylacetyl-, Di- oder Trialkylacetylreste einen Aktivitätsverlust mit sich bringt. Nur bei hohen, nahe der Giftigkeitsgrenze gelegenen Konzentrationen dieser Verbindungen konnten im Erbsentest schwache Krümmungen hervorgerufen werden, wie wir sie schon früher bei Anwendung einiger normaler Fettsäuren beobachtet hatten. Die Säuren mit verzweigter Kette wirken hier rascher als ihre normalen Isomeren. Ähnliche Zusammenhänge wurden bei einigen, strukturell garnicht verwandten Netzmitteln beobachtet, die im Erbsentest schwache Krümmungen hervorrufen, insbesondere wenn der hydrophile Teil in der Mitte der lipophilen Kette gelegen ist.

2. Die Bedeutung der räumlichen Abhängigkeit zwischen dem Ringsystem und der in der Seitenkette gelegenen Carboxylgruppe, die schon früher für die hochaktiven Substanzen abgeleitet wurde und an die Verhältnisse in der Gruppe der Netzmittel erinnert, wurde erneut für die Naphthalenacrylsäure und ihre Derivate bewiesen. Die Richtigkeit der diesen Verbindungen auf Grund ihrer physiologischen Aktivität zugeschriebenen räumlichen Anordnung wurde inzwischen auch mit Hilfe ihrer Ultraviolettabsorptionsspektren bestätigt (HAVINGA, NIVARD, 1948).

3. Ein Vergleich der Aktiväten in einer Reihe von Verbindungen führt zu der Schlussfolgerung, dass abgesehen von dem räumlichen Verhältnis zwischen polaren und nicht polaren Molekülteilen auch noch ein bestimmtes Gleichgewicht zwischen diesen hydrophilen und lipophilen Teilen (H.L.-Balanz) herrschen muss damit die Aktivität maximal sei. In diesem Sinne wird eine nun auch obere Grenze angedeutet für die Bedingung: "hohe Grenzflächenaktivität der nicht polaren Teile".

4. Die Wirkung einer Reihe von Wuchsstoffen und verwandter Verbindungen auf das Gewebe der roten Rübe (*Beta vulgaris rubra*) verläuft parallel mit der für ein Oleatkoazervat beobachteten (siehe vorhergehende Mitteilung)—durch eine Veränderung in der Membrane des Endo- und des Ectoplasmas verlässt der Farbstoff die Vakuole und kann quantitativ bestimmt werden. So ist also auch an diesem biologischen Objekt die Wirkung im quantitativen Sinne zum grössten Teil den Erwartungen entgegengesetzt wenn man sich auf die Annahme stützt, dass die primäre Wuchsstoffwirkung hauptsächlich die Protoplasmamembranen betrifft. (Beeinflussung der Permeabilität).

5. Findet die primäre Reaktion wirklich im Cytoplasma statt—ein weiteres Argument zu den in der vorhergehenden Mitteilung angeführten ist die rascherer Wirkung von einigen "Vorstufen" hochaktiver Säuren im Vergleich zur Wirkung dieser Säuren selbst — dann können die Ergebnisse des Rübentests verständlich gemacht werden. Überschreitet nämlich der lipophile Charakter einer Substanz einen gewissen Grenzwert, dann kann durch zu starke Wirkung auf die Membrane (Absorption), die, für eine maximale Aktivität nötige Konzentration im Protoplasma nicht mehr erreicht werden (dies ganz abgesehen von der direkten Auswirkung der veränderten Eigenschaften der Membrane).

6. Hieraus wird abgeleitet, dass die Wirkung eines "unterdosierten" Wuchsstoffes zusammen mit einer Säure, die aus den unter 5. angeführten Gründen inaktiv oder wenig aktiv ist, der maximalen Wirkung des allein zugeführten Wuchsstoffes gleich sein sollte, auch wenn die Gesamtzahl der Moleküle kleiner ist.

Solche synergetische Effekte gewisser Säuretypen konnten in der Tat mit dem Erbsentest festgestellt werden. Aus den hier gefundenen Proportionen schliessen wir, dass in einem biologischen System nur ein kleiner Teil der Wuchsstoffmenge für die primäre Reaktion verbraucht wird, während der grössere Teil an den Membranen absorbiert wird (physiologische Funktion), auf die Zellwand wirkt und möglicherweise unspezifisch an anderen Stellen absorbiert wird (Verlust).

7. Diese Ergebnisse werden in Verbindung mit den früher von F. W. WENT geäusserten Anschauungen (1939 "vorbereitende" Reaktion — primäre Reaktion) und den von BURSTRÖM (1941, 1942, 1945) gelieferten Beweisen für eine Wachstumsreaktion in zwei Fasen diskutiert.

8. Wir geben eine kurze Übersicht über die wichtigsten biologischen Untersuchungen die die Wirkung der Wuchsstoffe betreffen. Die Ergebnisse lassen zwischen den folgenden Faktoren einen Zusammenhang vermuten: Wuchsstoffwirkung / ein bestimmter Teil der Atmung (Sauerstoffaufnahme, man denke an den aeroben Charakter des Wachstums) / Wasseraufnahme / Zuckertransport / Veränderungen in den Eigenschaften der Zellwand. Was die Wirkung der Wuchsstoffe im Protoplasma betrifft so handelt es sich hier höchstwahrscheinlich um enzymatische Vorgänge.

9. Auf Grund der angeführten Argumente sollte die Ansicht verworfen werden, dass die Auxine (als prosthetische Gruppe) einen Teil eines Enzyms bilden; unserer Ansicht nach wirken die Auxine also nicht katalytisch als Coenzyme. Es bleibt dann die Möglichkeit einer Wirkung *auf* ein Enzym

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als Regulator der enzymatischen Aktivität. Auf Grund der chemischen Untersuchungen schlagen wir einige mögliche Mechanismen für diese Wirkung vor. In Zukunft wird daher die gegenseitige Wirkung von Wuchstoffen und Proteinen (Enzymen) besonders untersucht werden müssen.

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