Some Experiments on the Effects of Animal Hormones on Plants.

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With Plate XV and one Figure in the Text.

INTRODUCTION.

THE satisfactory experimental demonstration of the specificity of the action of the animal hormones in plants would be a valuable contribution to our knowledge of the parallelism between the physiological processes of plant and animal protoplasm. Various workers have from time to time examined some of the effects of animal hormones on plants, but the literature is in the main scanty, and such as there is somewhat contradictory. This lack of unanimity among the workers in this field is comprehensible, when one realizes the difficulties which attend the extraction and purification of the hormones which are at present available.

One of the earliest workers in this field was Budington (8 and 9), who studied the action of an extract of thyroid gland on the roots of *Allium* and of *Narcissus*. He found that the extract apparently had a stimulatory effect on the growth of the roots. Similar results were obtained by Niethammer (34), using a similar extract with germinating seeds of cereals and with dormant shoots of Syringa and Aesculus. Eyster and Ellis (15) found that low concentrations of insulin increased the growth of the roots and of the shoots of Maize seedlings, while higher concentrations had an inhibitory effect. Rebello (38), using hyacinths as his test plants, found stimulatory effects with extracts of suprarenal and of pituitary glands while the freshly grated substance of these glands had an inhibitory effect. His results with the thyroid extract are contradictory to those of Scaglia (40). According to this author, thyroid extracts have an inhibitory effect on the increase in the total weight of the plant, the inhibition being increased

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with increasing concentration, while they increase the growth rate and tissue differentiation. Marked differences were found in the effects of different extracts of the same gland. De Gaetani (16), using *Lemna minor* plants, found in the extracts of the suprarenal cortex a powerful stimulant of the development of the sex organs in these plants, while the growth in size was inhibited. Occhipinti (35), using *Azolla caroliniana*, in general confirmed these conclusions, but found that the response was most marked in plants which were very young at the beginning of the experiment. The response varied with the age of the plants, as to whether it was most marked on the floral or foliar tissues. Voss (46) and Griebel (18) found that extracts of tonsils, which had previously been found to have an inhibitory effect on the growth of tadpoles, had similar effects on the tissues of *Lupinus* sp. and of *Laminaria*. Griebel (18) confirmed the observations of Scheer (41) that the effect of extracts of the thymus or thyroid gland was conditioned to a considerable extent by the hydrogen-ion concentration of the medium. The experiments of Kustner (28), on the other hand, indicate that the effect of hormones may be increased by exposure to red light, not only in the Aschloim-Zondok test, but also on the germination of barley seeds treated with the urine of pregnancy.

An important contribution to the study of the action on plants of the sex hormones of animals was made by Schoeller and Goebel (42), who found that follicular hormone extracts increased the production of 'cobs' by maize plants, by increasing the number of pistillate heads, apparently at the expense of the staminate. These authors suggest that the hormone had a 'feminizing' effect on the plant. Madaus (31) was able to induce a similar effect on maize plants by using poultry hen manure as a fertilizer. All three authors have found that low concentrations of oestrogenic extracts have a stimulatory effect on the flowering of hyacinths and other plants, while high concentrations appear to increase the development of the leaf at the expense of the floral parts. Madaus has also noted a marked increase in the root-pressure of decapitated *Helianthus* plants, the roots of which were treated with adrenalin.

A very complete series of experiments was carried out by Wasicky, Brandner, and Hauke (48), who used all the readily available hormones, with hyacinths, beans, lupins, cabbages, and with the fungi *Aspergillus* and *Phycomyces*. They also repeated the experiments of Niethammer with the dormant shoots of *Forsythia*, *Prunus*, *Syringa*, *Cheiranthus*, *Crataegus*, and *Salvia*. In the main the results of these authors seem to reconcile a number of the apparently contradictory results of other workers.

The action of the hormones seems to vary to some extent with the plant and also with the concentration of the hormone itself. In high concentrations the effect of the extract may be more marked on organs and tissues other than those most affected by the same hormone in low con-

centrations. These authors found further that cod liver oil had a stimulatory effect on plant growth. They used this material because of its high content of Vitamin A. This year, however, Harden and Störmer (21) have repeated many of the experiments which have been outlined above and have found that their results have been almost entirely negative.

It is obvious from this survey that no definite conclusions on the action of animal hormones in plants can be drawn from the evidence presented in these papers and that the problem is very complicated. In the work detailed in the present paper many of the previous experiments have been repeated and a new technique adopted in an endeavour to throw fresh light on the action, if any, of these hormones on plants.

The experimental methods.

The previous work which has been summarized above was carried out, in the main, with plants which were grown in culture solutions containing the substances under investigation. In our experiments we adopted the same technique in many instances, and added to the water or culture solution in which the rooted plants were growing a suitable amount of the hormone-extract under examination. The obvious objection to this method, is that there is no certainty that the hormone in the solution enters the tissues of the plant, and if, as seems probable, some of the hormones may have a fairly large molecule, then the chance of it entering the root hairs is probably slight. It was felt that a more certain method of ensuring that the solutions entered at least into the water-stream within the plant body, if not into the actual tissues, was that used by one of the authors in earlier experiments. This consisted essentially of cutting a petiole and using the negative pressure in the water-stream to pull solutions into the plant through the petiolar stump. It has been found that this is a convenient method for introducing stains and other substances into the xylem, although it is not suggested that all substances which enter the xylem in this way are able to spread into the parenchymatous ground tissue of the plant.

In addition, a large number of experiments were set up in which the hormone substances were added to the culture solutions in which the plants were grown. For this purpose various plants were used—hyacinths, tomatoes, beans, and cabbages among others.

Experiments with oestrous hormone.

Extracts of crystalline oestrous hormone (Prolan A) from the urine of pregnant mares (glandubolin, Richter) were used in this group of experiments. A group of young tomato plants having 7–8 leaves were selected for the preliminary investigation. A leaf of each of the experimental plants was removed and the severed end of the petiole was introduced into a small test-tube of 5·5 c.c. capacity, containing either 5 c.c. of a

2 per cent. solution of glandubolin (= 100 mouse units of oestrin) or 5 c.c. of distilled water as controls. The pH of the glandubolin solution was found to be 7·24. The experiment was set up on the 23rd October. During the next few days additional glandubolin solution or water was added, as required, until by the 30th October, two treated plants had absorbed 16·2 c.c., another 18·2 c.c., and the fourth 13·2 c.c. of glandubolin solution, corresponding respectively to 327, 367, and 267 mouse units of hormone. For the first few days the uptake of the solution by the plants was fairly rapid, but it became slower as the experiment progressed. There was some evidence that the uptake of the solution was at a slightly higher rate than that of the distilled water.

The first appearance of any reaction of the plants to the treatment was manifested on the 27th October. On that date chlorosis round the veins of the leaves above the petiole was noticed. On the 1st November the top of the same leaflets showed signs of dying. The effect of the treatment was much more marked on the leaves directly above the treated petiole and on the half leaves on the same side. The leaves on the opposite side of the stem were apparently unaffected, and the leaflets in an intermediate position showed symptoms more or less severe, depending on their position with reference to the cut petiole. It was clear that the solution had moved up the stem and had entered one side of the intermediate leaves, had passed along that side to the leaflets and then had reached the other side of the leaf by way of the leaf tip. On the 6th November it was noticed that the chlorosis had largely disappeared and normal green tissues had developed right to the edge of the dead areas at the tips of the laminae.

The same plants were left under observation until the 13th November, when a second series of experiments was set up. The same method of introduction of the solutions was employed, but the leaf next above the first was removed and the cut end of the petiolar stump inserted as before into the solution. Plants 1 and 2 were given 4·5 c.c. of a solution of 2 per cent. glandubolin, this quantity corresponding to 83 mouse units of hormone, Plants 3 and 4 were given 5 c.c. each of a 10 per cent. solution of glandubolin—corresponding to 500 mouse units of hormone. Thereafter additional doses of 2 per cent. glandubolin (equivalent to 20 m. u. per c.c.) were given to all the plants, until by the 19th November the total amounts absorbed by the plants were respectively, No. 1, 373 mouse units, No. 2, 323 mouse units, No. 3, 770 mouse units, No. 4, 730 mouse units. The amounts of distilled water absorbed by the control plants was again found to be much smaller (about 50 per cent.) than those of the solutions.

The first appearance of the effect of glandubolin in this experiment was the 'burning' of the leaf-tips and the development of round, dead areas on the laminae. No yellowing, similar to the chlorosis above described,

was noted. All the treated plants, either with the large or small amounts of hormone-substance, show similar effects on the leaves in direct vascular connexion with the treated leaf. It is important to notice in these and in the other experiments dealing with the absorption of solutions into the xylem vessels, through a cut petiole, that proximity in space does not necessarily imply direct and effective vascular connexion between pairs of leaves (see 737).

It later became evident that the stronger doses had had a greater effect on the tissues than had the weaker, and the distortion and burning of the affected leaves in the plants with most hormone became very marked. A faint chlorosis was also noticeable, though not so pronounced as after the earlier treatments, but necrotic areas appeared on some of the laminae. Thereafter the dead areas became sharply delineated and the chlorotic condition disappeared. The areas immediately behind the burnt cells of the leaf-tips also made some growth, as was noted in the earlier experiment. Neither in the first nor the second experiment were the leaves below the treated petiole affected. The treated plants appear to recommence active growth at this stage and they were set aside in the glasshouse for observation. The details of the measurements made during the experiment are set out in Table I.

TABLE I.

Experiments on the Absorption of Glandubolin through Petiolar Stumps. (*Tomato Plants.*)

Treatment of plant.	Length of stem.			
	23rd Oct.	12th Nov.	18th Nov.	10th Dec.
	cm.	cm.	cm.	cm.
Glandubolin	14·5	68·0	82·0	153·0
Control	17·0	70·0	101·0	138·0

These figures indicate that the damage to the tissues by the large doses of glandubolin does, as would be expected, reduce the growth-rate of the plant, but that this retardation is temporary, and after the glandubolin has been immobilized, probably in the leaf-tips, the plant grows at least as fast as do the controls. There were not sufficient plants to establish whether or not there was a significant increase in the growth of the treated plants, after the initial effect of the hormone had been overcome.

In order to ascertain if the hormone or products of its toxic action on the plants were still present in the tomato plants after treatment, some leaflets of those plants which had received the large doses of glandubolin were crushed in water and the macerated material was rubbed on to the leaves of normal tomato plants. The leaf hairs and portions of the epidermis of these plants were broken by this process, and the death of areas of the leaves indicated that the toxic effect of the glandubolin was

still present. Similar experiments with crushed healthy leaves and with leaves which had been burnt by being touched with a hot iron, gave no evidence of any toxic effect on the leaves of other tomato plants.

Similar experiments, in which the macerated material or a glandubolin solution 1/100 was rubbed on tobacco leaves gave no indication that the glandubolin had any effect on the tissues of this plant.

The effect of glandubolin on tomato plants in water culture.

Some young tomato plants of about 11 cm. high were put into flasks containing 30 c.c. of a glandubolin solution which contained 330 mouse units of hormone. In one plant the main root was removed. This plant first showed evidence of the toxicity of the solution, but after ten days all the plants showed signs of wilting and were obviously seriously affected by the treatment. An attempt was made to induce recovery by keeping the plants in Knop's solution, but the tissues of the roots were immediately attacked by bacteria and the experiment had to be abandoned. The evidence indicated that the absorption of relatively large doses of glandubolin through the roots of tomato plants is possible and that glandubolin is as toxic to tomatoes when absorbed through the roots as when it is administered through a petiole by the method described above.

Experiments with adrenalin.

The first series of experiments were made with adrenalin hydrochloride (Richter) in 1/1,000 solution. The pH of the solution, was found electrometrically to be about 6·8. The formula for adrenalin is $(OH)_2$, C_6H_3, CHOH, CH_2, NH, CH_3, but in addition to the adrenalin itself the solution contained 0·9 per cent. of NaCl and 2·5 per cent. chloretone and traces of hydrochloric acid.

The object of the first experiment was to ascertain if adrenalin had an effect on the sap pressure of plants. Madaus (31) has suggested that the root pressure of decapitated plants was much increased by the addition of traces of adrenalin to the water culture in which the roots were immersed.

Four potted tomato plants with nine leaves each were chosen for the preliminary experiment. A leaf of each was removed and the end of the petiolar stump was inserted into a test-tube. In these, as in the earlier cases, the petiole was cut under water and precautions were taken to ensure that the xylem vessels of the petiole stump would not be filled with air bubbles. With the control plants, distilled water was substituted for the adrenalin solution. After 6 c.c. of a 1/100,000 solution of adrenalin had been absorbed by each of the test plants, all four plants were put under bell jars standing on trays filled with water. The atmosphere in the bell jar soon became saturated and hydathode activity of the tomato plants was observed. It was expected that had the root-action of these plants been increased by the administration of adrenalin the hydathodes of the treated

plants would have been more active than those of the control plants and would have functioned earlier. They did not, in fact, do so, and there was no evidence that the adrenalin had any effect on the 'root-pressure'.

Observations on the plants after treatment made it clear that the adrenalin had no toxic effect on the tissues. Even after as much as 27·5 c.c. of a 1/10,000 solution of adrenalin hydrochloride had been absorbed by the treated plants, there was no evidence of any toxic action, such as was observed after absorption of glandubolin. There was, however, some evidence to suggest that the adrenalin solution was more rapidly absorbed at the cut petiole than was distilled water. It was noticed, also, that the adrenalin treated plants flowered rather more freely than did the controls, but the conditions obtaining in the glasshouses at the time of the experiments were not very favourable and no conclusion is drawn on that account. The adrenalin treated plants, certainly did not grow any faster than the controls, nor was their size affected in any way by the treatment.

The experiments on the effect of adrenalin on the 'root-pressure' was repeated with small tomato plants having seven leaves each. These were put into small flasks surrounded with black paper to exclude light from the roots. In each flask was 100 c.c. of Knop's solution alkalinized with KOH to pH 8·0. To some of the flasks was added adrenalin hydrochloride to make a 1/1,000 solution, while the others were kept as controls. When these plants were put under a bell jar, as was the previous group, no difference in reaction between the adrenalin-treated and the control plants was noted. It was noted, when the plants had been in the solution for 24 hours, that the solution had developed a red colour which, however, was lost again after another two days. The addition of more adrenalin to the Knop's solution resulted in a repetition of the colouring, followed by decoloration. This coloration was not due to tomato plants alone, since similar results were obtained when cabbage and cucumber plants were substituted for them.

Neither these cabbage plants, nor detached leaves and shoots of tomato kept in adrenalin-hydrochloride solution (1/1,000) gave any evidence that the adrenalin had the effect of increasing the movement of water in the tissues. No differences of any kind could be detected between the treated and the control material, nor was there any evidence that the adrenalin was toxic to the roots or other tissues. The plants produced well-developed root-hairs in this concentration of adrenalin.

In another experiment the main root-tips of a tomato plant with ten leaves were cut off and the plant placed in a flask with 30·0 c.c. of Knop's solution with adrenalin hydrochloride added to give a concentration of 1/10,000. The plant wilted slightly and then absorbed all the liquid in the flask. Thereafter it was kept in Knop's solution, where it grew quite normally and showed no evidence of toxic effects of the earlier treatment.

A solution of adrenalin hydrochloride from the British Drug Houses was also used in some of the experiments. It was found to have a pH of 5·33 at a concentration of 1/1,000 in water. Young tomato plants were kept with their roots in flasks in 30 c.c. tap water for a few days and then to the water was added 0·1 c.c. or 1·0 c.c. to a 0·1 per cent. solution of adrenalin hydrochloride, corresponding to a concentration of adrenalin hydrochloride of 1 in 3,000 or 1 in 300. In these high concentrations the red colour was very markedly produced (the colour at the lower concentrations was safrano pink (Pl. II, 7-R-0, f) and at the higher concentrations grenadine (Pl. II, 7-R-0, c) of Ridgeway's Colour Standards). The colour disappeared again after a few days, and while at the higher concentration of adrenalin hydrochloride the roots appeared to be somewhat shrivelled, the new roots which developed later produced normal and plentiful root-hairs. No evidence was obtained which suggested that the adrenalin hydrochloride had any toxic effect on the plants.

Experiment with the extract of the anterior lobe of the hypophysis.

This extract is prepared by Richter under the name of 'glanduantin'. Four medium sized tomato plants were selected for the experiment. Two were treated with glanduantin and two were kept as controls. There were left on each plant three flower trusses, the lowest with three flowers, the second with four buds, and the uppermost with six very small buds developing. Through the severed petioles of a leaf on each of two plants 170 rat units of glanduantin was administered as an aqueous solution, while the controls received only distilled water. There was no sign of a toxic action of this substance, even at the very high concentrations in which it was used. Measurements were made of the plants and these are detailed in Table II.

TABLE II.

Plant.	Height.	No. of leaves.	Weight of fruits.	Weight of stem and leaves.	Weight of roots.
	cm.		gm.	gm.	gm.
No. 10 Treated	170·5	53	116	282	44
No. 12 ,,	155·0	57	135	264	35
No. 11 Control	169·0	52	102	255	72
No. 13 ,,	167·0	58	0*	267	61

* Ovules not fertilized.

It would appear that this extract, while it had no effect on the foliage of the plants, may have, as suggested by Wasicky, Brandner, and Hauke (48), who worked with French beans, an effect on the differentiation of tissue, and the very low weight of the roots of the treated plants is to be noted.

Before the plants were weighed another 100 rat units of glanduantin

was administered to each by cut petioles, so that in all they had each 270 rat units of glanduantin, which induced no toxic effects on the foliage.

Experiments with testis extract (orchitic, Richter).

Experiments were set up to discover whether testis extract would show the same toxic effect as did the extracts of follicular hormone. Medium sized tomato plants were selected and doses of 1 or 2 c.c. of testis extract in 4 or 3 c.c. of water respectively were introduced through cut petioles. The subsequent growth of the plants was apparently neither accelerated nor retarded by the treatment.

The experiment was repeated with very young rooted tomato plants, grown in water, which were equally unaffected by solution of testis material. The Richter extract was so prepared that 1 c.c. of extract was equivalent to 15 gm. fresh testis tissue.

Experiments with extract of the suprarenal cortex.

In an attempt to discover the effect of the hormone on the suprarenal cortex on the sap-pressure of tomato plants the experiments outlined above for adrenalin were repeated with cortex extract (Richter's 'cortigen'). This was used in a solution of 1/1,000 with water cultures, and 1/10 or more concentrated in experiments involving absorption through cut petioles. Cortigen Richter is so prepared that 1 c.c. is equivalent to 16 grm. of fresh suprarenal cortex. The pH of a 1/100 solution in distilled water was 7·3. In none of the experiments did cortex extract appear to have the slightest effect of any kind on the treated plants.

Experiments were set up to see whether very high concentrations of cortex extract had a toxic effect on tomato plants when introduced through cut petioles. Plants of various sizes were selected and were treated with cortigen solution of concentration of 1/10 or 2/5 in distilled water. As much as 10 c.c. of the solution in the higher concentration, i.e. 4 c.c. of cortigen have been absorbed without any apparent differences being manifested. So far, therefore, as tomato plants are concerned cortigen has no demonstrable toxic effects.

Similar results were obtained when broad beans (*Vicia faba*) were grown in Knop's solution containing cortigen in concentration of 1 in 125 and 1 in 10. At both concentrations the leaves appeared to grow as normally as did the controls in Knop's solution.

Experiments on the effects of gland extracts on the growth of Hyacinths.

A group of hyacinth bulbs of the variety 'L'Innocence' which had been prepared for forcing, were kept on damp moss, covered with pebbles in a dark cellar until the roots had begun to grow. They were put in the cellar on the 1st November, 1934, and had all developed roots

by the 13th November. On that date they were put on wide-necked bottles of 300 c.c. capacity. These were filled with tap water, covered with black paper and put in a warm greenhouse under a black shade. On the 19th November, the leaves having grown to some half-inch or so, the shade was removed and the bulbs were divided into five groups, all the groups being, so far as possible, identical as regards the development of roots, leaves, and the general appearance of the bulbs. Some days before (19th November) while the plants were still in the dark, solutions of glandular extracts were added to the bulbs as indicated in Table III.

TABLE III.

The Amount of Glandular Extract added to Cultures of Hyacinths.

Serial number.	No. of bulbs in series.	Extract.	Concentration of extract.	Amount per flask.	pH.
				c.c.	
I. a.	3	Glandubolin (follicular hormone)	100 M. U.	2·0	7·5–8
b.	3	,, ,, ,,		5·0	—
II.	6	Orchitic extract	16 gm./1 c.c.	1·0	7·5–8
III. a.	3	Thyroid gland	16 gm./1 c.c.	1·0	7·5–8
b.	3	,, ,,		2·0	—
IV. a.	2	Adrenalin	1/000 aq.	0·5	—
b.	1	,,	,,	1·0	6·8
c.	3	,,		0·3	—
V.	6	Control: (1) Peptone	1/1000	—	8·0
		NaCl	8/10000	—	—
		(2) Special meat extract		3·0	7–7·5

These solutions were made up on the 9th November and were changed on the 7th, 11th, and 17th December. On the 22nd December one-third of the usual concentration was used, and on the 27th December one-half. The usual treatment was repeated on the 3rd January 1935. It was found that the peptone-salt solution used as control was unsatisfactory as bacterial and fungal growth was extensive, even after a week, and a special meat extract—prepared by Richter and hormone-free was substituted.

A few hours after the first administration of the adrenalin, the solution was coloured pink, this colour being darkest in the most concentrated solution. Gradually this colour disappeared, as happened also with tomato, and was not observed in the subsequent treatments with adrenalin. On the 28th November, when the bulbs were placed on a glasshouse bench in the light, the leaves of all were approximately equal in development with those of the group receiving thyroid extract, perhaps slightly in advance. The flowering times of the bulbs is indicated in the following table.

It was noticed that the control and the treated plants developed, at first, at the same rate. After the appearance of the flower buds, however, the treated plants were found to be slightly in advance of the controls (see

Fig. 1). When fully grown, however, there was no marked difference in the size of the plants, though the leaves of the thyroid and adrenalin groups appeared to be rather less well developed than those of the 'orchitic' and 'glandubolin' groups, the difference between treatments manifesting itself rather in the rate of development. It is interesting to notice in this connexion that the whole inflorescence was completely wilted nineteen days after the opening of the first flower in all the plants, regardless of the stage in the experiment at which they opened. There was no evidence that differences in concentration of the hormone extracts had any effect on the growth of the experimental plants (see Table V and Graph).

TABLE IV.

Time of the Appearance of Flower Buds of the Hyacinth Bulbs.

Treatment.	Date.			
	7th Dec.	10th Dec.	12th Dec.	18th Dec.
Glandubolin (a)	1	2	2	3
(b)	0	1	2	2
Orchitin '	3	4	5	5
Thyroid extract (a)	2	2	2	2
(b)	2	2	3	3
Adrenalin (a)	0	0	0	1
(b)	0	1	1	1
(c)	0	2	3	3
Controls	2	2	2	3

TABLE V.

Effect of Hormone Extracts on Hyacinths.

(*a*) Height of plants in cm. 30/1/35.

Controls.	Adrenalin.	Thyroid.	Orchitic.	Glandubolin.
31·5	30·5	32·5	29·0	33·0
29·5	30·0	29·0	26·5	25·0
28·0	29.5	28·5	27·5	34·0
19·5	27·0	29·0	33·5	24·5
24·0	27·0	19·0	32·0	27·0
Average 26·5	29·2	27·6	29·7	28·7

(*b*) Weight of leaves. 30/1/35.

27·0	35·0	34·0	27·0	38·5
26·0	30·0	32·0	18·0	22·5
23·5	37·0	36·5	20·0	30·0
16·5	33·5	38·5	47·0	16·0
23·0	21·0	33·5	29·5	23·0
Average 23·2	31·3	34·9	28·4	26·0

To test the effect, if any, of the solutions on detached leaves of hyacinth, leaves from the control plants were cut off and put in the following solution—(1) 19 c.c. of distilled water and 1 c.c. glandubolin (= 1,000

mouse units), (2) 20 c.c. of a 1/50 solution of adrenalin hydrochloride (0—1 per cent.), (3) 20 c.c. of a 1/10 solution of the control extract (see below, and (4) 20 c.c. distilled water. The control extract damaged the

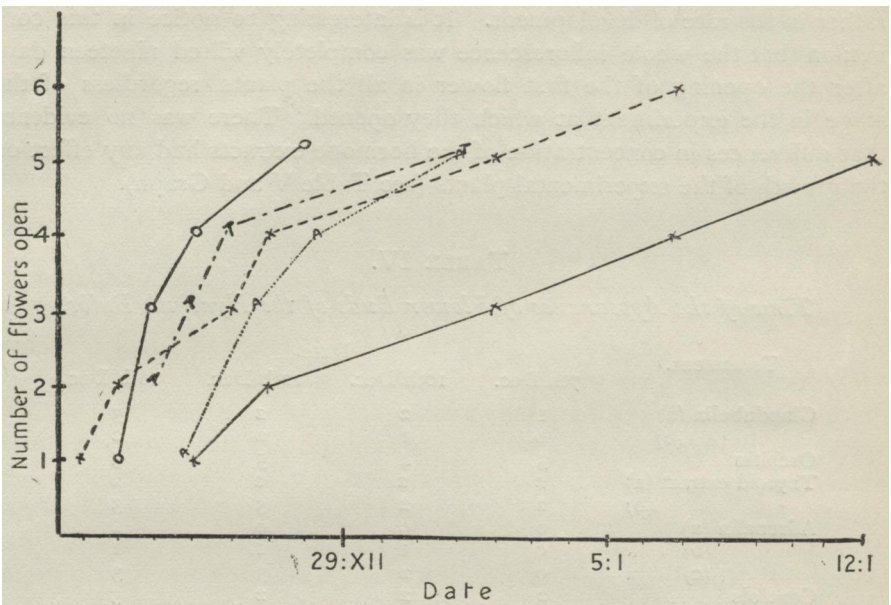

Chart illustrating time of flowering of the treated hyacinths. x —— x Control. x - - - - x Glandubolin. O——O Orchitic extract. A- - -A Adrenalin. T–.–.–T Thyroid extract.

cut surfaces of the leaves and the tissues around them, the adrenalin solution turned reddish and then discoloured. None, except the 'control extract' solution appeared to affect the leaves.

Experiments with 'Control Extract'.

It was thought desirable to set up a series of control experiments with an extract of muscle tissues which nearly resembled the hormone extracts in chemical composition. By the courtesy of Dr. Gerö of Richter Ltd., such a substance was prepared, and put up for convenience in 1 c.c. ampoules each ampoule containing the equivalent of 5 gm. of fresh muscle substance.

This material was used in methods similar to those employed with the hormone extracts. When concentrated doses of the extract were absorbed by cut petioles (e.g. 5 c.c. extract–0 c.c. H_2O, 2 c.c. extract–3 c.c. H_2O, and 1 c.c. extract–4 c.c. H_2O) the petiolar stump was plasmolysed and soon collapsed. The stem below and above the treated petiole also showed signs of disturbance round the associated vascular bundles, and deep grooves were formed for distances of as much as 80 cm. above the treated

petioles. These grooves extended a short distance into the petioles of other leaves in direct vascular connexion, but in no case examined did the leaflets appear to be affected by the treatment. When more dilute concentrations were used, the amount of damage was proportionately less, and at concentration as low as 1 c.c. in 500 c.c. of solution = 1 per cent. of original muscle tissues, no damage to, or stimulation of, the plants was recognizable. In all cases where damage was noted, the extent was limited in that the cells of the medulla and ground-tissue around the damaged area were stimulated into activity, and a layer of phellogen cells formed. Plants which, after the formation of the 'grooves' in the stem, were allowed to continue growth, while they did not regenerate the dead tissues, developed perfectly normally in respect of the rest of the plant.

In a series of plants, capsules having fine capillary ends, and filled with the undiluted extract, were inserted into the vascular tissues, and in spite of the fact that only very small quantities of the material was absorbed (e.g. 0·2–0·3 c.c.), the damage to the bundles was similar to, if not as extensive as, that discussed above.

Young rooted plants were put into solution of the extract of concentrations 1/10 and 1/100. In the weaker solution no effect was evident after some days' treatment, while in the more concentrated solution, the roots were shrivelled within twenty-four hours, and the plants ultimately died. In these, as in other experiments, fungi and bacteria tended to grow in the solutions, and prevented the extension of the observation over a period of more than a few days.

Discussion.

In a discussion of the experimental results outlined above, the limitation of investigation of the response of plants to animal hormones must be considered. The extent, and even the nature of the response of animals themselves to the artificial administrations of these hormones, is not always regular, and varies with a number of internal and external factors, many of which are as yet not comprehended.

Moreover hormones, with a few exceptions, are not synthetically prepared and obtainable in a chemically pure form, so that the composition of extract of glandular material must vary very much, depending on the method of extraction. This may well account for the many apparently contradictory results which have been obtained by workers, using, nominally, the same materials. At the same time, the relatively simpler organization of plants, may complicate the investigation and interpretation of the effects of substances which have only very markedly specific effects on certain of the highly differentiated animal tissues.

How far plants themselves secrete substances similar in character to the animal hormones has not been fully established. Various workers

from the time of Dohrr, Faure, Poll, and Bloteuogel to Blutenandt (4, 5) have found, however, that oestrogenic substances ('thelykinins') are present in a great many plants. These induce oestrus in mice (as shown by the Allen-Doisy test) in the same way as do oestrogenic substances of animal origin. Kogl and Haagen-Smits' 'auxin' (1931) is a further indication of possible parallelisms between the two groups.

It is reasonable to suppose, therefore, that some at least of the animal hormones might have a specific effect on the protoplasm of plants. It is hardly to be expected that the effects need be generalized, or that they would be manifested under every condition and after administration, for example, through the root system. It is hardly to be expected that the effect of adrenalin, for instance, need be the same when it is absorbed through the digestive tract as when injected intravenously into an animal, or that oestrogenic substances would act equally well if smeared on the epidermis of the experimental animal.

With these considerations in mind, we have to examine the data outlined above to discover if animal hormones affect plants at all, and if they do, does the effect show itself in the precocious development of tissues, the marked increase in size, or in an alternation in the proportion between the reproductive and vegetative parts.

The rather drastic method adopted of introducing relatively enormous quantities of hormone substance into a plant by means of a petiolar stump, resulted in considerable damage to the plant when oestrogenic substance (prolan A) was so administered. Similar concentrations of other hormone extracts, such as testis hypophysis, suprarenal and suprarenal cortex extracts, did not induce similar damage to the tissues. The preparations were clearly of different toxicity with reference to the same test plants.

It was clear from the experiments with oestrogenic substance that this extract moved freely with the water stream, and was able, moreover, to leave the xylem elements and to enter the parenchymatous ground-tissues. It is to be noted, on the other hand, that the toxic effects of concentrated solutions of hormone-free tissue extract were, in our experiments, limited in every case to the tissues round the xylem of the stem, and for some reason not yet clear, were not manifest in the tissues of the leaves of the treated tomato plants. It is further of interest to note that the toxicity of the prolan A group oestrogenic substance, was observed when the substance was introduced through the petiolar stump, or through the roots in water-culture.

Not all our results, however, gave indication that the effects described by other investigators were to be found in the plants used in our experiments.

The effects of the hormone extracts on growth.

The effect on growth of the oestrogenic substance is indicated by the fact that the tomato plants, after recovering from the tissue damage caused by its absorption, grew actively, and developed a larger root system than did the controls, while the size of the aerial parts was as great as that of the controls, even in spite of the check they had received. There was evidence, also, that the very large doses of thyroid extract given to the hyacinths induced an increase in size. On the other hand, extracts of testis and of the anterior lobe of the hypophysis, which in animals are supposed to induce increase in size, failed to produce a similar effect on the treated plants, while in the case of the hypophysis extract there were indications that the root systems had been decreased. The absence of effect on growth of the extracts of the suprarenal and suprarenal cortex in our experiments, while in accord with the findings of others in experiments with animals, are not in accord with those of many of the workers on plants, whose papers are noted in the introduction. As regards the extracts of the suprarenal cortex, some preliminary experiments on germination with peas and wheat seemed to indicate an inhibitory effect induced by this substance.

The effect of adrenalin on the root pressure.

Although adrenalin has a specific effect on the blood-pressure of animals, and has been said to increase the root-pressure of plants, our experiments with the substance have yielded negative results. Nor was there any evidence, from experiments with slices of the tissue of stems of tomato plants, that this substance had any effect on the respiratory mechanism when administered in considerable concentration (1 in 300). It was repeatedly noticed, however, that the enzymes in plant tissues had an interesting effect on this substance. When rooted plants, the petioles of detached leaves, or extracts of plant tissues were placed in adrenalin solutions, the solutions after a few hours developed a reddish coloration, which after a longer or shorter period disappeared. The reaction was very marked with extracts of potato and similar tissues which are known to have a high oxidase content, and it is suggested that the oxidase system acts on the adrenalin, and produces from it a coloured, and later a colourless substance similar to those produced by the oxidase systems on the catechol substance in the potato, for example.

The effect of the hormone extracts on the floral development.

It is recognized that some hormones have a very marked effect on the reproductive organs of animals. In our experiments with tomato plants, however, there was no indication that any of the hormones used, including

testis and ovary extracts, had any effect whatever on the flowering. There are indications, nevertheless, that some of the hormones used may have induced increased flower development in hyacinths. With hyacinths, on the other hand, the thyroid extract gave no evidence of any effect in either direction. It may be significant, being contrary to the findings of others, that it was noted that the tomato plants which had been treated with extracts of the suprarenal and the suprarenal cortex appeared to flower more freely than did some of the controls grown under similar conditions, the conditions in the glass-houses in winter not tending to the production of flower-trusses.

Conclusions.

The obvious objection to the administration of hormone extracts to plants, is that with the hormone itself is being given material which might have a definite nutritive value, making the interpretation of results difficult. To overcome this difficulty we have used the muscle-extract which was described above. This was prepared as a hormone-free substitute for the glandular extracts with which we worked. The experiments with this material did not give any indication that this substance actually increased the growth or had, in fact, any marked effect on the plants, except when used in concentrated solution, when it was actually toxic. It is to be noted, further, that the reactions observed are not necessarily specific to hormones but, this is not unexpected, since many chemical substances are known to induce in animals themselves reactions comparable with those of hormones.

From our experiments, therefore, it seems reasonable to conclude that while many hormone extracts apparently have no effect on the growth and tissue differentiation of plants, others may have effects which may be stimulatory or toxic.

Summary.

The present position of our knowledge of the effect on plants of animal hormone extracts is examined. Experiments are described in which different glandular extracts were administered to plants, either by the roots or by the petiolar stumps, and the results obtained and discussed. Glandubolin-Richter, a preparation of oestrogenic hormone, was found to be toxic to tomato plants when administered through a cut petiole or through the roots, but even concentrated extracts of the tissue of testis, ovary, pituitary, suprarenal, thyroid, and thymus appeared to have no marked effect, and certainly no toxic effect on the plants. The results obtained indicate that most animal glandular extracts have little or no effect on the growth and development of plants, but suggest one or two fruitful lines for further investigation.

We desire to express our gratitude to Sir John Russell and Dr. J. Henderson Smith for their kindness in offering facilities for the carrying out of this work, and to our colleagues for their help, especially to Fraulein Cunow and Dr. H. Nicol. Our thanks are due, also, to Messrs. Richter of Budapest, and to their Director in London, Dr. J. Gerö for the material furnished.

LITERATURE CITED.

1. AGOSTINI, A.: Azione di alcuni estratti endocrini su due felci acquatiche. Scritti biologici, v. 729, 1930.
2. BEALL, D., and MARRIAN, G. F.: A Rapid Method for the Isolation of Oestrone from the Urine of Pregnant Mares. Journ. Soc. Chem. Ind., liii. 309, 1934.
3. BLARINGHEM, L.: La parthénogénèse des plantes supérieures. Bull. Sci., France et Belgique, xliii. 2, 113, 1909.
4. BLUTENANDT, A.: Zur Biologie und Chemie der Sexualhormone. Naturwissenschaften, xxi. 49, 1933.
5. ————————: Ueber die Biochemie der Sexualhormone. Der Biologe, i. 219, 1932.
6. BOTTOMLEY, W. B.: Some Effects of Organic Growth-promoting Substances (Auximones) on the Growth of Lemna minor in Mineral Culture Solutions. Proc. Roy. Soc. London, Ser. B, lxxxix. 481, 1917.
7. BRANDT, A.: Sexualität—Eine biologische Studie. Dorpat-München (E. Reichardt), 1925.
8. BUDINGTON, R. A.: Effect of Thyroid Gland Substances on Protoplasm in General. Oberlin Coll. Lab. Bull. lxiv. 83, 1925.
9. ————————: A Suggestion as to the Effect of Thyroid Gland Substances on Protoplasm in General. Biol. Bull. Marine Biol. Lab. Woods Hole, xlviii. 83, 1925.
10. CALDWELL, J.: Studies in Translocation. II. The Movement of Food Material in Plants. New Phytol., xxix. 27, 1930.
11. COLLO, P. G., DAL: Sull'azione cicatrizzante degli estratti testicolari ed ovarici. Clin. Chirurg., xxix (8), 787, 1926.
12. DINGEMANSE, E., LAQEUR, E., und DE LONGH, S. E.: Die Anwesenheit des Menhormons im Tier- und Pflanzenreich unter verschiedenen Umständen. Nederl. Tijdschr. v. Geneesk., 767, 1929.
13. ————————, FREUD, J., and LAQEUR, E.: Difference between Male Hormone Extracts from Urine and from Testis. Nature, cxxxv. 184, 1935.
14. DOLK., H. E., and THIMANN, K. V.: Studies on the Growth-hormone of Plants. I. Proc. Nat. Acad. Sc., xviii. 30, 1932.
15. EYSTER, W. H., and ELLIS, M. M.: Growth of Maize Seedlings as Affected by Glucokinin and Insulin. Journ. Gen. Physiol., vi. 653, 1927.
16. GAETANI, L. DE: Azione della corticale surrenale su un vegetale (Lemna minor). Scritti biol. racc. da L. Castaldi, iv. 243, 1929.
17. GOMBOCZ, E.: Termeszettudományi Lexikon (K. M. Term. tud. társ. Budapest), 1934.
18. GRIEBEL, K.: Experimentelle Untersuchungen zur Physiologie der Tonsillen. Arch. Ohren-, Nasen- und Kehlkopfheilk. cxxi. 1/2 18, 1929.
19. HABERLANDT, G.: Zur Physiologie der Zellteilung. VI. Über Auslösung von Zellteilungen durch Wundhormone. Sitzungsber. Preuss. Akad. Wiss., 1921, 221.
20. ————————: Über Zellteilungshormone und ihre Beziehungen zur Wundheilung, Befruchtung, Parthenogenesis und Adventivembryologie. Biol. Zentralb., xlii. 145, 1922.
21. HARDEN R., und STÖRMER, J.: Blütenentfaltung und Hormonwirkung. Nachr. Ges. d. Wiss. Göttingen; Math. Phys. Kl. VI. Biologie, i. 3.
22. HARROW, B., and SHERWIN, C. P.: Chemistry of the Hormones. Baltimore. Williams Wilkins Co., 1934.

23. HELLER, M., und TESTSCHENKO, G.: Über die Wirkung des Thymusdrüsenextrakts auf die Hefezellen (Saccharomyces cerevisiae). Biochem. Ztschr. 217, 416, 465, 1930.
24. ISAACHSEN, H.: Effekten av husdyrjodsel Kontra Kunstjodsel. Tidsskr. f. d. Norske Landbr., 225, 1933.
25. KISSER, J.: Chemische Fernwirkungen (Hormone) im Pflanzenreiche. Die Natur, Ztschr. öster. Lehrerver. f. Naturkunde. 83, 97, 1925.
26. KLOBUSITZKY, D.: Hormónok es hormónhatasok. (K.M. Term. tud. társ. Budapest), 1934.
27. KRASNOSSELSKY-MAXIMOV, T. A.: Accumulation of Dry Substance and Time of Heading by Oats under Influence of Animal Hormones. Bull. Appl. Bot. and Genet. and Plant Breed. III. Ser. 3, 161, 1933.
28. KÜSTNER, H.: Hormonwirkung bei den Pflanzen und Hormonsteigerung durch rotes Licht. Klin. Wchnschr. S. 1585, 1931.
29. LOEWE, S., LANGE, F., und SPOHR, E.: Über weibliche Sexualhormone (Thelyotropine). XII. Mitt. Brunsterzeugendem Stoffe (Thelykinine als Erzeugnisse des Pflanzenreiches.) Biochem. Ztschr. 180, 113, 1, 1927.
30. MACDOUGAL, D. T., and SPOEHR, H. A.: Growth and Imbibition. Proc. Am. Phil. Soc., lvi. 1912.
31. MADAUS, G.: Sexualhormone. Jahrbuch (Dr. Madaus & Co.) Dresden, 1933.
32. NATH, B. V., and SURYANAYARANA, M.: Effect of Yeast Extract on the Growth of Plants. Nature, cxxxiv. 27, 1934.
33. NEMILOV, A. V.: Utilization of the Achievements of Endocrinology in Plant Industry. Bull. Appl. Bot. and Plant Breeding, A, No. v–vi. 48, 1933.
34. NIETHAMMER, A.: Stimulationswirkungen im Pflanzenreich. III. Die Thyroidea und Zinksulfat. Protoplasma, ii. 3, 392, 1927.
35. OCCHIPINTI, G.: Influenza di estratti endocrini su alcuni vegetali. Riv. Sanit. Siciliana, 1559, 1929.
36. PETRI, L.: Experienze sulla formazione del sughero delle ferite. Bull. p. Staz. Patol. Veget. (Firenze), ix, 4, 328, 1929.
37. RAAB, W.: Hormone und Stoffwechsel. Naturw. und Landw. Freising–München. (Dr. F. P. Datterer), 1926.
38. REBELLO, S.: Action des glandes à sécrétion interne et de leur extraits sur le développement des plantes. C.R. Soc. Biol., xc. 1095, 1925.
39. ROBSON, J. M.: Recent Advances in Sex and Reproductive Physiology. London, J. A. Churchill, 1934.
40. SCAGLIA, G.: Effetto di estratti tiroidei e dell'iodio sullo svilluppo e accrescimento di Hyacinthus orientalis. Primi risultati. Scritti biol raccolti di Luigi Castaldi, 261, 1927.
41. SCHEER, K.: Über die Einwirkung der Thymusdrüse und des Wachstumsvitamins auf das Wachstum. Klin. Wchnschr., 702, 1925.
42. SCHOELLER G., und GOEBEL, H.: Die Wirkung des Follikelhormons auf Pflanzen. Biochem. Ztschr. 240, 113, 1931.
43. SIMOLA, P. E.: Über insulinähnliche Körper in höheren Pflanzen und Mikroorganismen. Medic. fennica (Helsingfors), 1928.
44. THIMANN, K. V.: Studies on the Growth-hormone of Plants. VI. The Distribution of the Growth Substance in Plant Tissues. Journ. Gen. Physiol., xviii. 23, 1934.
45. TOCCO-TOCCO, L.: Contributo alla conoscenze del mecanismo di azione delle sostanze che determino glicosuria negli animali. Ricerche di Farmacologia vegetale. Biochem. e terapia sperim. 11, 1, 1924.
46. VOSS, D.: Zur Physiologie der Tonsillen. Arch. Ohr.-, Nasen- und Kehlkoptheilk. cxxi. (112) 1, 1929.
47. WALKER, B. C., and JANNEY, J. C.: Estrogenic Substances. II. An Analysis of Plant Sources. Endocrinology, xiv, 389, 1930.
48. WASICKY, R., BRANDNER, D., und HAUKE, C.: Beiträge zur Erforschung der Hormonwirkungen. Biol. generalis, ix. 331, 1933.

EXPLANATION OF PLATE XV.

Illustrating the paper by Mr. L. Havas and Dr. J. Caldwell on 'Some Experiments on the Effects of Animal Hormones on Plants'.

Fig. 1. Tomato plant after treatment with 'Glandubolin' (oestrogenic hormone, preparation). The hormone solution was absorbed at the petiolar stump X.

Fig. 2. Photograph taken on 15th Dec. of the treated hyacinths. The treatments were as follows: (*a*) Controls, (*b*) Glandubolin, (*c*) Adrenalin, (*d*) Thyroid extract, (*e*) Orchitic extract.

Fig. 3. Photograph taken on 25th Dec. of the treated hyacinths. The treatments were as follows: (*a*) Controls, (*b*) Adrenalin, (*c*) Thyroid extract, (*d*) Orchitic extract, (*e*) Glandubolin.

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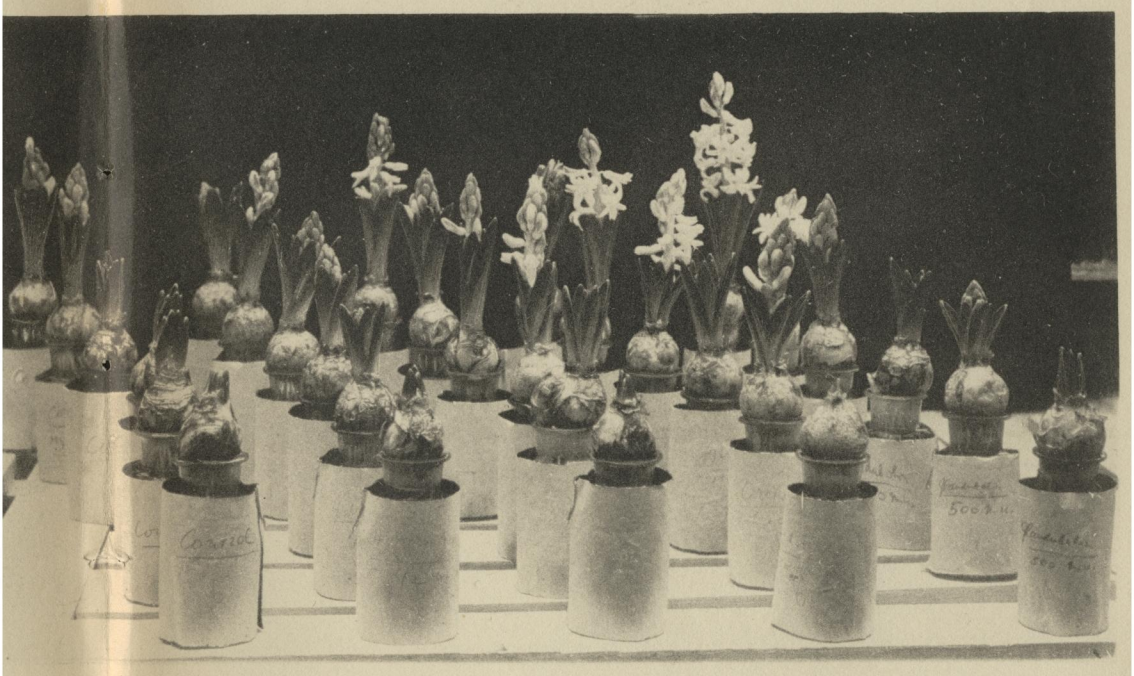
HAVAS AND CALDWELL — EFFECT OF ANIMAL HORMONES ON PLANTS.

2



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Huth, Stubbs X. Kent.