THE EFFECT OF AUXINS UPON PROTOPLASMIC STREAMING

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INTRODUCTION

Although the existence of growth substances, or auxins, which produce cell enlargement in plants has been known for some time, the mechanism by which they exert their effect is still little understood. A full account of this field is given in Went and Thimann (1937). Briefly, it has been shown within the last 4 years that auxins cause not only cell enlargement but also bring about root formation, inhibition of bud development, inhibition of root elongation, and other effects; this multiplicity of activities indicates that the auxins must exert some fundamental action upon the plant cell. Even in cell enlargement the action must be of a very deep-seated nature, for while Heyn and Söding have shown that the action of auxin results in an increase of cell wall plasticity, this does not appear to result from a direct reaction between auxin and cell wall material. Thus the results of Thimann and Bonner (1933) show that there is no stoichiometrical relation between the amount of auxin entering and the amount of cellulose or of total new wall material deposited as a result, nor is it possible for the auxin to produce a monomolecular layer upon the new cell wall. The action on the cell wall must therefore be realized in some indirect way, and is probably the result of a chain of reactions originating inside the cell.

Changes in growth rate are not in general measurable until some considerable time after the application of the auxin. Thus, in measurements of straight growth or of curvature of the *Avena* coleoptile, a period of 15 to 30 minutes usually elapses before the increased growth rate, or the curvature, becomes detectable. The first of the

series of reactions ultimately leading to increased wall plasticity and elongation, or to the other effects mentioned above, presumably begins very much earlier than this, most likely immediately the growth substance has penetrated into the cell. It is therefore desirable to find a method by which quantitative measurement of the *immediate* effect of auxin might be made; in view of the above considerations, such effect must be looked for not in the wall but in the contents of the cell, that is, in the protoplasm.

One of the most obvious properties of the protoplasm is its streaming, which in the *Avena* coleoptile, especially in the epidermal cells, is readily observed and measured. Immediate changes in the streaming have been found by various investigators to take place as a result of temperature change (Ewart, 1903, Bottelier, 1934), visible light (Bottelier, 1934), x-rays (Williams, 1923, 1925), and miscellaneous dissolved substances (Ewart, 1903). In an investigation with Prof. I. W. Bailey of the effect of auxin on cambium cells, one of us (K.V.T.) observed that streaming was very rapid in the preparations treated with auxin. There was also a change in the extent of the streaming, the moving particles being carried up to the extreme tips of the cells, which previously were stagnant. It was therefore decided to make a study of the protoplasmic streaming in favorable material, in order to determine whether auxin exerted any effect upon it and whether the rate of streaming could be used as a measure of the immediate physiological changes brought about by auxin within the cell.

Materials

Indole-3-acetic acid, the most active auxin readily available in pure state, was used for the bulk of the experiments. The sample used was prepared and purified more than 2 years ago (Thimann and Koepfli, 1935) and its activity is the same as when first prepared. For coumaryl-3-acetic acid the authors are indebted to Prof. T. Reichstein of Zurich; for pure allocinnamic acid to Prof. A. J. Haagen-Smit. These substances were used in concentrations ranging from 0.005 up to 10 mg. per liter, which include all those causing physiological response.

The water used for all solutions and in controls was redistilled in a pyrex still and stored in paraffined glass containers.

The Cornellian strain of oats was used; its properties, growth rate, dimensions, etc., are essentially the same as those of "Segerhavre" used in other experiments.

Methods

The seeds were husked, soaked for 2–4 hours, germinated on moist filter paper in a dark room with occasional red light, and planted in moist sand at 24° and 85 per cent relative humidity. The coleoptiles were slit lengthwise, removed from the plant, and spread out upon a slide with the cut face down, as described by Bottelier (1934). The slight pressure of the cover slip was sufficient to hold them in place. The zone studied was about 1 cm. below the tip. In this series of experiments coleoptiles 5 to 6 days old from the time of soaking were used; these measured 4 to 5 cm. in length. The effect of auxins on younger coleoptiles will be discussed in a later paper.

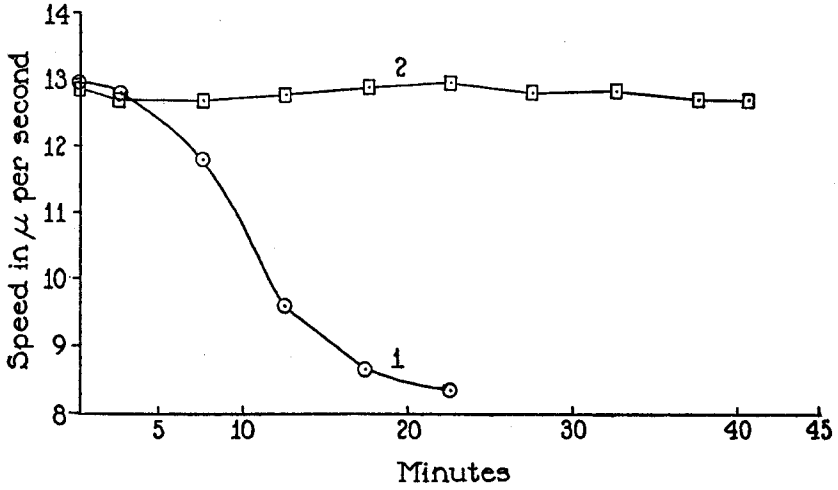

FIG. 1. Rate of streaming of epidermal cells of *Avena* coleoptiles in pure water. Curve 1, not aerated; curve 2, aerated, (mean of five experiments).

After cutting, the coleoptiles were allowed to recover in water for 5 minutes. Continuous readings were then taken for 5 minutes in water, after which the water was removed from under one side of the cover slip with filter paper and the solution to be tested was added to the other side. Further solution was added at frequent intervals during the experiment. Continuous readings were made of the motion of the smallest visible particles in a single epidermal cell, using a calibrated ocular micrometer and a stop-watch. The mean of about ten readings within a 3 minute period was taken to establish the streaming rate for that period.

Since Bottelier (1934) has shown that the streaming in *Avena* is sensitive to light, all readings were made in the dark room by red light, heating of the slide being avoided by the interposition of a water cell about 5 cm. thick.

Influence of Aeration

When the coleoptiles were mounted as described, in pure water, the rate of streaming showed a steady decrease (Fig. 1, curve 1), which became apparent in from 2 to 5 minutes. Bottelier (1935) noted a similar decrease, and found that it disappeared if a stream of fresh solution was passed under the cover slip. He attributed this fall in the rate of streaming to the rapidly diminishing amount of oxygen in the solution under the cover slip, and showed that oxygen is one of the principal factors controlling the streaming rate. That the cause of the decrease in rate in our experiments is oxygen deficiency is confirmed by the fact that no such decrease took place when aerated water was used (Fig. 1, curve 2). Under these conditions, the streaming rate, as can be seen, remained constant for at least an hour. Aeration was accomplished by bubbling air through the water for 20 to 30 minutes immediately before using it. All control experiments were therefore made with water thus aerated, and solutions to be tested were made up with the same water.

Qualitative Effect of Auxin

Dilute solutions of indole-3-acetic acid (0.005 to 0.5 mg. per liter) induced an increase in the rate of streaming, the extent of which was dependent on the concentration used. The rate increased from 13 to about 18 μ per second when the coleoptiles were treated with a solution containing 0.010 mg. per liter, about the optimum concentration (Fig. 2). The effect of immersing coleoptiles in the auxin solutions began to appear immediately, the increase in rate being observed well within the first 5 minutes of treatment. The maximum streaming rate was obtained at 10 to 15 minutes' exposure, after which the rate slowly fell again to reach that in water at 30 to 40 minutes from the time of application of the solution. This time relation was observed in all the experiments with auxin of any kind. In no case was the effect latent, nor was the maximum rate of streaming found earlier or later; and never was the effect found to last beyond 30 to 40 minutes. Finally, the streaming always returned to exactly the same rate as before auxin was applied.

When relatively concentrated solutions of indole-3-acetic acid were used (above 0.5 mg. per liter), the effect was to decrease the rate of

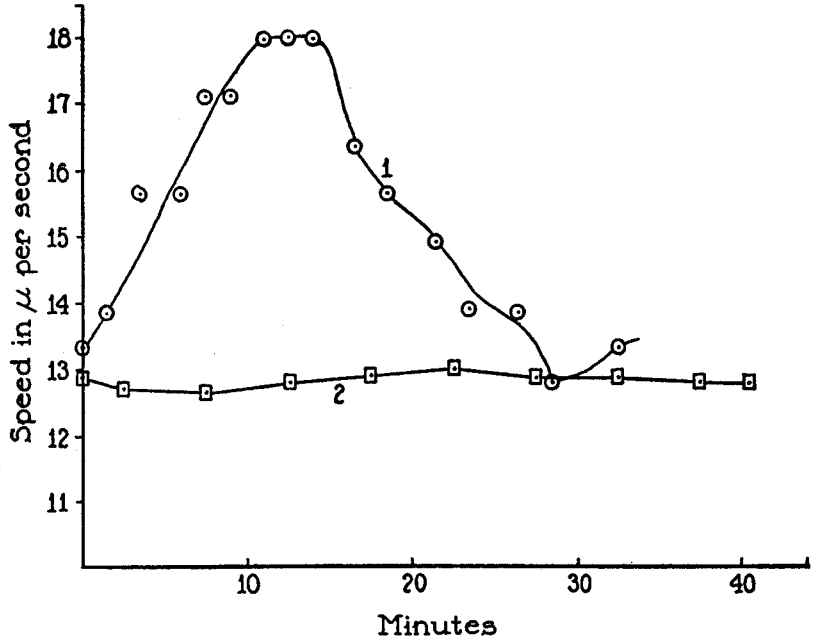

FIG. 2. Typical effect of low concentrations of auxin on the rate of streaming. Curve 1, 0.007 mg. indole-3-acetic acid per liter; curve 2, pure water.

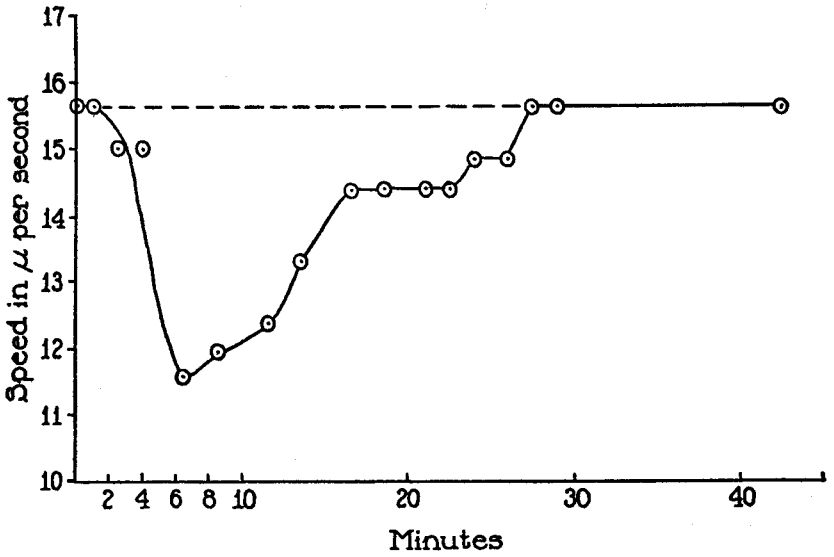

FIG. 3. Typical effect of high concentrations of auxin on the rate of streaming. Curve 1, 5 mg. indole-3-acetic acid per liter.

streaming. The curve thus obtained (Fig. 3) is the reverse of that of Fig. 2. The effect of high concentrations also takes place very rapidly, beginning in 2 to 5 minutes, reaching its maximum in 5 to 20 minutes, and returning to the rate in water at about 30 minutes.

The Quantitative Effect and Its Relation to Concentration

In the curves of rate against time, such as those in Figs. 2 and 3, a quantitative measure of the total effect of a given auxin concentration is evidently given by the *area* under the curve. The value thus obtained combines the maximum speed reached and the duration of the effect. It is expressed in units essentially of length, since it is a product of a velocity by a time ($LT^{-1} \times T = L$). Clearly this value represents the total extra distance travelled by one particle when it is acted upon by auxin. Since the effect of auxin on growth is also expressed in units of length (at least for coleoptiles), a comparison may justifiably be made between the two effects.

Each experiment, comprising 20 or 30 average velocities determined over a 40 minute period, thus gives a single value for the total effect of auxin at the concentration used. Since the values obtained from different experiments at the same concentration agree fairly well, the mean of several such values represents an accurate measure of the effect. It is to be noted that each final value for the effect of a given concentration is therefore founded on about 1000 actual velocity readings. Experiments were made in all at thirteen different auxin concentrations.

The mean final values are plotted in Fig. 4 against the logarithm of the auxin concentration. It may be seen that the effect, that is, the mean extra distance travelled by a given particle increases linearly with concentration from zero at 0.0025 mg. per liter to a maximum at 0.01 mg. per liter. From this point, the effect decreases with further increase in concentration to reach zero at about 0.5 mg. per liter, and negative values at still higher concentrations. The curve thus consists essentially of two straight lines separated by an intermediate region around the maximum.

It is of interest to compare the effect of auxin on streaming with its effect on growth. Measurements of elongation of coleoptiles immersed in solution are obviously the most nearly comparable with the data on

streaming. Such measurements, using short sections from coleoptiles, were first made by Bonner (1933) and have since been used by several workers. Jost and Reiss (1936, 1937), using longer sections dipping in solution only at one end, found that the maximum growth reaction in 24 hour tests took place at 10 mg. per liter. Higher concentrations gave less growth, though still more than controls in pure water. In order to have comparable data we have made measurements on 3 mm.

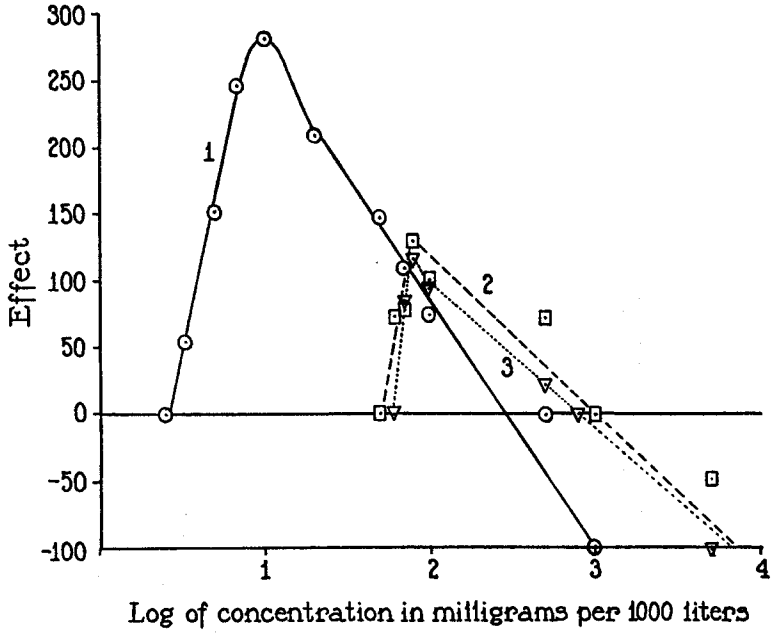

FIG. 4. Total effect of auxin on the rate of streaming, each point derived from the mean of several curves of the type of Figs. 2 and 3. Curve 1, indole-3-acetic acid; curve 2, allocinnamic acid; curve 3, coumaryl-3-acetic acid. The ordinates represent the effect in change of velocity over that of controls × time.

sections of 5 day old coleoptiles, immersed in shallow layers of solutions as described by Bonner (1933), using the zone about 1 cm. below the tip.

Fig. 5 summarizes the data from two such experiments, using coleoptiles with the primary leaf just broken through and protruding about 5 mm. The type of curve obtained is clearly the same as that obtained with streaming, and it is of interest to note that very high

auxin concentrations may cause an actual shrinkage of the coleoptiles, although the tissue appears normal and shows no increase in width. The peak of the growth effect is obtained at 10 mg. per liter or close to

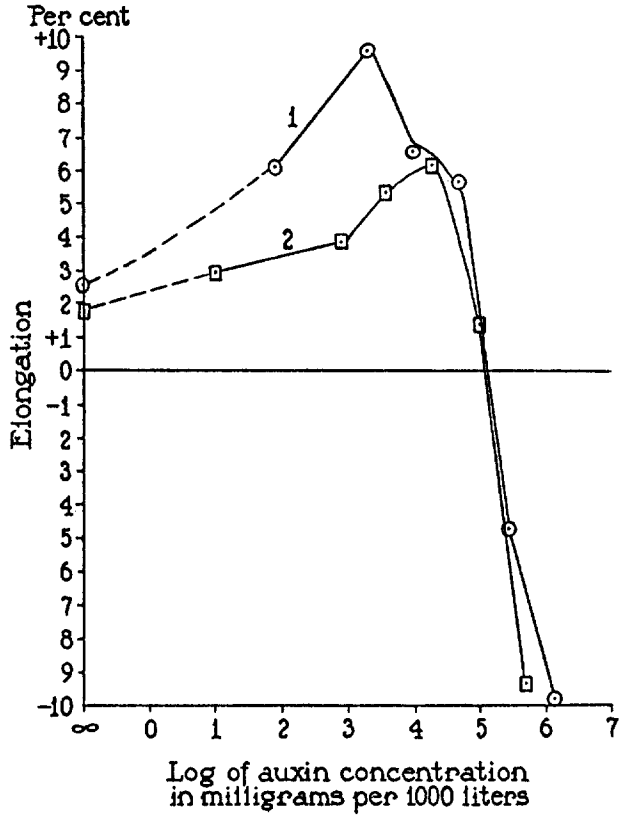

FIG. 5. Effect of auxin on the elongation of sections of coleoptiles immersed in solution. Abscissae, logarithm of the auxin concentration as in Fig. 4. Ordinates, elongation in per cent of original length after 24 hours' immersion. The abscissa marked ∞ refers to controls in water. Curves 1 and 2 represent two separate experiments, each point being the mean of 15–25 sections in each solution.

it, agreeing with the result of Jost and Reiss, while the maximum effect on streaming occurs at 0.01 mg. per liter. The streaming curve is then displaced to the left of the growth curve by about 3 logarithmic units. The significance of this displacement is not clear.

Specificity of the Auxin Effect

The question now arises whether this phenomenon of increase in the rate of streaming is a specific one for growth substances. In order to determine this, experiments of two kinds were made: (*a*) the effects of other auxins on streaming were studied, using the procedure as above; and (*b*) other substances, not growth-promoting, but previously known to affect streaming of protoplasm in various materials, were studied for their effect under our conditions.

(*a*) Coumaryl-2-acetic acid and allocinnamic acid were found to be active as growth-promoting substances by Thimann (1935) and Haagen-Smit and Went (1935) respectively. Instead of the former we have used for these experiments coumaryl-3-acetic acid, *i.e.* the oxygen analog of indole-3-acetic acid; its activity is somewhat higher than that of the 2-substituted derivative. Both coumaryl-3-acetic and allocinnamic acids have an activity on growth of immersed coleoptile sections of from a tenth to one hundredth of that of indole-3-acetic acid. Study of their activity on streaming showed that they both accelerate the rate at low concentrations. Plotting the total effect against concentration (Fig. 4, curves 2 and 3) shows that their activity reaches a peak at a concentration ten times the optimum concentration of indole-3-acetic acid. The curves for allocinnamic and coumaryl-3-acetic acids fall very close to one another and are approximately parallel to that for indole-3-acetic acid. The total increase in rate is, however, less than that caused by the latter. When it is borne in mind that both of these substances are what could be described as auxins of only moderate activity, it will be seen that their effect on streaming is qualitatively and quantitatively about what would be expected if this effect is related to their activity as auxins.

(*b*) Ethylene chlorhydrin has no auxin activity, although it has the effect of breaking dormancy of certain tubers (Denny, 1926, 1934). The streaming of the protoplasm of *Elodea* and *Nitella* was found by one of us (Marcy, 1937) to be stimulated by concentrations of 0.25 to 0.075 gm. ethylene chlorhydrin per liter, after immersion of the plant material in the solution for 24 hours. In the experiments here described, concentrations from 0.1 to 0.0001 gm. per liter were tested, and all failed to show any effect on streaming in *Avena* coleoptiles within the 30 to 40 minutes required to reach the total effect of indole-

3-acetic acid, coumaryl-3-acetic acid, or allocinnamic acid. The average of the results of three experiments at 0.01 gm. per liter is shown in Fig. 6, curve 1.

The extensive experiments of Fitting (1929, 1932) showed that α-amino acids, among them histidine, cause the renewal of active streaming in leaf cells of *Vallisneria*, in which streaming has previously been stopped by placing the plants in glass-distilled water. Histidine was found to be active in concentrations of one part in 80×10^6 or

FIG. 6. Curve 1, the effect on the rate of streaming of ethylene chlorhydrin, 10 mg. per liter, (mean of three experiments). Curve 2, effect of histidine, 0.02 mg. per liter.

0.0000001 M. Histidine was therefore tested in our experiments at concentrations of 0.005, 0.01, 0.05, and 0.1 mg. per liter. In no experiment did it have any effect on the rate of streaming in *Avena*. The results of one experiment are plotted in Fig. 6, curve 2. There was no consistent variation either with concentration or with time, the variations observed being no greater than those in single control experiments in pure water.

Urea, in concentrations of 10 and 0.1 mg. per liter, also was without effect on streaming in *Avena* coleoptiles.

Since the auxins used above are all weak acids the possibility remains that their effects on streaming might be due to changes in pH. The pH of the stock solution (100 mg. per liter) was found colorimetrically to be about 6.0. Now the maximum effect of indole-3-acetic acid was obtained in solutions containing 0.01 mg. per liter; *i.e.*, M/10^7. Even if the acid were as strong as hydrochloric acid, the pH of this solution would be, by definition, 7.0. However, dilute solutions of acetic acid were tested for the sake of completeness and found to have no effect on streaming either at 0.001 M or at 0.000001 M (Fig. 7).

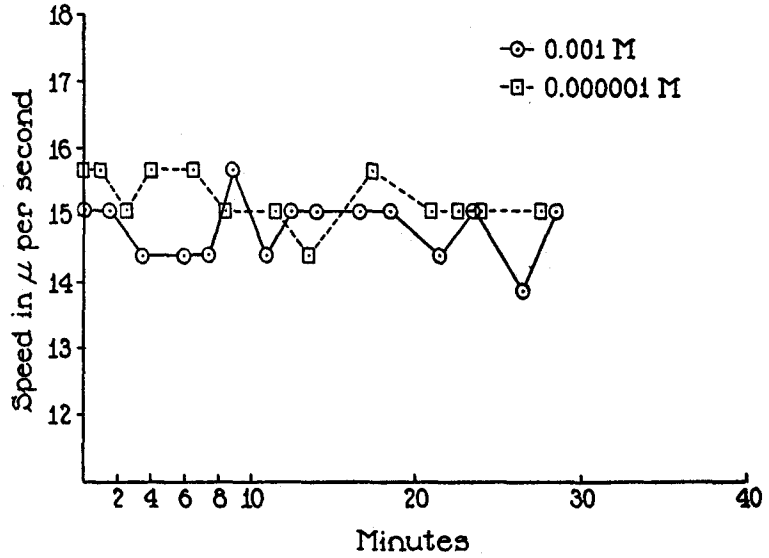

FIG. 7. Effect of acetic acid on the rate of streaming.

DISCUSSION AND SUMMARY

1. Evidence has accumulated that the action of auxins in promoting growth is exerted not upon the cell wall but upon the cell contents; *i.e.*, the protoplasm. Following indications previously obtained, therefore, the effect of auxins on the rate of protoplasm streaming in the *Avena* coleoptile was studied.

2. Indole-3-acetic acid, the most active auxin available in pure form, was found to increase the rate of streaming in the epidermal cells of the *Avena* coleoptile at concentrations between 0.5 and 0.002 mg. per

liter, the maximum increase being brought about at 0.01 mg. per liter. This concentration is approximately that which, applied in agar to one side of the decapitated coleoptile, would give a curvature of 1°; *i.e.*, it is well within the range of concentrations active in growth promotion. It is, however, much less than that which produces maximum elongation in immersed sections of *Avena* coleoptiles.

3. This accelerating effect is readily determined quantitatively by comparison with the streaming in control coleoptiles in pure water, which, if thoroughly aerated, maintain a constant rate for over an hour. The accelerating effect takes place immediately and is over within about 30 minutes.

4. Concentrations of indole-3-acetic acid greater than 0.5 mg. per liter inhibit the streaming, the effect being also over in about 30 minutes, and its extent increasing with increasing auxin concentration. This parallels the effect of high auxin concentrations in inhibiting elongation, although the inhibition of streaming is obtained at much lower concentrations than inhibit elongation.

5. The effects of indole-3-acetic acid on streaming are not specific for that substance, but appear to be common to auxins in general. Thus coumaryl-3-acetic acid and allocinnamic acid, both of which bring about cell enlargement, root formation, and bud inhibition, *i.e.* are typical auxins, also cause an immediate acceleration of the rate of streaming, and as with indole-acetic acid the effect is over in about 30 minutes. The concentrations of these two substances which produce the maximum effect are about ten times that of indole-acetic acid, which approximately corresponds with their relative auxin activities. The curves relating concentrations of these substances to their effects on streaming are very similar to that for indole-acetic acid.

6. On the other hand, certain substances which are known to affect streaming in other materials do not produce any effect comparable to that of auxin. Ethylene chlorhydrin, histidine, and urea in all concentrations were without effect on streaming in the *Avena* coleoptile within the first 30 minutes of treatment.

7. The effects produced by the auxins were not due to pH.

8. The action on streaming here studied is evidently quite different from the re-starting of streaming after its cessation, studied by Fitting in *Vallisneria*. Correspondingly histidine, which in Fitting's experiments showed activity down to 10^{-7} M, is inactive here.

9. *Per contra,* the effect of auxin here studied is on *normal* streaming. It takes place immediately and at concentrations in the same range as those which produce growth. The curve of effect against concentration parallels that for growth although the actual concentration values differ. It is therefore reasonable to suppose that the effect of auxin on streaming is closely connected with one of the first stages of its effect on the growth process.

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