PROTOPLASMIC STREAMING IN *TRADESCANTIA*

I. THE EFFECTS OF INDOLEACETIC ACID AND OTHER GROWTH-PROMOTING SUBSTANCES ON STREAMING

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Summary

The rate of protoplasmic streaming in the staminal hair-cells of *Tradescantia virginiana* is not affected by change of oxygen concentration in a hanging drop enclosing the hairs over the range 1·5-100 per cent. of oxygen in the surrounding gas phase. This allows experiments to be carried out without continuous renewal of the water drop.

Over the physiological range of concentrations, indoleacetic acid (IAA), indolebutyric acid (IBA), naphthaleneacetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D) all modify the rate of protoplasmic streaming in these cells.

These growth-promoting substances, added alone, bring about changes in the rate of streaming within 10 min; maximum effects are reached in 30 min, and the rates return to the normal in 60-70 min (exceptions to this rule are noted for NAA (high concentrations) and 2,4-D (low concentrations)).

Low concentrations of the auxins stimulate the streaming, high concentrations depress the rate, and intermediate concentrations are without effect. The auxins thus affect streaming much as they do the growth of stems, and the optimal concentrations for stimulation are similar for both processes. The "total effect" (T.E.) for each growth substance may be estimated as the total extra or diminished distance travelled by a protoplasmic particle in the presence of the applied substance. The maximum positive T.E. is given at 1 mg/l (1 p.p.m.) for IAA and IBA, and at 5 mg/l for NAA.

There is a marked pH effect for the reaction of streaming to IAA: increase in pH renders the applied solution much less effective over the whole range of concentration.

The temporary effects of indoleacetic acid on streaming, both stimulation and inhibition, are stabilized, near their maximal values, if fructose or malic acid are added with the auxin.

These results largely confirm those obtained by Thimann and Sweeney for the *Avena* coleoptile, except that the concentration for IAA giving maximal T.E. for *Avena* is as low as 0·01 mg/l. Moreover, for *Avena*, malic acid not only stabilizes the auxin effect but alters the threshold of response.

An improved apparatus is described for measuring the rate of protoplasmic streaming in cells.

I. INTRODUCTION

In 1937 Thimann and Sweeney reported a marked effect of indole-3-acetic acid (IAA) and other growth-promoting substances on the rate of protoplasmic streaming in the cells of the *Avena* coleoptile. Extension of this work (Sweeney

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and Thimann 1938, 1942; Sweeney 1944) led the authors to put forward a hypothesis that auxin effects on streaming, growth, and respiration are all related to the action of auxins on a 4-carbon respiratory cycle. Their results were questioned by Olsen and duBuy (1940), who failed to obtain stimulation of streaming with IAA, although, with higher concentrations, they obtained slight inhibition; they argued that the effects described by Thimann and Sweeney were due to impurities in the auxin. Sweeney (1941), however, attributed these negative findings of Olsen and duBuy to their use of coleoptile sections infiltrated with water; she found that under such conditions no acceleration of streaming was produced by IAA. While not adding further data on the effect of IAA on streaming, Showacre and duBuy (1947) countered Sweeney's argument indirectly by showing that infiltration of *Avena* coleoptile sections does not prevent the acceleration of growth by IAA. The matter appears to have rested here, and Audus (1949), on reviewing this field, remarked that "the experiments of Sweeney and Thimann should be repeated under the experimental conditions described in their papers." In 1947 we began to study this problem in another way, by the choice of a different species in which the complications due to the use of cells organized in tissues could be avoided. After some preliminary work with fungi, algae, and flowering plants, we selected the mature staminal hair-cells of *Tradescantia* as suitable material. The results presented in this paper are in accord with most of those obtained by Sweeney and Thimann, and will form the background for later papers on the effects of respiratory inhibitors on streaming, and on a marked effect of oxygen which has been demonstrated for the reaction of streaming to IAA.

II. Methods

Streaming was studied in single cells of the staminal hairs of *Tradescantia virginiana*. A few hairs were cut from stamens taken from open flowers, mounted in water (double-distilled in glass), or in the appropriate solutions on the surface of a circular coverslip 1¼ in. diameter, the ends of the hairs being kept in position with petroleum jelly. The coverslip was then inverted to form the top plate of a chamber made from a brass ring and glass slide. Gas could be passed through the chamber by means of two hypodermic needles sealed into the edges of the ring. The coverslips were sealed into position with petroleum jelly. All experiments were carried out in a constant-temperature room at 24°C, where the basal streaming rate showed relatively small fluctuation. The rate of streaming was not appreciably affected when the microscope lamp was switched on; nevertheless, the light was kept on continuously throughout an experiment.

During the first year of the experiments the streaming was observed through a monocular microscope at a magnification of × 625, and a stop-watch was used to time the movement of the smaller particles in the cytoplasm. Readings were made at 5-min intervals, each reading being the mean of 10 individual measurements of the time taken for a single small particle to travel past 10 scale divisions of the eyepiece micrometer. Unless otherwise stated, the

chamber contained air, and gas was not passed through it during measurements. In all experiments streaming rate was determined in distilled water for 15-25 min, by which time a steady rate of streaming had been established. Good uniformity was observed in the basal streaming rate in water of hair-cells from the one flower, but in most experiments on the effect of a given factor one cell was used as its own control, its basal rate of streaming being determined before the conditions were changed. The distilled water was then drawn off from the hairs, with filter paper, and the test solution added. All solutions were made up in distilled water, and brought to 24°C before use. The pH values of the solutions used were determined by means of a glass electrode, and adjusted when necessary with hydrochloric acid or sodium hydroxide.

During and after the second year of the experiments a device was used which saved eyestrain and probably led to a smaller subjective error in determination of streaming rate. However, the results obtained with this apparatus were not different from those obtained earlier using direct ocular measurements.

The apparatus is based upon that described by Sweeney and Thimann (1942). In their apparatus a moving bead belt is viewed at stage level with the streaming protoplasm, via a camera lucida. The bead belt is driven by a constant-speed motor and speed variations of 1-2 are obtained by altering the tension of the driving belt. Because we considered that it might be difficult to reproduce conditions accurately over a long period, with such a method of varying the speed, the following apparatus was devised and constructed for us by Mr. Matthaei of this Department. The principle applied is that the apparent speed of a given object varies with the viewing distance. The apparatus is illustrated in Plate 1. A small synchronous motor drives a disk at 1 r.p.m. and a fog-developed strip of 35-mm film is fixed to the periphery of the disk (5). A pilot lamp illuminates the perforations of the film from behind. A slit on the shield (4) permits 10 perforations to be seen. The image of this slit and the moving band of perforations of the film are superimposed on that of the specimen through a camera lucida (2) and mirror (3). The appearance of the streaming protoplasm (9) and of the film image is shown in the photomicrograph (Plate 1, inset). The apparent speed of the perforation images is altered every few minutes until it coincides with that of the main stream of protoplasmic particles. Streaming was observed with a 3-mm objective under a total magnification of × 525. Screen (4) with its accompanying motor and illuminated strip can be moved along the optical bench (6) by means of the endless belt (7). A pointer attached to (4) moves along a permanent fixed scale on the bench. The apparent streaming rate of the film perforation images is dependent on the distance of the film from the microscope. The instrument is calibrated from the formula

$$V^1 = V\frac{d}{mD},$$

where V^1 = velocity of the (matched) stream in mm/min,
V = surface speed of film drum (5) in mm/min,
d = 25 cm (unit distance of magnification),

m = magnification of microscope measured 25 cm from the exit pupil of microscope, and
D = distance between exit pupil of microscope and film drum.

The resulting speeds for various positions are marked off on a bench ruler in mm/min, and the scale extends from 0·51 to 0·14, giving a speed variation of about 1-3½.

The apparatus was tested by setting the pointer to a given speed after calibration according to the above formula, and then measuring the speed of a single moving light spot by direct observation and stop-watch. The two figures so obtained usually differed by less than 4 per cent.; occasional large discrepancies must be ascribed to personal errors in the direct method.

Critical illumination of the microscope was necessary for obtaining a clear contrasted image of the protoplasmic stream. A microscope lamp was used as the illuminant, with diaphragm adjusted according to the Köhler principle. Some difficulties were experienced in projecting the field stop of the lamp into the plane of the specimen chamber. This was overcome by removing the front lens of the condenser. The resultant numerical aperture of about 0·4 was enough for a good definition. A green filter was used in order to give clearer definition of the moving protoplasm.

The IAA (β-indolylacetic acid, BDH) was purchased at frequent intervals, only pure white crystalline material being used. The acid was dissolved in water, double distilled from glass at 20-25°C. Solutions were made up freshly for each set of experiments, or were kept between experiments in the dark at 0°C for not longer than 2 days; they were brought to the temperature of the constant-temperature laboratory (24°C) before use.

III. Results

(*a*) *Basal Rates of Streaming in Water*

Flowers, picked when freshly opened, were placed with their stalks in water and kept in a cool place. Such flowers were used, for a period not exceeding 8 hr, as the source of supply of fresh hair-cells. Occasionally the apparently healthy hair-cells of freshly opened flowers showed no cyclosis. These cells were discarded, as in these experiments we were not concerned with factors initiating streaming.

Generally the protoplasm of the hair-cells was found to be actively streaming. The basal rate in a given hair-cell, mounted in water over air, was measured either directly by observation of single particles, or (later) by means of the apparatus already described.

In the first year, when direct timing of particles was used, the rates obtained were some 30 per cent. lower than those found for comparable periods in later years, by matching the speed of the stream of protoplasm. It is impossible now to determine whether this was due to physiological differences between the plants of the first and later years, or to the earlier choice of the rather

larger and slower particles for measurement (see Sweeney and Thimann 1942). It is, however, significant that, over a 2-yr period, the basal rate, as measured by both methods, showed a marked rhythm. It was at its minimum in July (midwinter) and showed two pronounced maxima, one in autumn (April-May), and one in spring (October-November). The maximum rate (using the apparatus) was approx. 7 μ/sec and the minimum 6 μ/sec. Basal rates given by Sweeney and Thimann (1942) for *Avena* coleoptiles are between 12 and 15 μ/sec. They also found a seasonal variation in the basal rate, with a maximum in late summer.

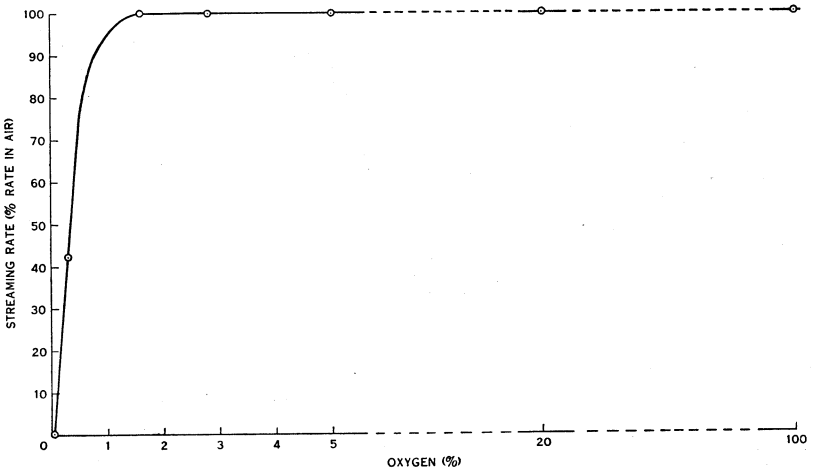


Fig. 1.—Relationship between oxygen concentration surrounding the hanging drop and the rate of protoplasmic streaming in the staminal hair-cells of *Tradescantia virginianum*. Each point on the curve is the mean of five separate determinations of the steady state rate, each expressed as a percentage of the rate in air.

(*b*) *Streaming as Affected by the Concentration of Oxygen*

Streaming in *Tradescantia* staminal hair-cells continues under conditions of low oxygen tension. Ewart (1903) reported that some workers were unable to stop streaming even by placing the hairs in pure hydrogen. However, in his own work with this species, streaming ceased in hydrogen within 15 min to 3 hr, the time varying with the temperature and with the age and condition of the cells. In our experiments, gases, including air, cylinder oxygen, cylinder hydrogen, and various mixtures of oxygen and nitrogen were passed through the experimental chamber and their effect upon the rate of streaming measured. The concentration of oxygen in each gas was determined in the Haldane gas analysis apparatus. The gases were bubbled through water before passing through the cell in order to prevent evaporation in the chamber. The results are graphed in Figure 1, similar results being obtained over 2 yr with various specimens of staminal hairs and using both ocular timing of particles and the apparatus described above. In all experiments the rate was first determined

in air and the change in rate, if any, measured when air was replaced by a different gas mixture. The new rate was measured for a period of at least 30 min, following the establishment of steady state, and rapid recovery to normal rate took place on return to air. All rates were expressed as percentages of the rate in air. It is clear that above about 1·5 per cent. oxygen (the critical value) the concentration of oxygen in the gas over the hanging drop has no influence upon the rate of streaming. Only when the concentration of oxygen was reduced below 1·5 per cent. was the rate of streaming reduced. In these cases the rate decreased immediately and reached a new steady state 15-20 min after the new gas was first introduced into the chamber.

A stream of hydrogen containing only 0·3 per cent. oxygen brought about a complete stoppage of protoplasmic streaming within 15 min. Recovery occurred almost at once when the current of hydrogen was discontinued, presumably owing to slight leakage of oxygen into the chamber, or to traces of oxygen in the water film. When cylinder hydrogen was again passed through the cell, streaming stopped within 3 min. Recovery was more gradual after prolonged passage of hydrogen.

The combination of the low critical value of oxygen for *Tradescantia* and the use of microscopic samples in hanging drops allows experiment in small closed vessels in which the water drop is in contact with air. In the work with *Avena* it had been found necessary to use specially aerated water for the longer experiments or to replace the liquid drop every few minutes or even continuously. If half a young coleoptile 4-5 cm long, split longitudinally, is placed under a coverslip, the resulting oxygen deficiency (due to respiration) leads to a decrease of streaming within a few minutes. The critical value of oxygen for *Avena* has not been clearly established; the results of Bottelier (1935) indicate that for young coleoptiles (grown for 96 hr at 26°C) the streaming rate is increased on passage from water charged with air to that saturated with oxygen. If, however, the critical value for the *cell* in *Avena* is low, the use of comparatively large (respiring) samples of tissue in each experiment must be responsible for the rapid decrease in streaming in unaerated or unchanged water drops.

(*c*) *Effect of IAA on Streaming*

In all the experiments described in this section the experimental chamber contained air. The rates of streaming for a given cell were first measured while the cell was in glass-distilled water, and this was then replaced, without change of temperature, by a drop of freshly made up IAA solution (measured pH 4-5·5, depending on the concentration). Rate measurements were made 3 min after the change and subsequently at 5-min intervals. In all, some 100 individual experiments of this kind have been made, and, with a few exceptions to be mentioned below, the results were consistent. Over the concentration ranges 0·1-10 mg/l and from 50-100 mg/l the IAA caused marked changes in the rate of streaming, these changes becoming apparent within 10 min of treatment. Figure 2 shows the results for a typical series of experiments with staminal hairs taken from the one flower.

(i) *Concentration Range*

(1) *IAA 0·1-10 mg/l : stimulation.*—These solutions all caused a transient increase in the rate of streaming, apparent within 3 min. Maximum rates, for each concentration, were reached within 20 min, and in all experiments the rates returned to the normal (i.e. to the rate in water) within 60-70 min. At the lowest effective concentrations (0·1 mg/l) the effect was small and simple,

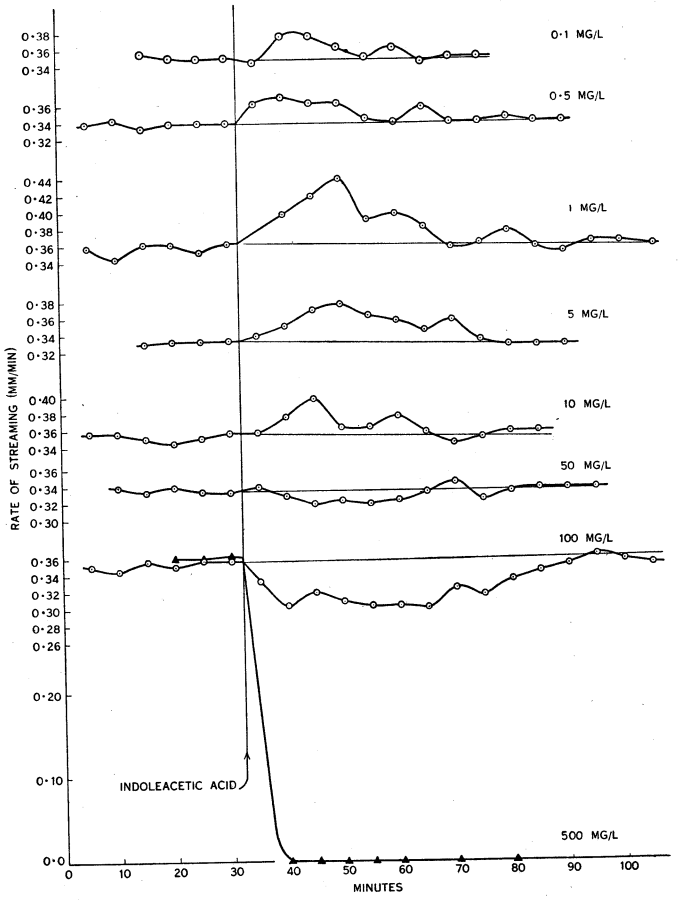


Fig. 2.—Effect of IAA solution, at various concentrations, on the rate of protoplasmic streaming. Each curve is obtained from measurements on a single hair-cell, all hair-cells from the same flower.

and it was over within 25 min. With concentrations near 1 mg/l the effect was greater and more prolonged. The curves often showed a curious double effect, a second brief stimulation interrupting the fall to the normal rate. The maximum increase was of the order of 20 per cent. of the basal rate and was given by the concentration of 1 mg/l. Effects given by 10 mg/l closely resembled those given by 0·1 mg/l, and concentrations between 10 and 50 mg/l had little effect on the rate.

(2) *IAA* 50-100 *mg/l* : *inhibition.*—These solutions caused an inhibition of streaming, which was not complete and not lasting. The time curves are in the form of mirror images of those described in section (1). The effects were

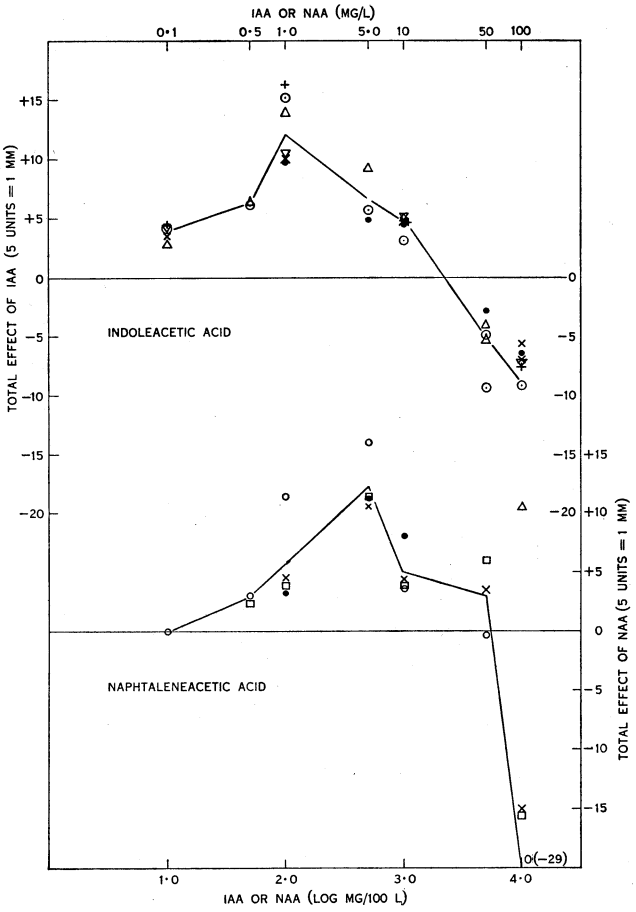


Fig. 3.—(Above) *Total effects* of various concentrations of IAA on the protoplasmic streaming. The lines join the means of points obtained in five separate series of experiments with different flowers. Each point represents the area under the rate/time curve (Fig. 2), using the extrapolated rate in water as the base line. (Below) The same, for α-naphthaleneacetic acid. The lines join the means for three separate experiments (see Fig. 6).

also apparent within a few minutes, maximal inhibition was reached within 20 min, and the rate again returned to the normal rate within 70 min. The greater the concentration, the greater was the inhibition.

(3) *IAA* > 100 *mg/l.*—Here the effect was a progressive inhibition, not followed by recovery, and eventually leading to a complete stoppage of all streaming.

These results show a striking parallelism with those obtained by Thimann and Sweeney (1937) with the *Avena* coleoptile. The general form of the curves is the same and the time scales are of the same order, recovery from auxin effects being somewhat slower for *Tradescantia,* and the stimulatory effect on the rate being smaller than demonstrated for *Avena.* The major difference is in the concentrations of auxin necessary to bring about similar effects in the two tissues. This quantitative difference is best shown if we follow Thimann and Sweeney and express the *total effect* of a given auxin concentration as the area under the curves of rate/time, using the rate in water as the base line. This is of value as giving a figure for the total extra or diminished distance (5 units = 1 mm) travelled by a protoplasmic particle when in the presence of applied auxin, and it is particularly useful in our experiments where a double-headed effect is given by the lower concentrations, making other comparisons difficult. In Figure 3 we plot the results obtained with five series of experiments, in each of which hairs from the same flower were tested with a range of concentrations of IAA. The values for the total effects are plotted against the concentration of IAA on a logarithmic scale, the means for the total effects at each concentration being joined by straight lines. The result is closely similar to that already described for the *Avena* coleoptile by Thimann and Sweeney, a major difference being that the curve for *Tradescantia* is shifted bodily to the right, the maximum effect of auxins being given by concentration of 1 mg/l, whereas for *Avena* the maximum effect is given by 0·01 mg/l. The results for *Tradescantia* are much more closely similar to those obtained by Thimann and Sweeney for the elongation of *Avena* coleoptiles plotted against the logarithm of the auxin concentration. The comparison is made clearer in the generalized summary of some auxin effects given in Figure 4.

In making these comparisons it may be pointed out that the growth tests take 24 hr, while the streaming effects subside within 1 hr; the *Tradescantia* hair-cells are fully grown and borne exposed to air on flowers, while the *Avena* cells are those of a rapidly growing organ with some cut surfaces. The curve for *Avena* streaming is shifted still further to the left if younger coleoptiles are used (Thimann). It is clear that the streaming of protoplasm in *Tradescantia* is markedly affected by IAA over a range of concentrations which is known to be of physiological importance in the growth of plant shoots.

The results described above were obtained consistently in experiments carried out during the first 2 years of the investigation. It seemed therefore that one might be able to use *Tradescantia* as a test material for the determination of auxin concentrations. Further work showed that this would be a possibility only if the *Tradescantia* plants were grown under standardized concentrations. In the great majority of our experiments the curve of Figure 3 applied, and it was often possible (for one of us) to identify an unknown IAA solution (e.g. to state whether its concentration was of the order of 0·1, 1·0, 10·0, or 100 mg/l) by using the streaming test. However, from time to time we have found that staminal hairs taken from apparently normal flowers react abnormally in the streaming test, being much less sensitive than normally. In such hairs inhibition of streaming was not given even by concentrations of 500 mg/l; they were

not used for the experiments under consideration. So far we have not sufficient data to enable an attempt to correlate these abnormalities with climatic data; these abnormal results were obtained in about 5 per cent. of the experiments, and with hairs taken from plants flowering at the end of the season or in greenhouses given supplementary illumination.

(*d*) *Hydrogen Ion Concentration and the Auxin Effect*

In most experiments we followed Thimann and Sweeney in adding IAA without additional buffer at a pH which varied between 4·0 (100 mg/l) and 5·5 (0·1 mg/l). As the distilled water of the control experiments was acid, not being free from CO_2, the change in acidity due to the auxin was not large.

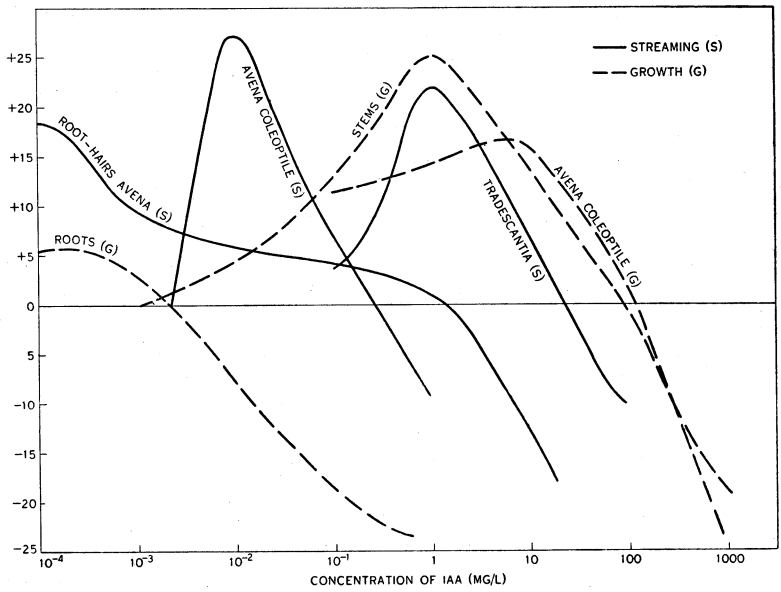


Fig. 4.—A generalized scheme showing the concentration ranges over which IAA exerts its effects, in different plant organs, on growth and on protoplasmic streaming. Data from Thimann and Sweeney (1937), Sweeney (1944), Audus (1953), and present work. Ordinates, increase or decrease in elongation or in rate of streaming; the scale for the ordinates is arbitrary, and comparisons of percentage effects are not attempted.

Two questions arise, however:

(1) Is the "auxin effect" merely due to pH change?

(2) Does the pH of the external solution markedly change the response to auxin?

(1) In our experiments change of pH in the external solution, in the absence of auxin, did not change the rate of streaming. Thus, with *Tradescantia* we found no change in the rate of streaming on passage of the hair-cells from water to phosphate buffers at pH 4 and pH 7. Thimann and Sweeney (1937) showed that, with *Avena,* acetic acid at concentrations between 0·001 and

0·000001M had no effect on streaming, while Sweeney stated that the addition of alkali to give a pH of 9 also did not affect the streaming rate in this plant. Becker (1936), working with *Tradescantia,* reported that there was no effect on streaming on the addition of various acids, including mineral acids and weak organic acids at pH 3·8. Lepow (1938) reported that the rate of streaming in *Physarum* was not altered by change of pH between 5 and 9. Like Thimann and Sweeney, we have found that the IAA effect is also given by certain other plant hormones, whereas malic acid and succinic acid had no effect on the rate of streaming when added alone at low pH. In spite, therefore, of a few records of a direct effect of pH on protoplasmic streaming (Seifriz 1943, mostly with green cells), we are of the opinion that our results must be explained in terms of the auxin and not of pH change *per se.*

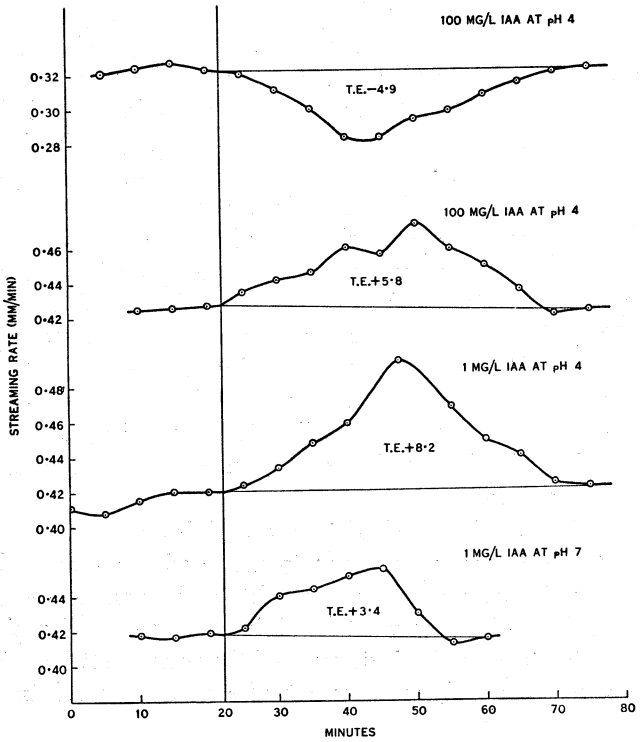


Fig. 5.—Effect of IAA on protoplasmic streaming, as modified by the pH of the solution. T.E. = total effects, the area under the curve with the control extrapolated as the base line.

On the other hand, we are in agreement with the Thimann school that the pH of the auxin solution is of great importance. A thorough investigation of this aspect has not so far been attempted but enough work has been done to show that, on changing the pH of the auxin solution from 4 to 7, there is a decrease in the effective concentration of the auxin. Thus (Fig. 5) a high concentration of auxin (100 mg/l, pH 4) caused an initial fall in the streaming

rate, followed by a rise to the normal, with a total effect of $-4{\cdot}9$. The same concentration applied at pH 7 brought about a temporary stimulation in the streaming (total effect $+5{\cdot}8$) similar to that given by a weaker auxin solution at pH 4. Similarly, a low concentration of auxin (1 mg/l) applied at pH 4 caused a marked transitory stimulus in the rate (total effect $+8{\cdot}2$), whereas the same concentration at pH 7 brought about a smaller increase (total effect $+3{\cdot}4$). Thus, although we have not enough data to plot the complete curve, there are strong indications that if we plotted total effect against log concentration of auxin at pH 7, a curve similar to that of Figure 3 would be obtained, except that it would be shifted bodily to the right by one unit of log concentration. Thimann and Schneider (1938) obtained a closely similar result for streaming (but not for growth) with the *Avena* coleoptile, and explained it as being due simply to the effect of pH on the dissociation of the auxin,—"at pH 7, when auxin consists of 1% acid and 99% salt, auxin action (on streaming) is as though its concentration has been reduced to 1/100 of its concentration at pH 4." This would imply that the pH effect is one largely concerned with the rate of entrance of the acid molecule into the protoplasm, and this hypothesis would also serve to explain the lack of a pH effect on growth, the measurement of which takes much more time than does measurement of change in streaming rate. However, it must be noted that while increase in pH (like simple dilution of an acid solution) changes the *total effect* of the auxin, it does not materially alter the rate at which the streaming reacts to the applied solution (Figs. 5, 2). This does not lend support to the explanation offered, which is probably an over-simplification of a very complex situation (see Hanly, Rowan, and Turner 1952). Reinhold (1954) has suggested that for one tissue, at least, the uptake of IAA probably involves both physical and metabolic processes, and while pH mainly affects a physical process, the fall in the rate of uptake with increasing pH is not parallel to the fall in the mean concentration of undissociated IAA molecules in the medium.

As stated above, we followed Thimann and Sweeney in using the weakly buffered pure IAA solutions in our experiments, and it seems probable that for both investigations the form of the curves obtained at the lower concentrations would be changed if all experiments had been done in phosphate buffer of pH 4 instead of in water at pH rising slightly with dilution. However, this change is likely to be small, and cannot affect the general interpretation of the results.

(*e*) *Effects of Auxins other than IAA*

Thimann and Sweeney (1937) showed for *Avena* coleoptiles that the growth-promoting substances IAA, coumaryl-3-acetic, and *allo*cinnamic acids all affect streaming in a similar way; quantitatively their effects are what would be expected from their activity as auxins. We have compared the effects of IAA on *Tradescantia* streaming with those produced by indolebutyric acid, indoleacetonitrile, α-naphthaleneacetic acid, and 2,4-dichlorophenoxyacetic acid.

The relative activity of indolebutyric acid, determined by four different growth tests (Thimann and Schneider 1939), is between 8 and 190 per cent.

of that of IAA. Its effect on streaming in *Tradescantia,* like its effect on the straight growth of *Pisum,* is quantitatively closely similar to that of IAA itself. Maximal temporary stimulation was given by a concentration of 1 mg/l; a solution at 10 mg/l stimulated very slightly, and one at 100 mg/l caused a temporary depression in the rate of streaming.

A few tests with indoleacetonitrile, which is more active than IAA in promoting cell elongation (Bentley and Bickle 1952), showed that it too modifies the streaming rate; a concentration of 10 mg/l stimulated streaming, and concentrations between 50 and 100 mg/l inhibited it. There were, however, indications that these effects were more permanent than those brought about by IAA itself, and further work with this substance is envisaged.

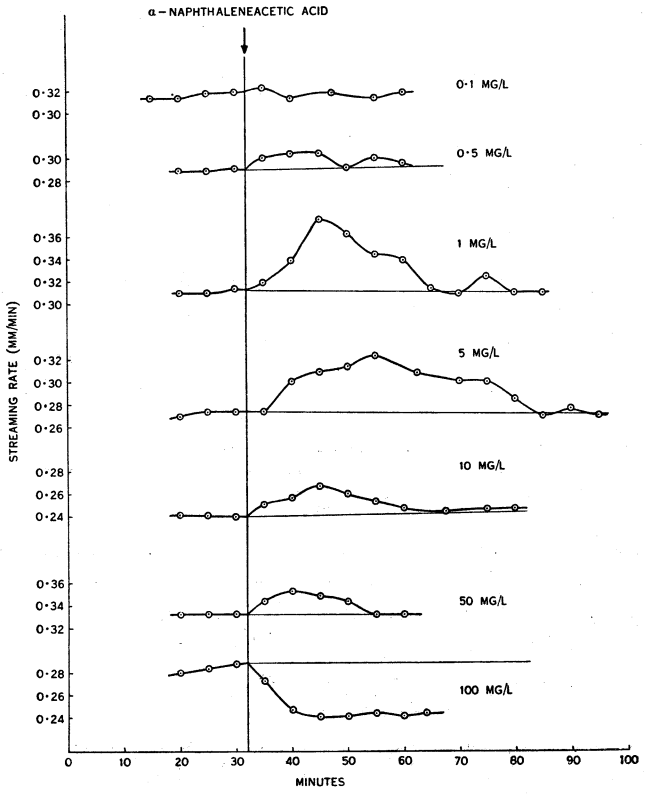


Fig. 6.—Effect of α-naphthaleneacetic acid solution at various concentrations on the rate of protoplasmic streaming in *Tradescantia.*

For α-naphthaleneacetic acid a more complete investigation has been carried out. This acid is reported as having between 2·5 and 23 per cent. of the growth-promoting activity of IAA (Thimann and Schneider 1939), except for the slit-stem *Pisum* test, in which it is nearly four times as active. Against streaming, in *Tradescantia,* it acts in a similar manner to IAA, with two qualifications. At concentrations between 0·1 and 50 mg/l it temporarily stimulates

streaming (Fig. 6); in the presence of the applied acid the rate returns to that of the water control within 40-50 min. The optimal concentration, however, was 5 mg/l, which caused a 20 per cent. stimulation in the rate. The "total effect" for three separate series of experiments is plotted against concentration in Figure 3, and comparison shows that α-naphthaleneacetic acid is less active than IAA in its effect on streaming. Another feature of difference so far found between these auxins, however, is that the partial inhibition of streaming brought about by concentrations of 100 mg/l is temporary for IAA and more permanent for NAA (Fig. 6). For this latter the inhibition reaches 70 per cent. within about 15 min; the new rate is maintained for a further 20-25 min, and then falls slowly to zero. We have not, with this acid, found recovery from inhibition such as is characteristic for the corresponding concentrations of IAA.

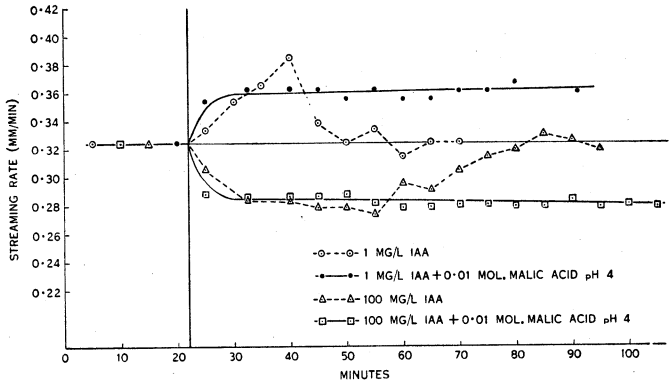


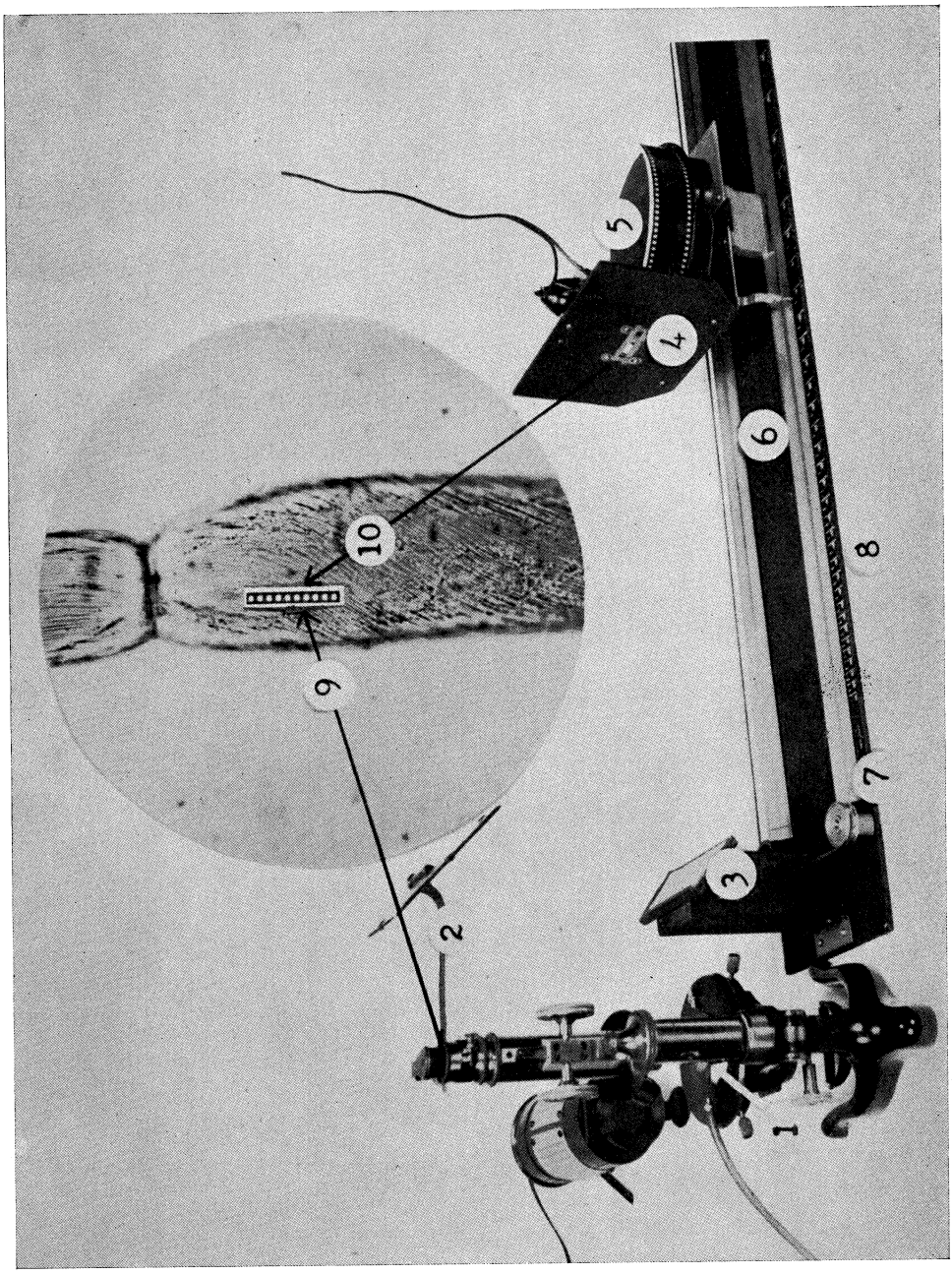
Fig. 7.—Effect of IAA on protoplasmic streaming as stabilized by the addition of malic acid (0·01M) with the auxin.

A few preliminary experiments with 2,4-dichlorophenoxyacetic acid show that this substance affects the rate of streaming, but its action is again different. A concentration of 100 mg/l caused a marked but only temporary depression in streaming rate, the effect being very similar to that due to the same concentration of IAA. Lower concentrations, between 0·5 and 50 mg/l of 2,4-D, brought about slight and apparently permanent stimulation of streaming, the maximum effect (10 per cent., due to 10 mg/l) being reached gradually over a period of 40 min. These stimulation effects, although smaller, resemble those obtained with IAA in the presence of sugar or malic acid.

(*f*) *The Role of Carbohydrate and Organic Acids*

A striking feature of the auxin effects in both *Avena* and *Tradescantia* is that in the great majority of experiments the streaming rate returns to that of the water control within 1 hr. For *Avena* the period of stimulation or inhibition is about 30 min. For *Tradescantia,* in 53 experiments, it varied over the range 20-60 min but seemed to be independent of the concentration of auxin. The mean for all concentrations was 38 min (S.D. 10·6 min). It must be emphasized

PROTOPLASMIC STREAMING IN *TRADESCANTIA*



Photograph of apparatus used for measuring the rate of protoplasmic streaming; description in text. *Inset*, photomicrograph of *Tradescantia* hair-cell showing the appearance of the projected image of the band of film perforations.

that recovery from maximum stimulation (1 mg/l) took the same time as recovery from inhibition (100-200 mg/l).

Sweeney and Thimann (1938) obtained recovery from stimulation even although fresh auxin solutions were continually supplied; under such circumstances there was only a "second slight upward trend which suggested a return of the auxin effect" an hour after the first stimulation had subsided. Moreover, if, after the rate had returned to normal, the cells were washed with water, a second stimulation by auxin could be obtained if a period of 30 min in water was allowed to elapse, or, alternatively, if sugar were added with the auxin. They explained the transient nature of the auxin effect as due to the exhaustion of a substrate; and supporting this view is their observation that the initial auxin effects on streaming could be stabilized by adding carbohydrate or malic acid with the auxin.

For *Tradescantia* our findings are:

(1) Fructose alone (0·03M) and malic acid alone (0·01-0·1M, pH 4) have no effect on the rate of streaming, at least during 1 hr after their addition.

(2) When added either with IAA or indolebutyric acid at pH 4, fructose (0·03M) and malic acid (0·05M) modify the auxin effect on streaming in the same way. They stabilize the effect for each concentration of auxin, whether it be stimulation or inhibition. They do not alter the threshold of response, nor do they shift the position of the optimum, maximum stimulation still being given by 1 mg/l auxin. Malic acid, but not fructose, significantly increases the rate at which maximum stimulation is attained.* The stable rates are quickly reached and maintained for at least 1 hr; in one experiment with 0·1 mg/l auxin and 0·01M malic acid, for at least 2 hr. The percentage stimulation or inhibition reached in the presence of fructose or malic acid is of the same order as the maximum figure for the transient effects caused by the same concentrations of auxin added alone. Typical results are plotted in Figure 7.

The most effective concentration of malic acid in our experiments was between 0·01 and 0·1M. At 0·001M malic acid prevented the complete recovery from stimulation due to 1 mg/l auxin, but was not fully effective in stabilizing the response. Sweeney and Thimann used this concentration (0·001M) with *Avena* as it had an optimal effect on respiration. It lowered the threshold of response to auxin some 10 times; moreover, in long-soaked sections the response to auxin alone was small, the response in the presence of malic acid was normal. Such results have not so far been obtained with *Tradescantia,* for which, in

* Times required for maximum stimulation from addition of solution:
Auxin alone (53 experiments) 15·4 min; S.D. 7·1 min.
Auxin and fructose (4 experiments) 15·5 min; S.D. 6·5 min.
Auxin and malic acid (8 experiments) 4·4 min; S.D. 2·1 min.
Difference auxin acid and malic and auxin alone highly significant at 0·1 per cent. level.

this respect, fructose and malic acid are interchangeable. Moreover, the combined effects of auxin and fructose are not enhanced if malic acid is also added. Thus, a mixture of auxin (1 mg/l) with 0·5 per cent. fructose gave a steady enhanced rate of streaming; when this solution was replaced by one containing auxin (1 mg/l), 0·5 per cent. fructose, and 0·01M malic acid there was no change in the rate of streaming.

The recovery from the effects of auxin (added alone) cannot be due to the rapid removal of all auxin from the external solution. Several experiments have shown that the same drop of IAA solution will bring about similar streaming effects (e.g. stimulation followed by recovery) in successive experiments with two different hair-cells. This result is in agreement with that mentioned

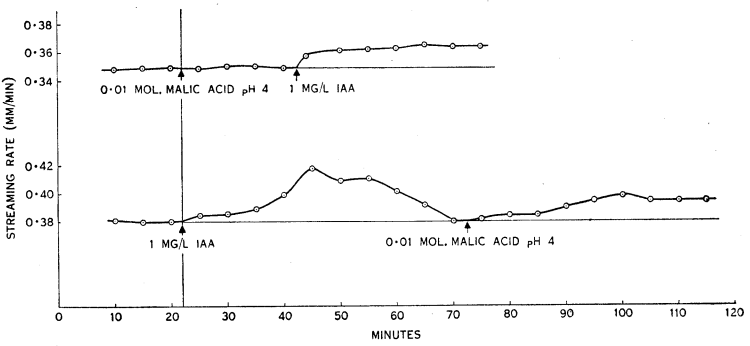


Fig. 8.—Effects on the rate of protoplasmic streaming of malic acid and IAA, added alone and consecutively. Description in text. At the first arrows the reagent added alone; at the second arrows the second reagent is added after the removal of the first.

above for *Avena*, in which recovery occurs even when the bathing solution is continuously renewed. Nor is recovery due to the destruction of auxin within the cell. In three separate experiments maximum stimulation was given when IAA was added alone at 1 mg/l, pH 4 (T.E. + 8·1, + 6·3, + 6·6); when the rate had returned to normal, after 50 min, the IAA solution was withdrawn, the cells momentarily washed with water, and then malic acid alone (0·01M, pH 4) was added (Fig. 8). In each experiment the streaming rate increased, rather slowly, to a new steady state. This was lower than would be expected for the concentration of auxin originally added, but these results suggest that after stimulation has subsided auxin is still present in the cell, and can exert its effect if malic acid is added. A somewhat similar experiment shows also that malic acid can pass into the protoplasm, without affecting its streaming rate, and become available to stabilize the effects of auxin added later. Thus (Fig. 8) when malic acid (0·01M, pH 4) was added alone there was no effect on streaming; when, however, after 20-25 min, the solution of acid was removed, the cells rinsed with water and mounted in IAA solution (1 mg/l), then a *stabilized* stimulation of streaming resulted.

One hypothesis, already mentioned, is that these results may be explained if the sugar and the malic acid are themselves the substrate (or provide the

substrate) for an auxin reaction. We have some evidence, however, which conflicts with this view, and full discussion of the cause of recovery from the varied effects of auxins on streaming will be left to a later publication.

The earlier findings of Thimann and Sweeney as to the effects of IAA and other growth-promoting substances on the rate of protoplasmic streaming have therefore been confirmed. It seems likely that further work in progress along these lines will add to our knowledge, not only of the nature of protoplasmic streaming, but also of the mode of action of auxins in the plant cell.

IV. Acknowledgments

This work was carried out while the junior author (J.M.K.) was in receipt of a research scholarship in the University of Melbourne. We are most grateful to Mr. E. A. F. Matthaei, lecturer in Microscopy in this Department, for the design and construction of the apparatus described.

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