In connection with the analysis of the action of auxins on plant tissues, we have made a study of the relation between auxin and the streaming of protoplasm. It was shown in the preceding paper (Thimann and Sweeney, 1937) that very dilute solutions of indole-3-acetic acid increase the rate of streaming in the epidermal cells of the Avena coleoptile. The effect is proportional within certain limits to the concentration of indole-3-acetic acid used; and it is probably connected with the fact that it is an auxin, since two other substances having auxin activity behave in the same way, while substances which are not auxins produce no increase in streaming rate. However, the effect of these auxins on streaming was found to differ from their effect on growth in two important particulars:

1. While low concentrations of auxin accelerate the streaming, higher concentrations retard it. Nevertheless, these higher concentrations are those which give the greatest growth of immersed sections of coleoptiles.

2. The acceleration (or retardation) is transient, being over within 20–30 minutes, while the effect on growth lasts for 24 hours or more.

The present paper is mainly concerned with the reasons for these two differences. It is believed that the explanation which our experiments yield throws additional light on the mechanism of auxin action.

The methods used were the same as those previously described. However, "Segerhavre" (Victory) oats were used in the majority of the experiments in place of the Cornellian strain previously used. In several series of observations the two types have been found strictly comparable, and in fact curve I of Fig. 11 represents means of values obtained with both strains.
The influences mainly studied have been those of carbohydrates, oxygen tension, and age.

The Role of Carbohydrates

As mentioned above, the effect of auxins on streaming is characteristically of short duration, i.e., the rate of streaming returns to normal within about 30 minutes after the first application of auxin, although fresh auxin is being continually applied.

![Graph](image)

**Fig. 1.** The recovery of the ability to react to auxin. All auxin solutions 0.01 mg. per liter. Curve 1, auxin replaced at arrows by fresh auxin solution. Curve 2, auxin replaced at first pair of arrows by water and 15 minutes later by fresh auxin. Curve 3, auxin replaced at first pair of arrows by water and 30 minutes later by fresh auxin. 5 cm. coleoptiles: aerated water.

If, however, the readings in auxin solution, 0.01 mg. per liter, are continued for an hour or more, the rate of streaming in the epidermal cells of the coleoptiles shows a second slight upward trend which suggests a return of the auxin effect. This indication led to the following experiments. About 30 minutes after the beginning of application of auxin, or as soon as the rate had returned to normal, the auxin solution was removed and replaced by fresh, aerated water. The streaming then continued at the normal rate. After a definite interval of
time, auxin was again introduced. If this interval were about 30 minutes or more, at 24°C., an acceleration of streaming was again observed, approximately equal in amount to that obtained during the first auxin treatment (Fig. 1). The coleoptiles have thus completely recovered their ability to react. This recovery must be due to the regeneration of the factor which has been exhausted; the subsequent experiments show that this factor is sugar.

If the streaming rate be allowed to return to normal, and, instead of pure auxin solution, a solution of the same concentration of auxin in aerated sugar solution be now applied, an immediate rise in streaming rate is obtained. The period of about 30 minutes required for recovery thus completely disappears if sugar is present.

Fig. 2 shows the effect of treatment with different concentrations of fructose, and indicates that 1 per cent is sufficient to cause the complete return of auxin sensitivity.

Fig. 3 compares the activity of a number of different carbohydrates in 1 per cent solution. It will be seen that fructose causes an immediate rise, sucrose and maltose a slower rise, which, however, reaches the same final rate. Curve E shows that soluble starch acts in the
same way which, in view of its large molecular size and consequent slow rate of entry, is rather surprising. It is noticeable, however, that its effect resembles that of maltose, and may perhaps be ascribed to the maltose present in such heat-treated starch. The effect of

![Graph showing recovery in solutions of different carbohydrates.](image)

**Fig. 3.** Recovery in solutions of different carbohydrates. Experiments as Fig. 2. At second arrow, solutions replaced by (curve A) fresh plain auxin; (curve B) auxin in 1 per cent fructose; (curve C) auxin in 1 per cent sucrose; (curve D) auxin in 1 per cent maltose; (curve E) auxin in 1 per cent soluble starch; (Curve F) auxin in 0.33 per cent NaCl. 5 cm. coleoptiles: aerated water.

the sugars is not simply osmotic, for curve F shows that the same molar concentration of NaCl has no such effect.

Further, so long as the sugar-auxin solution is supplied, the increased rate is maintained. This is made clearer by the experiments
of Fig. 4, in which the auxin is applied in sugar solution from the start. Under these conditions, the streaming rate rises in the usual way to its maximum value and remains there. The increased rate was found to remain constant for at least 2 hours. Fig. 4 also shows that the application of fructose alone to the coleoptile is without any effect. The fructose curve shows a higher initial streaming rate because carried out later in the year (see section IV).

![Fig. 4](image_url)

**Fig. 4.** Curve 1, auxin 0.01 mg. per liter in 1 per cent fructose (mean of two experiments). Curve 2, 1 per cent fructose alone. 5 cm. coleoptiles: aerated water: the rate at time 0 is the rate in water.

![Fig. 5](image_url)

**Fig. 5.** Curve 1, auxin 1 mg. per liter; curve 2, auxin 1 mg. per liter in 1 per cent fructose. 5 cm. coleoptiles: aerated water.

This action of sugars in maintaining the increased rate of streaming makes it at once possible to correlate the effect of auxin on streaming with its effect on growth. In the growth experiments, which are always conducted over periods of more than 30 minutes, the coleoptile is able to regenerate its carbohydrate supply (from storage products) continuously, and thus to respond to auxin over long periods.
The carbohydrate reserve is probably starch, since it has been shown that statolith starch is usually present in *Avena* and other coleoptiles even when grown in the dark (von Guttenberg, 1912; Zollikofer, 1918). The stored carbohydrate could not be sucrose, since sucrose itself gives a very rapid effect (see Fig. 3), while the coleoptile evidently requires 15 to 30 minutes to obtain a fresh supply of active carbohydrate from the stored form.

Since concentrations of indole-3-acetic acid greater than about 0.5 mg. per liter cause a decrease in streaming rate, it was of interest to determine whether, in the presence of sugar, this decrease was also maintained. As Fig. 5 shows, it is maintained. Thus auxin concentrations which, alone, cause a temporary decrease in rate, cause a lasting decrease if they are applied together with fructose.

II

The Role of Oxygen

The experiments described above, as well as those in the preceding paper, were all carried out with coleoptiles 5 cm. long. When the experiments were extended to younger coleoptiles, it was found that in these the streaming rate does not remain constant in aerated water, but falls steadily just as does that of 5 cm. coleoptiles in unaerated water. If, however, oxygenated water was used, the rate remained constant. The 3 cm. coleoptiles therefore have a greater oxygen requirement. Now Bonnet (1934) has shown that the rate of oxygen uptake of 5 cm. coleoptiles is not a great deal lower than that of 3 cm. coleoptiles. Thus, at 77 hours, the $Q_{O_2}$ (i.e. cubic millimeters $O_2$ consumed per hour) was 0.31 per mm. coleoptile length, while at 100 hours, the corresponding figure was 0.21. Hence, it is possible that the oxygen requirement of 5 cm. coleoptiles is only just being satisfied by aerated water. The experiments were therefore repeated in oxygenated water, which was prepared by bubbling oxygen from a tank through redistilled water cooled in ice. Using auxin solutions made up in this water, the decrease in rate of streaming caused by higher concentration is not obtained. Instead, there is an increase in rate about equal to that given by a concentration of 0.01 mg. per liter. Fig. 6 compares the “total effect” curves for coleoptiles in aerated and in oxygenated solutions. The total effect, as defined in
the preceding paper (Thimann and Sweeney, 1937) is a measure both of the extent of the acceleration and of its duration, and is obtained by measuring the area between the curve for auxin-treated plants and the curve for controls. The curve for aerated solutions is similar to that given in the preceding paper, but the values obtained in July and August are somewhat lower than those previously obtained in the winter (see section IV). Higher concentrations than were previously used were included in this series.

![Graph](image_url)

**Fig. 6.** The total effect of auxin on streaming. Curve 1, auxin in aerated water; curve 2, auxin in oxygenated water; curve 3, auxin in aerated water containing 1 mg. per liter DNP. Each point the mean of 1–12 determinations. Observations made July–August, 1937; 5 cm. coleoptiles.

It follows from Fig. 6 that the decrease in rate of streaming in auxin concentrations between 0.5 and 10 mg. per liter in aerated water is due to a deficiency in oxygen. No deficiency of oxygen occurs when the coleoptiles are not treated with auxin, since the controls maintain a constant rate in aerated water or inactive solutions. Hence, *the higher concentrations of auxin must increase the oxygen consumption of the coleoptile.* This deduction was confirmed by artificially increasing the oxygen deficiency still more.

Numerous workers have shown that 2,4-dinitrophenol (DNP) increases the oxygen consumption both of animal and of plant cells (see, for instance, Plantefol, 1932). *Avena* coleoptile sections were
therefore placed in solutions of DNP and their streaming rates followed. Fig. 7 shows that with 5 cm. coleoptiles, the streaming rate, which remains constant in aerated water, decreases steadily in aerated DNP solutions. The fall is extremely rapid at 100 mg. per liter (curve 1), moderately rapid at 10 mg. per liter (curve 2), and is not observable at 1 mg. per liter (curve 3). Immediately upon removing the DNP solution, the streaming rate returned to normal. Hence the action of DNP is in no way permanent, and evidently consists simply of an increase in the oxygen consumption. This point is borne out by the experiments on 3 cm. coleoptiles described in the next section.

Since a concentration of 1 mg. per liter DNP just causes no observable decrease in streaming, it presumably increases the oxygen consumption only to the point of balance, at which any further increase in the oxygen consumption will immediately become observable as an oxygen deficiency. Auxin solutions were therefore made up in aerated water containing DNP at this concentration. It was at once found that auxin concentrations which in pure water would
cause an increase in streaming, cause a decrease when in 1 mg. per liter DNP. Fig. 8 shows the action of 0.01 mg. indole-3-acetic acid alone and in presence of DNP. It is clear that 1 mg. DNP per liter reverses the sign of the auxin effect, while the lower DNP concentration gives an intermediate result.

The total effect of auxin on streaming over a series of auxin concentrations in the presence of 1 mg. per liter DNP is plotted in Fig. 6, curve 3. All the auxin concentrations studied decreased the streaming rate when in DNP. It follows, therefore, from this and the preceding data, that all auxin concentrations, except those above 10 mg.

Fig. 8. Modification of the effect of auxin by DNP. Curve 1, auxin 0.01 mg. per liter alone; curve 2, auxin 0.01 mg. per liter plus DNP 0.2 mg. per liter; curve 3, auxin 0.01 mg. per liter plus DNP 1 mg. per liter. 5 cm. coleoptiles: aerated water.

per liter, increase the oxygen consumption of the coleoptile tissue. This provides further proof that the decrease of streaming observed above in aerated water is due to oxygen deficiency.

We have attempted to bring about the opposite effect of DNP, i.e. to reduce the oxygen consumption of the tissues in general, by treatment with dilute cyanide solutions. However, cyanide, at $1.10^{-4}$ and $5.10^{-4}$ molal, reduced the streaming rate. The effect of cyanide is immediate and is therefore probably a direct effect on the streaming process itself. Simply draining off the cyanide does not immediately restore the initial rate, as in the case of oxygen deficiency, but 5–10 minutes elapse before the normal rate is again ap-
proached. That cyanide should exert a direct effect on streaming seems significant in view of Bonner's finding (1936) that cyanide inhibits growth of the *Avena* coleoptile.

Since, then, the retarding effect of high auxin concentrations is due to oxygen deficiency, the curves for growth and streaming may now be reconsidered. It is clear that they are approximately parallel. The effect of auxin increases steadily not only up to 0.01 mg. per liter, but up to about 2 mg. per liter, this latter increase being shown not by increased rate of streaming, but by an increased oxygen consumption.

The effects of still higher concentrations of auxin will be considered below (see Discussion, sections E, F, and H).

Two small points remain to be mentioned. Since oxygenation of the water has so marked an effect, it seemed possible that still higher concentrations of oxygen might further increase the streaming rate. Accordingly, observations were made on sections supported at the surface of an auxin solution in such a way that only the under half of the cylinder was immersed, and the cells under observation were actually in air. The concentration of oxygen in air is about eight times as high as in oxygenated water. However, the total effect of a given auxin concentration was no greater under these conditions than in oxygenated water under the coverslip. Further, the maximum streaming rate, which is reached 7–15 minutes after the application of auxin, is about 20μ per second; i.e., is also no greater. Hence, the maximum rate is not limited by oxygen.

Secondly, the maximum rate is also not limited by sugar. This follows from numerous measurements with auxin plus fructose in oxygenated water, which gave no consistently higher rates than those without the sugar. At the maximum rate the streaming is therefore limited by some other factor.

In experiments with sugar, since the acceleration or retardation is maintained for a long time, it is difficult to assign values to the total effect. We have, however, plotted the mean maximum rates reached against auxin concentration, and in this way have obtained curves very similar to the total effect curves. Using this procedure, it was found that the presence or absence of sugar does not affect the range of auxin concentrations over which acceleration, or retardation, is
produced. The results with DNP also furnish maximum rate curves similar to those of the total effect.

III

The Behavior of Younger Coleoptiles

(A) The Dependence of the Streaming Rate on Oxygen.—As mentioned above, the response of 3 cm. coleoptiles (77-78 hours old) was studied for comparison. Since the plants have a much higher oxygen requirement, their streaming rate does not remain constant in aerated water, but does so in oxygenated water. It has already been shown by Bottelier (1935) that the younger the coleoptile, the more readily can its streaming rate become limited by oxygen, and this, as mentioned in section II, is borne out by Bonner's measurements of respiration rates. In our experiments, the solution is ordinarily kept continuously changed by diffusion so that the oxygen deficiency may be partially made up. In order to determine the relative extents of oxygen deficiency at different ages, readings were taken on sections placed in just as much aerated water as the space under the coverslip would hold; i.e., 0.4 cc. This water was not changed. Fig. 9 shows that the rate in coleoptiles 72 hours old falls off with great rapidity, in 100 hour coleoptiles very slowly, and in 77 hour coleop-
tiles (3 cm. long) at an intermediate rate. This decrease in rate is solely due to oxygen deficiency, for as soon as the solution is removed, the rate returns to normal. This return is so rapid that intermediate streaming rates cannot be measured. Fig. 10 illustrates a series of experiments in which the solutions were changed in this way. At the first arrow, when the streaming rate had fallen to an approximately constant value, the water was removed, giving instantaneous recovery of the normal rate. After 5 minutes, fresh water (curve 1), auxin solution (0.05 mg. per liter) in the same water (curve 2), and auxin solution (1.0 mg. per liter) in the same water (curve 3) were applied. It will be seen that the decrease in rate begins in the auxin solution after 2 minutes, but in the water only after 5 minutes. The slope for the coleoptiles in auxin is also steeper than in water. The effect of the auxin in increasing the oxygen consumption is thus clearly seen. When a constant low streaming rate had been reached, the solution was again removed from one coleoptile, and, after recovery, dinitrophenol (2 mg. per liter) in the same water was applied. Here the decrease in rate begins before any reading can be taken, the slope is still steeper than before, and the rate reaches a very low value. Hence the effect of auxin is in this respect exactly comparable to that of DNP, only not so great. In the light of the dis-
cussion below, this is probably because DNP increases the whole of the respiration, while auxin increases only part of it.

(B) The Effect of Auxin on Streaming.—Using oxygenated water, therefore, the effect of auxin on 3 cm. coleoptiles was determined, the total effect being evaluated as previously described. The duration of the accelerating effect and the maximum value obtained were the same as in 5 cm. coleoptiles.

Curve 1 of Fig. 11 shows that the active auxin concentrations are very much lower than those for 5 cm. coleoptiles, the lowest effective concentration being about 0.0014 mg. per liter, as against 0.0033 for the latter. The entire curve is thus displaced to the left. The right hand arm of the curve differs from that of the 5 cm. coleoptiles, however, in that the higher auxin concentrations do not retard the streaming below that of coleoptiles in water (compare curve 1 of Fig. 11 with curve 1 of Fig. 6). The reason for this lies in the fact that even at high auxin concentrations, these coleoptiles, in oxygenated water, are only partially oxygen deficient; i.e., their “oxygen surplus” is greater than that of 5 cm. coleoptiles in aerated water. This is

Fig. 11. The total effect of auxin on streaming. Curve 1, oxygenated water: 3 cm. coleoptiles. Curve 2, oxygenated water: 5 cm. coleoptiles (cf. curve 2 of Fig. 6). Curve 3, aerated water: 3 cm. coleoptiles. Curve 4, oxygenated water: 3 cm. coleoptiles decapitated 90 minutes (triangles) and 180 minutes (squares) before start of experiment. Auxin 1 mg. per liter = 3 on abscissa.
shown by a comparison with the curves for the same material in the same range of concentrations, but made in aerated water (curve 3 of Fig. 11). Here the left hand arm is the same as in oxygenated water, i.e. the material is sensitive to lower concentrations of auxin than are the older coleoptiles. The right hand arm, however, shows close similarity to the curve for 5 cm. coleoptiles in aerated water (curve 1 of Fig. 6); i.e., higher auxin concentrations cause a retardation of rate. When the oxygen supply is reduced, therefore, application of auxin brings about a definite oxygen deficiency.

If it were possible to determine the effect of higher concentrations of auxin on streaming rates in still greater oxygen tensions, the total effect curve would doubtless closely parallel that for 5 cm. coleoptiles. This is shown by the one determination we have been able to make on 3 cm. coleoptile sections supported at the surface of the solution in air. At 0.05 mg. per liter, the value obtained (mean of two experiments) was 124, which, as will be seen, falls exactly on the curve for 5 cm. coleoptiles in oxygenated water (curve 2 of Fig. 11).

(C) The Effect of Decapitation.—A remarkable feature of the response of 3 cm. coleoptiles is the great effect of decapitation. Curve 4 of Fig. 11 shows that if the coleoptiles are decapitated 90 minutes before the start of measurements, the total effect curve is quite different from that of intact 3 cm. coleoptiles; the range of effective concentrations is now equal to that of 5 cm. coleoptiles. In this curve, the values for plants decapitated 90 and 180 minutes are plotted separately, but since they agree closely, a mean curve is drawn through them all. Thus, depriving the coleoptiles of their normal stream of auxin and other exudates from the tip for 1½ hours brings them approximately into the physiological condition of older coleoptiles, which have been naturally deprived of these secretions by the physiological senescence of the tip.

(D) Histidine: A Comparison with Fitting’s Experiments.—In a series of experiments, Fitting (1929, 1936) showed that, when Valisneria leaves are kept in pure water, their streaming ceases. If leaf extract, or any one of a number of pure substances, be now applied, the streaming recommences; one of the most active of such substances

Since under these conditions no constant rate is obtained in the controls, the points on this curve are only approximate.
is L-histidine. In our experiments, previously reported (1937), it was shown that histidine has, however, no effect on the normal streaming of 5 cm. coleoptiles. The experiments on 3 cm. coleoptiles, however, suggest an explanation of the rôle of histidine, because when the streaming rate under oxygen-deficient conditions is at a low value, it could be brought back to normal, either by increasing the oxygen supply, or by supplying histidine. Thus histidine presumably acts by decreasing the respiration. In the experiments of Fig. 12, coleoptiles 72 hours old were allowed to remain in 0.4 cc.

![Diagram of streaming rate in limited quantities of water.](Fig. 12)

Fig. 12. Effect of histidine on the streaming rate in limited quantities of water. Coleoptiles 72 hours old, in 0.4 cc. aerated water, replaced at second arrow by 0.4 cc. aerated histidine $6 \times 10^{-7} M$ (CE) or by 0.4 cc. aerated water (CD); again replaced at fourth arrow by 0.4 cc. aerated histidine $6 \times 10^{-7} M$ (FG). In histidine, oxygen deficiency appears only after 8 minutes, in water after 2-4 minutes.

aerated water, and the rate at which they became oxygen deficient was determined (cf. also Fig. 9). The curves show how closely the decrease in rate is repeated from experiment to experiment. The water was then withdrawn and after recovery in air, an aerated solution of pure L-histidine 0.1 mg. per liter, or $6 \times 10^{-7} M$, was applied to one of the coleoptiles, and fresh aerated water to the other: the rate at which the coleoptiles became oxygen deficient was again determined. The solutions were then once more withdrawn and, after recovery in air, histidine solution was applied to the coleoptile
which had previously been only in water. Thus the effect of histidine is given by comparing FG with CD and AB, or CE with CD (Fig. 12). It is clear that in the presence even of this low concentration of histidine—which is about equal to the threshold concentration active in Fitting’s experiments—the rate of onset of oxygen deficiency is much less than normal and hence histidine must reduce the respiration of the coleoptiles. In other experiments, it was found that when the streaming rate had fallen to a low value, the application of histidine, at the same concentration, increased the rate almost to normal. Clearly if the stoppage of streaming in immersed Vallisneria leaves be also due to oxygen deficiency, then the streaming would be restarted by reducing the respiration. Whether all the substances which start streaming in Vallisneria act by reducing the respiration rate is of course not demonstrated, but it seems highly probable that this is the effect of histidine.

Recently Fitting (1937) has shown that also auxin restarts the streaming in Vallisneria, but only in concentrations above 0.0001 M; i.e., 18 mg. per liter. If restarting is due to a decrease of respiration, then auxin would not be expected to bring it about except at very high concentrations. Reference to curve 2 of Fig. 6 shows that 18

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² In unpublished experiments Bonner (1934) found that histidine \(3 \times \times 10^{-3}\) molal reduced respiration of coleoptile sections by 25 per cent.
mg. per liter falls well on the downward slope of the curve, where auxin appears to be decreasing the respiration. This satisfactorily supports the above explanation.

IV

The Variation of Streaming Rate with Time of Year

During the course of these experiments, it was found that the streaming rate of controls in pure water became steadily faster. In November, 1936, the mean was 12.4μ per second and in August, 1937, 17.6μ per second. Table I summarizes the data from all our observations. With the exception of June, 1937, the means are derived from at least twenty observations each. If the results are classified by time of day, they show no consistent variation, a fact which was also noted by Bottelier (1934). Coincident with the higher values from May to August, the effect of added auxin was found to be less, both at low and at high concentrations. The total effect curve (Fig. 6) determined in July and August is noticeably flatter than that determined in the winter and given as Fig. 4 of the preceding paper (Thimann and Sweeney, 1937).

DISCUSSION

The experiments raise a number of new points, not all of which can be immediately explained. Some call, however, for special consideration.

In the experiments with carbohydrates, one of the most surprising conclusions is that simple sugars evidently enter the cell with extreme rapidity. The effect of fructose is observed within a minute of application. This stands in clear contrast with classical experiments on permeability, in which the sugars are always the slowest substances to enter. Whether this contrast is due to the use of mature, non-growing cells in permeability experiments, as opposed to the young, growing cells of coleoptiles cannot be said. The sugar must actually penetrate the cell in these experiments, since the inactivity of NaCl shows that the effect cannot be a simple osmotic one. Permeability experiments, however, depend upon the entry of sugar into the vacuole; our data only indicate its entry into the protoplasm. Nevertheless, this may be physiologically more significant than entry into the vacuole.
solutions. This discrepancy is easier to understand if we consider the following three facts. First, the increase of respiration caused by auxin may not be very large. It is great enough to cause coleoptiles which had an adequate oxygen supply, when in aerated water, to become oxygen deficient when auxin is added. It is not, however, great enough to induce an oxygen deficiency when the 5 cm. coleoptiles are in oxygenated water. It is possible, indeed, that the decrease in streaming rate provides a very delicate indicator of increased respiration. Second, the increase in the absence of added fructose is transient, and is mostly over within 20–30 minutes. Since the rate rises to a peak in 10–15 minutes with an immediate subsequent fall, the mean increase of rate over a 30 minute period is only 14–20 per cent of the rate in the controls (see Fig. 2 of Thimann and Sweeney, 1937). Third, concentrations of auxin up to 0.01 mg. per liter (equal to 1 on the log abscissa) evidently cause little increase of respiration; a marked increase, as measured by development of a marked oxygen deficiency, is caused only by concentrations above 0.1 mg. per liter. This concentration is about 10 units per cubic centimeter. Neither Bonner nor van Hulsen used auxin concentrations higher than this.

While it is hoped to carry out respiration measurements at a later date, we feel at present that the failure to detect an increase of respiration on treating coleoptile sections with pure auxins is sufficiently explained by these three considerations.

The only fact which does not fit in with this interpretation is the failure of auxin concentrations above 0.05 mg. per liter to increase the oxygen deficiency (as measured by retardation of streaming) of coleoptiles in dinitrophenol. Up to about 0.05 mg. auxin per liter the oxygen deficiency in DNP increases regularly with increasing auxin concentration (curve 3 of Fig. 6), but beyond this point the curve in DNP (1 mg. per liter) approaches the curve in plain aerated water, meeting it at 1 mg. auxin per liter. At this concentration, however, there are equal numbers of molecules of auxin and DNP present per unit volume (molecular weight of indole-acetic acid = 175; molecular weight of DNP = 184), and this suggests that some interaction is taking place between auxin and DNP, whereby the action of the DNP is inhibited. That such an interaction occurs is supported by observations on more dilute DNP solutions. In con-
centrations of 0.2 mg. DNP per liter, which, as Fig. 8 shows, still exert considerable influence on the respiration, the total effect curve in DNP meets the curve in plain aerated water at an auxin concentration of 0.2 mg. per liter. That is, while 1 mg. DNP is rendered ineffective by 1 mg. auxin, if only 0.2 mg. DNP is present it is rendered ineffective by 0.2 mg. auxin. At auxin concentrations above this point of ineffectiveness, the streaming response is not altered by the presence of DNP (Fig. 6, curve 3).

There is also some chemical evidence that compounds might be formed between DNP and indole-3-acetic acid (see the discussion in Sidgwick, 1937).

We can now return to consideration of the fundamental problem—the mechanism of the action of auxin. It was shown above that while low concentrations of auxin accelerate streaming, higher concentrations in presence of a limited oxygen supply actually retard it. This may be interpreted as follows:—

The respiration of the coleoptile comprises the oxidation of several substrates, of which sugar is doubtless one of the most important. One of these oxidation processes controls the rate of protoplasmic streaming, and is itself evidently accelerated by auxin. Its oxygen consumption must be only a small fraction of the whole. If now we make one assumption, namely that auxin also accelerates another oxidation process, which does not control the rate of streaming, the observed facts can be satisfactorily explained. We have the following system:

I. Sugar + O₂ auxin-sensitive protoplasm streaming and growth
II. Sugar + O₂ auxin-sensitive no effect on streaming
III. Other substrates + O₂ auxin-insensitive no effect on streaming

The discussion will be made clearer by reference to the lettered sections of the diagrammatic Fig. 13.

Section A.—Low concentrations of auxin accelerate reactions I and II, the acceleration of I causing an acceleration of streaming. It is important that these concentrations (0.0033 to 0.01 mg. per liter for 5 cm. coleoptiles) are also the lowest which cause an increase in growth rate. The 3 cm. coleoptiles, which give a growth response to
somewhat lower auxin concentrations, correspondingly give a streaming response down to 0.0014 mg. per liter.

Section B.—Higher auxin concentrations bring about a greater acceleration of reaction I, but since both I and II consume oxygen, there will be a competition for oxygen. The result will be that the effect of auxin on I cannot be fully exerted—we get a smaller acceleration of streaming rate.

Section C.—Still higher auxin concentrations accelerate reaction II to such an extent that oxygen is actually withdrawn from I, which is therefore retarded.

Section D.—This retardation does not, of course, occur in oxygenated water; instead, the acceleration of I is maintained over a considerable range of auxin concentrations, being limited here by some other factor, such as perhaps the amount of the enzyme for this reaction. The observations at the end of section 2 make it clear the limiting factor cannot be oxygen or sugar.

The fact that the total effect does not increase with increasing auxin concentration above 0.01 mg. per liter, i.e. that section D is parallel to the axis, agrees with some measurements of the growth of coleoptile sections floating on auxin solutions for 1 hour. In this short period the agreement between streaming and growth should be at its best. Correspondingly the measurements show about the same increment for all auxin solutions from 0.01 to 10 mg. per liter.

Section G.—In the presence of dinitrophenol the effect of auxin is the opposite of that in section A. Here reaction III, which comprises other oxidative systems of the cell, is accelerated and conse-

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**FIG. 13.** Total effect curves: diagrammatic. ABCFH in aerated water; ADEH in oxygenated water; GF in aerated water plus DNP 1 mg. per liter. 5 cm. coleoptiles. Auxin 1 mg. per liter = 3 on abscissa.
quently the tissues are at the verge of oxygen deficiency; any acceleration of I and II leads to an increased demand for oxygen and I is retarded on account of it. This explanation, as also that of sections B and C, depends on the "affinity" of reaction II for oxygen being greater than that of reaction I, so that II can withdraw oxygen from I. This may be due to a difference between the enzymes of I and II, a larger amount of its enzyme present, or other reasons. Although II thus differs from I in its oxygen affinity, its auxin-sensitivity is evidently the same as that of I.

The fact that section G meets section C at about 1 mg. auxin per liter has been discussed above. By contrast with DNP, the action of cyanide is evidently exerted directly on reaction I, although it may involve reactions II and III as well.

Section E.—At high auxin concentrations, above 1 mg. per liter, the streaming in oxygenated water is accelerated less. These, then, are the concentrations at which the effect of auxin on reactions I and II begins to be reduced; i.e., the "supramaximal" zone.

Section F.—Correspondingly, in aerated water, since II is less accelerated the oxygen demand is less and hence I is retarded less; i.e., the "aerated" and "oxygenated" curves both approach zero.

Section H.—At auxin concentrations above about 50 mg. per liter the system is evidently damaged, for the streaming rate falls irreversibly. This final lowering is not reversed by the presence of oxygen and is evidently due to some toxic effect of high auxin concentrations. It is noteworthy that these concentrations are those which cause actual shrinkage of the coleoptile. This may be seen by comparing Fig. 6 with the growth curve given as Fig. 5 in Thimann and Sweeney (1937).

In the range of sections E and F, it is possible that a discrepancy exists between the curves for growth and for streaming. Auxin concentrations of 20 and 100 mg. per liter cause a considerable increase in growth during the 1st hour, but have only little effect on streaming. Since the amount of growth in the period covered by the streaming measurements is very small, however, it is hard to obtain satisfactory data for it. Curvature measurements, by the agar technique, certainly show decreased angles in this range. Further experiments will be necessary to decide this point.
In conclusion, the close parallel between the effect of auxin on streaming (in presence of sufficient oxygen) and the effect of auxin on growth—a parallel which the present experiments have greatly extended—indicates that the reactions giving rise to accelerated streaming and to growth are either the same or are mutually interdependent. While streaming may go on without growth, as in resting cells, old coleoptiles, or other non-growing tissues, it is probable that growth cannot go on without streaming. Hence in the chain of auxin-induced reactions, the effect on streaming doubtless precedes that on growth. This is also supported by the extremely rapid onset of the effect on streaming.

The experiments and the theory outlined above provide an explanation for the close relationship between growth and respiration elucidated by Bonner (1936). They fit in well with the findings of Schneider (1938) that the action of auxin on the coleoptile is largely dependent on the presence of sugar, and that under suitable conditions growth may be limited either by auxin or by sugar. They suggest also that the effect of light on streaming, studied by Bottelier (1934) may be exerted through an effect of light on auxin relations. They give a tentative explanation for the remarkable results of Fitting. Finally, they provide a satisfactory basis for the known fact that auxin brings about a number of apparently independent responses in the cell, since an acceleration of protoplasmic movement, through accelerating the movement of nutrients or other substances in the protoplasm, might bring countless other effects in its train.

SUMMARY

1. A further study has been made of the effect of indole-3-acetic acid (auxin) on protoplasmic streaming in the epidermal cells of the *Avena* coleoptile.

2. The transient nature of the effect of auxin, both in accelerating and retarding streaming, is due to the temporary exhaustion of carbohydrate from the tissues. In presence of 1 per cent fructose or some other sugars the acceleration or retardation of streaming by auxin is not transient, but is maintained for at least 2 hours.

3. The retardation of streaming brought about by concentrations of auxin above 0.5 mg. per liter is due to oxygen deficiency. This has been confirmed in several ways.
4. It follows that the effect of auxin is to increase the respiration of the coleoptile tissue.

5. Younger coleoptiles, 3 cm. long, are sensitive to lower concentrations of auxin than those 5 cm. long, and more readily exhibit oxygen deficiency as a result of the action of auxin. However, after decapitation their response to auxin more closely resembles that of 5 cm. coleoptiles.

6. The retardation of streaming in such coleoptiles, resulting from oxygen deficiency, is delayed by very dilute solutions of histidine. On this basis an explanation is suggested for the results of Fitting on streaming in Vallisneria leaves.

7. The mean rate of streaming in control untreated coleoptiles in pure water varies with the time of year, but not with the time of day.

8. The results support the view that auxin accelerates an oxygen-consuming process which controls the rate of protoplasmic streaming, and that the latter controls growth. The substrate for this process is probably sugar.

9. It is suggested that auxin also accelerates another oxygen-consuming process, which may withdraw oxygen from the process which controls streaming rate and hence cause retardation of the latter.

REFERENCES

Schneider, C. L., 1938, Am. J. Bot., 26, in press.