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AUXIN-SYNTHESIS INHIBITION BY SUGARS, NOTABLY BY GALACTOSE

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SUMMARY

In decapitated *Avena* coleoptiles the regeneration of the physiological tip is inhibited by glucose, galactose, lactose and raffinose. Galactose is the strongest inhibitor, and a further analysis of its action indicates that it inhibits auxin synthesis. The galactose effect is not antagonized by other sugars.

Sucrose, maltose, fructose, rhamnose and ribulose have no influence on the regeneration.

1. INTRODUCTION

The literature on the regulation of the synthesis of the regulatory substances themselves is negligibly small. This state of affairs is of special inconvenience to those investigators who try to interpret the growth and the development of the whole plant. It cannot be denied that the effects of auxins on these processes are numerous and diverse, and it is known from the research on the tropisms that light and gravity operate by changing the metabolism, the transport and the distribution of auxins. It is also true that the growth and the development of the whole plant are being regulated by nutritional factors like carbohydrates and nitrates, and the question arises whether these factors exercise their influence in the same way, that is by changing the metabolism and the distribution of hormones.

In connection with this problem it was thought worth examining the influence of a variety of sugars on the synthesis of auxin. For that purpose the coleoptile seemed to be the proper test object, since the capacity of this organ to produce auxin has often been studied before. This is connected with the fact that the removal of the tip (decapitation) causes the distal cells of the stump to regenerate a new auxin production centre, a process that can be influenced in different ways (ANKER 1973).

It appeared from previous investigations (ANKER et al. 1973) that the time required for the regeneration of the physiological tip is not a matter of precursor supply since neither tryptophane nor tryptamine could bring about an earlier resumption of the growth. No more did addition of sucrose or changing the pH speed up the regeneration, whereas glucose caused a little delay. The regeneration processes, however, were completely inhibited by the presence of the end product itself (IAA), even in concentrations as low as 0.01 mg/liter, whereas the auxin analogues 2,4-D and NAA were inactive in this respect.

From these results it follows that the time required for the regeneration is

determined by the falling of the concentration of the residual auxin to a critical level, and by the production of those enzymes which catalyse the synthesis of auxin.

In connection with the above-mentioned want of information on the regulation of auxin synthesis, and encouraged by the earlier observation that glucose had some influence, a number of sugars, all common in plant cells, was tested in this respect. Sucrose, maltose, fructose, rhamnose and ribulose had no influence, glucose, lactose and raffinose caused a transitory inhibition, whereas galactose appeared to be a very strong inhibitor of the regeneration. The effect of this sugar was analysed in greater detail.

2. MATERIAL AND METHODS

As in previous work on this subject the regeneration of the physiological tip was studied with the *Avena* coleoptile. After decapitation, which means the cutting off of a tip of exactly 1 mm length, 12 coleoptile cylinders, comprising the apical 18 mm of the stump, were put on pins and then submerged in the solution the activity of which was determined by examining the course of the growth rate of the sections over 5 hours. These determinations were done by taking shadowgraphs at intervals with phototropically inactive light. The first shadowgraph, giving the initial length, was made after an accommodation time of 15 minutes in the solution, the following ones were made at intervals of 1, ½, ½, ½, ½, 1 and 1 hour.

Submerging the sections in the solution to be tested has the advantage over supplying the substance in agar blocks to coleoptiles in air, that the concentration of the added substance practically remains constant, since the volume of the solution was one liter. The solution was constantly aerated and submersion does not injure the growth.

The experiments were carried out in weak incandescent light filtered by red selenium glass, at 23 °C. The method has been described in detail by ANKER (1954).

3. RESULTS

The present work is not a systematic investigation of the effect of all sugars, known to be present in plants, on the auxin synthesis. The choice was partly made for reasons becoming evident with the description of the results.

3.1. Sucrose, maltose, fructose, rhamnose and ribulose

In *fig. 1* the results have been brought together of experiments with the sugars that had no influence on the regeneration of the physiological tip, the course of the growth in the sugar solution being similar to that in water. During the first hours the growth rate decreased rapidly due to exhaustion of the residual auxin. At about the beginning of the third hour the minimum level was arrived at. The length of the period of minimum growth was different at different oc-

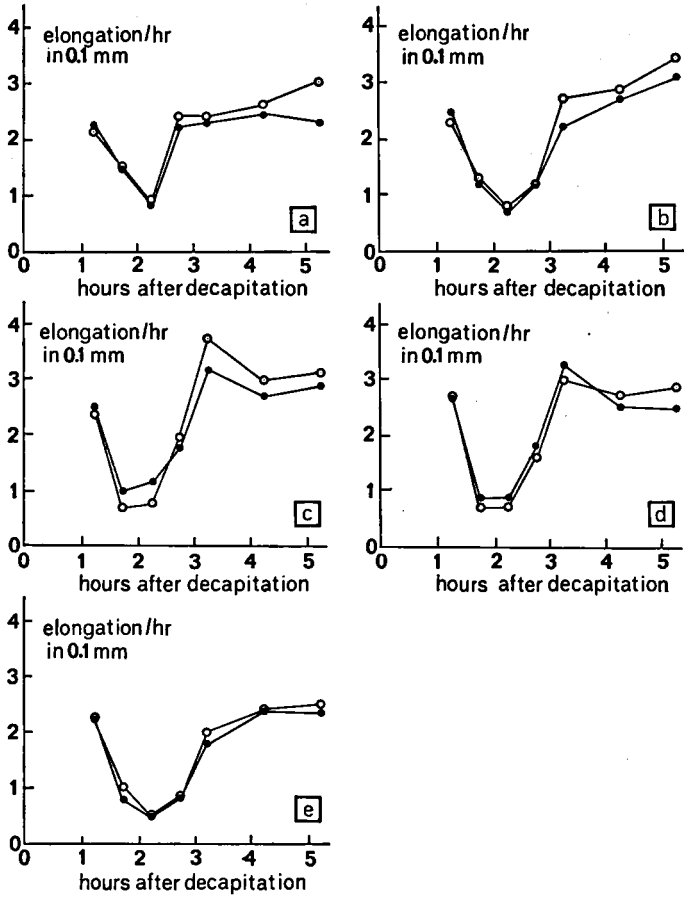

Fig. 1. The regeneration of the physiological tip is not influenced by 1% sucrose (a), 0.5% fructose (b), 1% maltose (c), 0.5% rhamnose (d) and 0.42% ribulose (e). ●–● course of growth in water; ○–○ idem in the sugar solution.

casions, and the cause of the differences is obscure. It was not connected with the habitus of the coleoptiles at the moment of decapitation (long, short, slender, thick etc.) so that the type of growth was not predictable. After this period the growth increased more or less rapidly to the level observed at the beginning of the experiment.

During the first three hours of each experiment the course of the growth of the sections in water ran parallel to that of the sections submersed in the sugar solution, which is obviously due to the internal IAA concentration being the limiting factor. After the regeneration all sugars except ribulose caused a small promotion of the growth. It may not be excluded beforehand that these sugars promoted the auxin synthesis once it had begun, but is seems simpler to ascribe the stimulation to their well-known nutritional value.

3.2. Glucose

Glucose, added in concentrations down to 0.1 %, caused a transitory inhibition of the regeneration of the physiological tip. This confirms the observation of Anker et al. that the geotropic reaction of coleoptile sections is retarded by glucose if the curvature was dependent on the auxin formed in the new tip. From the observations represented in *fig. 2* one notices that the delay of the auxin synthesis was about half an hour to one hour. In the concentration range from 0.1 to 1 % the activity was not proportional to the concentration of the sugar. The variation of the inhibitions observed with different glucose concentrations was not greater than the differences found in duplicate experiments with the same concentration. Things became different after the third hour of

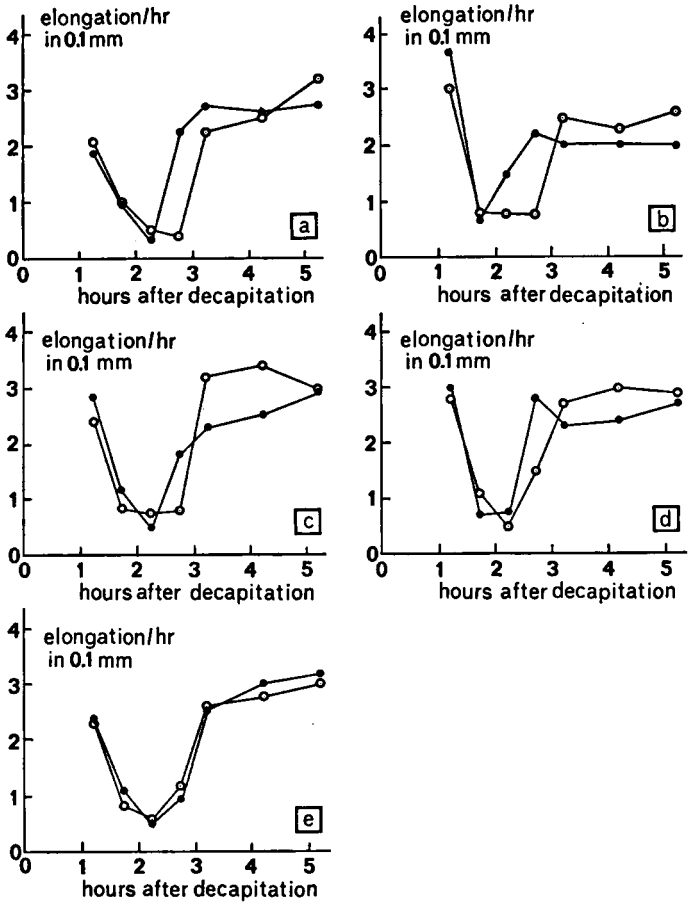

Fig. 2. Delay of the regeneration of the physiological tip by glucose in the 1 %, 0.5 %, 0.25 % and the 0.1 % concentration (a–d, resp.), but not in the 0.05 % concentration (e). ●–● course of the growth in water; ○–○ idem in the glucose solution.

the experiments, that is when the regeneration of the physiological tip had been completed. From that moment the rate of growth evidently was influenced by the glucose concentration. The 0.25% concentration seemed to be optimal, but this value is not of general significance. The optimum glucose concentration for section growth in IAA solutions, for instance, is higher and it differs with the length of the experiment (RIETSEMA 1950).

3.3. Galactose, lactose and raffinose

Each of these three sugars inhibited the increase of the growth rate of the sections normally attending the regeneration of the physiological tip. Galactose showed the highest activity in this respect, and it is imaginable that the activities of lactose (= galactose + glucose) and of raffinose (= galactose + fructose + glucose) were arising from the galactose moiety released by hydrolysis.

Fig. 3 makes clear that galactose is a potent inhibitor, its action being already manifest at the 0.05% concentration. In contrast to the observations with glucose, there was a clear proportionality between the extent of inhibition and the concentration.

Fig. 4 illustrates the effects of lactose and raffinose. As with galactose, but contrary to glucose, the inhibiting action of lactose increased with the concen-

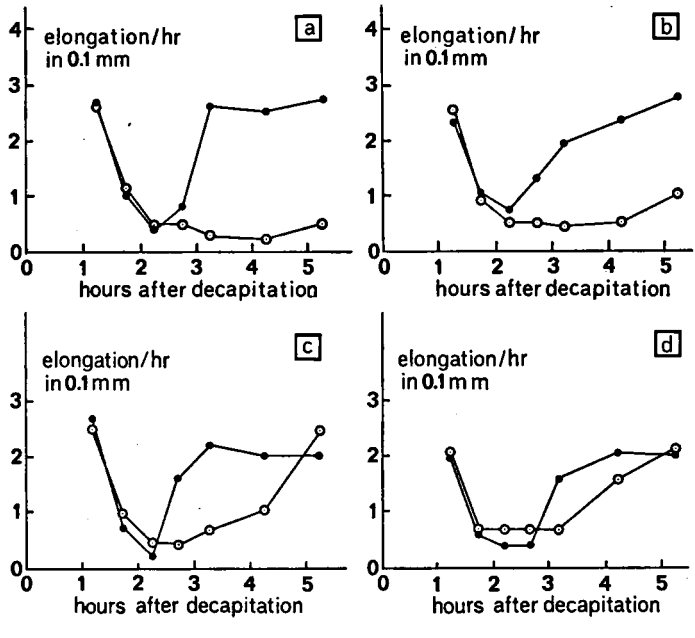

Fig. 3. The inhibition of the regeneration of the physiological tip by galactose in the 0.5% (a), 0.25% (b), 0.1% (c) and the 0.05% (d) concentration. ●–● course of growth in water; ○–○ idem in the galactose solution.

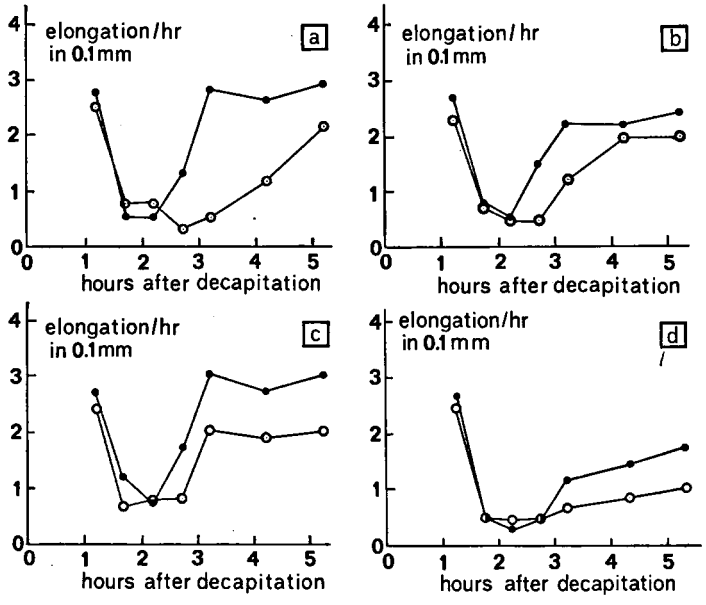

Fig. 4. The inhibition of the regeneration of the physiological tip by 2% (a) and 1% (b) lactose, and by 1.6% (c and d) raffinose. ●–● course of growth in water; ○–○ idem in the sugar solutions.

tration. The activity of raffinose was studied only in the 1.6% concentration, being osmotically equivalent to a 0.5% galactose solution. The reason why the results of both experiments with the same raffinose concentration have been presented in *fig. 4* is to demonstrate the influence of the age of the coleoptiles on the power of regeneration. The coleoptiles available for the experiment of *fig. 4d* were near the moment the primary leaf comes through, and it is seen from the course of the growth of the control sections in water that the regeneration became deficient.

3.4. Further analysis of the galactose effect

Because galactose appeared to be the strongest inhibitor, the effect of this sugar was studied in greater detail. The observation that the inhibitory effect of galactose begins at the moment the control coleoptiles in water resume their growth due to the regeneration of the physiological tip, suggests that galactose inhibits auxin synthesis. With galactose, however, one has to be very cautious because other mechanisms of action have been proposed in the literature. The results of ORDIN & BONNER (1957), for instance, suggest that in the *Avena* coleoptile galactose specifically inhibits cell elongation by interfering with cellulose synthesis. Besides, it was found in own experiments that the galactose inhibition of the growth also appeared in the presence of IAA *(fig. 5)*.

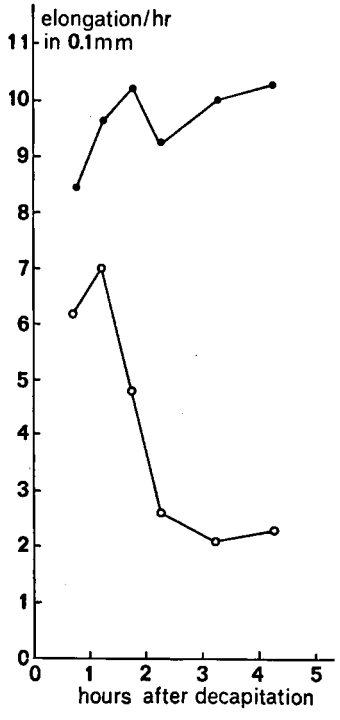

Fig. 5. Growth inhibition by galactose in the presence of IAA. ●–● section growth in 0.5 mg/l IAA; ○–○ idem in 0.5 mg/l IAA + 0.5% galactose.

For these reasons additional experiments were required in order to make sure that the inhibitions described in the previous section were not due to other effects. The following result *(fig. 6)* indicates that the inhibitions observed in the experiments of *fig. 3* were really due to a lack of auxin. Addition of IAA when the growth was at its minimum caused a sharp increase of this process in spite of the presence of galactose in the concentration that caused a lasting inhibition in the absence of IAA (see *fig. 3a*).

Further support is derived from the observation that transfering the sections

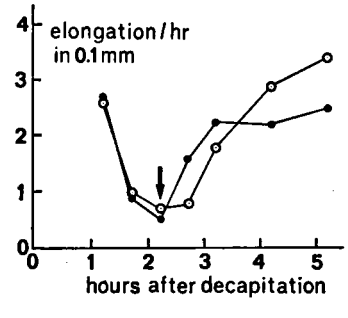

Fig. 6. Cancelling the growth inhibition due to galactose by addition of IAA. ●–● section growth in water; ○–○ idem in 0.5% galactose with addition of IAA to the final concentration of 0.25 mg/l after the second hour.

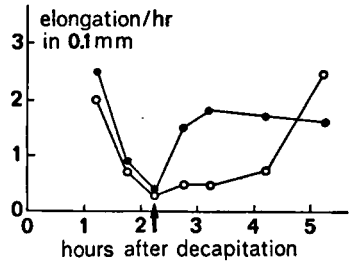

Fig. 7. Not until two hours had passed, the replacement of galactose by sucrose cancelled the growth inhibition due to galactose. ●–● section growth in water; ○–○ idem in 0.25% galactose, and, after the second hour, in 0.5% sucrose.

from a 0.25% galactose to a 0.5% sucrose solution, two hours after the decapitation, was not immediately followed by a rise of the growth. The increase of the growth occurred not until another two hours had passed *(fig. 7)*. The length of this period was not determined by galactose inhibiting the permeation of sucrose, nor by a competion between these sugars inside the cells. These possibilities are excluded by the fact that a favourable effect of sucrose on the growth rate did not stay out long if the growth was rendered possible by the presence of IAA *(fig. 8)*.

These possibilities then being excluded, the result of the experiment of *fig. 7* indicates that at the moment of replacing galactose by sucrose no appreciable amount of auxin was present in the tip of the stump, and that the production of the enzyme system responsible for the synthesis of auxin was not yet in progress.

On account of the presence of data in the literature on a reversal of galactose

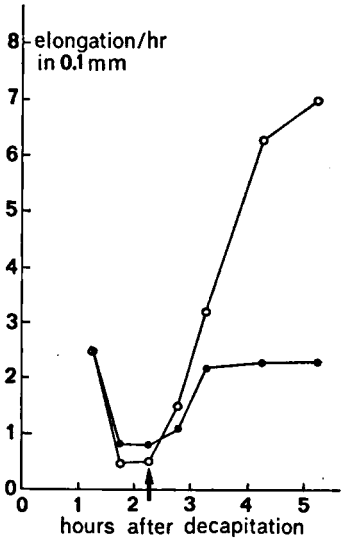

Fig. 8. The replacement of galactose by sucrose + IAA is immediately followed by a rise of the growth. ●–● section growth in water; ○–○ idem in 0.25% galactose, which was replaced by 0.5% sucrose + 0.5 mg/l IAA at the end of the second hour.

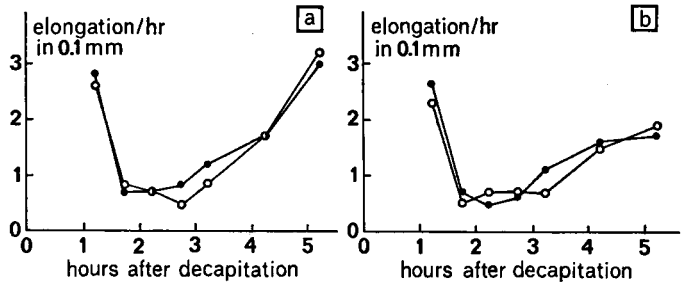

Fig. 9. No cancelling of the galactose-induced growth inhibition by the simultaneous addition of glucose. Fig. 9a. ●–● section growth in 0.1% galactose; ○–○ idem in 0.1% galactose + 0.4% glucose. Fig. 9b. ●–● section growth in 0.1% galactose; ○–○ idem in 0.1% galactose + 0.9% glucose.

effects by glucose, it was tried wether the present galactose effect could also be neutralised by glucose or fructose. KNUDSON (1915) was the first to observe an inhibiting effect of galactose on the growth of whole plants belonging to different genera. The toxic effect of galactose could be counteracted by glucose.

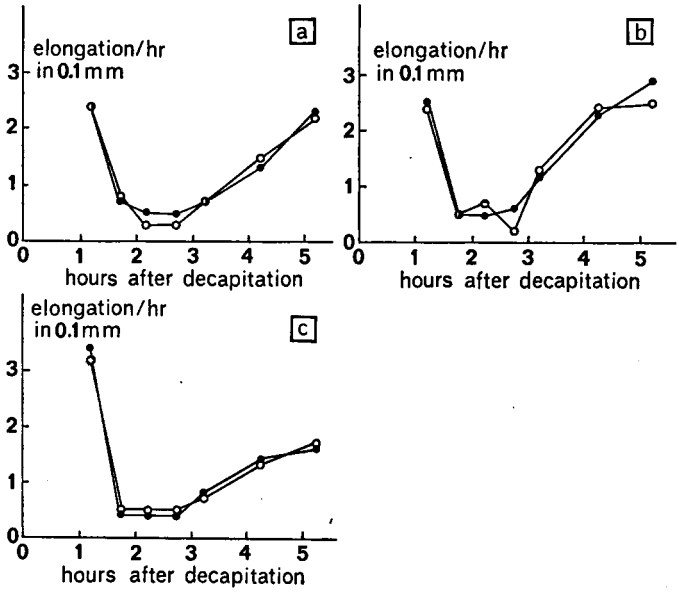

Fig. 10. No cancelling of the galactose-induced growth inhibition by the simultaneous addition of fructose. Fig. 10a. ●–● section growth in 0.1% galactose; ○–○ idem in 0.1% galactose + 0.4% fructose. Fig. 10b. ●–● section growth in 0.1% galactose; ○–○ idem in 0.1% galactose + 0.9% fructose. Fig. 10c. ●–● section growth in 0.15 % galactose; ○–○ idem in 0.15% galactose + 0.6% fructose.

FERGUSON, STREET & DAVID (1958), experimenting with tomato roots, found that the inhibitory effect of concentrations up to 0.15% galactose could be fully antagonised by glucose if these sugars were added in the 5:1 ratio (glucose : galactose).

In the present investigation no action of glucose as an antidote against galactose was perceptible when these sugars were applied in the 5:1, nor in the 9:1 ratio *(fig. 9)*. This result was not too surprising since it had been found that glucose by itself caused a short-lasting inhibition of the growth *(fig. 2)*. But no more than glucose, fructose, a sugar which did not influence the regeneration *(fig. 1)* could counteract the inhibition caused by galactose, neiter in the 4:1 nor in the 9:1 ratio (fructose : galactose). This is illustrated by *fig. 10*.

This new aspect of the galactose activity: its high efficiency as an inhibitor of auxin synthesis, together with the absence of any influence of the more common sugars, suggests the possibility that galactose and galactose containing sugars have a special function in the regulation of the growth and the development of the whole plant.

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