Botany. — On the mechanism of auxin action. I. The influence of indole-3-acetic acid on the respiration of Saccharomyces cerevisiae. By L. ANKER. (Communicated by Prof. V. J. KONINGSBERGER.)

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Introduction.

In a recent publication called "The mechanism of auxin action" AUDUS (1949) reviewed critically the suggestions put forward in the last decade on the way in which growth substances affect the rate of cell elongation. According to one of these suggestions, originated by BONNER (1933) and supported by COMMONER and THIMANN (1941) and others, the primary action of growth substances consists in stimulation of the aerobic respiration.

Promotion of the rate of oxygen uptake, however, could not be confirmed in all cases. VAN HULSSEN (1936, in Avena-coleoptiles), BURSTRÖM (1942, in wheat roots) and others showed, that respiration was not altered by adding indole-3-acetic acid.

In the present investigation the influence of indole-3-acetic acid (hetero-auxin) on the rate of oxygen uptake of Saccharomyces cerevisiae in pure culture is studied.

The experimental part is divided in four sections:

a. experiments in which hetero-auxin did not influence aerobic respiration;

b. experiments in which acceleration of the oxygen uptake was observed;

c. effect of various concentrations of hetero-auxin;

d. microscopical examination of yeast cells treated with hetero-auxin.

From these experiments it is concluded, that hetero-auxin promotes respiration in an indirect way, namely by stimulating the breakdown of reserve carbohydrates into respirable substrate.

Material and methods.

Cells of Saccharomyces cerevisiae, inoculated in a dilute malt extract, were cultivated in continuously shaken flasks during two days at a constant temperature of 25°C. After centrifuging and washing a suspension was made in a 2½ % KH₂PO₄ solution (pH = 4.5), two ml. of which were pipetted into Warburg vessels in order to determine manometrically the rate of oxygen uptake by endogenous respiration (consumption of reserve food). Next, after about three quarters of an hour, hetero-auxin and — if necessary — glucose were added (end volume of the medium: 2½ ml).
The subsequent readings were divided by the initial value of endogenous respiration. This ratio was plotted on the graph.

The experiments were carried out at 25°C.

Experimental part.

a. The effect of hetero-auxin on exogenous respiration, the substrate occurring in excess.

These experiments were made in order to study whether hetero-auxin affects respiration if glucose is added in concentrations at which the enzyme system is saturated.

When for this purpose glucose was present in concentrations ranging from 3 to 0.5 per cent no significant effect of hetero-auxin (10 mg/l), in any case no promotion, could be observed. Fig. 1 shows the results with 1.0, 0.75 and 0.50 % glucose.

In accordance with data of Geiger-Huber (1934) a sugar concentration

![Diagram](image_url)

**Fig. 1.** No promoting influence of hetero-auxin on respiration, if substrate-concentration is supra-optimal.
of about 0.2 per cent was found to be optimal concerning the respiration of yeast cells. In lower concentrations glucose proved to become limiting factor in the process.

From the above mentioned experiments, in which substrate was abundant, one may conclude, that hetero-auxin does not increase the respiration capacity.

b. The effect of hetero-auxin when substrate concentration is the limiting factor in respiration.

When, however, glucose was added in suboptimal concentrations a marked promoting effect of hetero-auxin on respiration appeared immediately after the beginning of the experiment (see fig. 2, glucose concentration: 0.125 %). At the moment when the rate of oxygen uptake in the control started to decrease (after about 1½ hour), the respiration of the treated cells (10 mg/l hetero-auxin) was still increasing. In the next 4 hours of the experiment the difference between treated cells and controls became maximal.

The same effect was obtained at a double glucose concentration (0.25 %) but only after the moment at which substrate became limiting factor (fig. 2, after about 3 hours). In the first hours, when this concentration was still supraoptimal, no promotion of respiration by 10 mg/l hetero-auxin was found. The course of these curves will be dealt with in the discussion.

In this experiment, as well as in that of fig. 1, no significant influence was shown at the 0.50 % glucose concentration.

A strongly promoting effect of hetero-auxin (10 mg/l) was observed in experiments without glucose (fig. 3).
These results show, that hetero-auxin is able to promote the respiration of glycogen containing yeast cells when the amount of added substrate is the limiting factor in the process.

Fig. 3. Influence of hetero-auxin in four different concentrations on endogenous respiration.

c. Effect of various concentrations of hetero-auxin.

The results of an experiment concerning the question which concentration of hetero-auxin causes maximal stimulation of respiration is represented in fig. 4.

During the first hour of this experiment, in which glucose was added in a very low concentration (0.03 %), hetero-auxin in a concentration of 10 mg/l seemed to function optimally.

A similar experiment, without addition of glucose, confirmed this result (fig. 3).

In the subsequent part of the experiment of fig. 4, however, the influence of the concentration of 10 mg/l vanished gradually, whereas the stimulation by the highest concentration remained constant.
Hardly any influence of the lowest concentration of hetero-auxin used (0.1 mg/l) could be observed.

Fig. 4. Influence of hetero-auxin in four different concentrations on respiration (at the start of the experiment 0.03 % glucose was added).

A possible explanation of the existence of an optimal hetero-auxin concentration will be presented in the discussion.

d. Microscopical observation of yeast cells treated with hetero-auxin.

Glycogen containing yeast cells were suspended in a buffered solution (pH: 4.5) of hetero-auxin (100 mg/l) without substrate. Microscopical examination on the next days showed, that glycogen disappeared much sooner from the hetero-auxin treated cells than from the untreated ones. On the second day, after staining with jodine, the treated ones turned yellowish whereas the controls still coloured brown. In other cases a marked difference was apparent on the first day after inoculation.

This result seems to indicate that hetero-auxin accelerates reserve carbohydrate mobilisation in yeast.

Discussion.

From these results it may be concluded, that the promoting influence of hetero-auxin on the oxygen uptake of Saccharomyces cerevisiae is not the primary effect of this growth substance. Only when respiration is dependent on the presence of reserve substances the effect can be observed. Hence it is suggested, that the hetero-auxin induced increase of the rate of respiration is caused by an acceleration of the glycogen mobilisation.

This view is supported by Reinders (1938), Bausor (1942), Mitchell and Brown (1945), Rasmussen (1947), Smith (1948) and others who
showed a rapid increase of the amount of reducing sugars at the expense of starch present in the cell after application of growth substances.

From such observations, however, it is not permitted to conclude, that improved diastase- or phosphorylase activity is to be visualized as the primary effect of growth substances. WEINTRAUB and WOOD (1947) and the author (unpublished experiments) failed to demonstrate a direct stimulation of purified diastase by hetero-auxin in vitro. SMITH, LANGELEND and STOTZ (1947) did not succeed in confirming EYSTER'S results (1943, 1946), who showed a releasing of diastase from the bound state on charcoal by growth substances. These negative results might indicate, that improved hydrolysis (or phosphorolysis) of reserve carbohydrates is effected by a more fundamental process.

According to the opinion of PFLÜGER (1905), PRZYLECKI (1934), WILLSTÄTTER and RHODEWALD (1934) glycogen in the cell in some way is either bound to- or surrounded by proteins. TSAI (1937) and MEYER and PRESS (1941) showed, that glycogen, present in extracts of organs is attacked by α- and β-amylase in vitro only when the albumin had been precipitated. NORTHEN (1942) ascribed the decrease in structural viscosity of protoplasm, caused by hetero-auxin in his experiments to dissociation of cellular proteins. Thus, an influence of hetero-auxin on physico-chemical properties of protoplasm, resulting in an unlocking of glycogen from its bound state might be the cause of increased phosphorolysis.

The occurrence of an optimal hetero-auxin concentration in stimulating respiration might be explained by assuming, that higher concentrations are injurious to the cell. Remembering, however, that hetero-auxin (concentration 10 mg/l) just like 0.2 % glucose (optimal glucose concentration) is able to bring about the maximal oxygen uptake, one might imagine that a tenfold of this concentration releases so much substrate that its concentration becomes supraoptimal. In this way the hetero-auxin optimum might be explained as a consequence of the existence of a substrate optimum.

The remarkable course of the curves 3 and 4 of fig. 2, namely the descent in two steps separated by a nearly horizontal stretch, might be explained by assuming a temporary (re)synthesis of glycogen from glucose-1-phosphate or from the added glucose. In the first descent the amount of free substrate becomes the limiting factor; next, the new formed glycogen is used (horizontal part) at a rate equalling that of endogenous respiration at the beginning of the experiment (level 100); lastly the amount of free glycogen becomes the limiting factor causing a decrease of the rate of oxygen uptake to that of the use of bound glycogen.

The experiments of fig. 2 and fig. 4 show, that the duration of hetero-auxin action is limited. This substance is either used or inactivated. The highest concentration of hetero-auxin used (100 mg/l) proved to stimulate respiration during a considerable longer period than a concentration of 10 mg/l did (fig. 4).
Part of the contradictory results reported in literature on stimulation of respiration by growth substances possibly may be ascribed to the different organisms or tissues used. From experiments, which show an increased respiration upon addition of hetero-auxin one is not allowed to conclude to a stimulation of the respiratory enzymes unless substrate is in excess at the moment of adding. In case of a negative response of respiration one is not permitted to draw the conclusion, that growth substances do not stimulate the enzymes unless it is demonstrated, that substrate occurs sufficiently at the moment of adding this substance. From the present results it may be obvious, that in case of limitation of respiration by substrate concentration the amount of reserve carbohydrates in the cell determines whether the results will be positive or negative.

Summary.

Endogenous respiration of starving glycogen containing yeast cells is stimulated by hetero-auxin. Since exogenous respiration could not be stimulated by this growth substance it is stated that this stimulation results from an increased glycogen mobilisation, a process, which supplies substrate to the endogenous respiration.

About 10 mg/l hetero-auxin causes maximal stimulation.

Microscopical observation showed increased glycogen mobilisation on addition of hetero-auxin (100 mg/l) to the medium.

The experiments are continued.

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