been sensitized with *E. coli* antigen and which subsequently were exposed to *E. coli* antiserum (tubes D and E). Fixation of complement by these cells during the incubation in the ice bath was followed by their lysis on subsequent exposure to 37°C (Column 11, tubes D and E). Sheep cells which had been sensitized with *E. coli* antiserum failed to fix significant amounts of complement when they were exposed to dilutions of *E. coli* antigen solution (Column 9, tubes A and B). Subsequent incubation of these cells at 37°C failed to result in their lysis. (Column 11, tubes A and B).

*Summary.* The data presented in this paper confirm and extend the recent observations of Fisher and Keogh(2). Erythrocytes from sheep, rabbit, and man, which have adsorbed extracts from *E. coli* and *Salmonella typhosa,* or human plasma, are lysed in the presence of complement and of corresponding immune sera for the adsorbed antigens. Evidence for the specificity of the reaction has been presented. Neither appreciable complement fixation, nor lysis occurred when erythrocytes first sensitized with non-hemolytic immune sera were then exposed to complement and the corresponding antigens.

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## A Direct Relationship between Indoleacetic Acid Effects on Growth and Reducing Sugar in Tobacco Tissue.* (17973)

FOLKE SKOOG AND BETTY JANE ROBINSON (Introduced by W. H. McShan)
*From the Department of Botany, University of Wisconsin, Madison, Wis.*

Numerous attempts have been made to relate the effects of auxin (indole-3-acetic acid (I.A.A.); naphthalene acetic acid; and related compounds) on growth of plants with specific changes in metabolism and cellular constituents. In the case of toxic effects of 2,4-dichlorophenoxyacetic acid (2,4-D) decreases or temporary rises and subsequent depletion of available carbohydrates have been reported(1-4). An increase in reducing capacity in tissues, measured by staining, has been obtained from treatments of bean stem cultures with 2,4-D(5).

Recently Christiansen and Thimann(6) have demonstrated a decrease in reducing sugar in *Pisum* stem sections and *Avena* coleoptile sections grown for short periods of time in solutions of various compositions with auxin concentrations which promote growth. However, they report a similar decrease in controls without I.A.A. and even greater decreases of sugar from the addition of respiratory inhibitors, which completely stop growth, such as iodoacetate, arsenite, and fluoride. In general, tests on changes in composition associated with auxin treatments which promote growth have been carried out in short time experiments under conditions leading to net decreases in dry weight of the tissues, and where their continued growth was precluded. It was of interest, therefore, to carry out analyses for changes in cell constituents in tissues supplied with I.A.A. in media adequate for continuous growth over periods of several months. The experiments reported here, with tobacco stem segments

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1. Mitchell, J. W., and Whitehead, M. R., *Bot. Gaz.,* 1940, v101, 668.
2. Mitchell, J. W. and Brown, J. W., *Bot. Gaz.,* 1945, v107, 120.
3. Sell, H. M., Luecke, R. W., Taylor, B. M. and Hamner, C. L., *Plant Physiol.,* 1949, v24, 295.
4. Weller, L. E., Luecke, R. W., Hamner, C. L., and Sell, H. M., *Plant Physiol.,* 1950, v25, 289.
5. Gall, H. J. F., *Bot. Gaz.,* 1948, v110, 319.
6. Christiansen, G. L., and Thimann, K. V., *Arch. Biochem.,* 1950, v26, 230.

grown *in vitro*, show that under these conditions I.A.A. added to the medium markedly increases the tissue concentrations of reducing sugars. This effect is a function of the I.A.A. concentration and closely parallels its effect on increasing the growth of the tissues.

*Materials and methods.* Stem segments obtained from young internodes of tobacco (Wisconsin No. 38) grown in the green house were sterilized and cultured as described by Skoog and Tsui(7) on White's nutrient medium with 2% sucrose and 0.7% agar. I.A.A. was added to the medium in concentrations of 0, 0.01, 0.1, and 10.0 mg/l and the pH was adjusted to 4.0 before autoclaving. The segments were grown in 125 cc Erlenmeyer flasks with 50 cc of medium and 3 stem pieces per flask and were kept in cupboards in a hallway of the building with temperature fairly constant at 25°C. They received weak diffuse illumination from the electric ceiling lights. In the first experiment, analyses were made after 65 days; in the second, samples were taken at the start and at intervals during a 52 day period. Analyses for reducing sugar and sucrose were carried out by the method of Forsee(8) and starch was extracted and converted to reducing sugar by the method of Pucher, Leavenworth and Vickery(9).

*Results.* The results obtained in the first experiment are presented in Fig. 1. It may be seen from curve 1 that the average dry weight per piece increased with the concentration of I.A.A. up to 1 mg/l and was somewhat lower for the 10 mg/l treatment. The values for fresh weights (curve 2), which may be more indicative of total growth, are plotted on one-tenth the scale and correspond closely with the values for the dry weights. The concentrations of sucrose plus reducing sugar (curve 3), reducing sugar (curve 4), starch (curve 5), and sucrose (curve 6) are all plotted as per cent of dry weight. The sucrose concentration of the tissues (curve 6)

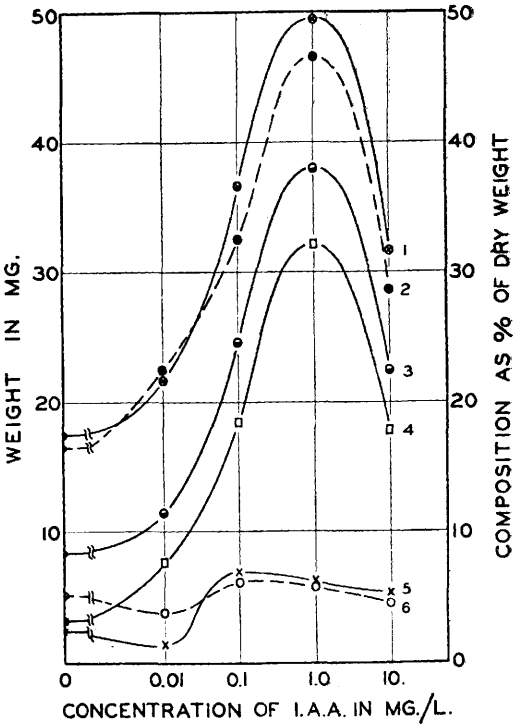


FIG. 1.

Effects of I.A.A. concentrations on growth and changes in carbohydrate concentrations of tobacco stem segments cultured *in vitro*. Experiment started 11/14/49, harvested 1/18/50. Curve 1, average dry weight; curve 2, average fresh weight × 1/10 in mg/segment. Curves 3, sucrose + reducing sugar; 4, reducing sugar; 5, starch, and 6, sucrose concentrations of segments expressed as % of dry weight. All data are values from 27 to 42 segments.

was practically unaffected by the I.A.A. concentration and remained at approximately 5%, which is about 0.5% of the fresh weight or only one-fourth of the sucrose concentration of the external medium at the beginning of the experiment. On the other hand, the reducing sugar concentration showed a striking increase with increasing concentrations of I.A.A. up to 1 mg/l and then decreased, so that the curve for the reducing sugar concentration in the tissues closely parallels the curve for the weights of the stem segments. The starch concentrations were much lower than those of sugar. In this experiment the starch concentrations increased somewhat with the I.A.A. treatments, but the correlation with weights of pieces and concentrations of I.A.A. is less definite than

7. Skoog, F., and Tsui, C., *Am. J. Botany*, 1948, v35, 782.

8. Forsee, W. T., *Ind. Eng. Chem., An. Ed.*, 1938, v10, 411.

9. Pucher, G. W., Leavenworth, C. S., and Vickery, H. B., *Anal. Chem.*, 1948, v20, 850.

TABLE 1. Effects of IAA on Weights, Sugar, and Starch Concentrations in Tobacco Stem Segments Grown *in Vitro*. Experiment started 2/10/50.

| Age of cultures (days) | Treatment | No. of pieces per sample | Fresh wt | | Dry wt | | Reducing sugar | | Sucrose | | Starch | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Avg per piece (mg) | Diff. from control | Avg per piece (mg) | Diff. from control | % of dry matter | Diff. from control | % of dry matter | Diff. from control | % of dry matter | Diff. from control |
| 0 | | 48 | 77 | | 5.7 | | 8.1 | | 9.4 | | 4.1 | |
| 21 | Control | 15 | 134 | | 10.0 | | 7.2 | | 9.0 | | 1.9 | |
| | Low IAA | 26 | 132 | —2 | 10.0 | 0.0 | 7.6 | +0.4 | 11.9 | +2.9 | 2.1 | +0.2 |
| | High IAA | 21 | 206 | +72 | 13.0 | +3.0 | 13.8 | +6.6 | 13.0 | +4.0 | 0.6 | —1.3 |
| 36 | Control | 15 | 104 | | 8.2 | | 5.6 | | 11.9 | | 3.7 | |
| | Low IAA | 23 | 138 | +34 | 11.1 | +2.9 | 7.8 | +2.2 | 8.9 | —3.0 | 1.9 | —1.8 |
| | High IAA | 18 | 263 | +159 | 16.7 | +8.5 | 21.7 | +16.1 | 14.0 | +2.1 | 1.0 | —2.7 |
| 52 | Control | 15 | 163 | | 13.6 | | 5.3 | | 10.1 | | (lost) | |
| | Low IAA | 26 | 129 | —34 | 11.1 | —2.5 | 6.5 | +1.2 | 10.8 | +0.7 | 3.3 | |
| | High IAA | 21 | 339 | +176 | 24.0 | +10.4 | 23.7 | +18.4 | 15.5 | +5.4 | 2.2 | |

Low IAA = Mean for treatments with 0.01 and 0.1 mg/l of IAA. High IAA = Mean for treatments with 1.0 and 10.0 mg/l of IAA.

for reducing sugars. Since the values are low, the discrepancies may be within the experimental error. These curves demonstrate a definite correlation between the applied auxin concentrations, the extent of growth of the tissues, and their reducing sugar concentrations. Furthermore, since the tissues with most growth had the highest concentrations of both sugars and starch, the high reducing sugar content cannot have been derived from storage products but must have been formed by the tissues in response to the treatments with I.A.A.

In a second experiment samples were analyzed at successive stages of growth. The data obtained at the start and after 21, 36, and 52 days of growth are shown in Table I. In this experiment there was no significant difference in growth between cultures with 0.01 and 0.1 mg/l of I.A.A. added to the medium or between those with 1.0 and 10 mg/l of I.A.A.; therefore mean values for the former two treatments are presented in the table as "low I.A.A." and mean values for the latter two treatments are presented as "high I.A.A."

The data for fresh and dry weights indicate that control pieces and those supplied with low I.A.A. concentrations increased in weight to about the same extent, but much less than did the pieces supplied with the high I.A.A. concentrations. The reducing sugar content was 8.1% at the start and decreased to about 5.5% in the controls during the growth period. With the low I.A.A. treatments the mean decrease in reducing sugar was less than in controls, while with the high I.A.A. treatment the reducing sugar markedly increased with time to a mean value of 24% of the total dry weight. Comparisons of the data for weights of pieces and their reducing sugar concentrations again show a close agreement between increase in size and reducing sugar concentration. Furthermore, it may be stressed that the values for reducing sugar obtained after long periods show net losses in control pieces, which grew but little, as against marked increases in tissues supplied with high I.A.A. concentrations, which grew rapidly. The sucrose con-

centrations of the tissues with low I.A.A. treatments and of controls did not change appreciably during the growth period, but showed a definite, small increase with high I.A.A. treatments. The starch concentrations of the tissues were again relatively low, and in contrast with the results after 65 days growth in the first experiment, there was no increase from treatments with I.A.A.

*Discussion and conclusions.* The present results demonstrate a definite correlation between the effects of different concentrations of I.A.A. on growth and on reducing sugar concentrations in the tissues. To our knowledge this is the first case of such a relationship between a metabolic constituent and growth of tissues in response to treatments with an auxin.

We have no explanation for the contrast between these results and reports in the literature other than that differences in methods and plant materials may be responsible. The tobacco tissues we used have a low endogenous auxin content and they were analyzed after long growth periods with added I.A.A.; however definite increases in reducing sugar from treatments with I.A.A. may be obtained within the first week. Furthermore, the tissues were growing continuously and particularly those treated with I.A.A. continued to increase both in size and dry weight, whereas earlier tests have been performed on tissues capable of only limited growth in length and undergoing marked losses in dry weight regardless of the treatments. It should be pointed out that in the present work a correlation is demonstrated between increase in weight, rather than growth rate of the pieces, and the concentration of reducing sugar. This distinction may not be important but will be investigated. The reducing material has been expressed in terms of glucose but has not yet been characterized, and may not be simply glucose.

*Conclusion.* Auxin has a catalytic function in carbohydrate metabolism which is related to its effect on growth but which is not primarily concerned in the respiratory degradation of sugars. The above results are, therefore, reported at this time as they may be of general interest in studies of the mechanism of auxin action in the growth of plants.

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## Effect of Carbohydrate on Growth Response to Vitamin $B_{12}$ in the Hyperthyroid Rat.* (17974)

U. J. LEWIS, D. V. TAPPAN, U. D. REGISTER, AND C. A. ELVEHJEM

*From Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.*

A rat assay for the estimation of vitamin $B_{12}$ in biological materials previously reported (1) makes use of a corn-soybean meal basal ration containing iodinated casein. Ershoff (2), however, has noted that no increase in growth is obtained with vitamin $B_{12}$ in rats fed thyroid active materials when a purified sucrose-casein diet is used. Whole liver, on the other hand, gives a maximum response. Betheil and Lardy(3) report that vitamin $B_{12}$ will partially overcome the growth retardation in the hyperthyroid rat on the sucrose-casein ration. In his latest report Ershoff(4) demonstrates that full-fat soybean

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1. Register, U. D., Ruegamer, W. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1949, v177, 129.

2. Ershoff, B. H., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 209.

3. Betheil, J. J., and Lardy, H. A., *J. Nutrition,* 1949, v37, 495.

4. Ershoff, B. H., *J. Nutrition,* 1949, v39, 259.