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Some experiments performed with IAA-C^{14} on the Auxin transport in relation to the role of Sodium Glycocholate (Na-G)

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Introduction

The discussion about the role of TIBA on auxin transport started with the statement of Kuse (1953–1954) still continues. Because, beside the observations which support this statement (Niedergang, 1954; Vardar, 1955; Niedergang-Kamien and Skoog, 1956; Keitt, 1956; Zwar and Rijven, 1956; Hay, 1956; Niedergang-Kamien and Leopold, 1957; Kuse, 1958 and 1961), it is also reported that the role of TIBA on the growth can be interpreted in other ways (Galston, 1947; Thimann and Bonner, 1948; Åberg, 1950 and 1953; Street, 1955; Audus and Thresh, 1956; Vardar and Acarer, 1957).

Moreover the experiments of Vardar (1959) performed with IAA-C^{14} put forward the difficulty in accepting the blocking effect of TIBA on auxin transport.

On the other hand Champagnat and Pigeret (1957) conducted some experiments with the technique of Kuse (1953) by using Sodium glycocholate instead of TIBA. As a result of their observations on the alternation in the growth of cotyledonary buds with the treatment of Na-G, these authors accepted the blocking effect of Na-G in auxin transport also.

The aim of this paper is to test the validity of the conclusion arrived by Champagnat and Pigeret concerning the Na-G blocking effect on auxin transport by using IAA-C^{14}.

Method and material

As previously used by Vardar (1959) for the autoradiographical and dark bending reaction experiments, the 45 mm *Ipomea* hypocotyls were used. In the experiments of quantitative comparison of radioactivity, the 45 mm tall *Helianthus* hypocotyls were preferred. Both *Ipomea* and

Helianthus hypocotyls were grown and prepared for experiments completely in the same manner as previously done by Vardar (1959). The Na-G was applied as a lanoline paste in the concentration which was found convinient for blocking effect by Champagnat and Pigeret (100mg/L or 500 mg/L). The radioactive auxin (IAA-C^{14}) used was a sample prepared in 1958 by Waltham Tracer laboratory in 3-Indole-Acetic acid-α-C^{14} ($C_8H_6NC^{14}$-H_2-COOH) form. The specific activity of the IAA-C^{14} sample used was 1,1 mc/mμ and the concentration of the solution used was 2 μm/ml, the quantity applied to the plants was 0,2 μg per plant and the activity was 1×13 cpm/per plant. For the measurements of the radioactivity the same technique was used as previously followed by Vardar (1959). The radioactivity measurements were made with a Nuclear Chicago M5 Gas-flow counter.

In the chromatographic experiments, performed for verification that the radioactivity measured in the upper and lower parts of Na-G ring in *Helianthus* hypocotyls, Watman No. 2 paper was used and the Nitsch and Nitsch (1955) technique was followed.

In chromatography the solvent used was water-amonium-butanol and the extraction was made with methanol by keeping the sections for a while in ice or in some experiments by boiling in a waterbath. For the determination of spots on the chromatograms Salkowski reagent was used.

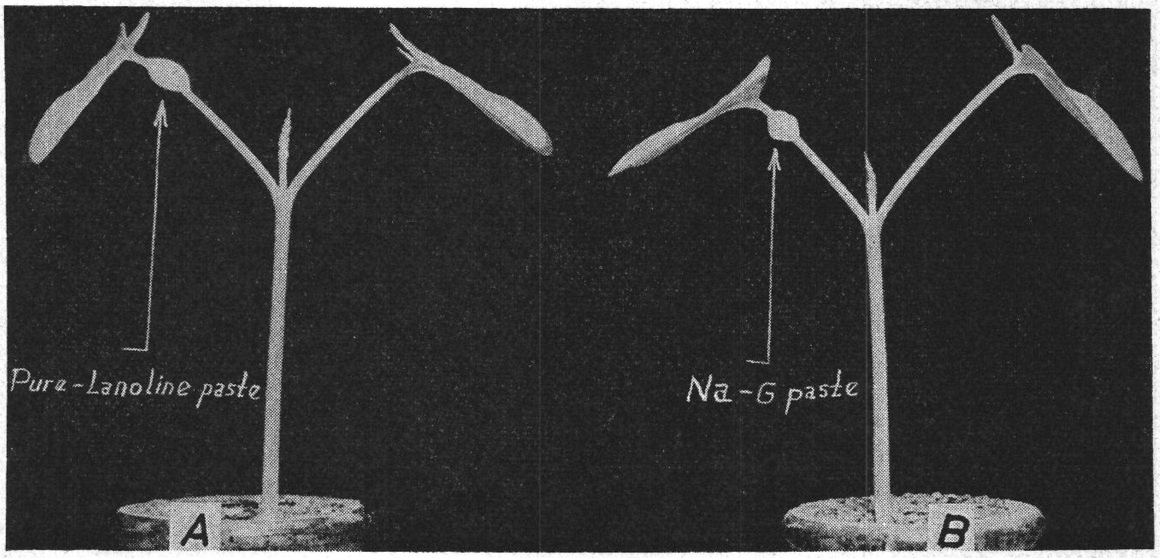

Figure 1

The behaviour in the dark conditions of Ipomea hypocotyls. A) Control hypocotyl, B) Experimental hypocotyl; one of its cotyledonary stalk ringed with Na-G. (In both cases no bending reaction.)

Experimental Part

a) Dark bending reactions of Ipomea hypocotyls:

When asymmetric auxin distribution is functioning in an organ, even in the complete darkness the occuring of bending reaction, is well known. If the Na-G applied to the cotyledon stalk had blocked the auxin flow coming from the cotyledon, a typical bending reaction must occure in the hypocotyl even in the dark conditions. But as seen in fig. 1 we did not observe any bending reaction in the treated hypocotyl in comparison with untreated ones. This result shows that no asymmetrical distribution in growth substances occurs with the treatment by Na-G. This indicated that Na-G does not block the auxin transport on the applied stalk. This conclusion is against to the suggestion of Champagnat and Pigeret (1957).

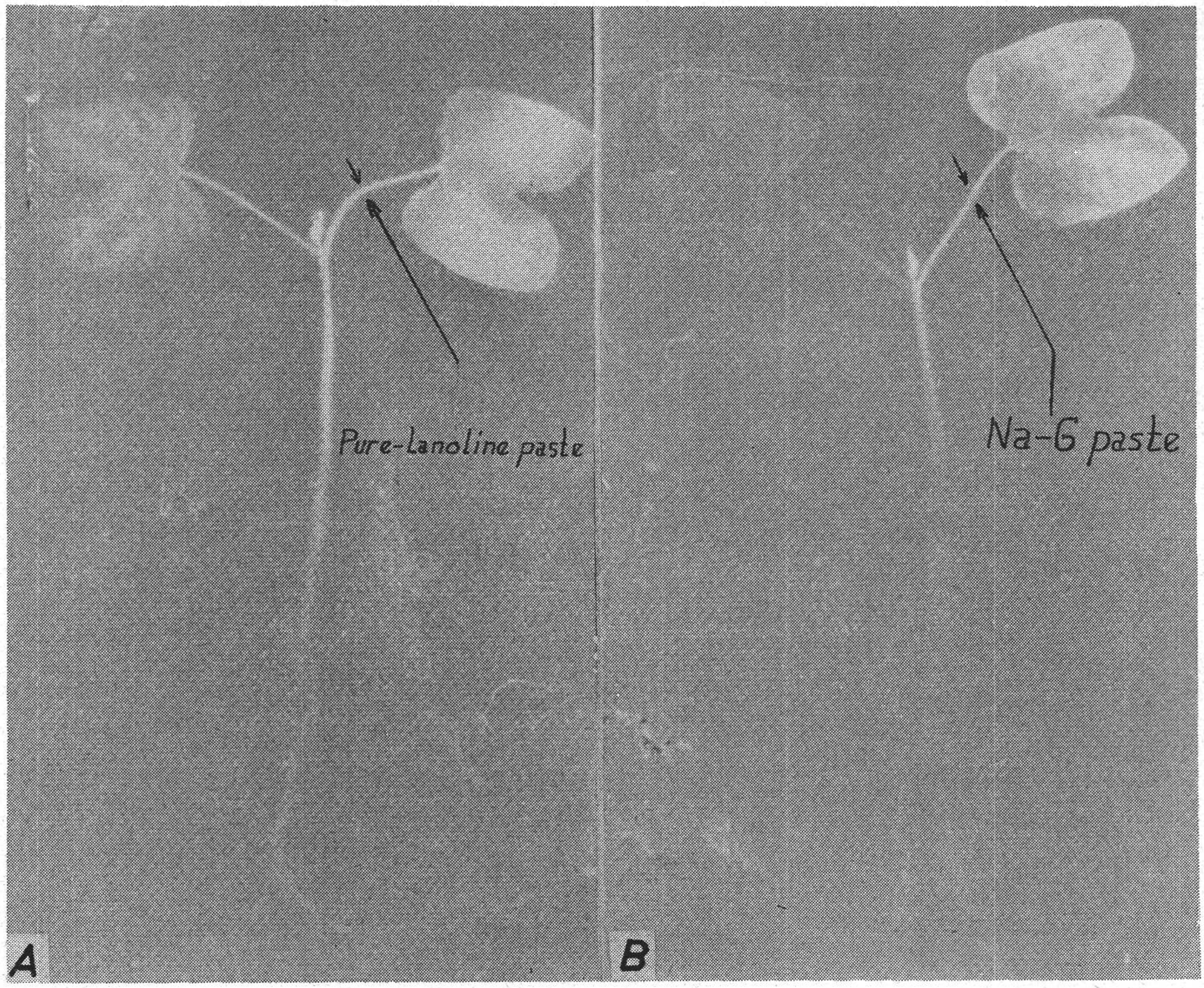

Figure 2

The autoradiographs of Ipomea hypocotyls. A) Control hypocotyl, B) Experimental hypocotyl; one of its cotyledonary stalk ringed with Na-G. (IAA-C^{14} was applied 6 h. after ringing with Na-G paste and the plant fixation X-Film was made 15 h. after the application of IAA-C^{14}. Time of exposure 3 months.)

b) Autoradiographical verification:

It seemed interesting for us to verify the results observed in the dark bending reactions with autoradiographic experiments. However if the Na-G has a blocking effect on auxin transport, the applied IAA-C^{14} on the cotyledons must be blocked by the Na-G at the ringed point and no radioactivity must be found below it.

As seen in fig. 2, there were no differences in the distribution of the radioactivity in the treated and control *Ipomea* hypocotyls. These again indicate that it is difficult to accept a blocking effect for Na-G on auxin transport.

c) The quantitative comparison of radioactivity:

By using the technique of Vardar (1959) some experiments were conducted for quantitative measurements of the radioactivity in the upper and lower parts of Na-G ring of treated hypocotyls and were compared with the radioactivity in the same parts of untreated (control) hypocotyls.

Table 1

The distribution of the radioactivity in the different segments of Na-G ringed and control untreated *Helianthus* hypocotyls. The IAA-C^{14} solution was applied 6 hours after ringing with Na-G paste and the measurement of the radioactivity in segments was made 15 hours after the application of IAA-C^{14}. Average of 10 experiments (31.1.–14.2.1962)

Experimental hypocotyls ringed with Na-G paste		Control hypocotyls ringed with pure lanoline	
Counted activity per segment in the upper part of Na-G ring	Counted activity per segment in the lower part of Na-G ring	Counted activity per segment in the upper part of pure lanoline ring	Counted activity per segment in the lower part of pure lanoline ring
85 cpm/Segment	103 cpm/Segment	86 cpm/Segment	102 cpm/Segment

d) Chromatographic analysis:

One can say that the results shown in Table 1 indicate essentially the distribution of C^{14}, but perhaps not the presence of IAA-C^{14}. Therefore these results may not prove completely the blocking effect of Na-G on the auxin transport. In the light of this argument we thought it will be much more satisfactory to show that the radioactivity measured in the lower and upper section of Na-G ring of treated hypocotyls is due to IAA-C^{14}. For this reason some chromatographic analysis were made. In these experiments radioactive and non-radioactive auxin were applied to the cotyledons of treated (experimental) and untreated (control) hypocotyls. Then the chromatographic separation of the extractions obtained from the upper and lower parts of Na-G ring and from the same sections of the control plants was followed.

The comparison of Rf value, radioactivity in spots of the chromatograms obtained from the lower and upper section of the Na-G ringed extraction with those of the control plants show us no differences in respective points (fig. 3). Thus, it is easy to understand that the radioactivity followed even in the lower parts of Na-G ring is due to the IAA-C^{14}. Therefore it is difficult to accept the suggestion that the Na-G has a blocking effect on auxin transport.

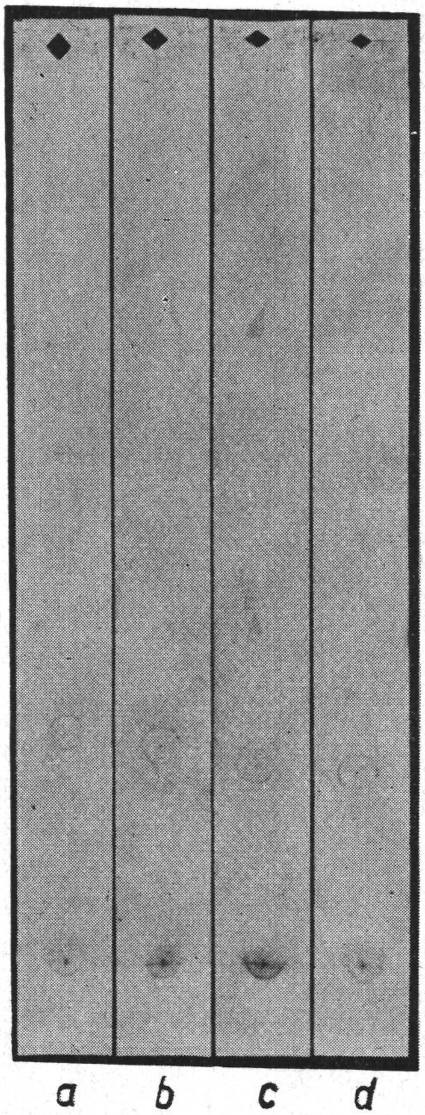

Figure 3

The chromatograms of extracts obtained a) from the upper, b) from the lower part of control, and c) from the upper, d) from the lower parts of experimental Helianthus hypocotyls.

Conclusion

In 1938 Clark according to his results obtained from the experiments on the effect of glycocholate on the auxin transport, reported that the «glycocholate completely abolishes the auxin transport». Champagnat and Pigeret (1957) conducted some experiments on the role of the Na-G in relation to correlative growth changes of buds. In the light of their investigations on the effect of glycocholate on the bud growth correlation, these authors came to the conclusion also that the Na-G has a blocking effect on the auxin transport.

Our results obtained by using IAA-C^{14} indicate that the Na-G has no effect on the auxin transport (c. f. autoradiographic results and the experiments of quantitative measurements of radioactivity). Moreover with chromatographic separation it is shown that radioactivity found even in the lower parts of Na-G of treated hypocotyls is due to the IAA-C^{14}. Therefore, it is concluded that the role played by Na-G on growth must be other than the blocking of auxin transport.

Summary

In this paper the validity of the conclusion concerning the Na-G blocking effect on auxin transport is investigated by using IAA-C^{14}.

According to the observations on dark bending reactions, autoradiograms, and the results obtained from the comparison of radioactivity and chromatographic separations, it is concluded that Na-G has not a blocking effect on auxin transport.

Zusammenfassung

In vorliegender Arbeit wurde mit Hilfe von IAA-C^{14} die Gültigkeit der Feststellung der Sperreffekt von Na-G auf den Transport von Auxin untersucht.

Auf Grund von Beobachtungen an Krümmungsbewegungen bei Dunkelheit, von Autoradiogrammen und Ergebnissen, die beim Vergleich der Radioaktivität und der Trennung mittels Chromatographie erhalten wurden, kommen die Verfasser zum Ergebnis, daß das Na-G keine Sperrung des Auxintransportes verursacht.

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