## Spermine as a New Growth-Promoting Substance for *Helianthus tuberosus* (Jerusalem Artichoke) in vitro

Polyamines are present in vegetable embryos[1] and in small amount in seeds of various plants[1,2]; also an enzyme was found which forms putrescine from agmatine in barley leaves[3]. Nevertheless, the biological significance of polyamines is still unknown in plants and particularly in vegetable embryos.

The effects of polyamines on nucleotide metabolism and protein synthesis[4,5] on the growth of some bacteria and fungi[6,7] and their presence in dormant organs (embryos), which are destined to an elevated metabolism and cell division, lead us to consider them involved in mechanisms inducing the first vegetative phase.

In order to clarify the biological role of polyamines in higher plants, we have studied the effects of spermine on cellular division of tissues, whose growth is closely related to the presence of auxines[8].

Dormant tubers of *Helianthus tuberosus* were utilized. Cylindrical explants (3 mm diam., dry weight ~10 mg) of an homogenous vascular parenchyme were placed in sterile culture in vitro in a nutritive medium[9] with glucose 5% and agar 1.2%.

Agar was washed overnight in tap water, rinsed with distilled water, dehydrated with acetone and finally washed in ethyl ether to eliminate also the traces of 3-indoleacetic acid (IAA)[10].

Concentrations of spermine between $10^{-3}$ and $10^{-6}M$ were utilized with a control in basal medium alone and basal medium plus IAA $0.5 \cdot 10^{-7}$ ($\sim 0.5 \cdot 10^{-6}M$).

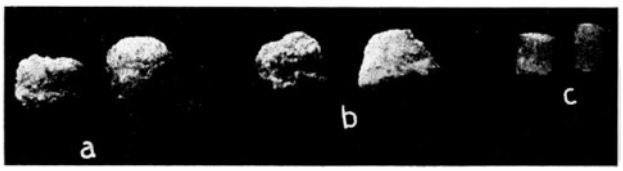
Spermine was obtained from Fluka AG, Buchs (Switzerland) and a further purification was carried out by means of successive recrystallizations.

Twenty replications were utilized for every concentration. The explants were randomized and grown in a culture room at 23°C, light of ~1000 lux, for 12 h pro die. After 5–7 days the explants with spermine ($10^{-4}$ and $10^{-5}M$) were grown almost as those with IAA: no visible growth in other concentration.

The experiment was stopped after forty days: fresh and dry weights, RNA and DNA were determined. The experiment was repeated thrice in different times with similar results.

In the Table the results refer to a single experiment.

It is evident that the greatest growth was with spermine $10^{-4}M$, similar to optimal concentration of IAA (see Figure). The chlorophyll was determined only for spermine $10^{-4}M$ and IAA and referred to fresh weight corrected according to the surface: the pigment was in no detectable trace in the control, while no significant differences were observed in spermine $10^{-4}M$ and IAA. Microscopic sections of spermine explants $10^{-4}$ and $10^{-5}M$ showed that callus was not fundamentally different from IAA. No proliferation was microscopically observed in the control, while some new assemblages of cells were seen for spermine $10^{-3}$ and $10^{-6}M$, even if they showed at first small necroses. Experiments to reveal possible interactions between IAA and spermine were made, but results with a statistic value were not obtained, at first. RNA and DNA dosage[11] perfectly confirmed microscopic observations and dry weight determination. Very small traces of




Explants of *Helianthus tuberosus* (Jerusalem artichoke) treated with: a, spermine; b, IAA; c, nothing.

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Fresh and dry weight, nucleic acids in explants of Jerusalem artichoke treated with spermine

| | 0 | Spermine $10^{-3}M$ | Spermine $10^{-4}M$ | Spermine $10^{-5}M$ | Spermine $10^{-6}M$ | IAA $0.5 \cdot 10^{-7}$ |
|---|---|---|---|---|---|---|
| Fresh weight[a] | 39.9 | 49.9 | 101.7 | 74.5 | 55.2 | 141.2 |
| Dry weight[a] | 11.6 | 13.5 | 15.8 | 14.9 | 13.2 | 17.1 |
| % dry weight | 28.3 | 27.1 | 15.5 | 20.0 | 23.7 | 12.5 |
| Growth index | 100.0 | 116.3 | 136.2 | 129.3 | 112.9 | 147.4 |
| RNA | | | | | | |
| mg/g dry weight | 18.0 | – | 21.0 | – | – | 22.5 |
| DNA | | | | | | |
| mg/g dry weight | 3.6 | – | 5.2 | – | – | 6.8 |
| RNA/DNA | 5.0 | – | 4.0 | – | – | 3.3 |

[a] Average of 20 explants.

spermidine alone were revealed on 100 g fresh weight of Jerusalem artichoke dormant tubers.

Our data clearly show that spermine is a growth substance able to take the place of IAA to cause the cellular division. It is interesting to note that, in the literature, no substance having aliphatic structure was found to be able to act in a similar way. Our researches are continuing in order to decide at which metabolic level spermine acts.

*Riassunto.* È stato per la prima volta dimostrata che la spermina ($10^{-4}M$) ha un effetto di crescita simile a quello dell'acido indolacetico sugli espianti di *Helianthus tuberosus* in vitro.

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## A Comparison of Methods for the Inactivation of Third Component of Guinea-Pig Complement[1]

For many years after the discovery of complement (C') the consensus was that it consisted of four components, based on the fact that hemolytically active serum can be fractionated or inactivated to give reagents which are non-hemolytic when used alone, but which are fully active hemolytically when combined in pairs. The assumption was made that each reagent was deficient in one component, without regard to the possibility that more than one component might be absent from a given reagent and that the hemolytic system might therefore involve more than four components.

In recent years evidence has been obtained clearly establishing the complexity of $C'_3$ (third component of C')[2-6]. It is possible, therefore, that an $R_3$ reagent, believed heretofore to lack $C'_3$, may in fact be deficient in more than one component. A study of various methods for preparing an $R_3$ reagent was therefore undertaken.

$R_3$ reagents were prepared as follows using guinea-pig serum: (1) by the inactivation of serum by 'Liquoid'[7] or formaldehyde, and (2) by the absorption of serum with Zymosan[8-9].

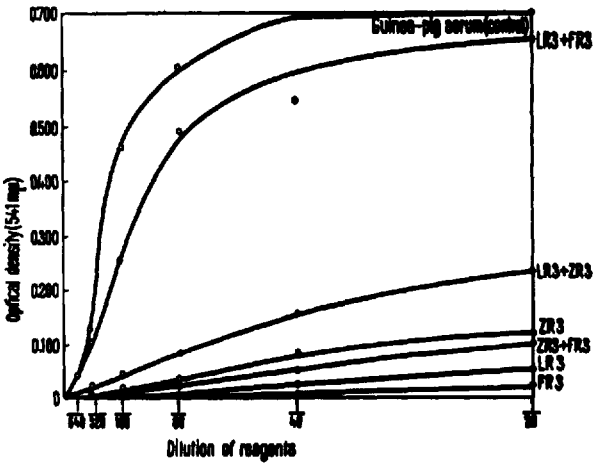
The $R_3$ reagents were assayed to ascertain whether or not they were deficient in more than one component and whether or not the missing components were the same.

Each of the $R_3$ reagents was non-hemolytic by itself and cross-titrated with reagents $R_1$, $R_2$ and $R_4$, which are deficient in $C'_1$, $C'_2$ and $C'_4$, respectively. The $R_3$ reagents were therefore lacking in one hemolytic component at least, presumably a component of the $C'_3$ complex, and contained $C'_1$, $C'_2$ and $C'_4$.

In order to learn whether the missing component in the three preparations of $R_3$ was the same or not, they were cross-titrated with each other in pairs over a wide range of concentrations. The results are shown in the Figure. Each $R_3$ preparation was essentially non-hemolytic, but $LR_3$ ('Liquoid' preparation) and $FR_3$ (formaldehyde preparation) taken together had virtually the same hemolytic activity as the untreated guinea-pig serum used as a control.

This finding suggests that the missing $C'_3$ factors in one preparation are different from those in the other, and although it gives no clue as to the exact number of components involved, the minimum number is certainly two. The Zymosan preparation ($ZR_3$) did not cross-titrate significantly with either $LR_3$ or $FR_3$, an indication that it must have lacked the components absent from both $LR_3$ and $FR_3$.

A further comparison of $LR_3$ and $FR_3$ was made with respect to those components of complement that require either $Ca^{++}$ or $Mg^{++}$ for immune hemolysis. For this purpose was used the reaction of these reagents with EA (sensitized sheep cells) to form $EAC'_{1,4,3}$, that is, complemented cells that are lysed by guinea-pig serum in the presence of Versene. Because neither reagent was hemolytic by itself, the reaction was carried out at room temperature, whereas the reaction with the untreated guinea-pig




Cross-titrations of $R_3$ reagents for hemolytic activity of complement. The reagents were tested either alone or in combinations taken two at a time; using guinea-pig serum as a control. Optical Density at 541 mμ is proportional to the fraction of cells lysed and is a measure of the hemolytic activity of complement.

[1] Supported in part by Contract DA-49-007-MD-696 with the Office of the Surgeon General, Department of the Army.

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