cells by the manometric Warburg technique according to DE SANDRE et al.[10].

Results are summarized in Tables I and II. It is noted that erythrocytes hemolyse in acidified serum when AChE inhibition is more marked. A similar finding has been observed by PERONA et al.[8] in normal red cells altered with trypsin; these authors postulate that 'a decrease in AChE content may be a necessary, but not sufficient requirement to sensitize red cells to acid lysis'. However, erythrocytes treated for 9 min with a 4% AET solution, although maintaining their normal AChE activity, hemolyse in the Crosby thrombin test. Therefore under these experimental conditions, AChE inactivation and susceptibility to acid lysis do not appear to be interdependent. This agrees with the finding of some workers that destruction of AChE activity does not result in erythrocytes developing the PNH defect[11].

Table I. AChE activity and susceptibility to acid lysis of normal red cells incubated with 8% AET solution over different periods

	Period of incubation (min)		
	3	6	9
AChE activity (% inhibition)	47	60	80
Ham's test	—	—	+
Crosby's test	—	+	+

Table II. AChE activity and susceptibility to acid lysis of normal red cells treated for 9 min with AET solutions of progressive concentrations

	Concentration %			
	2	4	6	8
AChE activity (% inhibition)	0	0	55	80
Ham's test	—	—	—	+
Crosby's test	—	+	+	+

Riassunto. Emazie umane normali trattate con AET mostrano, similmente alle emazie dell'emoglobinuria parossistica notturna, una diminuzione della loro attività acetilcolinesterasica ed una suscettibilità all'emolisi acida. Nel presente lavoro vengono studiati i rapporti esistenti tra questi due parametri e viene concluso che essi non sono interdipendenti.

S. FERRONE, A. ZANELLA, and G. SIRCHIA

Istituto di Semeiotica Medica dell'Università di Milano (Italy), July 11, 1966.

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Aliphatic Amines and a Growth-Factor of Coconut Milk as Stimulating Cellular Proliferation of *Helianthus tuberosus* (Jerusalem Artichoke) in vitro

First, we have shown[1] that spermine, an aliphatic polyamine, is a growth-promoting substance able to take the place of 3-indoleacetic acid (IAA) causing the cellular division of *Helianthus tuberosus* in vitro.

In this experiment we have studied the effect of other aliphatic amines (1,4-diaminobutane, 1,5-diaminopentane and spermidine), also taking into consideration their probable occurrence as growth factors in coconut milk, whose growth-stimulating properties have been studied for a long time without definite conclusions[2].

Dormant tubers of *H. tuberosus* (Jerusalem artichoke) were utilized. No evident traces of spermidine, putrescine or cadaverine were revealed in dormant tubers. Cylindrical explants (3 mm diameter, 3 mm height) of a homogeneous vascular parenchyme were placed in sterile culture in vitro in a nutritive medium[3] with glucose 5% and previously purified agar 1.2%[4].

Concentrations of amines between 10^{-4} and $10^{-6}M$ were utilized with a control in basal medium alone and basal medium plus naphthaleneacetic acid (NAA) $0.5 \cdot 10^{-7}M$ ($\sim 0.5 \cdot 10^{-6}M$). Putrescine (1,4-diaminobutane) and spermidine were obtained from Fluka AG, Buchs, and cadaverine (1,5-diaminopentane) from Calbiochem. Further purifications were carried out by means of successive recrystallizations and the purity verified with paper chromatography[5], especially to discover possible indole compounds. 16 replications were utilized for every concentration. The explants were randomized and grown in a culture room at 25 °C, ~1800 lux, for 12 h pro die. After 8–10 days the explants with spermidine (10^{-4} and $10^{-5}M$), putrescine (10^{-4} and $10^{-5}M$), and cadaverine ($10^{-4}M$) had grown almost as much as those with NAA: no visible growth in other concentrations or in basal medium. Afterwards, the other amine explants began to grow too. The experiment was stopped after 40 days: fresh and dry weights, RNA and DNA were determined. The experiment was repeated at three different times with similar results.

In Tables I and II the results refer to a single experiment. From Table I it is evident that the greatest growth was with spermidine $10^{-5}M$, similar to optimal concentration of NAA, less however than spermine $10^{-4}M$[1]. Putrescine $10^{-5}M$ and cadaverine $10^{-4}M$ (Table II) also have an action similar to optimal concentration of NAA.

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Microscopic sections of spermidine $10^{-5}M$, putrescine $10^{-5}M$ and cadaverine $10^{-4}M$ explants showed that callus was not fundamentally different from NAA. No proliferation was microscopically observed in the control, while new assemblages of cells were seen from the other concentrations of amines. Experiments to reveal possible interactions among IAA, putrescine and spermidine were made, but results with a statistic value were not obtained.

Table I. Fresh and dry weight of nucleic acids in explants of Jerusalem artichoke treated with spermidine

	0	Spermidine			NAA
		$10^{-4}M$	$10^{-5}M$	$10^{-6}M$	$0.5 \cdot 10^{-7}M$
Fresh weight[a] (mg)	48.1	70.0	95.1	78.2	174.2
Dry weight[a] (mg)	14.1	18.6	25.6	21.0	28.1
% dry weight	29.3	26.5	26.9	26.8	16.1
Growth index	100.0	131.9	181.5	148.9	199.2
RNA mg/g dry weight	12.1	13.0	17.5	15.4	22.3
DNA mg/g dry weight	2.4	3.8	4.2	3.6	5.9
RNA/DNA	5.0	4.3	4.1	4.2	3.7

[a] Average of 16 explants.

Table II. Fresh and dry weight of nucleic acids in explants of Jerusalem artichoke treated with putrescine and cadaverine

	0	Putrescine		Cadaverine		NAA
		$10^{-4}M$	$10^{-5}M$	$10^{-4}M$	$10^{-5}M$	$0.5 \cdot 10^{-7}M$
Fresh weight[a] (mg)	67.2	265.1	276.6	282.5	241.5	311.1
Dry weight[a] (mg)	21.0	36.8	38.1	36.3	32.1	39.3
% dry weight	31.2	13.8	13.7	12.8	13.2	12.6
Growth index	100.0	175.2	181.4	172.8	152.8	187.1
RNA mg/g dry weight	11.8	18.3	19.3	19.9	18.9	21.0
DNA mg/g dry weight	2.1	6.0	6.3	6.0	5.8	7.2
RNA/DNA	5.4	3.0	3.0	3.3	3.2	2.9

[a] Average of 16 explants.

Table III. Fresh and dry weight of explants of Jerusalem artichoke treated with 3 amine bands separated by paper electrophoresis from coconut milk (see text)

	0	Band 1	Band 2	Band 3 (putrescine)	NAA $0.5 \cdot 10^{-7}M$
Fresh weight[a] (mg)	62.3	66.6	67.1	116.3	299.6
Dry weight[a] (mg)	11.2	11.4	11.3	16.5	38.3
% dry weight	17.9	17.1	16.8	14.1	12.7
Growth index	100.0	101.7	100.8	147.3	341.9

[a] Average of 16 explants.

RNA and DNA dosage[6], modified for vegetable tissues[7], perfectly confirmed microscopic observations and dry weight determination.

The data are much more interesting if compared with our preliminary investigations on the growth-stimulating properties of coconut milk. Especially diamines and polyamines were investigated according to the method of RAINA[8]. The identification of amines was carried out with paper electrophoresis separation with sulphosalicylic acid buffer $0.065M$ at pH 3.5[8]. The amido black method for quantitative determination of polyamines[8] allows us to exclude the presence of these substances in coconut milk. Spraying paper strips with 0.1% ninhydrin in *n*-butanol containing 1% glacial acetic acid[9], three distinct amine bands were detected. These were compared with authentic diamines: the third band, more rapidly moving fraction, was identified as true putrescine; the other two bands were not identified. The putrescine content, after elution, was evaluated with spectrophotometric determinations at 420 nm after reaction with 2,4-dinitrofluorobenzene[10]. In coconut milk putrescine is present at a concentration of $\sim 0.25 \cdot 10^{-5}$ ($\sim 10^{-6}M$). Bands were then used to assay the possible cellular proliferation of Jerusalem artichoke dormant tubers. Three bands were quantitatively obtained from 150 ml of coconut milk, according to the method previously described, and were diluted in 1000 ml of nutritive medium[3] with a control in basal medium alone and basal medium plus NAA $0.5 \cdot 10^{-7}$. After 5–7 days the explants with the third band (putrescine) had grown almost as much as those with NAA; no visible growth in other bands or in basal medium. The experiment was stopped after 40 days and fresh and dry weights were determined. In Table III the proliferation caused by the third band (putrescine) is evident, although less than NAA.

In short, our research has shown that not only spermine[1] but also other aliphatic diamines and polyamines cause the cellular division of *H. tuberosus* in vitro. These results allow for an increase in the number of non-aromatic compounds able to act as growth factors. We think the first finding of putrescine in coconut milk is very interesting, since this diamine must be considered one of the natural growth factors present in liquid endosperm of coconut[11].

Riassunto. Putrescina, cadaverina e spermidina, amine alifatiche strettamente collegate alla spermina, agiscono come fattori di crescita su espianti di *Helianthus tuberosus* in vitro. L'azione della putrescina è particolarmente interessante essendo stata trovata per la prima volta nel latte di cocco.

N. BAGNI

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