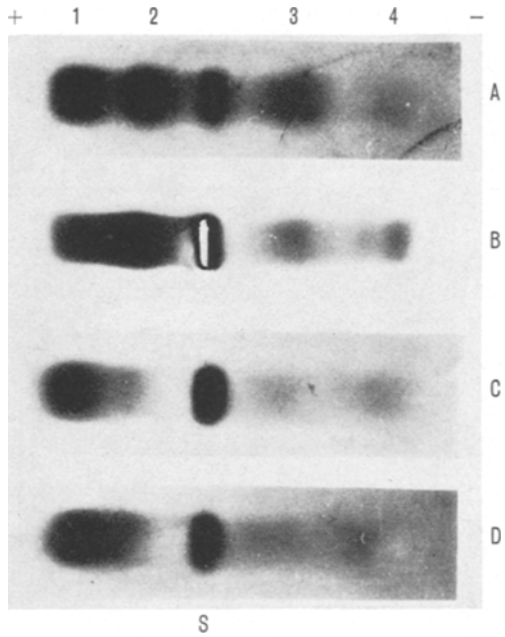
mit Esteraseaktivität: zwei intensivere Zonen mit anodischer Migration und zwei mit kathodischer Wanderung. In den leukämischen Zellen ist die elektrophoretische Verteilung im wesentlichen ähnlich, wie in den normalen reifen Zellen. ANDERSEN und SÖLVSTEN[10], welche nur drei




Elektrophoretische Trennung der Esteraseaktivität auf Agargel: (a) normale Granulozyten, (b) leukämische Myelozyten, (c) normale Lymphozyten, (d) leukämische Lymphozyten. Die leukämischen und die normalen Zellen zeigen ein analoges Elektrophorese-«Pattern».

Leukozytäre Esteraseaktivität in $\mu M \cdot 10^{-11}$/min/Leukozyt ± = Standard Error

| Zahl der Fälle | | Zahl der Fälle | |
|---|---|---|---|
| 4 | Granulozyten = 19,3 ± 0,38 | 5 | normale Lymphozyten = 15,4 ± 0,34 |
| 4 | Myelozyten = 26,3 ± 1,29 | 12 | leukämische Lymphozyten = 21,0 ± 1,16 |
| | $p < 0{,}001$ | | $p < 0{,}001$ |

Fraktionen mit Esteraseaktivität fanden, konstatierten ebenfalls keine Unterschiede im Elektrophorese-«Pattern» der Esterasen zwischen normalen und leukämischen Zellen.

*Summary.* A quantitative and qualitative analysis of the carboxylic esterases of normal granulocytes, normal lymphocytes and leukemic cells was performed. In the myeloid leukemic cells and in the lymphatic leukemic cells, the esterase activity was significantly increased. By means of electrophoretic fractionation, four zones with esterase activity were detected in normal granulocytes and lymphocytes; the electrophoretic pattern of the leukemic cells was similar to that of the normal cells.

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## Normal Human Red Cells Treated with 2-Aminoethylisothiouroniumbromide (AET). Acetylcholinesterase Inactivation and Susceptibility to Acid Lysis in vitro

Among the few enzymes recognized in the red cell membrane, acetylcholinesterase (AChE) is one of the most investigated. A deficiency in erythrocyte AChE activity has been reported in a variety of hematologic disorders. The only disease so far characterized by reduction in erythrocyte AChE is paroxysmal nocturnal hemoglobinuria (PNH)[1–3]. Another peculiarity of this disease is the in vitro hemolysis caused by acidified normal serum; however, there is no proof that AChE deficiency by itself leads to increased susceptibility to hemolysis. It has been demonstrated that normal human erythrocytes treated with proteolytic enzymes or sulfhydryl compounds resemble those of PNH in the property of acid hemolysis[4,5] and have a remarkable deficiency of stromal AChE activity[6–9].

We have investigated the relationship between hemolysis by acidified serum and loss of AChE activity in normal erythrocytes treated with AET. The blood of healthy adults was drawn with heparin; the red cells, separated by centrifugation and washed thrice with saline, were treated with AET as previously described[5], but for various periods of incubation or with solutions of different concentrations. After treatment the erythrocytes were subjected to the tests of Ham and of Crosby. The red cell AChE activity was determined in normal and treated red

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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.

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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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