with abscisin II turned almost completely yellow and indicated very little chlorophyll. On the other hand, the leaf discs treated with kinetin appeared perfectly green and fresh. Kinetin was also successful in reversing the senescence-accelerating effect of abscisin II. This is indicated by the fact that the leaf discs reared in abscisin II (2 ppm) in presence of kinetin (5 ppm) showed as much as 8 times more chlorophyll than those floated on 2 ppm solution of abscisin II alone. Not only in leaf discs but even when whole leaves were used, similar results were obtained.

Recently, OSBORNE[3] observed that pretreatment of leaf discs with abscisin II reduced the subsequent incorporation of (^{14}C)leucine into protein. On the contrary, earlier results[2] have clearly shown that kinetin delays leaf senescence by maintaining or enhancing protein synthesis. Thus, in view of the above results, it would appear that kinetin probably reverses the senescence-accelerating effect of abscisin II by exerting its influence on protein and RNA synthesis. However, whether these results indicate genuine interaction between abscisin II and kinetin, or simply represent essentially independent effects which the plant is 'adding' or 'subtracting', is not clear at present, and remains to be decided on the basis of further experimental evidence[7,8].

Zusammenfassung. Abscisin II, eine natürlich vorkommende, die Abtrennung beschleunigende Substanz, erhöhte das Altern von Blattscheiben von *Arabidopsis* sehr. Dieser Effekt von Abscisin II blieb jedoch beinahe vollständig unterdrückt, wenn Kinetin gleichzeitig zugefügt wurde.

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[7] Acknowledgments. We are grateful to Prof. K. EGLE for providing facilities, to Prof. J. W. CORNFORTH for his kind donation of a sample of abscisin II, Dr. KRANZ for his gift of seeds of *Arabidopsis* and to Deutscher Akademischer Austauschdienst for financial support.

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Fluorescein Staining of Human Lymphocytes Induced by *Echis colorata* Venom

Echis colorata (EC) venom was shown to render guinea-pig lymphocytes susceptible to fluorescein-staining in vivo and in vitro[1]. The present study is concerned with a similar action of the venom on human lymphocytes in vitro. Leucocytes were separated from venous blood collected from healthy donors over $^1/_9$ volume 0.115 *M* Na_2 EDTA, pH 7.4, using polyvinylpyrrolidone and ammonium chloride[2]. The leucocytes were spun down for 3 min at 1000 rpm and the concentrated suspension of unwashed cells was used in the experiments. EC venom, stored in freeze-dried form, and 2 of its chromatographically separated fractions[3] – procoagulant fraction devoid of protease (gelatinase) and esterase (substrate *N*-benzoyl L-arginine ethyl ester HCl, NBC), and protease (gelatinase) fraction devoid of procoagulant but containing esterase, were used. The venom procoagulant induces thrombin formation in native as well as in EDTA-plasma[4]. The fractions were applied in concentrations having biological activity similar to that of the whole venom. Serum was prepared from spontaneously clotted blood incubated at 37 °C for 1 h, and defibrinated plasma from EDTA-plasma clotted with thrombin (2 U/ml) subsequently inactivated by similar incubation. Fluorescein (Fluorescite) was obtained from Moore Kirk Laboratories, Inc., Worcester, Mass., and bovine thrombin (Thrombin, Topical) from Parke, Davis and Co., Michigan, USA. Fluorescence was observed with a Philips-Mercury-High Pressure Lamp CS 150 in smears prepared from experimental mixtures incubated at 37 °C for 1 h, as described previously[1].

In the standard experimental mixture containing 0.1 ml leucocyte suspension (1.5×10^8 leucocytes/ml), 0.015 ml 5% fluorescein-saline solution, 0.1 ml EC venom saline solution (1000 µg dry weight, protein content 600 µg/ml), 0.1 ml thrombin-saline solution (200 U/ml), and serum up to a final volume of 1.6 ml, all lymphocytes became fluorescent. EC venom or thrombin alone did not induce fluorescence (results summarized in the Table). Inactivation of thrombin by incubation with human serum prior to addition to the EC venom-containing system prohibited lymphocyte fluorescence. Neither trypsin (twice crystallized, Worthington Biochemical Corporation, Freehold, N.J.) nor papain (twice crystallized, Sigma Chemical Company, St. Louis, Miss.) when added, instead of thrombin, in final amounts of 200 µg and 500 µg, respectively, caused fluorescence in the presence of EC venom.

Lymphocyte fluorescence induced by *Echis colorata* (EC) venom

	Medium	Lymphocyte fluorescence
ECV[a] + thrombin	serum	+
ECV	serum	–
Thrombin	serum	–
ECV + trypsin	serum	–
ECV + papain	serum	–
ECV	defibrinated plasma	+
EC procoagulant	defibrinated plasma	–
EC protease	defibrinated plasma	–
EC procoagulant + EC protease	defibrinated plasma	+

For experimental mixture see text. [a] ECV, whole venom.

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The requirement of both EC venom and thrombin for the induction of human lymphocyte fluorescence in the above in vitro system led to the assumption that, in the causation of lymphocyte fluorescence in EC-venom inoculated guinea-pigs[1], thrombin, formed in vivo by the action of the venom procoagulant[4], plays an essential role. Supportive evidence for such a mechanism was provided by the prevention of lymphocyte fluorescence by heparinization of the guinea-pigs prior to injection of EC venom and fluorescein. Furthermore, subsequent intravascular administration of a large amount of thrombin to such heparinized envenomated animals caused the appearance of lymphocyte fluorescence.

The verification of the role of the venom procoagulant in lymphocyte fluorescence induction in an in vitro plasma medium is wrought with the difficulty that the leucocytes are trapped in the clot induced by the venom procoagulant. This obstacle was overcome by using plasma defibrinated by thrombin. In a mixture containing defibrinated plasma instead of serum, EC venom produced lymphocyte fluorescence. Addition of thrombin to this system, in which thrombin is produced by the procoagulant, was not required to obtain cell fluorescence, in contrast to the experiments in which serum was used as medium. Venom procoagulant (0.1 ml, 100 μg protein content/ml) or venom protease fraction (0.1 ml, 1200 μg protein content/ml), each alone, did not induce cell fluorescence in defibrinated plasma medium, but they were able to do so by joint action. Evidently, the fluorescein-staining promoting action of EC venom is due to a combined action of its procoagulant, inducing thrombin formation, and an additional venom component contained in the protease fraction[5].

Résumé. Le venin de l'*Echis colorata* produit la fluorescence des lymphocytes humains dans un mélange contenant du plasma défibrinogéné et de la fluorescéine. L'effet du venin est causé par l'action conjointe du thrombin produit par le procoagulant du venin et d'un autre facteur, qui peut être une protéase.

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[5] This study was supported by a grant from the Israel Cancer Association.

The Effect of the Anticoagulant EDTA on Oxygen Uptake by Bone-Marrow Cells

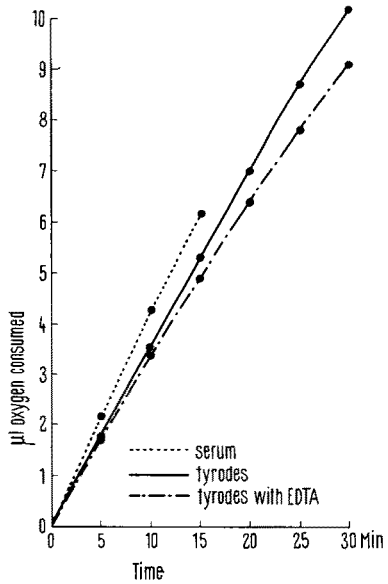
It is generally assumed that low concentrations of anticoagulants such as heparin and ethylenediaminetetraacetate (EDTA) are not excessively damaging to blood cells[1-3]. Studies in this laboratory using a modified Clark oxygen electrode apparatus have demonstrated that the uptake of oxygen by bone-marrow cells is significantly reduced in balanced salt solution containing a low concentration of disodium EDTA. The results of this experimental work are outlined in the present communication.

Methods. Male Holtzman rats, 42 $\pm$ 2 days of age, were used in all experiments. Animals were killed by a sharp blow to the base of the skull and the 2 femora and tibiae removed. The bones were split lengthwise and the marrow teased apart in Tyrode balanced salt solution. The composition of the Tyrode solution was: NaCl 8.0; KCl 0.2; $MgCl_2 \cdot H_2O$ 0.1; $NaH_2PO_4 \cdot H_2O$ 0.5; $NaHCO_3$ 1.0; glucose 1.0 g/l distilled water. In experiments using anticoagulants, the concentration of NaCl in Tyrode solution was reduced by 1.0 g/l and a corresponding amount of disodium EDTA dihydrate powder ($Na_2EDTA \cdot 2H_2O$) was added. This gave a concentration of Na_2EDTA of 1 mg/ml and served to maintain osmolarity of the suspending medium at 310 mOsm/l as measured with a Fiske osmometer.

Cell suspensions were obtained by passing the marrow successively through two 100-mesh stainless steel screens.

Determination of cell counts were made using a Coulter Counter Model B. Aliquots of the marrow cell suspension were diluted to 5 ml with either isologous serum or Tyrode solution with or without Na_2EDTA to obtain the desired number of cells for study.

Oxygen consumption of the bone marrow cells was determined at 37 °C with a Model 53 Biological Oxygen Monitor (Yellow Springs Instrument Company, Ohio).



Comparison of oxygen consumption of rat bone-marrow cells (cell concentration 8×10^7) in different suspending media. Each line represents the mean of 10 experiments.

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