

Since GAARDEN et al.⁵ had revealed for the first time the predominant role of ADP in thrombocyte adhesion, this concept has been largely accepted. Although other substances such as thrombin and collagen cause also thrombocyte adhesiveness, ADP would appear to be always the responsible factor for such adhesion.

ADP liberation originating from thrombocyte ATP (the concentration of which in the thrombocytes is very important) is a general phenomenon which appears whenever the thrombocytes are subjected to a change in their environment causing thereby an alteration of their membrane^{6,7}.

The negative charge of the thrombocytes results from the difference in ionic concentration between the plasma and the thrombocytes. Adhesion results from a depolarization or from a decrease of their zeta potential. The stability of a colloidal suspension depends on this potential which varies from 0–35 mV with 3 degrees of stability. Since the zeta potential of the blood cells falls between –15 and –20 mV, it confers only a very precarious stability on blood as a suspension⁷. Whenever the physicochemical characteristics of blood are altered, blood cells are subjected to degenerative changes which are preceded by an alteration of membrane permeability. The accumulation of H⁺ ions within the cytoplasm causes a drop in pH. The ion accumulation causes the penetration of Na⁺ and a K⁺ depletion. The lesions of their protein matrices are followed by a dissociation of the lipoprotein complexes and also by a shift of the Ca²⁺ ions. Since the production of membrane and cell energy is disturbed, protein synthesis no longer takes place and the enzymatic system activity decreases with the exception of those involved in the cell breakdown or lysis liberated at that very

moment. We believe that L-lactic acid is no exception to the general rule and causes – depending on the concentration – either an immediate depolarization of the membrane and an irreversible adhesiveness, at least in vitro, or the blocking of the glycolysis brought about by the reversal of the equilibrium reaction between pyruvic and lactic acid, which corresponds to a blocking of ATP synthesis. In the case of an essentially glycolytic cell structure (or the A structure of Laborit), as is the case for thrombocytes (a cell which never reaches the advanced state of cell differentiation, of early senescence and short life), the interruption of energy production speeds up its degenerative evolution which then facilitates its adhesiveness in the areas where its accumulation is possible.

Résumé. Les auteurs, après avoir montré le rôle probable de l'hyperlactacidémie dans l'apparition du sludge, du blocage du SRE, de l'œdème des cellules endothéliales des vaisseaux et de l'œdème intragial, montrent dans le présent travail sa responsabilité probable dans l'aggrégation des plaquettes sanguines de l'homme et du rat, en dehors de toute influence de pH.

H. LABORIT and M. R. ORNELLAS

*Cephepe, Laboratory of Eutonology,
Boucicaut Hospital,
Paris (France), 1969*

⁵ G. V. R. BORN, *J. Physiol.* 162, 67P (1962).

⁶ E. F. LÜSCHER, in *Exp. Biol. Med.* 3, 112 (Karger, Basel/New York 1968).

⁷ A. LARCAN and J. F. STOLTZ, *Agressologie* 9, 4, 481 (1968).

Aging in Relation to Auxin and RNA

The study of the aging of plant cells has been focused essentially on the action of exogenous factors¹. Few assays have, however, brought to light the change of endogenous compounds associated with the aging process². The aim of this work is to analyze the relationships between auxin and RNA levels – in connection with the biodegradation of such substances – and the aging of root cells prepared from etiolated *Lens culinaris* seedlings. The advantage of working with root tips is that they have both very young tissues (meristem and quiescent centre) and older ones (root cap)³.

With a special guillotine, 2 series of sections (from 0–200 μ , mainly old cells; from 200–500 μ , essentially young cells) were prepared from 18 mm roots of etiolated seedlings. For the auxin content, the acid fraction of the ethylacetate extracts were separated by thin layer chromatography⁴ and submitted to a *Lens* root and stem sections test⁵. The auxin biodegradation was studied with crude extracts and spectrophotocolorimetric (535 nm) analyses of the β -indolylacetic acid (IAA) destruction⁶. The total RNA was determined by the orcinol method previously discussed for a similar material⁷. The RNA biodegradation is based on the extraction of the RNase, tested by spectrophotocolorimetric (260 nm) analyses of RNA destruction⁸. All results will be expressed both per unit of protein nitrogen determined by UV-absorption (280 nm) after elimination of the interferences of nucleic acids⁹ and per unit of cells according to a method based on the use of the Navachine reagent and a pectinase incubation¹⁰.

Table I. Auxin content and auxin biodegradation in young and old cells prepared from the 18 mm roots of *Lens culinaris* seedlings

	Young cells	Old cells
Auxin content (in μ g IAA)		
per 10 mg N-protein	706.07 \pm 60.39	1.78 \pm 0.62
per 10 ⁷ cells	35.4	0.06
Auxin biodegradation ^a	68.5 \pm 7.1	94.4 \pm 10.2

^a In μ g IAA destroyed per 1 mg N-protein and 60 min of enzyme incubation.

As shown in Table I, the auxin level in young cells is significantly higher than in older ones and the IAA biodestruction is greater in old cells than in younger ones. As previously discussed¹¹, enzymes which control the

¹ H. M. WOOLHOUSE, XXIst Symposium Soc. exp. Biol. 21, 269 (1967).

² A. W. WHEELER, *J. exp. Bot.* 19, 102 (1968).

³ P. E. PILET and A. NOUGAREDE, *Bull. Soc. fr. Physiol. vég.* 11, 187 (1965).

⁴ G. COLLET, J. DUBOUCHET and P. E. PILET, *Physiol. vég.* 2, 157 (1964).

⁵ P. E. PILET, *Rev. gén. Bot.* 65, 605 (1958).

⁶ P. E. PILET, IV intern. Conf. on Plant Growth Regul. (Iowa Press 1961), p. 167.

⁷ P. E. PILET and R. BRAUN, *Physiologia plant.* 20, 870 (1967).

⁸ T. A. TRUELSSEN, *Physiologia plant.* 20, 1112 (1967).

⁹ O. WARBURG and W. CHRISTIAN, *Biochem. Z.* 310, 384 (1941).

¹⁰ E. C. HUMPHRIES and A. W. WHEELER, *J. exp. Bot.* 11, 81 (1960).

¹¹ R. C. HARE, *Bot. Rev.* 30, 129 (1964).

Table II. RNA content and RNase activity in young and old cells prepared from the 18 mm roots of *Lens culinaris* seedlings

	Young cells	Old cells
RNA content (in μg)		
per 0.1 mg N-protein	93.4 ± 8.0	52.9 ± 4.7
per 10^7 cells	456	223
RNase activity ^a	0.019	0.687

^a In OD₂₆₀ nm per 0.5 mg N-protein.

auxin catabolism determine the endogenous auxin content: high enzymes activity, for the old cells, means low auxin level, and vice versa for the young cells.

Values as regards the RNA are presented in Table II from which one can conclude that the concentration of the total RNA is greater in young cells than in older ones, and the RNA biodegradation is higher in old cells than in younger ones. Thus, the young cells which have a high auxin content, have a high RNA level. A positive correlation between endogenous IAA and RNA is consequently confirmed¹². It is well known that IAA can induce an increase of the RNA concentration by increasing the rate of RNA synthesis¹³, but it is also demonstrated that RNase activity is inhibited by IAA¹⁴.

Without asserting that only these interactions between auxin and RNA cause the cell aging, present data do suggest that enzymes, controlling the auxin biodegradation, are increasingly active as the cell grows older. Then the level of endogenous auxin is decreasing and therefore the RNase activity is enhancing, which consequently produces a decline of the RNA content as cell age increases.

Résumé. Sur des extraits de racine de *Lens culinaris*, il est observé qu'au cours du vieillissement cellulaire les enzymes qui contrôlent le catabolisme auxinique sont progressivement plus actives, ce qui a pour conséquence d'entraîner une diminution du taux en auxines endogènes. Par ailleurs, l'activité des systèmes RNasiques – inhibés par les auxines – va s'élever d'une façon significative dans les cellules âgées ce qui explique, partiellement du moins, la diminution de le teneur en RNA.

P. E. PILET

*Institut de Biologie et de Physiologie végétales
de l'Université, Lausanne
1000 Lausanne (Switzerland), 18 June 1969*

¹² Y. MASUDA and S. WADA, *Physiologia plant.* 19, 1055 (1966).

¹³ A. TREWAVAS, in *Progress in Phytochemistry* (Interscience Publishers Inc., New York 1968), vol. 1, p. 114.

¹⁴ P. E. PILET and R. BRAUN, *Physiologia plant.*, in press (1969).

The Presence of Carotenoids in Eggs Deposits of the *Chironomus annularius* Meig (Diptera: Chironomidae)

It is known that the eggs of mosquitoes are grouped together in so-called egg deposits of varying sizes. The deposit is enclosed in a transparent cover, the eggs being suspended in a colourless substance inside. The colour of the eggs and consequently the colour of the whole egg deposit depends on the colour of the yoke. The eggs are usually yellowish in colour, pinkish or sometimes greyish-green.

The purpose of these investigations was to determine which known carotenoids give this colour to the *Chironomus annularius* eggs.

The egg deposits were collected from the pond on the Bialystok aerodrome¹ and from a pond in Bialystok city. The columnar and thin-layer methods of chromatography were employed, various systems of solvents being used². The identification of the various fractions of the columnar chromatogram was based on the absorption maxima and the spots of the thin-layer chromatogram on the Rf³⁻¹⁰ values.

The absorption curves of extracts of the *Ch. annularius* eggs are given in Figure 1 and the absorption maxima of

these curves in Table I. As regards the columnar chromatography, by means of various solvent systems, the extract was divided into 6 fractions (Table II).

By means of thin-layer chromatography, employing A, B and C solvent systems (see Table III and Figure 2), the extract was separated into 5 spots, with solvent system D (benzene-ethyl ether-methanol in volumetric proportions of 17:2:1) only 4 spots were obtained.

On comparing the various absorption maxima and the Rf values with the data given in literature, it was possible to identify most of the carotenoids. The Rf value of the

Table I. Absorption maxima of extracts from eggs deposits of the *Ch. annularius* Meig

Absorption curve	Maximum absorption	Solvent
1	459–460; 480; 500; 520; 540; 550; 580; 600; 620; 660	Ethanol absolute
2	470; 500; 530; 580; 600; 620; 665	Acetone

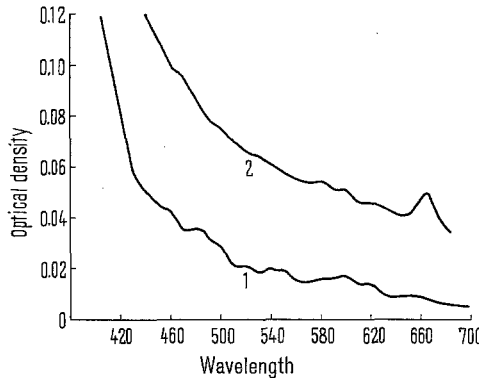


Fig.1. The absorption curves of extracts of the *Ch. annularius* Meig eggs.

¹ B. CZECZUGA, E. BOBIATYŃSKA-KSOK and E. NIEDŹWIECKI, *Zoolog. Pol.* 18, 317 (1968).

² B. CZECZUGA and R. CZERPAK, *Comp. Biochem. Physiol.* 24, 37 (1968).

³ B. CZECZUGA and R. CZERPAK, *Comp. Biochem. Physiol.* 14, 523 (1966).

⁴ B. CZECZUGA and R. CZERPAK, *Comp. Biochem. Physiol.* 24, 37 (1968).

⁵ B. CZECZUGA and R. CZERPAK, *Experientia* 24, 218 (1968).

⁶ B. CZECZUGA and R. CZERPAK, *Comp. Biochem. Physiol.* 25, 547 (1968).

⁷ B. CZECZUGA and R. CZERPAK, *Comp. Biochem. Physiol.* 26, 101 (1968).

⁸ B. CZECZUGA and R. CZERPAK, *Europ. J. Biochem.* 5, 429 (1968).

⁹ B. CZECZUGA and R. CZERPAK, *Comp. Biochem. Physiol.* 28, 221 (1969).

¹⁰ R. CZERPAK and B. CZECZUGA, *Marine Biol.* 4, 122 (1969).