Hyperlactacidemia as a Factor of Thrombocyte Adhesiveness

It has been shown experimentally that hyperlactacidemia plays a predominant part in the development of sludge, in the inhibition of the reticulo-endothelial system, in the oedema of the endothelial cells of vessels and in the increase in sedimentation rate[1,2]. It became, therefore, of interest to find out what would be the consequence of this same hyperlactacidemia on thrombocyte adhesiveness.

Materials and methods. Since it was not possible to reproduce exactly the techniques published by other investigators, we were first led to use a miscroscopic technique and then the spectrophotometric technique proposed by O'BRIEN[4] without constant stirring.

The mixing of the reagents was performed directly in the cuvettes of the apparatus with a plastic stirrer. It was then incubated at 37 °C. The thrombocyte-rich human and rat plasma (PRP) was citrated (final concentration: 0.38 per 100). The number of thrombocytes was around 200,000/mm³ for human and 150,000/mm³ for rat plasma, according to our count. In agreement with the results of others, our experiment shows that the adhesiveness is more rapid at 37 °C and depends on the rate of stirring, as also on the ADP concentration (or that of other reagents) and on the number of thrombocytes; the absence of any one of these factors slows down the process.

Results and discussion. Adhesiveness begins with 20 mg of L-lactic acid/100 ml of plasma and is complete with 80 mg/100 ml (Figures 1 and 2), (a concentration reached during conditions of metabolic acidosis). The final pH varies from 6.55–6.35, according to the concentration. However, the latter pH is the same for epinephrine ($M \times 10^{-7}$). The addition of hydrochloric acid ($M \times 10^{-4}$), with a final pH of 6 does not cause any adhesiveness (Figure 3). Conversely, potassium chloride ($M \times 10^{-4}$; pH 7.35) and NAD ($M \times 10^{-3}$; pH 7.35) causes an immediate decrease of the O.D. in the first case, and a slower and more regular decrease in the second (Figure 4). The microscopical technique was applied to human thrombocytes and packed rat placed between a slide and cover glace. The various reagents were introduced by capillarity.

Adhesiveness caused by L-lactic acid is accelerated by the rapid diffusion and it is almost complete. ADP does not cause the same type of adhesion; it appears in various areas and the thrombocytes are swollen. Epinephrine, which is considered as non-aggregating for thrombocytes of rats, causes a slight aggregation, an increase in adhesiveness, and the thrombocytes assume the characteristic thin rod shape. It should be noted that the in vitro experimental conditions are completely artificial.

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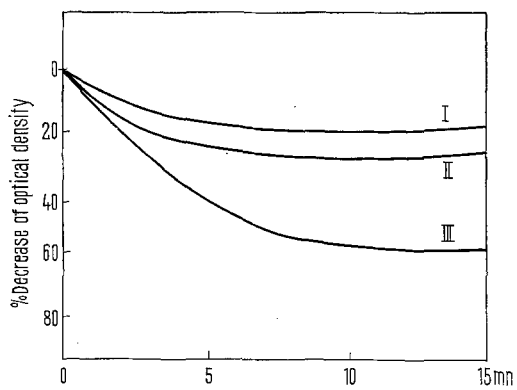

Fig. 1. Curve I. At zero time addition of L-lactic acid (final concentration 20 mg/100 ml of plasma). Curve II. At zero time addition of L-lactic acid (final concentration 40 mg/100 ml of plasma). Curve III. At zero time addition of L-lactic acid (final concentration 80 mg/100 ml of plasma).

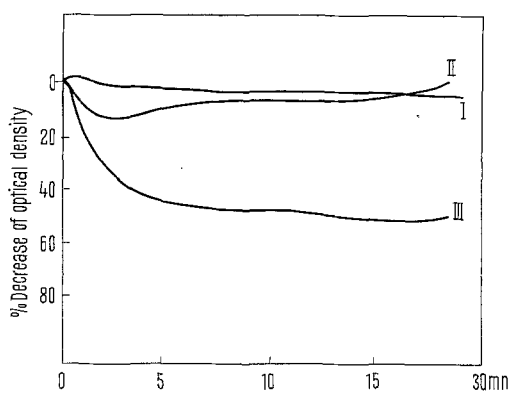

Fig. 3. Curve I. At zero time addition of buffer pH 7.35. Curve II. At zero time addition of hydrochloric acid (final concentration $M \times 10^{-4}$; pH 6. Curve III. At zero time addition of L-lactic acid (final concentration 80 mg/100 ml of plasma; pH 6.35).

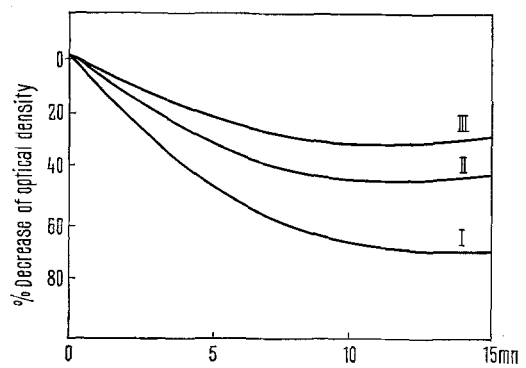

Fig. 2. Curve I. At zero time addition of L-lactic acid (final concentration 80 mg/100 ml of plasma). Curve II. At zero time addition of ADP (final concentration $M \times 10^{-6}$). Curve III. At zero time addition of epinephrine (final concentration $M \times 10^{-7}$).

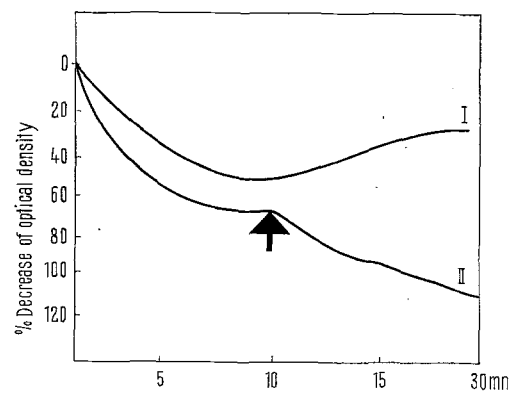

Fig. 4. Curve I. At zero time addition of NAD^+ (final concentration $M \times 10^{-3}$; pH 7.35). Curve II. At zero time addition of L-lactic acid (final concentration 80 mg/100 ml of plasma). At the arrow, addition of KCl (final concentration $M \times 10^{-4}$; pH 7.35).

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 suspension[7]. 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 Ca^+ 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 intraglial, montrent dans le présent travail sa responsabilité probable dans l'agrégation des plaquettes sanguines de l'homme et du rat, en dehors de toute influence de pH.

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Aging in Relation to Auxin and RNA

The study of the aging of plant cells has been focused essentially on the action of exogenous factors[1]. Few assays have, however, brought to light the change of endogenous compounds associated with the aging process[2]. 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 chromatography[4] and submitted to a *Lens* root and stem sections test[5]. The auxin biodegradation was studied with crude extracts and spectrophotocolorimetric (535 nm) analyses of the β-indolylacetic acid (IAA) destruction[6]. The total RNA was determined by the orcinol method previously discussed for a similar material[7]. The RNA biodegradation is based on the extraction of the RNase, tested by spectrophotocolorimetric (260 nm) analyses of RNA destruction[8]. All results will be expressed both per unit of protein nitrogen determined by UV-absorption (280 nm) after elimination of the interferences of nucleic acids[9] and per unit of cells according to a method based on the use of the Navachine reagent and a pectinase incubation[10].

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 µg IAA)		
per 10 mg N-protein	706.07 ± 60.39	1.78 ± 0.62
per 10^7 cells	35.4	0.06
Auxin biodegradation[a]	68.5 ± 7.1	94.4 ± 10.2

[a] In µg 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 discussed[11], enzymes which control the

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