Cytotoxic activity and binding capacity of rabbit anti-rat cervical ganglion and anti-rat thymocyte sera for rat thymocytes and B-lymphocytes

Cells used in test	Cells used for absorption	Cytotoxic index (%) Antiserum dilution (log_2) 1	2	3	4	5	6	7	Percent of fluorescein-positive cells
		Anti-rat cervical ganglion serum							
Thymocytes	None	82.6	62.4	27.1	10.5	3.0	0	0	58.2
	Thymocytes	3.4	0	0	0	0	0	0	0.2
B-lymphocytes	None	3.1	1.9	2.4	0	0	0	0	0.3
	Thymocytes	1.4	0	0	0	0	0	0	0.2
		Anti-rat thymocyte serum							
Thymocytes	None	99.1	97.9	97.1	91.0	47.5	16.4	3.8	99.6
	Thymocytes	2.4	0	0	0	0	0	0	0.2
B-lymphocytes	None	58.2	46.8	24.5	6.7	0	0	0	60.3
	Thymocytes	2.8	0.5	0	0	0	0	0	2.1

Each figure represents mean value of 3 independently performed experiments. At least 1000 lymphocytes/preparation were counted in cytotoxicity and immunofluorescence assays.

for anti-CG serum. The cytotoxic activity[10] of anti-CG and ATS was tested against rat thymocytes and B-lymphocytes. B-cells were obtained from the femoral bone marrow of 6-week-old rats thymectomized at birth and irradiated at the age of 2 weeks with 700 R. Paraffin sections[11] of the rat cervical ganglion and viable thymocytes[9] were employed in the indirect fluorescent staining[12] using a sheep fluorescein-conjugated anti-rabbit IgG serum[13]. Several controls were set up in order to distinguish clearly specific green fluorescence from nonspecific fluorescence[14].

Results and discussion. The cytotoxic activity and binding capacity of rabbit anti-rat cervical ganglion and anti-rat thymocyte sera are shown in the table. Anti-CG serum was cytotoxic for thymocytes at a 1:8 dilution, and induced specific fluorescence of about 58% of thymocytes, whereas this antiserum was completely inactive for B-lymphcytes. On the other hand, ATS killed or stained specifically about 100% of thymocytes and about 60% of B-lymphocytes. Absorption of anti-CG and ATS with 10^9 thymocytes/ml of antiserum removed all of antibody activity to thymocytes and B-lymphocytes.

The cervical ganglion sections exposed to anti-CG (figure, a) or ATS (figure, b) and fluorescein-conjugate exhibited specific fluorescence of neuronal membranes, whereas neuronal cytoplasm and nuclei remained unstained. This fluorescence was significantly stronger with tissue sections treated with anti-CG serum. As for thymocytes, ATS induced a bright specific fluorescence of the ring-type (figure, c). Thymocytes exposed to anti-CG serum displayed a specific staining of irregular shape, most of fluorescein-conjugate molecules being concentrated on a portion of the surface membrane (figure, d). All control tissue sections and thymocytes preparations were negative.

These results show the presence of common membrane antigens on the surface of rat thymocytes and superior cervical ganglion cells, thus indicating that the thymus and the peripheral nervous tissue share at least some antigenic determinants[2].

10 S. Konda, E. Stockert and R. T. Smith, Cell Immun. *7*, 275 (1973).
11 G. Sainte-Marie, J. Histochem. Cytochem. *10*, 250 (1962).
12 E. J. Holborow and G. D. Johnson, in: Handbook of Experimental Immunology, p. 18.8. Ed. D. M. Weir. Blackwell, Oxford 1973.
13 O. Aalund, J. W. Osebold and F. Murphy, Archs Biochem. Biophys. *109*, 142 (1964).
14 B. D. Janković, J. Horvat, K. Mitrović and M. Mostarica, Immunochemistry *14*, 75 (1977).

Electrical stimulation induces clot retraction after previous in vitro platelet aggregation

D. Bottecchia, G. P. Fantin, P. Gruppo, G. Nassuato and M. Barbera[1]

Istituto di Fisiologia Umana, 1° Cattedra, Facoltà di Medicina, Università di Padova, via Marzolo 3, I–35100 Padova (Italy), 2 May 1977

Summary. The spontaneous clot retraction of platelet-rich plasma is inhibited by previous in vitro ADP-induced platelet aggregation. The electrical stimulation of the clot always restores a maximal clot retraction, even after a prolonged previous in vitro platelet aggregation.

Earlier work showed that previous in vitro platelet aggregation inhibits spontaneous clot retraction[2]; the degree of inhibition is related to the degree of aggregation. However, if the aggregation is followed by a rapid and complete disaggregation, subsequent spontaneous clot retraction is not inhibited[2].

These facts suggested that platelets have not to be aggregated before or during clotting in order to support a normal clot retraction. It was supposed that the inhibition of clot retraction by previous platelet aggregation could be due to the lack of a random distribution of the

1 Acknowledgment. We thank Mr Renzo Ferronato for his technical assistance and Miss Maria Tommasini for her secretarial aid.
2 G. de Gaetano, M. B. Donati, J. Vermylen and M. Verstraete, Thromb. Diath. haemorrh. *26*, 449 (1971).

Electrical stimulation	Time of previous in vitro platelet aggregation 2 min No	Yes	4 min No	Yes	6 min No	Yes	8 min No	Yes	10 min No	Yes
Clot reaction (%)	74.2	79.0	79.7	78.0	43.7	70.5	25.0	56.5	24.1	79.1
	76.3	78.2	78.4	79.2	41.5	78.6	23.1	77.3	23.4	78.4
	72.4	75.6	79.1	78.1	40.0	81.0	27.2	73.0	25.0	85.1
	77.0	72.0	77.1	77.2	39.0	79.3	23.0	83.3	22.2	77.1
	76.1	77.2	78.3	78.3	44.7	75.0	24.5	72.1	23.3	73.2
	75.7	75.8	77.5	77.9	42.1	77.6	22.6	73.5	22.4	77.6
					40.0	79.1	21.3	79.0		
Mean ± SD	75.2 ± 1.69	76.3 ± 2.48	78.3 ± 0.96	78.1 ± 0.65	41.8 ± 1.84	77.3 ± 3.51	23.8 ± 1.92	73.5 ± 8.48	23.4 ± 1.04	78.41 ± 3.86

Percent clot reaction (following, or not, electrical stimulation) after previous in vitro platelet aggregation induced by ADP. Clotting of PRP, electrical stimulation and evaluation of clot retraction, were performed as described in 'materials and methods'.

platelets as a consequence of their clumping, or to a diminution of the platelet surface available for fibrin fibres[2].

In this paper we present evidence that electrical stimulation of PRP is able to induce clot retraction even if coagulation was preceded by platelet aggregation.

Materials and methods. Human blood was collected from the anticubital vein of healthy donors. Platelet-rich plasma (PRP), platelet-poor plasma (PPP) and platelet count were performed as described previously[3]. Adenosine-5'-diphosphate (ADP) trisodium salt (Boehringer, Mannheim, Federal Republic of Germany) was dissolved in buffered saline pH 6.8 at a concentration of 10^{-3} M and stored at −20 °C until use.

The ADP-induced platelet aggregation (final concentration of ADP 2×10^{-4} M) was performed in PRP with a standard platelet concentration (300,000/mm³) by an aggregometer (169 Platelet Aggregation Meter, Evans Electroselenium Ltd) and recorded by Speedomax XL 690 Series Recorder (Lees and Northrup, North Wales and Philadelphia) as usual[3]. At 2, 4, 6, 8 and 10 min after the addition of ADP, each sample of PRP was divided in 2 equal parts which were transferred into 2 glass tubes and clotted by thrombin (Topostasine Roche, Milano, Italy) at a final concentration of 3 units/ml, and incubated at 37 °C. 3 min after clotting, one of the 2 samples was stimulated electrically as described previously[4]. The electrical stimuli were applied for 6 min and had the following characteristics: intensity: 150 V; frequency: 10/sec; duration of each stimulus: 50 msec.

Clot retraction was evaluated 60 min after the end of the electrical stimulation, by measuring the amount of serum expressed[5].

Results. Figure 1 reports a typical tracing of platelet aggregation induced by ADP at a final concentration of 2×10^{-4} M. The platelet aggregation is very rapid and irreversible, and it reaches its maximal value in about 3–4 min. The spontaneous clot retraction is progressively less marked as more as the time of the previous in vitro platelet aggregation increases. After an aggregation of 2 or 4 min, the spontaneous clot retraction reaches maximal values (figure 2, A), while after an aggregation prolonged for 6 min, the spontaneous clot retraction is reduced (figure 2, A); more marked inhibition can be observed after 8 or 10 min of platelet aggregation (figure 2, A).

3 D. Bottecchia and M. G. Doni, Experientia *29*, 211 (1973).
4 G. Fantin, D. Bottecchia, G. Nassuato, P. Gruppo and P. Zatti, Thromb. Res. *8*, 523 (1976).
5 D. Bottecchia, G. Fantin, P. Gruppo, G. Nassuato and P. Zatti, Scand. J. Haemat. *16*, 75 (1976).

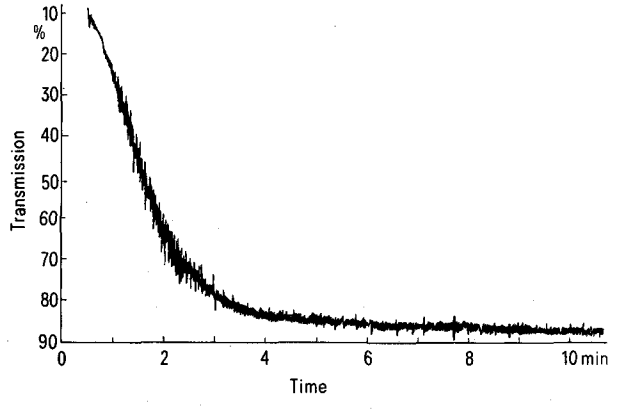

Fig. 1. Typical tracing of platelet aggregation induced by ADP (final concentration 2×10^{-4} M) on human PRP.

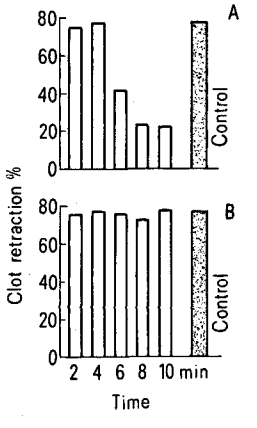

Fig. 2. Average clot retraction evolving spontaneously (A) or induced by electrical stimulation (B), in PRP clotted by thrombin (final concentration 3 units/ml) after previous in vitro platelet aggregation prolonged of different times. The values are deduced from the data reported in the table.

The electrical stimulation of the clot restores always a maximal clot retraction, even after a prolonged (8 or 10 min) previous in vitro platelet aggregation (figure 2, B); this result was constantly found in all the experiments.
The table reports all the results of clot retraction (following previous in vitro platelet aggregation prolonged of different times), occurring in clots submitted or not to electrical stimulation.
Discussion. Present results confirm that previous in vitro platelet aggregation inhibits spontaneous clot retraction. However, the degree of inhibition seems more dependent on the duration than on the degree of aggregation: 4 min after the addition of ADP, the degree of aggregation was the same as after 10 min, but the subsequent spontaneous clot retraction was inhibited only in the latter case. Moreover the electrical stimulation of the clot provokes clot retraction even after a previous prolonged (10 min) platelet aggregation, thus demonstrating that suitable condition (s), like electrical stimulation, can trigger off platelet modification leading to clot retraction.
These facts show clearly that the inhibition of spontaneous clot retraction, by previous in vitro platelet aggregation, is not related to the altered distribution of the platelets, nor to a diminution of the platelet surface available for fibrin fibres, as was supposed by de Gaetano et al.[2]; therefore it is possible that the previous in vitro platelet aggregation provokes an altered metabolic state of the platelets, which become unable to supply energy for spontaneous clot retraction, even if their responsiveness to the electrical stimulation is not decreased.
An earlier paper from our laboratory indicated that electrically induced retraction of reptilase clots is inhibited by aspirin and by indomethacine[5], which are ineffective on the reptilase clot retraction occurring in the presence of aggregating agents[6].
Present experiments showed clearly another difference between clot retraction occurring after electrical stimulation or in the presence of aggregating agents[6].

6 G. de Gaetano, D. Bottecchia and J. Vermylen, Thromb. Res. *2*, 71 (1973).

Leukocyte mobilization by epinephrine and hydrocortisone in patients with chronic renal failure

G. Perescenschi, V. Zakouth, Z. Spirer and A. Aviram

Department of Nephrology and the Paediatric Immunology Unit, Rokach (Hadassah) Hospital, Tel-Aviv Medical Center, Tel-Aviv (Israel), 23 May 1977

Summary. The mobilization of WBC from the bone marrow, as judged from hydrocortisone-induced leucocytosis, is markedly impaired in dialyzed and nondialyzed uremic patients. The release of WBC from the marginal pool by epinephrine was found to be normal.

Uremia is commonly considered to be an acquired immune-deficiency state, but the exact mechanism(s) of this impaired immunity is still not clear[1-4]. As polymorphonuclear leucocytes play an important role in the first line defence in the immunological process, it seemed important to study their function in uremia. We investigated the mobilization of leucocytes from the bone marrow and the marginal pool in patients with chronic renal failure.
Material and methods. 16 patients with chronic renal failure treated conservatively participated in the study. They had creatinine clearance below 30 ml/min. All were well maintained and none was in the terminal stages of uremia. Additional 18 patients were treated by hemodialysis. They were well dialyzed in accordance with accepted criteria, 15–18 h a week on coil dialyzer. A group of volunteer medical students and hospital employees served as normal controls. Age and sex were equally distributed among the 3 groups. None of the patients received steroids or immunosuppressive drugs.
Epinephrine test: 0.01 mg/kg b.wt of epinephrine solution was injected s.c. Capillary blood samples were drawn before and at 5, 15, 20, 30 and 60 min following the injection, for total white blood counts[5]. This test was performed in 10 of the normal subjects, 13 uremic and 8 dialysis patients.

1 M. C. Jordan, W. E. Rousseau, G. E. Tegtmeier, G. R. Noble, R. G. Muth and T. D. Y. Chin, Ann. intern. Med. *79*, 790 (1973).
2 D. P. S. Sengar, D. B. Hyslop, A. Reshid and J. E. Harris, Cell. Immun. *20*, 92 (1975).
3 C. A. Kauffmann, A. D. Manzler and J. P. Phair, Clin. exp. Immun. *22*, 54 (1975).
4 L. J. Quadracci, O. Ringden and M. Krzymanski, Kidney Int. *10*, 179 (1976).
5 A. J. Samuels, J. clin. Invest. *30*, 941 (1951).

Table 1. Mean leucocyte counts (± SE) following epinephrine injection*

	Min after epinephrine injection						
	0	5	10	15	20	30	60
Normal subjects (n = 10)	6 645 ± 518	9 435 ± 1 317	14 415 ± 1 921	15 333 ± 1 806	14 083 ± 1 105	11 485 ± 875	9 365 ± 898
Uremic patients (n = 13)	7 438 ± 593	9 020 ± 828	12 659 ± 1 744	12 938 ± 1 124	13 526 ± 1 274	12 065 ± 1 252	10 042 ± 800
Dialysis patients (n = 8)	7 206 ± 599	8 175 ± 394	11 762 ± 1 019	12 850 ± 1 128	14 512 ± 1 339	12 788 ± 1 273	8 781 ± 1 162

*Differences between the means of the 3 groups were not statistically significant at any time.