Phagocytosis index, activities of acid and alkaline protease plasminogen, plasminogen activator, and spontaneous fibrinolytic activity of the leucocytes in control and irradiated guinea-pigs

No. of animals	Time of PMN induced after irradiation (days)	Phagocytosis index	Acid protease[a]	Alkaline protease[a]	Plasminogen[b]	Plasminogen activator[b]	Spontaneous fibrinolytic activity[b]
9	Control	11.8 (4.3–13.4)	66.0 (35–129)	99.0 (59–102)	82.0 (35–124)	578.0 (397–877)	78.0 (30–96)
3	1	9.4 (5.8–10.5)	20.0 (19.5–20.0)	27.0 (18.5–41)	46.0 (26–64)	156.0 (100–200)	72.0 (68–78)
3	14	11.6 (11.4–11.8)	13.0 (12.0–14.5)	24.0 (19.5–29)	0 (0–0)	282.0 (208–357)	30.0 (15–44)
2	20	6.5 (5.9–7.2)	20.5 (20–21)	28.5 (11–46.5)	7.0 (0–14)	246.0 (240–253)	8.4 (7–10)

[a] Δ OD $\times 10^3$. [b] mm^2 of area of digested fibrin. No. in brackets denotes minimal and maximal values.

No correlation between the degree of inhibition of phagocytosis index and the decrease of activities of fibrinolytic and proteolytic enzymes can be stated in particular animals. It is of interest to notice that in some leucocyte samples plasminogen and the other proteases considerably decreased while the phagocytosis index was not significantly changed. Similar results were obtained in guinea-pigs treated by cytostatic agents[1]. It has been suggested that there is an excess of proteolytic enzymes and their significant decrease may be without any effect on the phagocytosis index.

The question arose of why the irradiated animals are so susceptible to the infection. Several authors stated that some leucocytes functions of irradiated animals are disturbed. In these cells the ability to destroy intracellular bacteria[8] and intracellular digestion of chicken red cells[2] is depressed. SELVARAJ and SBARRA[3] stated that, following irradiation, there was a decrease of lactic acid formation, which may be a cause of the impairment of acid protease activity. The formation of hydrogen peroxidase is also diminished. This factor is known to be the antimicrobial agent[9].

It is possible that the decrease of proteolytic enzymes may also be responsible for the decrease of intracellular digestion of bacteria in the granulocytes of irradiated animals.

Résumé. Après l'exposition du cobaye aux rayons X, nous avons constaté l'affaiblissement des enzymes granulocytaires suivants: la protéase acide, la protéase alcaline, le plasminogène, l'activateur du plasminogène, l'activité fibrinolytique spontanée. L'index de la phagocytose n'a diminué qu'au 20e jour après l'irradiation.

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[8] E. L. NELSON and J. R. BECKER, J. infect. Dis. *104*, 13 (1959).
[9] G. Y. N. IYER and J. H. QUASTEL, Can. J. Biochem. Physiol. *41*, 427 (1963).

Hageman Factor (Factor XII) Activity in Synovial Fluid of Rheumatoid Arthritis Patients and its Possible Pathogenic Significance

It is well known that the activation of factor XII in the blood triggers intrisic clotting and induces the formation of kinins and a pain-producing substance[1,2]. Recently it has been found that factor XII is activated by uric acid crystals[3,4].

Several authors[5,6] suggested that this phenomenon may play a significant role in the pathogenesis of gouty arthritis. It seemed of interest to investigate factor XII activity in the synovial fluids of patients with rheumatoid arthritis.

The synovial fluid was aspirated from patients with rheumatoid arthritis by puncture of the knee joint and immediately mixed with 0.1 *M* sodium oxalate (1 + 9 vol. aspirate). At the same time, samples of oxalated blood were obtained from the patients.

In the first part of the experiments the following blood clotting factors were simultaneously determined in the oxalated plasma and in the synovial fluid samples of 30 patients, prothrombin (II) factors V, VII + X, VIII, IX, XI + XII. These factors were determined using one-stage methods. As a substrate for the factor XI + XII determinations 'exhausted plasma'[7] was used. Plasma samples of a patient with hemophilia A and B were used for testing factor VIII and IX. The protein concentration in the plasma and in synovial fluid was determined using the biuret method. The results of the determinations of the specific activities of these clotting factors in synovial

[1] O. D. RATNOFF, Prog. Hemat. *12*, 204 (1967).
[2] J. MARGOLIS, J. Physiol. *144*, 1 (1958).
[3] R. W. KELLERMEYER and R. T. BRECKENRIDGE, J. Lab. clin. Med. *64*, 873 (1964).
[4] R. W. KELLERMEYER, J. Lab. clin. Med. *67*, 455 (1966).
[5] D. J. MCCARTHY JR. and J. L. HOLLANDER, Ann. intern. Med. *54*, 462 (1961).
[6] J. E. SEEGMILLER, R. R. HOWELL and S. MALAWISTA, J. Am. med. Ass. *180*, 469 (1962).
[7] B. A. WAALER, *Contact Activation in the Intrinsic Blood Clotting System* (Oslo University Press, Oslo 1959).

fluids is presented in Figure 1 in comparison with those in plasma accepted as 100%.

It can be seen that the specific activity of factor XI + XII in the synovial fluid is on the average 2.5 times higher than in the plasma. The specific activities of other clotting factors in the synovial fluid were slightly lower than in the plasma. A marked decrease of factor V activity was noted.

In the second series of experiments, leucocytes were isolated from the synovial fluid by centrifugation and washed several times with saline. Factor XI + XII was tested using exhausted plasma. Hageman factor-deficient plasma (kindly supplied by Dr. E. LOELIGER, Leiden) was used for determining factor XII activity in the synovial fluid supernatant and in a leucocyte suspension.

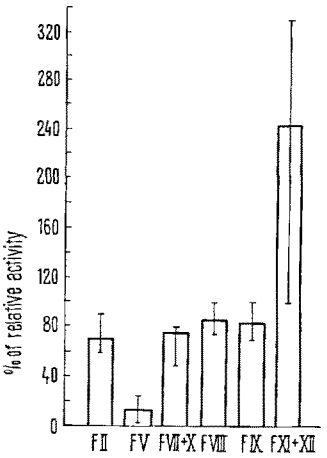

Fig. 1. Specific activities of various clotting factors in synovial fluids as compared with their specific activities in plasma accepted as 100%. Mean values from 30 determinations and ranges of variation are given.

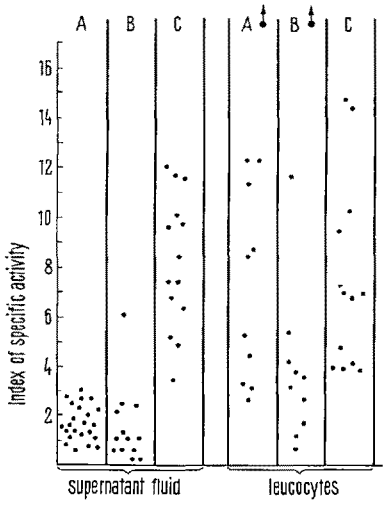

Fig. 2. Indices of the specific activities of factor XI + XII and of factor XII in supernatant of synovial fluids and in leucocytes suspensions. Experimental system: (A) Factor XI + XII, 0.1 ml of fluid or leucocyte suspension, 0.1 ml exhausted plasma, 0.1 ml to 0.025 *M* $Ca Cl_2$. (B) Factor XII, 0.1 ml of fluid or leucocyte suspension, 0.1 ml Hageman factor deficient plasma, 0.1 ml–0.025 *M* $Ca Cl_2$. (C) Isolated factor XII activity, 0.1 ml of isolated contact factor, 0.1 ml Hageman factor deficient plasma, 0.1 ml–0.025 *M* $Ca Cl_2$.

Furthermore, the activated contact factor was isolated from the synovial fluid supernatant and from the leucocyte homogenate obtained after freezing and thawing. This procedure of isolation consisted in adsorption of the tested material on kaolin and on subsequent elution at alkaline pH[8]. The results presented in Figure 2 are expressed in indices of specific activity. ($I = 1000/t$ sec/mg protein). It can be seen that the specific activities of factor XI + XII and of factor XII in the supernatant synovial fluid are of the same order of magnitude. The specific activity of factor XII increases when it is isolated from the fluid by means of adsorption on kaolin and elution. The specific activities of factor XI + XII and of factor XII are higher in leucocyte suspensions than in the supernatant fluid and the activated contact can be isolated from the leucocyte homogenate. It should be emphasized, however, that the activities of factor XI + XII and of factor XII in the leucocytes showed greater variations than those in the supernatant fluid.

It can be concluded from the results of the present experiments that there is a considerable Hageman factor activity in the synovial fluid. This factor shortens the clotting time of Hageman deficient and of exhausted plasmas and it may be adsorbed and eluted from kaolin.

The Hageman factor contained in the synovial fluid of patients with rheumatoid arthritis may be specifically adsorbed on the cell surface of leucocytes or may originate from these cells.

It has recently been stated by several authors[9,10] that granulocytes contain kinin-forming enzymes. Therefore it may be postulated that the activated Hageman factor triggers kinin formation within the cells. Another role of the Hageman factor in the synovial fluid may consist in triggering intraarticular coagulation leading to the deposition of fibrin in the joint cavities. Several authors[11,12] emphasize the role of this process in the pathogenesis of rheumatoid arthritis. It is possible that Hageman factor plays a role both in gouty arthritis and in the rheumatoid inflammation of the joints.

The mechanism of the activation of factor XII in synovial fluid is not clear. It has been found that purified insoluble collagen activates factor XII[13]. Therefore it is possible that collagen fibres of the joint tissue may also participate in this process.

Résumé. Nous avons constaté que l'activité spécifique du facteur de Hageman dans le fluide synovial de malades atteints de polyarthrite chronique évolutive est beaucoup plus elevée que celle d'autres agents de coagulation. L'activité du facteur de Hageman a été isolée du fluide synovial et de la suspension de leucocytes par adsorption sur kaolin et élution au pH alcalin.

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15 March 1968.

[8] S. NIEWIAROWSKI, J. STACHURSKA and Z. WEGRZYNOWICZ, Thromb. Diath. haemorrh. *7*, 514 (1962).
[9] M. J. CLINE and K. L. MELMON, Nature *213*, 90 (1967).
[10] L. M. GREENBAUM and K. S. KIM, Br. J. Pharmac. *29*, 238 (1967).
[11] L. E. GLYNN, Bull rheum. Dis. *14*, 323 (1963).
[12] M. I. BARNHART, I. M. RIDDLE, G. B. BLUHM and C. QUINTANA, Ann. rheum. Dis. *26*, 206 (1967).
[13] S. NIEWIAROWSKI, E. BAŃKOWSKI and I. ROGOWICKÁ, Thromb. Diath. haemorrh. *14*, 387 (1965).