

the ATP concentrations after the second period of stimulation were larger, and the amount found with a resting interval of 100 sec was close to the value immediately after a single period of stimulation. For comparison, the ATP after one period of stimulation is also plotted in Figure 3.

The experiments demonstrate the utilization of ATP during electrical activity and during the post-tetanic period. The peaks of ATP degradation and ADP synthesis correspond approximately to the peaks of heat production and oxygen consumption. The experiments further show that ATP is quickly resynthesized. This rapid synthesis is suggested also by the experiments of GREENGARD and STRAUB⁷, who measured the ATP concentration in vagus nerve trunks immediately after a 15 sec tetanus, and found no significant change in ATP with stimulation frequencies of 6 and 15 c/sec. Our experiments with 2 periods of stimulation demonstrate that the ATP concentration can be depressed to extremely low levels. This large depression suggests that, in addition to the non-myelinated axons, myelinated fibres as well as Schwann cells participate in the increased utilization of ATP after electrical activity⁸.

Résumé. Le métabolisme des esters phosphorés riches en énergie a été étudié dans des fibres non-myélinisées

pendant la période post-tétanique. Après une période de stimulation, le stock d'ATP est utilisé dans une proportion de 70%, puis, une rapide resynthèse a lieu en 90 sec. On constate également une augmentation concomitante de la teneur en ADP. Les pics de dégradation de l'ATP et de synthèse de l'ADP correspondent approximativement à ceux du dégagement de chaleur et de la consommation d'oxygène. Les expériences effectuées avec 2 périodes de stimulation montrent que le contenu en ATP peut s'abaisser à un niveau très bas. Cette importante diminution suggère une participation possible des cellules de Schwann au métabolisme post-tétanique.

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⁸ These experiments were supported by a grant from the Swiss National Fund for Scientific Research (No. 4540).

Effect of X-Rays on the Proteolytic Enzymes of Granulocytes and on Phagocytosis Index in Guinea-Pigs

In a previous communication¹ it was reported that the cytostatic agents decrease fibrinolytic and proteolytic enzymes of granulocytes but they do not affect phagocytosis index.

It is generally accepted that irradiated animals are more susceptible to the infection than non-irradiated ones. Numerous reports have been undertaken to elucidate the effects of irradiation on phagocytosis²⁻⁴. BERCOVICI⁴ reported that irradiation depresses the phagocytic activity of leucocytes in both in vivo and in vitro experiments but it did not affect the proportion of cells exhibiting phagocytosis.

Migratory capacity of phagocytic cells was not affected by irradiation, but the proliferation of these cells was depressed⁵. No reports appeared which describe the effect of irradiation on the activities of fibrinolytic and proteolytic enzymes of the leucocytes. The purpose of this work was to investigate this problem.

Seventeen guinea-pigs of both sexes (weight 300-400 g) were used in these experiments. Peritoneal exudates were induced by injection of 0.1% solution of glycogen in amounts of about 70 ml. The exudates were collected 15 h after injection of glycogen. Phagocytosis index was calculated according to DAVIES' methods⁶, using light microscopy. PMN leucocytes from exudates were mixed in tube with opsonized strain of staphylococcus 209 P. 100 cells were counted from each sample.

Whole-body irradiation of guinea-pigs was carried out in plastic cages at a target distance of 50 cm operated at 160 kVp, 10 mA, with a filter of 5 mm Cu and 10 mm Al at the rate of 23.2 rpm. Whole dose was 350 r.

The control group comprised 9 animals. PMN exudate obtained from these animals were considered as controls.

The leucocyte count of all samples amounted to 10,000 per mm³. Homogenate of the leucocytes were dialysed against phosphate buffer at 4°C for 18 h. The activity of alkaline and acid leucocyte protease was determined by the caseinolytic method. The fibrinolytic enzymes: plasminogen, plasminogen activator and spontaneous fibrinolytic activity were studied, using ASTRUP and LASSEN plate method. Details concerning preparation of leucocyte homogenate and proteolytic and fibrinolytic methods are presented elsewhere⁷.

The Table shows that irradiation induces a significant decrease of the activities of all investigated fibrinolytic and proteolytic enzymes. This decrease was statistically significant $p < 0.01$ when 9 samples of control leucocytes were compared with 8 samples of leucocytes collected from irradiated animals.

Phagocytosis index was slightly decreased but this change is not statistically significant, except in animals in which leucocytes were collected 20 days after irradiation.

¹ J. PROKOPOWICZ, L. REJNIAK and S. NIEWIAROWSKI, *Experientia* 23, 813 (1967).

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⁶ G. E. DAVIES, *J. Path. Bact.* 63, 149 (1951).

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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 mm² 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 agents¹. 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 bacteria⁸ and intracellular digestion of chicken red cells² is depressed. SELVARAJ and SBARRA³ 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 agent⁹.

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 20^e jour après l'irradiation.

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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 intrinsic 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

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