Table II. Rate of clearance from peripheral blood and organ uptake of $^{51}$Cr-labelled *E. coli* in irradiated and control mice[a]

| Experimental group | Rate of clearance $K \times 100$ | % of injected radioactivity | | | | | No. |
|---|---|---|---|---|---|---|---|
| | | Liver | Spleen | Lungs | Kidney | Total recovered | of mice |
| 7 days after 1000 rads | $14.6 \pm 1.1$ | $74.8 \pm 0.7$[b] | $0.47 \pm 0.05$[b] | $2.3 \pm 0.2$[b] | $0.34 \pm 0.03$[b] | $77.9 \pm 0.8$ | 6 |
| 14 days after 800 rads | $15.8 \pm 0.9$[b] | $74.6 \pm 1.3$[b] | $0.74 \pm 0.20$[b] | $2.8 \pm 0.3$[b] | $0.61 \pm 0.13$ | $78.7 \pm 1.0$ | 8 |
| Controls | $11.9 \pm 0.2$ | $66.1 \pm 0.6$ | $2.64 \pm 0.32$ | $6.4 \pm 0.3$ | $0.62 \pm 0.03$ | $75.7 \pm 0.8$ | 8 |

[a] All values are means ± standard errors. [b] Significantly different from controls at the 0.01 level.

which had been irradiated with 800 and 1000 rads 14 and 7 days earlier respectively, as well as unirradiated control mice, were injected i.v. via a tail vein with 3.2 to $4.2 \times 10^9$ organisms. Blood samples were obtained under light ether anaesthesia from the orbital venous plexus 2 and 20 min later, since it had been found in a preliminary experiment that labelled bacteria disappeared exponentially from the circulation for at least 25–30 min after injection. The rate of clearance $K$ was calculated from the equation $C_{t_2} = C_{t_1} e^{-K(t_2 - t_1)}$, where $C_{t_1}$ and $C_{t_2}$ are the concentrations at times $t_1$ and $t_2$ (in min) respectively. 40 min after the injection mice were killed by cervial dislocation and liver, spleen, lung and one kidney removed and digested with nitric acid. Radioactivity of blood samples and organ digests was measured by gamma counting in a scintillation well-type counter.

The results are summarized in Table II. In both groups of irradiated mice the rate of clearance of bacteria from the blood was greater than in controls, and so was the uptake of radioactivity by the liver. The uptake by the spleen and lung was reduced in irradiated mice. However, the uptake of radioactivity by the rest of the body other than liver, spleen and lung was not changed, suggesting that increased vascular permeability, if it was present, had little if any effect.

The greater uptake by the liver suggests that the increased rate of clearance of particles from the blood is the result of a greater phagocytic activity of liver RE cells. There is evidence from other experiments indicating that the higher rate of clearance of colloidal particles from the blood of irradiated mice is a consequence of increased intestinal permeability for bacteria and/or their products (ŠLJIVIĆ[9]). These are known to be able to stimulate RES activity[10].

No single explanation can be offered at present for the reduced uptake of radioactivity by spleen and lung. If on the basis of in vitro studies a direct effect of radiation on macrophages is excluded, some indirect effects operating in vivo could be considered. In the whole animal the uptake of particles from the blood stream must be affected by haemodynamic conditions and particularly by the rate of blood flow through specific organs. Greater activity of liver RE cells could reduce the uptake by other organs through competition for a limited number of particles.

Evidence presented here indicates that faster clearance of particles from the blood stream of irradiated mice is the result of greater phagocytic activity of liver RE cells rather than increased vascular permeability. A full account of changes of the rate of clearance of colloidal carbon after irradiation and the mechanisms by which the increased activity of the RES is brought about will be given elsewhere[11].

*Résumé.* Chez des souris irradiées, le carbone colloidal et les bactéries *E. coli* marquées au $^{51}$Cr ont été éliminés plus rapidement de la circulation. En étudiant la distribution de la radioactivité dans les divers organes, on conclut que cette évacuation plus rapide résulte d'une activité phagocytaire plus intense des cellules réticuloendothéliales du foie plutôt que d'une augmentation générale de la perméabilité du système vasculaire.

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*Medical Research Council, Radiobiology Unit, Harwell, Didcot (Berkshire, England), 25 July 1969.*

[9] V. S. ŠLJIVIĆ, in preparation.

[10] D. S. NELSON, *Marcophages and Immunity* (North-Holland Publishing Company, Amsterdam, London 1969) p. 129.

[11] Acknowledgements. I wish to thank Dr. J. F. LOUTIT, F.R.S., for his interest in this work, Dr. B. A. BRIDGES for providing bacteria and Mr. T. E. F. CARR for suggestions on radioactivity measurements.

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## Radioprotective Effects of Phytourease

The attempt therapeutically to increase the natural radioresistance of the organism is related to the protection of the individual against harmful effects of ionizing radiation. The natural radioresistance may be increased before irradiation by the administration of radioprotective pharmaceuticals. An important pre-requisite for a favourable action of chemical radioprotectives is their early application, a sufficient concentration in the organism, minimum toxicity and a long-lasting protective effect[1,2].

We have tested in our laboratory in recent years some radioprotective effects of several compounds with antigenic character in experimental animals. KALINA and DIENSTBIER[3] have found the favourable effects of applied human serum albumin in mice and rats before X-irradiation. We have proved, on the one hand, the mechanism of protective effect of HSA and, on the other hand, we have examined some further compounds which appear to be strong antigens in experimental animals[4].

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[4] V. HLAVATÝ, M. ŽÁK and V. MARTÍNEK, Research Report No. NM-6/4, 1967-31A.

In this paper we would like to point to the favourable radioprotective effect of urease applied as an antigen.

*Material and method.* Urease, as an enzyme, breaks the molecule of urea into $CO_2$ and $NH_3$, has a protein character, with low water-solubility, and is easily soluble in weak acid and alkalic solutions.

For the immunization of white male rats of Děčín breed, we have used urease of American provenance (Jack Bean), produced by Mann Research Lab., Inc., New York, activity 2 × N.F. and urease of Italian production (Carlo Erba, Milano). Urease dissolved in physiological solution was used for the immunization of experimental animals. The animals were immunized s.c. and some of them also i.p., so that the initial dose corresponded to 0.20 mg/10 g body weight in a volume of 0.1 ml. The second immunization followed 7 days after the first one and 0.4 mg/10 g was administered. The third immunization was performed 14 days after the first one and the dose of urease was followed by Ouchterlony method, by precipitation on agar medium. The precipitation lines were present in 85% of cases after initial immunization and in 95% after secondary application of the antigen. The irradiation of the immunized mice was carried out by X-rays on the 7th day after the last antigen-administration. The therapeutic apparatus TUR was used under the following conditions: 200 kV, 15 mA, 12 R/min, HVL = 1 mm Cu, filtration = 0.5 mm Al + 0.5 mm Cu. The exposure dose was 400 or 700 R. The survival of the irradiated animals was followed 28–56 days after irradiation.

*Results and discussion.* The results of survival in individual groups of irradiated and immunized animals are stated in the Table. Statistical significance was estimated by percentage *t*-test and was followed on the level of significance 1, 2 and 5% as compared with controls. As seen from results in the Table, the immunization of animals by means of urease shows a favourable action on their survival after X-irradiation. The radioprotective effect of immunization appears most significantly in animals which were irradiated a second time with the dose of 500 R at the end of the 4th week after primary exposure. There is a much higher number of surviving animals in the period between the 5th–8th week after primary irradiation.

We have also observed an improvement in survival of animals which were irradiated with 700 R, when the hyperimmune rabbit gamma-globuline against urease was administered to white mice before and after irradiation. We do not publish these results in our Table since they are still in the stage of verification.

We cannot explicitly elucidate the mechanism of radioprotective effect of the immunization by urease at the present time. One of the possible mechanisms of protective effect may be the effect of specific antigens as inhibitor of urea-splitting in the gastrointestinal tract of experimental animals. A direct consequence might be the reduction of $NH_3$, which affects the vitality of cells in the gastrointestinal tract unfavourably in post-irradiation period, according to the reports of some authors[5].

During the application of hyperimmune $\gamma$-globuline, in which we have undoubtedly proved the inhibitory effect in the complex urea-urease and where we expected a significant protective effect, the results in irradiated animals were not proportional to the amount of administered $\gamma$-globulin.

On the other hand, we cannot neglect the effect of the immunization itself carried out by means of ureases before irradiation where, as in other antigens of protein character, a significant activation of the reticuloendothelial system in the animal and simultaneously an activation of immunocompetent cells occurs.

The organism treated in this way is easily capable of reacting after sublethal and lethal doses of irradiation to

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| Group | No. of animals | First irradiation dose (R) | % of surviving animals Weeks after first irradiation | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Urease 0.2 mg/10 g | | | | | | | | | | |
| Control | 19 | 700 | 89 | 36 | 31 | 31 | Second | 26 | 15 | 10 | 5 |
| 4 × immunized i.p. | 20 | 700 | 95 | 75 | 75 | 75 | irradia- | 70 | 60 | 50 | 30 |
| | | | | $p$ 0.02 | $p$ 0.01 | $p$ 0.01 | tion | $p$ 0.01 | $p$ 0.01 | $p$ 0.02 | $p$ 0.02 |
| Urease 1% solution | 42 | 700 | 85 | 42 | 38 | 38 | with | 33 | 21 | – | – |
| Control | 43 | 700 | 97 | 43 | 37 | 34 | 500 R | 32 | 30 | – | – |
| 1 × immunized s.c. | | | | | | | | | $p$ 0.05 | | |
| Urease 1% solution | | | | | | | | | | | |
| Control | 22 | 400 | 100 | 95 | 95 | 95 | | 13 | 0 | – | – |
| 1 × immunized s.c. | 23 | 400 | 100 | 100 | 100 | 100 | | 100 | 100 | – | – |
| | | | | | | | | $p$ 0.01 | $p$ 0.01 | | |
| Urease 2% solution | | | | | | | | | | | |
| Control | 65 | 700 | 92 | 46 | 40 | 40 | | 36 | 20 | – | – |
| 1 × immunized s.c. | 67 | 700 | 91 | 62 | 59 | 59 | | 58 | 58 | – | – |
| | | | | $p$ 0.05 | $p$ 0.05 | $p$ 0.05 | | $p$ 0.01 | $p$ 0.01 | – | – |
| Urease 2% solution | | | | | | | | | | | |
| Control | 44 | 400 | 100 | 95 | 95 | 95 | | 13 | 0 | – | – |
| 1 × immunized s.c. | 43 | 400 | 100 | 97 | 97 | 97 | | 93 | 87 | – | – |
| | | | | | | | | $p$ 0.01 | $p$ 0.01 | | |

various antigens, including bacterial ones. It means that in this area the immunodepressive effect of ionizing radiation is depressed and therefore the immune resistance of irradiated animals is increased.

The authors assume that the radioprotective effect induced by immunization of urease is realized probably by the two mechanisms described above. The further possible pathways of the radioprotective effect of the above-mentioned compound are being studied further.

*Zusammenfassung.* Die Problematik der biologischen Radioprotektion wird diskutiert und die radioprotektiven Einwirkungen von Urease demonstriert.

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## The Effect of Angiotensin II on the Platelet Aggregation Induced by Adenosine-diphosphate, Epinephrine and Thrombin

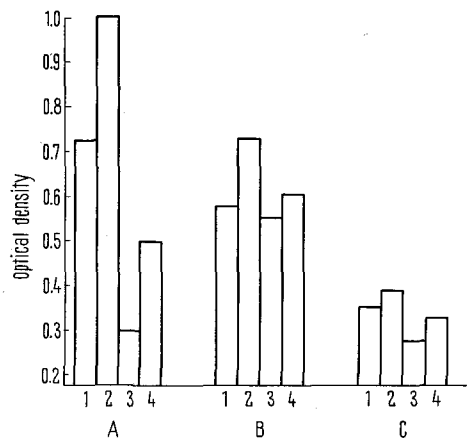
It is generally accepted that blood platelets play a main role in hemostasis, especially in the first step[1]. Formation of the hemostatic plug is initiated by the platelet aggregates on the surface of which fibrin threads may be formed[2]. Platelet aggregation in vitro can be induced by many substances, e.g. adenosine-di-phosphate (ADP), thrombin, epinephrine and serotonine[3,4].

The risk of the incidence of thromboembolic complications in patients with hypertensive cardiovascular disease is higher in comparison to normal persons[5]. Previously we have stated that in hypertension the adhesiveness of platelets is considerably increased[6]. On the other hand it is known that, in hypertension, the level of angiotensin is often elevated[7]. The purpose of this study was to investigate the effect of angiotensin II on the platelet aggregation induced by thrombin, epinephrine or ADP.

The experiments were carried out on human platelet-rich plasma (PRP). Platelet aggregation was induced by the method of BORN[8] in the following systems: 3.6 ml of PRP + 0.2 ml of angiotensin II (CIBA, Basel) and 0.2 ml of thrombin (Wytwórnia Surowic i Szczepionek, Łublin, Poland) or 0.2 ml of ADP (Sigma, USA) or 0.2 ml of epinephrine (Polfa, Poland).

The results are presented in the Figure. It has been found that angiotensin II very significantly increased the aggregating effect of epinephrine (A). This effect was observed to a lesser degree when ADP was used (B) with angiotensin II. Almost no changes were observed in the system thrombin + angiotensin II (C).

Recently we have shown that injection of angiotensin II to dogs increased the number of platelets aggregating twofold[9]. During stress, catecholamines, e.g. epinephrine, may be released into the circulation. On the basis of our results we suggest that release of epinephrine, with simultaneously increased level of angiotensin II, may be at least in part responsible for arterial thrombosis in such cases. The presence of ADP and thrombin together with angiotensin II does not seem to be so important as in the case of epinephrine in patients[10].




The effect of angiotensin II on the platelet aggregation induced by epinephrine, ADP and thrombin.

(A) (1) 3.6 ml of PRP + 0.2 ml of epinephrine $2\times10^{-5}$ mg/ml + 0.2 ml of 0.9% NaCl; (2) 3.6 ml of PRP + 0.2 ml of epinephrine $2\times10^{-5}$ mg/ml[a] + 0.2 ml of angiotensin II 0.005 μg/ml[a]; (3) 3.6 ml of PRP + 0.2 ml of epinephrine $2\times10^{-7}$ mg/ml + 0.2 ml of 0.9% NaCl; (4) 3.6 ml of PRP + 0.2 ml of epinephrine $2\times10^{-7}$ mg/ml + 0.2 ml of angiotensin II 0.005 μg/ml.

(B) (1) 3.6 ml of PRP + 0.2 ml of ADP 0.5 μg/ml + 0.2 ml of 0.9% NaCl; (2) 3.6 ml of PRP + 0.2 ml of ADP 0.5 μg/ml + 0.2 ml of angiotensin II 0.005 μg/ml; (3) 3.6 ml of PRP + 0.2 ml of ADP 0.25 μg/ml + 0.2 ml of 0.9% NaCl; (4) 3.6 ml of PRP + 0.2 ml of ADP 0.25 μg/ml + 0.2 ml of angiotensin II 0.005 μg/ml.

(C) (1) 3.6 ml of PRP + 0.2 ml of thrombin 30 u/ml + 0.2 ml of 0.9% NaCl; (2) 3.6 ml of PRP + 0.2 ml of thrombin 30 u/ml + 0.2 ml of angiotensin II 0.005 μg/ml; (3) 3.6 ml of PRP + 0.2 ml of thrombin 10 u/ml + 0.2 ml of 0.9% NaCl; (4) 3.6 ml of PRP + 0.2 ml of thrombin 10 u/ml + 0.2 ml of angiotensin II 0.005 μg/ml.
[a] These concentrations are final concentrations.

*Zusammenfassung.* Angiotensin erhöht die Plättchen-Aggregation verschiedener aktueller Stoffe.

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