cutaneous raction was expected[3], the mice were injected i.v. with 0.2 ml of 0.5% Evans blue (E. Merck AG., Darmstadt) solution in physiological saline. They were killed by cervical dislocation about 30 min later. Skin was excized and scraped clean of adjacent tissue. At the site of cell injections, the increased vascular permeability was manifested as round or oval blue spots. These spots were scored by calculating their surface areas. Differences between the experimental and control sites were evaluated statistically according to the *t*-test.

The results presented in the Table show that 13 out of 16 mice immunized to tumor had blue areas at the site of tumor cells injection. On the other hand, only 4 of these mice exhibited positive reaction at the site of spleen and liver cell injections. Also, the surface of blue areas was significantly larger at the sites injected with tumor cells ($P < 0.01$). In control group, however, only 1 out of 8 mice had positive reaction, both at the site of tumor and of spleen and liver cell injections.

Thus, we have found increased permeability of blood vessels at the site of tumor cell injection in mice sensitized with syngeneic tumor cells. This was significantly less pronounced in normal mice and it therefore seems that the phenomenon is immunological in nature. Since the recipients were syngeneic, the observed phenomenon should be ascribed to an immunological reaction against tumor-specific antigen(s), being in fact an expression of homograft reaction. It has been shown by others[1,2], and in our previous report[3], that homograft reaction can be expressed as a cutaneous reaction of the delayed type, detectable by Evans blue skin test 24 h after antigenic challenge. It is not probable that the increased vascular permeability was caused by serum antibodies, because at 4 to 6 h after intradermal injection of tumor cells there was no visible sign of Arthus reaction. Furthermore, it is well known that Arthus phenomenon is hard to produce in mice. Therefore, the observed increase of vascular permeability could be considered as a consequence of an immunological reaction of the delayed type. The results described confirm the findings of other investigators showing that tumor-specific antigens may produce delayed hypersensitivity reaction in their hosts[4–8]. Experiments dealing with timing of development and histology of the reaction and with correlation of Evans blue skin test with foot pad test are in progress.

Résumé. Chez les souris C57BL sensibilisées, on observe après une injection intradermique des antigènes spécifiques de tumeur (fibrosarcome) l'apparition d'une réaction cutanée du type retardé. Les lieux d'injection furent surveillés et 24 h après, la réponse cutanée a été evaluée par la mesure du diamètre de la tache bleue (bleu d'Evans) qui apparaît à l'endroit où l'on a injecté des cellules de la tumeur.

L. Milas, D. Dekaris and M. Horvat

Department of Biology, Institute 'Rudjer Bošković', Zagreb (Yugoslavia), 11 May 1970.

Atractyloside does not Inhibit the Release Reaction of Blood Platelets[1]

Abdulla[2] has reported recently that atractyloside inhibits the release of ADP[3] from blood platelets during aggregation induced by several agents in platelet-rich plasma. Atractyloside specifically inhibits the translocation of adenine nucleotides across the inner membrane of mitochondria, presumably by interacting with a permease[4]. In the course of the release reaction of blood platelets, the content of certain organelles is discharged in bulk to the exterior of the cell. In human platelets, these organelles, the dense bodies, contain large amounts of ADP, ATP and serotonin. These compounds are released together within minutes of induction[5]. This mechanism is fundamentally different from the transport of nucleotides in mitochondria, and it therefore seemed justified to reexamine the findings of Abdulla and to extend the investigation to the release of serotonin.

Human PRP was prepared from the buffy coats of citrated, 1-day-old blood[6,7]. It was adjusted to pH 6.8 and incubated with gentle shaking for one hour at 18 °C with serotonin-^{14}C[8] at a final concentration of 0.7 μM and 0.04 μC/ml. A sample of this PRP, supplemented with KCl, $CaCl_2$, *Tris*-buffer, platelet-free plasma and varying amounts of atractyloside[9] was stirred in the sample tube of a spectrophotometer at 37 °C. The sample contained in a volume of 5 ml: 0.4 ml of labelled PRP containing about 1.5×10^9 platelets; 1.1 ml of citrated plasma; *Tris*, 70 μmoles; KCl, 60 μmoles; $CaCl_2$, 10 μmoles; NaCl, 300 μmoles; atractyloside, 0–2.5 μmoles. The pH was 7.4 at 37 °C.

After 2 min of prewarming, either 5×10^{-8} moles of ADP or 0.1 mg of collagen[10] were added. A third series of samples with the same concentrations of atractyloside was treated in the same way, except that the aggregating agent was omitted. Platelet aggregation was assessed by recording the transmission at 600 nm. After 5 min, the mixture was cooled in ice and 22 μmoles of EDTA were added. The sample was centrifuged and the radioactivity of 0.2 ml of the supernatant measured by liquid scintillation counting[11]. Counting of a sample of untreated diluted PRP provided the total activity, and a sample of the supernatant of the ^{14}C-labelled PRP provided the activity not taken up by the platelets, which amounted to 5–7% of the total activity. From these figures the percentage of release of serotonin was calculated[12].

[1] Supported by the Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung.

[2] Y. H. Abdulla, J. Atheroscler. Res. *8*, 855 (1968).

[3] Abbreviations ADP, adenosine-5-diphosphate; PRP, platelet-rich plasma; *Tris*, *tris*-(hydroxymethyl) methane; EDTA, ethylene diamino tetra-acetate.

[4] For a review, see M. E. Pullman and G. Schatz, A. Rev. Biochem. *36*, part II, 539 (1968).

[5] For a review, see H. Holmsen, H. J. Day and H. Stormorken, Scand. J. Haemat., Suppl. *8* (1969).

[6] Obtained from the Central Laboratory of the Swiss Red Cross Blood Transfusion Service in Berne.

[7] M. Bettex-Galland and E. F. Lüscher, Thromb. Diath. haemorrh. *4*, 178 (1960).

[8] Obtained from the Radiochemical Centre, Amersham (U.K.).

[9] Calbiochem, Lucerne (Switzerland).

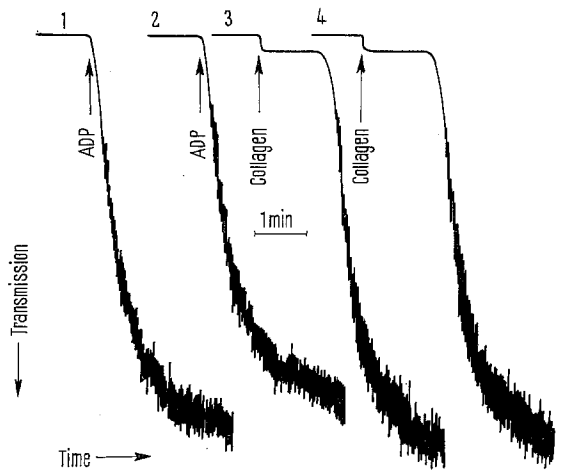
[10] Laboratoire Stago, Asnières-sur-Seine (France); repolymerized according to their instructions, but using 0.15 M sodium acetate instead of Michaelis buffer because of the UV-absorption of barbital.

[11] Scintillator used: 5.0 ml of methanol + 10.0 ml of 1% butyl-PBD (CIBA, Basle, Switzerland) in toluene.

[12] Z. Jerushalmy and M. B. Zucker, Thromb. Diath. haemorrh. *15*, 413 (1966).

Influence of atractyloside on the release of serotonin and of nucleotides by human platelets, induced by ADP or collagen

Concentration of atractyloside (M)	Release of serotonin (%)			Release of nucleotides (%)		
	ADP	Collagen	No agent	ADP	Collagen	No agent
0	59	60	0	33	44	0
10^{-6}	61	60	0	40	55	0
5×10^{-6}	61	61	0	37	48	0
2×10^{-5}	58	60	0	40	58	0
10^{-4}	59	61	0	41	53	0
5×10^{-4}	60	60	0	41	56	0



Recorder tracings of the transmission curves of stirred human PRP, induced to aggregate by $10^{-5}M$ ADP or 20 µg/ml collagen. Curve 1: ADP — control. Curve 2: ADP + $10^{-4}M$ atractyloside. Curve 3: collagen-control. Curve 4: collagen + $10^{-4}M$ atractyloside.

The release of nucleotides was estimated by measuring the difference of extinction at 260 nm between the perchloric acid extracts of treated and untreated sedimented platelets[13]. This method is justified by the similarity between the absorption spectra of ADP and perchloric acid extracts of human platelets and of the material released by them[14].

Results. The Figure shows some of the curves of the transmission change of stirred PRP during aggregation by ADP and by collagen, with and without addition of $10^{-4}M$ atractyloside. The curves of the samples containing 10^{-6} to $5\times10^{-4}M$ atractyloside were very similar to those shown in the Figure. The compound had no effect on the aggregation, in accordance with the finding of ABDULLA[2]. Atractyloside alone caused no aggregation or change in the transmission of the sample.

The Table shows that atractyloside had no influence on the release of serotonin and of nucleotides under the conditions of our experiments at concentrations of up to $5\times10^{-4}M$. This supports the current view that nucleotides and serotonin are released together by a mechanism which is different from the translocation of nucleotides through the mitochondrial membrane. Since the platelet release reaction has many characteristics in common with other release reactions[15], these findings may be of general importance[16].

Zusammenfassung. Atractylosid hat bei menschlichen Blutplättchen keinen Einfluss auf die Aggregation und auf die Freisetzungsreaktion durch ADP und Kollagen in vitro.

P. MASSINI

Theodor Kocher Institute, University of Berne, CH-3012 Berne (Switzerland), 27 April 1970.

[13] M. G. DAVEY and E. F. LÜSCHER, Biochim. biophys. Acta *165*, 490 (1968).
[14] R. KÄSER-GLANZMANN and E. F. LÜSCHER, Thromb. Diath. haemorrh. 7, 480 (1962).
[15] H. STORMORKEN, Scand. J. Haemat., Suppl. 9 (1969).
[16] Miss M. SCHNEIDER gave competent technical assistance. Prof. H. AEBI kindly made available his TriCarb liquid scintillation counter.

Differential Neuronal Radiosensitivity as a Tool for the Study of Short Connections

The purpose of this note is to present a new experimental approach to the study of short neuronal connections that will complement the rather sparse methodology now available for that purpose. The rationale behind the method here presented is the possibility of provoking considerable and rather selective damage to a given neuronal population, that of the small interneurons, while almost sparing – at least from a connectional view point – all other neuronal elements. For this purpose, advantage was taken of the differential neuronal radiosensitivity[1,2] that for a given dose and time of evolution prevails between granule cells and all other neuronal components of the olfactory bulb.

Body shielded rats were irradiated on the head with a dose of 20,000 r X-rays. 24 h post irradiation, the brains were excised after perfusion with osmic or glutaraldehyde solutions. The olfactory bulbs were processed for electron microscopy observation. Degenerating terminal endings of the electron dense type were observed at the level of the plexiform layer, in synaptic contact with ramifications of the accessory dendritic branches of the mitral cells (Figure 1). In some sections the synaptic site and the synaptic vesicles at the mitral side of the contact were easily identified. The terminals exhibited graded alterations from crowding of synaptic vesicles and disruption of mitochondria to an extreme densification of the whole synaptic sac. However, in some of the latter terminals, mitochondria, although extremely electron

[1] R. F. DE ESTABLE, J. F. ESTABLE-PUIG and W. HAYMAKER, J. appl. Phys. *35*, 3098 (1964).
[2] J. F. ESTABLE-PUIG, Diss. Stanford University (1968).