physiological correlation, but the physiological implication was not demonstrated. BULLOCK and HORRIDGE[12] point out that much evidence exists on the presence of clusters of peripheric sensory cells in gastropods. The possibility that plexuses play a convergence way role has also been postulated[12], and this would be their probable meaning. The recording of evoked potentials in central neurons after mechanical stimulation of the mantle collar, the amount of free ending processes and the presence of several cellular types leads us to assume the sensory role played by any of these elements in the mantle collar. However, more detailed physiological studies are necessary to support further conclusions[13].

*Resumen.* Se describen en la membrana y collar del manto próximos al poro respiratorio del molusco *Cryptomphallus aspersa* (Gasteropoda, Pulmonata) la presencia de varios tipos de neuronas y plexos. Estas observaciones morfológicas se discuten en relación a hallazgos previos con técnicas electrofisiólogicas obtenidos por otros autores.

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## Delayed Cutaneous Hypersensitivity Reaction to Tumor-specific Antigens of Fibrosarcoma in Sensitized Mice

Homograft immunological reaction in animals sensitized to grafts of allogeneic tissue can be manifested as a cutaneous hypersensitivity reaction of the delayed type[1–3]. Tumor-specific antigens of some experimentally induced tumors produce delayed hypersensitivity reactions[4–8]. These reactions to tumor-specific antigens can be detected by the foot pad test[7,8], by inhibition of macrophage migration from capillary tubes[6], and by the passive transfer reaction[5]. Concomitant with the cutaneous reactions of the delayed hypersensitivity is a local increase of vascular permeability[3,9]. In this report we describe such an increase evoked in mice by tumor-specific antigen(s) of a fibrosarcoma induced by methylcholanthrene.

Studies were performed on C57BL mice with the third isotransplant generation of the methylcholanthrene-induced fibrosarcoma. Male C57BL mice, 3 months old, were immunized to the tumor. Immunization was performed by 3 consecutive injections of $2 \times 10^7$ irradiated tumor cells at 7-day intervals. Tumor cells were irradiated with 10,000 R with a 250 KV Phillips X-ray machine. For the first injection, cells were mixed with an equal volume of the complete Freund's adjuvant (Difco, Detroit) and then injected s.c. into both axillar and inguinal regions. The second and third injections were administered i.p. without adjuvant.

A suspension of single tumor cells was prepared from non-necrotic tumor pieces minced in physiological saline and passed through nylon gauze. The suspended cells were washed 3 times by centrifugation and finally resuspended in a desired volume of physiological saline. 7 days after the last immunizing injection, the mice were clipped on both flanks and injected intradermally with syngeneic spleen and liver cells mixed together and tumor cells. Each mouse received the tumor cells on the left side and the spleen and liver cells on the right side. The number of injected cells was $3 \times 10^6$ suspended in 0.05 ml of physiological saline. Spleen and liver cells from C57BL male mice were prepared in the same manner as tumor cells. Controls were normal C57BL mice injected intradermally with tumor and syngeneic spleen and liver cells.

24 h after the intradermal inoculation of cells, when the increase of vascular permeability due to delayed

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Delayed cutaneous hypersensitivity reactions in C57BL mice injected intradermally with syngeneic spleen and liver or fibrosarcoma cells

| Recipients | Challenge (i.d.) | No. of mice with positive reaction over number of injected mice | Mean surface of blue areas at the site of injection ($mm^2 \pm$ S.E.) | |
|---|---|---|---|---|
| Immunized to tumor | Tumor cells | 13/16 | $2.57 \pm 0.86$ | $P < 0.01$ |
| | Spleen and liver cells | 4/16 | $0.19 \pm 0.08$ | $P < 0.01$ |
| Normal | Tumor cells | 1/8 | 0.09 | $P < 0.01$ |
| | Spleen and liver cells | 1/8 | 0.09 | |

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.

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## 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 $\mu M$ and 0.04 $\mu$C/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 $1.5 \times 10^9$ platelets; 1.1 ml of citrated plasma; *Tris*, 70 $\mu$moles; KCl, 60 $\mu$moles; $CaCl_2$, 10 $\mu$moles; NaCl, 300 $\mu$moles; atractyloside, 0–2.5 $\mu$moles. The pH was 7.4 at 37 °C.

After 2 min of prewarming, either $5 \times 10^{-8}$ 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 $\mu$moles 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].

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

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