phages[9]. It is related to the smooth muscle antibody which appears in malignant disease[10]. The immunosuppressive globulin produced by tumour is absorbed via the tumour capillaries into the tumour vasculature. It binds to the smooth muscle fibres in its path, i.e. to the muscle fibres of the post-capillary venules. Thus tumour vessels, or graft vessels that have formed anastomoses with tumour vasculature, become coated with 'macrophage repellent' globulin. The presence of this peptide in the vessel wall effectively blocks diapedesis.

In the case of widespread tumour, there are large amounts of alpha globulin in the circulation[11]. These bind to vessel walls other than tumour walls. Serum globulins also coat circulating monocytes and abrogate their cytotoxicity[12].

Hence the non-specific generalized anergy in patients with advanced cancer[13]. Hence also the reversal of such anergy after extirpation of tumour[14], after washing of leucocytes[15] or after infusion of streptokinase[16] – an enzyme which specifically cleaves alpha globulin[17].

Re-implantation of an i.t. ovary into a non-malignant environment restores its vulnerability to immune attack as the alpha globulin required to coat the vessel wall is no longer available. This concept suggests a new approach to problems of organ transplantation or cancer immunotherapy.

Organ transplantation: Perfusion of the donor organ with immunosuppressive macroglobulin before and after grafting[18].

Cancer immunotherapy: Removal of alphaglobulin from the vessel wall and from the blood stream by the following means: a) Eradication of the source of macroglobulin, i.e. extirpation of accessible tumour[11]; b) Prevention of neoformation of this peptide by specific inhibitors[19] and c) Digestion of globulins already formed[16].

It is perhaps only then that conventional immunotherapy can become fully effective.

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Ultrastructural study of vanadocytes in Ascidia malaca[1]

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Summary. The ultrastructural aspects of vanadocytes, found in the circulating blood of Ascidia malaca, were studied in formaline-fixed material. The results indicate that the diverse morphological aspects of 'vanadophores' reported here, are probably concerned with different metabolic stages of vanadine.

In the blood of tunicates, vanadium is found in certain particularly acidic cells, called vanadocytes[2]. Fine structural study of such cells in Ciona intestinalis revealed the presence of certain cytoplasmic areas with special electron absorbance faculty[3]. This type of absorbance, observed also in Phallusia mammillata, is found in both osmium-fixed and formalin-fixed material[4]. On the basis of such observations, it was suggested that this particular type of electron absorbance in some cytoplasmic areas is due to the accumulation of vanadium. These areas have been termed 'Vanadophores'. In the present work Ascidia malaca vanadocytes were subjected to an ultrastructural study to obtain a better understanding of certain morphofunctional aspects of the 'vanadium-filled' cytoplasmic areas.

The blood of Ascidia malaca, collected through a puncture in the heart, was centrifuged at 800 × g for 10 min. The sediment, containing cells, was fixed in 10% neutral formaline prepared in filtered sea water. This type of fixation was preferred to that in OsO_4, since this is reduced in the cells containing hemovanadine (trivalent vanadium) rendering the vanadophores highly electron-dense. Osmium-fixed material is, furthermore, not suitable, because OsO_4 increases the consistency of vanadophores and thus hinders in the penetration of resin. The blood cell pellets, obtained as above and fixed in neutral formaline, were dehydrated and included in Epon according to the method of Luft[5]. Sections, obtained on L.K.B. Ultrotome III, were stained with lead citrate and uranil acetate and then studied at the electron microscope Philips EM 200.

The vanadocytes of Ascidia malaca (4–6 μm in diameter), stained as above, contain highly electron dense cytoplasmic inclusions of 1.5 μm, around the nucleus (figure 1). Similar electron dense cytoplasmic areas can be observed also in unstained sections, although less intense than those of stained preparations (figure 2). On the basis of these observations the electron-dense cytoplasmic areas in the blood cells of Ascidia malaca can be con-

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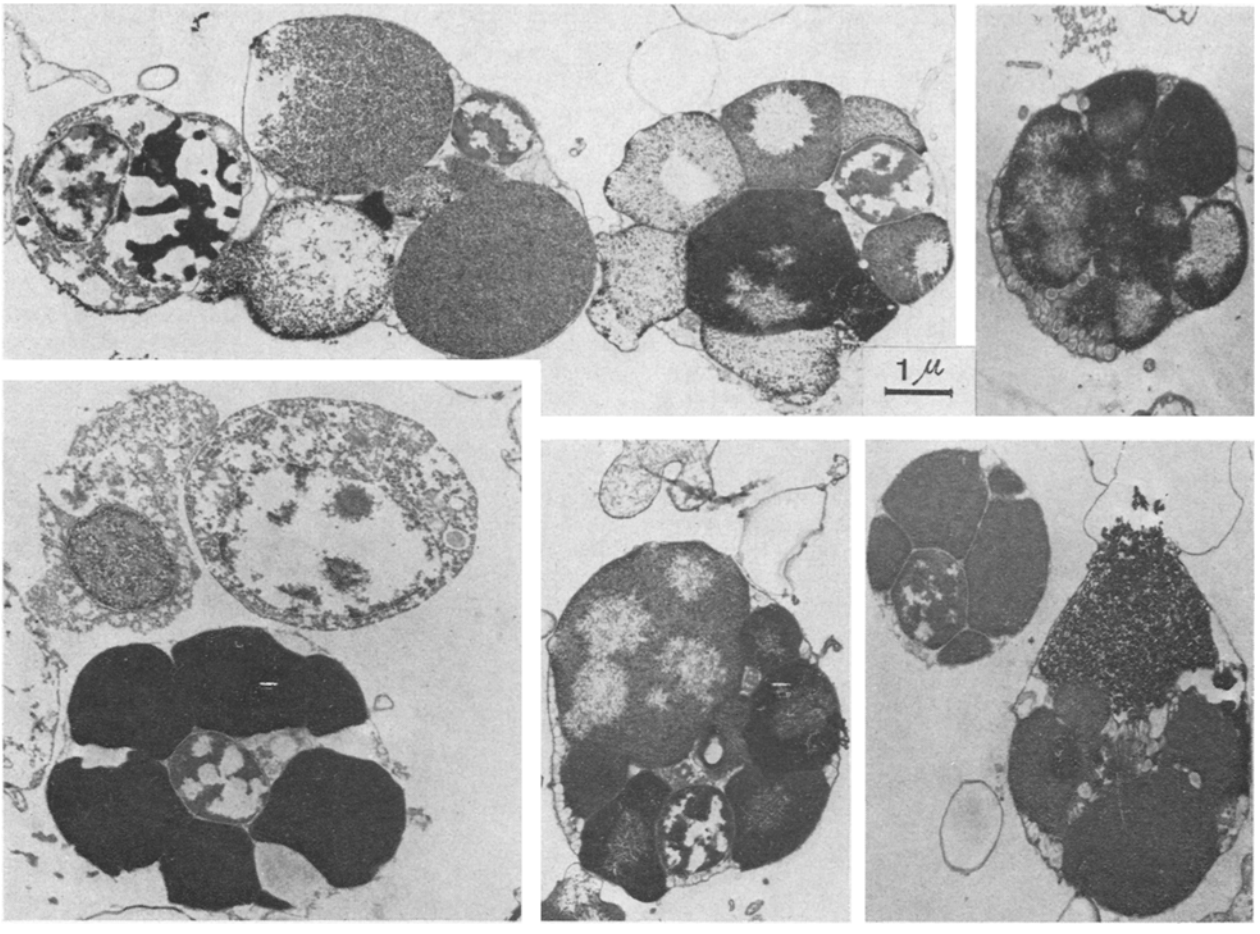

Fig. 1. Vanadocytes from the blood of Ascidia malaca. Double staining. In some the 'vanadophores' are homogeneously electron-dense, while others show a granulo-filamentous organization. × 12,000.

sidered homologous to the 'vanadophores' reported in Ciona intestinalis and Phallusia mammillata. The vanadophores of each vanadocyte show a diverse ultrastructural organization. The vanadophores prevalently manifest a granular filamentous component, which in a vanadophore may be highly concentrated (and thus not easily resolved), in another scarce, and still in another may disappear totally from the matrix. Sometimes this type of variable organization can be observed in a single vanadophore (figure 1) in which light, granular-filamentous areas are encircled by more compact and highly electron dense areas. These variable ultrastructural appearences may probably represent different metabolic stages of vanadium.

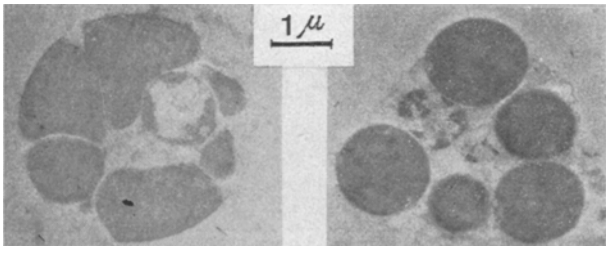

Fig. 2. Fine structure of vanadocytes from the blood of Ascidia malaca without staining. Note that the 'vanadophores' are electron-dense. × 12,000.

It is known that pentavalent vanadium, present in sea water, is captured through the branchial epithelium and assimilated in particular blood cells (compartment cell). Here it is reduced into trivalent from which on binding with a glycoprotein forms the highly electron-dense hemovanadine[6]. In Phallusia mammillata, the vanadocytes which migrate in the test, where they release vanadium with cellulose formation, show a reduction of electron-dense cytoplasmic vacuolizating and appearence of filamentous structures.

Similar morphological characteristics at the ultrastructural level were observed in the vanadocytes found also in the circulating blood of Ascidia. The present observation led us to consider that the ultrastructural variations in the vanadophores of circulating vanadocytes of Ascidia malaca, probably express different metabolic stages (probably bound to catabolic stages) of vanadine, as observed analogously in the test in Phallusia mammillata.

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