Specific inhibition by lipids and mucoproteins of pig sera

Antisera	Sera from which extracts were prepared	Test erythrocytes	Titers of specific inhibition	
			Lipids	Mucoproteins
Anti-Na	Na^+ (NaNa)	NaNa	0	$^1/_{16}$
	Na^+ (NaNb)		0	$^1/_{16}$
	Na^- (NbNb)		0	0
Anti-Nb	Nb^+ (NbNb)	NbNb	0	$^1/_4$
	Nb^+ (NaNb)		0	$^1/_4$
	Nb^- (NaNa)		0	0
Anti-A (-Ac)	A^+ (Ac)	Ac^+	$^1/_{32}$	$^1/_{16}$
	A^+ (Ap)		$^1/_2$	–
	A^- (O)		0	0

proteins, not to lipoproteins, and that the determinant of porcine serum A antigen is probably bound both to a mucoprotein and to a lipoprotein, if results of similar investigations on the bovine J substance[18] are taken as the basis. Thus, the A antigen of porcine serum resembles the J antigen of bovine serum in 3 respects: they exhibit immunological cross reactions[19], they are absorbed from the serum onto the erythrocytes[20], and they show similar diversities of carriers[21].

Zusammenfassung. In Schweineseren finden sich die Blutgruppenantigene A, Na and Nb in gelöster Form. Aus den entsprechenden Schweineseren wurden einerseits die Totallipide, andererseits die Mucoproteide extrahiert und in immunologischen Hemmungstests auf ihre Aktivität im A- und N-System untersucht. Es ergab sich, dass die determinanten Gruppen der Na- und Nb-Antigene an Mucoproteide gebunden sind, während die determinante Gruppe des A-Antigens sowohl an ein Mucoproteid als auch an ein Lipid gebunden ist.

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Detection of Early Changes in Cockroach Hemocytes During Coagulation with 8-Anilino-1-Naphthalene Sulfonic Acid

In many insects[1], the coagulation mechanism involves both cell-serum interactions and special forms of cellular adhesions. In spite of the considerable amount of attention that the morphology of these blood cells have received, their individuality is not completely clear. Even when these cells display different appearances, it has not been established for most cases that these are not manifestations of short-lived physiological responses.

Recently DYCKMAN and WELTMAN[2] applied the fluorescent probe of hydrophobic groups, 8-anilino-1-naphthalene sulfonate (ANS), to the study of human leucocytes and reported that with the exception of nucleoli in plasmacytes from multiple myeloma cells, fluorescence was confined to the cytoplasm. This probe has also been used to demonstrate conformational changes in nerve cell membranes during the transmission of the action potential[3]. In light of the foregoing, it seemed reasonable to apply this technique to insect hemocytes.

Materials and methods. Insect hemocytes were obtained from mature, female specimens of *Blatta orientalis* (L.). Antenna tips were excized and drops of hemolymph were allowed to flow directly into insect saline containing $10^{-3}M$ ANS (Sigma Scientific, St. Louis, Mo., USA). Since small volumes of both saline and hemolymph were used, it was not possible to insure exact concentration relationships. In practice, higher levels of ANS appeared

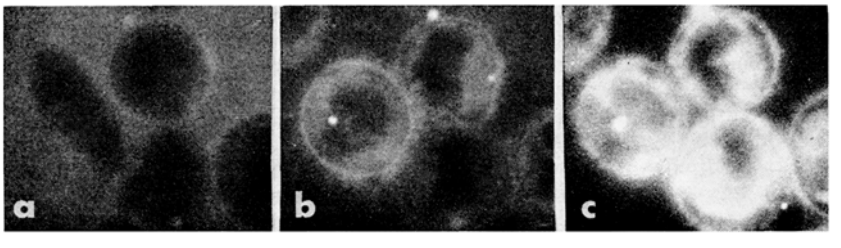

Fig. 1. Miscellaneous hemocytes at varying time intervals after introducing the probe. a) 40 sec; b) 3.5 min, and c) 6.5 min.

only to increase the background which became relatively high in any case when cells were surrounded by as yet undispersed hemolymph protein. In the interest of speed, preparations were covered with a cover-slip without sealing and placed immediately under the fluorescence microscope. The microscope employed in these studies was a Zeiss Photomicroscope II equipped with an epicondenser and head containing a 460 nm cut-off dichroic mirror. Excitation was with a HBO-200 mercury arc with a UG-1 primary filter. A secondary 530 nm cut-off filter was also employed. All photographs were taken using Kodak Plus-X 35 mm film. Attempts were made to photograph sequential changes in the same field over a 6 to 7-min period. An achromatic 40X, n.a. 0.85 immersion objective was used throughout these studies.

The general results were clearly in agreement with the findings of DYCKMAN and WELTMAN[1]. Only the cytoplasm displayed fluorescence, not the nuclei. Within 6 to 7 min, cytoplasmic fluorescence had developed in virtually every cell in the preparation to some degree.

Of perhaps more interest, however, it also became evident that some cells immediately displayed cytoplasmic fluorescence, while in others, the cytoplasmic fluorescence developed at varying rates over several min (Figure 1a, b, c). An easily identifiable, and the predominant, cell type that fluoresced immediately was the expanded, fragile hyaline hemocyte (coagulocyte) (Figure 2). This is the cell always associated with the formation of islands of coagulation in unfixed living preparations viewed with phase contrast optics and in fixed and stained preparations. One of the cell types that fluoresced at varying rates over the first several min of the clotting process has been tentatively identified, from comparison with phase optical studies and fixed and stained preparations, as the hyaline hemocyte. This is a particularily interesting finding since some of these hyaline cells were observed to expand and become coagulocytes while others did not during examination of the clotting process with phase contrast optics.

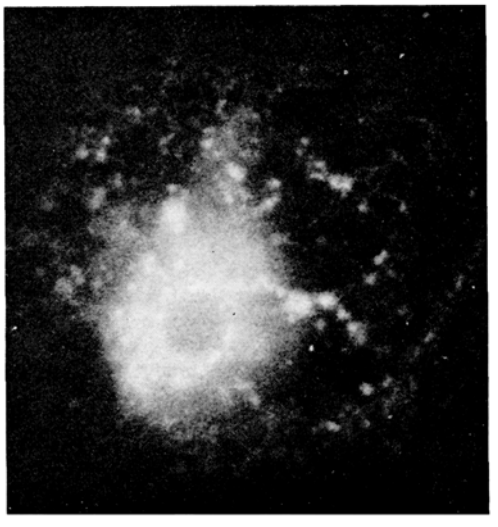

Fig. 2. Expanded, fragile hyaline hemocyte (coagulocyte), 40 sec after introducing the probe.

This represents the communication of preliminary findings. We feel that use of the fluorescent probe (ANS) indicates that early changes in hemocytes, or rather hemocyte membranes, can be detected by the action of this probe. It is significant that early changes can be detected in some cells, but develop at varying rates in others. Early fluorescence is considered to be due to immediate membrane alterations in the instantaneously fluorescent cells while the slower development of fluorescence in others in the presence of ANS is related to either maintainence of membrane integrity or slower movement of the dye through the membrane.

We feel that this technique may assist in the development of new insights into the cellular physiology of the insect coagulation response as well as many other intracellular functions involving exposure of hydrophobic groups. More detailed studies of roach hemocytes in coagulation are underway in this laboratory which will be reported in detail later.

Zusammenfassung. Gewisse Hämozyten von *Blatta orientalis* L. zeigten in Gegenwart fluoreszierender 8-Anilin-1-Naphthalensulfonsäure im Lebendpräparat zytoplasmische Fluoreszenz, während die Entwicklung dieser Fluoreszenz in anderen Hämozyten beträchtlich variiert und bis zu 7 min benötigt. Es besteht die Möglichkeit, mit dieser Fluoreszenzprobe Membranveränderungen in Hämatozyten zu erfassen.

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Immunpräzipitation Insulin-verwandter Proteine mit Ziegen-anti-Insulinserum

Nach der Entdeckung des Proinsulins[1] als Precursor in der Insulinbiosynthese gelang es, Proinsulin und weitere Insulin-verwandte Proteine aus kommerziellen Insulinpräparaten zu isolieren[2,3].

Bei Anwendung von Anti-Insulinserum und radioaktiv markiertem Insulin als Tracer konnten Kreuzreaktionen von Proinsulin, Intermediär (Proinsulin ohne Lys_{59}-Arg_{60}) und Dimer (zwei kovalent gebundene Insulinmoleküle) mit Insulinantikörpern nachgewiesen werden[4]. Verglichen mit Insulin zeigte Proinsulin ungefähr die halbe Hemmwirkung hinsichtlich der Bindung des radioaktiv markierten Insulins durch das Anti-Insulinserum. Die antigene Reaktivität von Intermediär und Dimer war grösser als die des Proinsulins.

Ziel der vorliegenden Untersuchungen war es, zu prüfen, ob präzipitierendes Ziegen-anti-Insulinserum (GAIS) auch mit dem Proinsulin und Intermediär Immunpräzipitate bildet.

Material und Methoden. Proinsulin und Intermediär wurden aus kristallinem Rinderinsulin (VEB Berlin-