leukaemia, which is a grafted leukaemia arizing from a leukaemia induced in $C_{57}Bl/6$ mice by the Gross virus. These animals were divided at random into 4 groups: group a) comprised 15 controls; group b) 12 mice which received 24 h after the graft of the leukaemia 1 mg living B.C.G. i.p.; this injection being repeated every 4 days for 16 days; group c) comprised 12 mice which received 48 h after the graft of the leukaemia a peritoneal injection of formalized 10^7 isogenic leukaemic cells; this injection being repeated every 4 days for 16 days; group d) comprised 12 mice which received the combination of both treatments.

Results and discussion. Figure 1 shows the results obtained in E ♀ K1 leukaemia: B.C.G. given alone had no significant action; formolinized isogenic leukaemia cells given alone have a very powerful action; the association of both had a weak, but statistically significant effect (S to 4%).

Figure 2 shows the results obtained in RC 19 leukaemia: the effect of B.C.G. given alone is considerable (S to 1 p. 1000); the effect of formolinized tumour cells is very weak, but the effect of the combination of both is similar to the effect of B.C.G. given alone (S to 1 p. 1000).

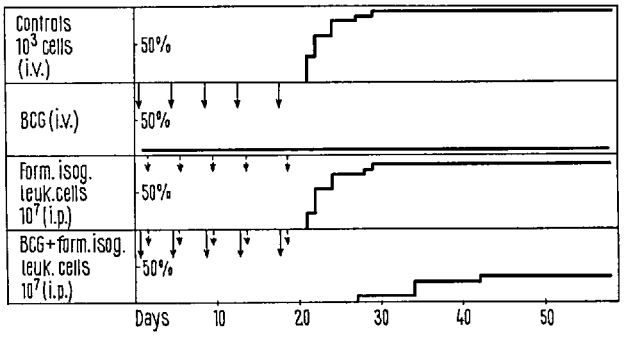

Fig. 2. Cumulative survival of mice carrying RC 19 leukaemia, not treated, or treated by B.C.G., or formolinized isogenic leukaemic cells, or by combination of both.

In conclusion, active immunotherapy applied after the intravenous graft of tumour cells from 2 leukaemias originally induced by virus and of which the tumour associated antigens are well known, can be active, as it has been shown active previously by us on experiments with L 1210 leukaemia, which justified a clinical trial that we have conducted on acute lymphoblastic leukaemia in man, the results of which are very encouraging[6,7].

Résumé. L'immunothérapie active non spécifique ou mixte, appliquée 24 h après la greffe isogénique de la leucémie RC 19, possède une action considérable; appliquée dans les mêmes conditions après la greffe de la leucémie E ♀ K1, elle possède une action modérée mais significative. Ces résultats confirment l'effet antileucémique de l'immunothérapie active appliquée après le début de la maladie et montre qu'elle est même efficace sur les cellules tumorales disséminées.

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The Chemical Nature of the Pig Blood-Group Substances Dissolved in the Serum

It is now well established that the determinants (haptenic sites) of the classical human blood-group substances are sequences of various sugars and/or N-acetylhexosamines[1,2]. There is a possible diversity of macromolecules carrying identical determinants. Thus, the cellular ABH-substances of man seem to be bound to lipids[3], the soluble ABH-substances present in the secretions of about 80% of individuals, however, are bound to glycoproteins[1]. The Lewis antigens in human erythrocytes are also bound to lipids[4], while the Lewis substances present in secretions occur in the form of glycoproteins. Some peculiar blood-group substances are also found dissolved in the serum, e.g. the Lewis antigens of man[5], the J antigen of cattle[6], the A antigen of pig[7,8] and the Na and Nb antigens of pig[9]. Since it has been demonstrated that the bovine J antigen in the serum[10] and recently also the human Lewis antigen in the serum[11] are bound to lipids, it appears reasonable to extend similar investigations to the other above-mentioned blood-group substances dissolved in sera.

With respect to the occurrence of dissolved antigens, the pig sera used were termed A^-Na/a, A^-Nb/b, A^+Na/b and A^+Nb/b. In one experimental series the total lipids were extracted with ethanol/diethyl ether (3:1, v/v)[12] and purified by the Folch procedure[13], in another experimental series they were extracted with methanol/chloroform (1:1, v/v) according to the description given[14] for the extraction of red cells and subsequently purified by passing the lipid solution through Sephadex G-25 fine[15]. The mucoproteins were extracted with cold phenol/water (3:1, w/w)[16] and purified by precipitation with ethanol containing potassium acetate[17]. The yield of total lipids was about 180–320 mg/100 ml serum, the yield of mucoproteins was about 15–20 mg/100 ml serum.

The total lipids were emulsified in isotonic (0.15*M*) saline by use of glass homogenizers giving a concentration of 10 mg lipid/ml. The mucoproteins were readily soluble in isotonic saline; a solution of 5 mg/ml was prepared. The immunological activities of those preparations were tested by their abilities to inhibit the specific antibody-antigen-reactions. The inhibition tests with the incomplete anti-Na and anti-Nb sera were carried out as described earlier[9]. The activities in the A system were measured by using hemolysis-inhibition tests. The titers of the 3 antisera used were $^1/_4$ each.

We found (Table) Na and Nb activities in the corresponding mucoproteins only, while both total lipids and mucoproteins extracted from A pig serum showed specific activities. These results indicate that the Na and Nb determinants are bound to porcine serum muco-

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

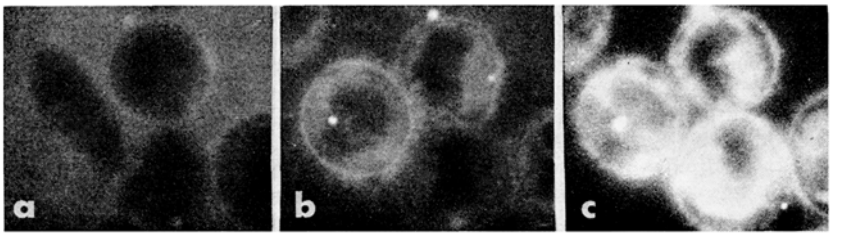

Fig. 1. Miscellaneous hemocytes at varying time intervals after introducing the probe. a) 40 sec; b) 3.5 min, and c) 6.5 min.