prol-D-arg-OH, hemoglobin, casein and catalyzed the conversion of human plasminogen to plasmin (Table I).

Mammary carcinoma cells were allowed to grow in the Eagle's essential medium alone (Figure A), and in the medium, to which was added 10 µg of the purified protease per ml (Figure B), and growth was examined at 48 h intervals. Clusters of round cells predominate in Figure A, while fusiform cells predominate in Figure B.

An antigenic glycoprotein was prepared and purified from the cell surface of the carcinoma cultured cells described earlier[9]. When added to cultured peripheral blood lymphocytes of patients with mammary carcinoma the glycoprotein released a protease which also inhibited migration of guinea-pig peritoneal macrophages, while it had no effect on peripheral blood lymphocytes of normal donors. The data summarized in Table I indicate similarities between the protease activities of the 2 cell-free media. In the experiments presented in Table II, the lymphocytes were first incubated at 37 °C for 120 °C in Eagle's essential medium containing 10 µg of the carcinoma associated glycoprotein, washed 3 times with the fresh medium, and then cultured for 120 h. The cell-free media were harvested and the protease was separated. The series of protease inhibitors listed in Table II blocked the migration inhibition of peritoneal macrophages caused by the 2 proteases. At concentrations of 5 to 25 µg/ml, insulin inhibited the proteolytic activity of the proteases in the 2 types of cell-free media.

Discussion. There is accumulating evidence for cell-mediated immune reactions against antigens associated with breast cancer. These reactions are revealed by reactivity of lymphocytes from breast cancer patients against tissue culture cells derived from breast cancer in the microcytotoxicity assay[10,11], inhibition of the migration of autologous leukocytes with crude extracts of breast cancers, and stimulation of autologous lymphocytes of breast cancer patients with tumor cell-membrane extracts[9,12–17]. Combined with the data presented, these observations are in strong agreement with the release of biologically active substances during stimulation of sensitized lymphoid cells either with non-specific mitogens, or with the specific sensitizing antigen. The results reported identify the macrophage migration inhibition factor with a wide spectrum of enzymatic activities, which explain several observations: The destructive properties of tumors, the decreased mutual adhesiveness of cells and a variety of local and systemic pathological reactions in cancer and inflammatory diseases.

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Hemagglutination of Neuraminidase-Treated Human Erythrocytes by *Leishmania enriettii* Infected Guinea-Pig Sera

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Summary. Sera from guinea-pigs infected with the protozoan parasite *Leishmania enriettii* showed higher hemagglutination (HA) titres for neuraminidase treated human erythrocytes than those of normal guinea-pig sera. This HA activity was associated mostly with the 19S fraction of the immune serum and could be absorbed out with an antigenic fraction of the parasite membrane. Antigenic determinants involved in this HA reaction consisted of, at least, β-D-galactosyl or lactosyl residues.

Treatment of human erythrocytes and lymphocytes with *Vibrio cholera* neuraminidase (VCN) removes cell surface sialic acids and exposes hidden antigenic determinants (Thomsen or T-antigens) which have been shown to react with normal human and various animal sera as well as with plant lectins[2–9]. Although the earlier workers[2–4] used the term Thomsen or T-agglutinins to refer to these activities in normal sera, more recently these are shown to be due to the presence of naturally occurring antibodies of IgM class[6,7]. The mechanism for the production of such antibodies and their functional significance still remains to be discovered, though several possibilities exist[10,11]. In the present communication, direct experimental evidence has been presented to show that these antibodies can be produced due to an immune response against a discrete set of antigenic determinants present on the surface of a microorganism. The system described here is the infection of guinea-pig with the protozoan parasite *Leishmania enriettii* and agglutination of VCN-treated human erythrocytes by normal (NGPS) and immune (IGPS) sera.

[1] Major part of this work was done at the W. H. O. Immunoglobulin Lab., Lausanne, and the author acknowledges the award of a W. H. O. Research Training grant for the period 1973–74. The author is grateful to Dr. D. S. ROWE for laboratory facilities and encouragement.

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Table I. Hemagglutination titres of some normal and *Leishmania enriettii* immune guinea-pig sera using untreated and neuraminidase-treated human erythrocytes (0 Rh^+)

Serum tested	Fluorescence antibody titre[a] (reciprocal)	Hemagglutination titre[a] (reciprocal)	
		Untreated erythrocytes	Neuraminidase treated erythrocytes
NGPS(1)	—	—	—
NGPS(2)	4	—	—
NGPS(3)	8	—	2
NGPS(4)	8	—	4
NGPS(5)	32	2	4
NGPS(6)	16	—	8
NGPS(7)	16	—	16
NGPS(8)	32	2	32
IGPS(1)	64	—	32
IGPS(2)	128	—	32
IGPS(3)	256	2	32
IGPS(4)	512	—	32
IGPS(5)	128	—	64
IGPS(6)	512	—	64
IGPS(7)	1536	4	64
IGPS(8)	256	—	128
IGPS(9)	512	2	128
IGPS(10)	512	—	128
IGPS(11)	1024	2	128
IGPS(12)	2048	—	128
IGPS(13)	512	—	256
IGPS(14)	1024	4	256
IGPS(15)	1536	—	256
IGPS(16)	1024	2	512

[a] Variations within the FAT and HA titres of immune guinea-pig sera may be attributed to the differences in bleeding time during the course of infection as well as to the individual variations in antibody response.

Table II. Hemagglutination inhibition of neuraminidase-treated human erythrocytes (0 Rh^+) with normal and immune guinea-pig sera by various sugars

Sugar (0.2 *M* final concentration)	Hemagglutination titre (reciprocal)		
	NGPS(7)	IGPS(6)	IGPS(14)
Without sugar	16	64	256
Glucose	8	32	128
Galactose	4	8	16
Lactose	2	4	8
Melibiose	8	16	128
Mannose	16	64	128
α-Methyl-D-mannoside	8	64	128
NAc-D-glucosamine	8	32	64
NAc-D-galactosamine	8	32	128

Methods. Human red cells (0 Rh^+) were washed 3 times with saline and were suspended in 0.01 *M* NaAc-saline buffer containing 10^{-4} *M* $CaCl_2$, pH 5.8. To 5 ml of a 2% (by volume) erythrocyte suspension 0.5 ml (250 IU) of neuraminidase (*Vibrio cholera*, Behringwerke AG, BRD) solution was added and the mixture was incubated at 37 °C for 1 h. Next, the treated cells were washed 3 times with saline and, finally, suspended in phosphate-buffered saline (PBS) to a 2% (by volume) suspension. Outbred guinea-pigs were infected intradermally in the nose with the protozoan parasite *Leishmania enriettii* (10^6 amastigotes) and immune sera were collected during various stages of infection (2–10 weeks after initial innoculation) as well as after the healing of the lesion. Normal guinea-pig sera were obtained from young guinea-pigs. All sera were decomplemented by heating at 56 °C for 30 min. 7S and 19S antibodies of guinea-pig serum were separated by sucrose density gradient ultracentrifugation method[12]. Hemagglutination (HA) experiments were done in microtitre plates (Takatsy) with serial dilution of sera in PBS. Final volume of each agglutinating mixture was 100 µl. Inhibition experiments were carried out by, first, incubating guinea-pig sera of various dilutions with appropriate sugars and by the subsequent addition of VCN treated erythrocytes. Antibody titres of normal and immune guinea-pig sera to *L. enriettii* were determined by fluorescence antibody technique (FAT) using *L. enriettii* promastigotes as antigen.

Results. Altogether 20 normal and 30 immune guinea-pig sera from different animals were included in this study. Table I shows some of the hemagglutination results obtained with untreated and VCN-treated human erythrocytes using normal and immune guinea-pig sera. For comparison, FAT titres of these sera to *L. enriettii* are also presented. It can be seen that these sera did not generally exhibit any HA titre with untreated erythrocytes, whereas most normal and all immune sera did agglutinate VCN-treated cells. HA titres of normal sera varied between 0 to 1:32, whereas for immune sera these ranged between 1:32 to 1:512. A defininite correlation is lacking between the FAT and HA titres, although both of these were rising in the first few weeks of infection. Hemagglutination titres with VCN-treated group 0 red cells were a few fold higher than those obtained with group A or B cells. Moreover, normal and immune guinea-pig sera exhibited only very weak hemagglutination activities with pronase- or trypsin-treated group 0 cells.

HA activities of normal and immune guinea-pig sera for VCN-treated red cells were found to be associated mostly with the 19S fraction, and no significant change of this activity from 19S to 7S fraction was observable during the course of infection. An antigenically active material was isolated from the membrane of *L. enriettii* promastigotes (A.C. GHOSE et al., under preparation) and this material showed the ability to absorb out HA activities from normal and immune guinea-pig sera. The specificities of the hemagglutination reactions were determined by HA inhibition studies using various sugars (Table II). It can be seen that among the different sugars used only galactose and lactose exhibited significant inhibitory capacities. In fact, the inhibitory capacity of lactose was even higher than that of galactose.

Discussion. Most of the sialic acids of erythrocyte are localized in the form of clusters of negative charges on the cell surface[13]. Neuraminidase treatment of red cells removes terminal sialic acids, thus exposing hidden saccharide residues of the polysaccharide chains. Inhibition data (Table II) suggest that at least β-D-galactosyl or lactosyl(gal β 1–4 Glc)-like residues are exposed on

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such treatment. These results are in agreement with earlier results with VCN-treated human erythrocytes and lymphocytes, using various homologous and heterologous sera as well as plant lectins[4,9,10]. The presence of naturally occurring antibodies in human and various animal sera with specificities for β-D-galactosyl or lactosyl determinants raises an important question regarding their functional significance[10]. Results presented here demonstrate that these antibodies could be the product of an immune response to a discrete set of antigenic determinants present on the surface of some microorganisms. Low titres of such antibodies in normal sera may reflect a residual activity of a previous immune response, or mere contact of the host with the microorganism. It is of interest to note that β-D-galactosyl determinant is present on the surface of *pneumococcus* type XIV polysaccharide[14], while lactose is the immunodominant sugar of *Streptococcus faecalis*[15].

Various workers[10,11] have shown that these antibodies which react with VCN-treated cells are mainly of IgM class. Results presented here agree with these findings and demonstrate further that the immune response does not mature during the course of infection so far as the switch from IgM to IgG class of antibody synthesis is concerned. IgM synthesis is generally considered as T-cell-independent response, and it is possible that some of the polysaccharide determinants of *L. enriettii* membrane are T-cell independent in nature in guinea-pigs. Similar type of IgM response is characteristic of several other polysaccharide antigens[16–18].

The lack of any striking correlation between the FAT and HA titres (Table I) demonstrates the heterogeneity of the antigenic determinants of the system. Guinea-pig immune response was probably directed against a variety of determinants of the parasite, only a few of which were being picked up by the hemagglutination assay system described here.

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Vermipodia – a New Type of Cell Process

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Summary. A new kind of cell process is described in leukemic cells in two cases of histiocytic malignancies. They were evident both with light microscopy and scanning electron microscopy. As they have a tubular, worm like appearance, the name vermipodia has been given to them.

During the course of our studies on two cases of histiocytic malignancies we have observed an unusual and hitherto undescribed variety of cell process in the leukaemic cells in the peripheral blood (Figures 1–3). The morphology of malignant histiocytosis[1,2] and histiocytic lymphoma[3,4] has been previously described, including the scanning electron features[5] but cell processes such as those shown here were not noted.

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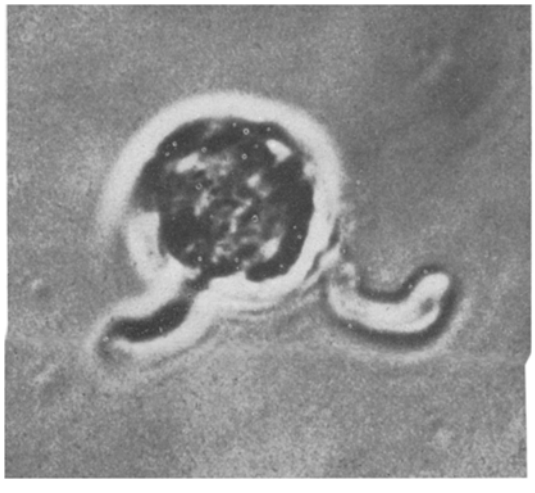

Fig. 1. Vermipodia as seen in a wet preparation. × 950.

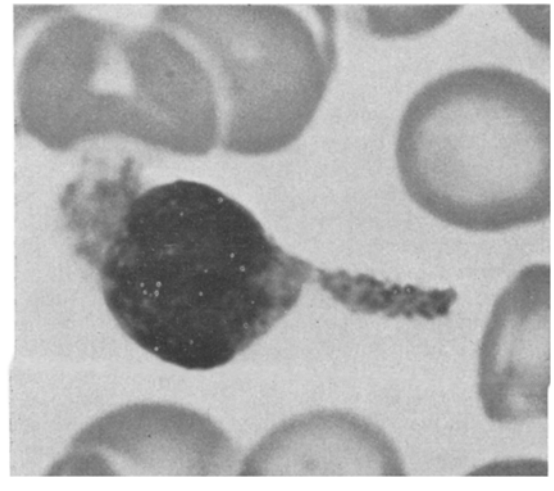

Fig. 2. Wright-Giemsa stained blood smear showing a leukaemic cell with a vermipodium. × 2,700.