

nor did they develop tumor during the 364 day observation period. This showed lack of *in utero* infection following maternal immunization with the virus.

Discussion. Experimental studies of virus-induced neoplasia in animals support the theory of virus etiology in human cancer and indicate that immunoprophylaxis might be of practical value in preventing the disease once viruses responsible for human neoplasia are identified. The present studies revealed passive transfer of SV₄₀ antibody to the offspring and consequent protection of the progeny against tumor induction by SV₄₀ virus administered early following birth. Immunization of the mothers with live SV₄₀ virus prior to conception did not result in infection of the offspring as revealed by tests for presence of SV₄₀ virus following delivery or by evolution of the tumor. The passive SV₄₀ antibody was sharply reduced in amount within 1 month after birth. Tumor antigen CF antibody was not produced in the adult hamsters, indicating lack of significant neoplastic transformation by the agent.

Similar passive protection of the newborn against virus-induced neoplasia by maternal immunization has been shown for polyoma, Friend leukemia, adenovirus 12, and avian visceral lymphomatosis viruses under experimental or normal ecological conditions (5-12). Such procedure holds promise for effective vaccination against neoplasia in man caused by viruses.

Summary. Administration of live SV₄₀ virus vaccine to adult female hamsters prior to conception afforded protection of the progeny

against tumorigenesis by the homologous virus given shortly following birth. The mechanism appeared to be passive transfer of maternal antibody which prevented infection. There was no apparent infection of the fetus with SV₄₀ virus *in utero* and there was no detectable CF antibody in the mothers or the progeny against SV₄₀ tumor antigen. Maternal immunization holds promise as a means for preventing tumor induction in man caused by hypothetical viral neoplastic agents.

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Studies on Chemotaxis IX. Migration of Rabbit Leucocytes Through Filter Membranes.* (32538)

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Filter membranes are widely used in tissue culture experiments. In the diffusion chamber

culture technique(1) the filters serve to separate the implanted cells from the host organism. On the other hand, the filters used in Boyden's technique for measuring chemotaxis *in vitro*(2) have to be permeable for

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cells. While the proper pore size of filters used in the diffusion chamber culture technique has been evaluated by Capalbo, Albright and Bennett(3), there is no systematic study on the permeability of filter membranes for leucocytes in chemotaxis experiments. A study of the migration of rabbit granulocytes or mononuclear cells through different filters was therefore performed in order to evaluate a) the proper type of filter for chemotaxis experiments and b) the pore size which prevents entry of cells into the filters.

Materials and methods. Chemotaxis of rabbit granulocytes and macrophages from peritoneal exudates has been measured by the technique developed by Boyden(4). The method involves the use of a chamber with two compartments separated by a filter membrane, which is permeable to migrating cells. The presence of a chemotactic agent in the lower compartment leads to the migration through the filter of the cells present in the upper compartment. The extent of the chemotactic effect is scored by counting the number of cells reaching the lower side of the filter. Filter membranes of 130-150 μ thickness and the following chemical constitution and porosity were purchased from Millipore Filter Corp., Bedford, Mass. Mixed esters of cellulose: 0.01 μ (VFWP); 0.45 μ (HAWP); 0.65 μ (DAWP); 1.2 μ (RAWP); 3 μ (SSWP); 5 μ (SMWP); 8 μ (SCWP); polyethylene 10 μ (OSWP); teflon: 10 μ (LCWP). Two batches of 8 μ filters (SCWP) were found to differ in their physicochemical properties as follows. The first batch had a tare weight of 5.2 mg/cm², while the second batch weighed 3.3 mg/cm². The first batch of filters deteriorated in 100% ethanol within 30 seconds, whereas the second batch of filters was resistant. The second batch of filters wetted immediately upon filling the test compartment, whereas 2-3 seconds were required in the case of the second batch of filters. According to the manufacturers, the differences between these two batches were "slight adjustments rather than substitution of entirely new material." 5 μ polyvinylchloride membranes (V-1 Metrical) were obtained from Gelman Instrument Co., Ann Arbor, Mich.

Ten percent normal rabbit serum incubated with antigen-antibody complexes was used as

the chemotactic agent for granulocytes and 10 mg casein/ml Gey's solution was employed in the case of macrophages.

Results. *Passage of granulocytes or mononuclear cells through filters of different pore size.* Filters made of mixed esters of cellulose but differing in their pore size were compared in chemotaxis experiments. When granulocytes were used, no cells were seen to enter filters of 0.01 μ pore size. Processes of these cells enter filters of 0.45 μ pore size, but entire cells are never seen within the filter. With 0.65 μ filters, however, a considerable number of granulocytes migrated a short distance into the filter but none succeeded in migrating through. Many more cells entered 1.2 μ filters and a few of them even migrated completely through. Massive migration through the filter is observed at a pore size of 3 μ , 5 μ , or 8 μ . The chemotactic values are summarized in Fig. 1. The corresponding values of the

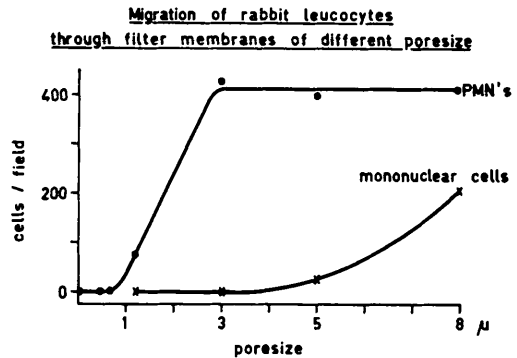


FIG. 1.

negative controls (Gey's solution as test agent) were 0 cells/field with 0.01 μ to 1.2 μ filters and 4, 7, and 32 cells/field for 3 μ , 5 μ and 8 μ filters respectively.

The results obtained with mononuclear cells (mostly macrophages) were different from those obtained with granulocytes. Very few of these cells were seen to enter 1.2 μ filters, whereas numerous mononuclear cells were seen within 3 μ filters although they did not migrate through. A few mononuclear cells are able to migrate through 5 μ filters and many more through 8 μ filters. The data for this set of experiments are also summarized in Fig. 1.

Passage of cells through filters with dif-

fering physicochemical properties. The foregoing experiments demonstrated that filters with a pore size of 3 μ or more permit massive migration of granulocytes through them. In the following experiment filters of 5 μ and 10 μ pore size differing in their physicochemical properties were used for measuring chemotaxis of granulocytes. In view of their pore size, massive migration of granulocytes through all these filters could be expected. Using filters of mixed esters of cellulose or of polyvinylchloride (Table I) a pronounced chemotactic response to antigen-antibody treated serum was indeed observed. No migration, however, was detectable using filters made of polyethylene or Teflon.

TABLE I. Migration of Rabbit Granulocytes Through Filter Membranes with Different Chemical Composition.

Type of filter	Test solution	
	Cells/Field	
	Gey's solution	NRS + Ag-Ab
5 μ Mixed esters of cellulose	2	500
5 μ Polyvinylchloride	7	508
10 μ Polyethylene	0	0
10 μ Teflon	0	0

Similarly, differences in migration of mononuclear cells have been observed when two batches of 8 μ filters (both mixed esters of cellulose) were compared. These two batches differed in their physicochemical properties. In the case of filter batch 1, pronounced migration of mononuclear cells was measured, whereas migration was small or absent in batch 2 filters (Table II).

TABLE II. Migration of Rabbit Mononuclear Cells Through Filter Membranes with Different Physicochemical Properties.

Type of filter	Mononuclear cells
	Field
8 μ First batch (SCWP)	207
8 μ Second batch (SCWP)	10
8 μ Second batch washed (SCWP)	7
5 μ (SMWP)	24

Since even 5 μ filters SMWP allowed consistently higher migration of mononuclear cells than 8 μ filters of batch 2, the differences between the two batches of 8 μ filters can be attributed to their physicochemical properties

rather than to variations in pore size. One possible explanation would be that batch 2 filters contain more detergents than batch 1 filters. Elution of detergent from batch 2 filters according to Cahn(5) however did not lead to increased migration of mononuclear cells through these washed filters (Table II).

Discussion. The experiments presented show that migration of cells through filter membranes depends on the following factors: 1) Cell type, 2) pore size of the filter, and 3) physicochemical properties of the filter.

The data indicate that the pore size permitting passage of cells through the filters differs for the different cell types (Fig. 1). For measuring chemotaxis, the pore size should be at least 3 μ for granulocytes and 8 μ for mononuclear cells. Other workers(6) prefer to use 0.65 μ filters for measuring chemotaxis because they observed pronounced migration in the negative controls with filters of larger pore size. As shown previously and confirmed in the present experiments (Table I), provided suitable experimental conditions are chosen(7), migration of granulocytes is slight with Gey's solution or normal serum in the lower compartment as negative control. On the other hand, the use of 0.65 μ filters renders the test less sensitive and evaluation of chemotaxis is made difficult. The cells do not reach the lower surface of the filter and therefore can not be easily counted on a well defined level.

In chemotaxis experiments the cells are subjected to a stimulus inducing their migration through filters provided the properties of the latter allow the cells to pass through. Empty diffusion chambers implanted *in vivo* do not necessarily stimulate immigration of cells. It seemed therefore adequate to evaluate the pore size preventing entrance of cells into filters by chemotaxis experiments. These experiments showed processes of cells but no entire cells entering the filters of 0.45 μ pore size. Filters of 0.01 μ pore size completely prevented entrance of cells. This indicates that filters used in diffusion chambers should have a pore size not larger than 0.45 μ and preferably a smaller one.

Furthermore, the data suggest that in addition to the pore size, the physicochemical properties of the filter are of significance for

chemotaxis experiments. Granulocyte migration was observed with filters made of polyvinylchloride or of mixed esters of cellulose but not with filters made of polyethylene or Teflon. It was found that filters made of Teflon or polyethylene are hardly wettable. No diffusion of the test solution into the upper compartment could be observed within 5 hours. Absence of migration in these filters may therefore simply be due to lack of contact between cells and chemotactic solution. The difference in migration of macrophages when the two different batches of 8 μ filters were used can not be explained on a similar basis. Both types of 8 μ filters are readily wettable. Since no precise information on the chemical composition and the physicochemical properties of these filters are available the different behaviour in chemotaxis experiments can not be explained.

Summary. Migration of rabbit granulocytes or mononuclear cells from peritoneal exudates

through filter membranes has been studied using Boyden's technique for measuring chemotaxis. Migration depends on 1) the cell type, 2) the pore size of the filter, and 3) the physicochemical properties of the filter. For measuring chemotaxis the pore size should be at least 3 μ for granulocytes and 8 μ for mononuclear cells.

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Aleutian Disease of Mink: Infectious Virus-Antibody Complexes in the Serum.* (32539)

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Aleutian disease (AD) of mink is characterized by a marked systemic plasma cell proliferation, hypergammaglobulinemia, and renal and arterial lesions(1-4). The disease is transmissible by cell-free extracts of affected mink tissue, urine or serum; infectivity is sedimentable in the ultracentrifuge and will pass through a 0.45 μ membrane filter, strongly implicating a viral agent(5,6). Previous attempts to demonstrate antibody to the agent have been unsuccessful, and have been hindered by the presence of the virus in

tissues and serum of the mink throughout the course of the disease.

Materials and methods. Infectious serum was pooled from 4 mink of genotype Aa with elevated levels of G immunoglobulin (IgG) that had had AD for 4 months. This serum was incubated with rabbit antibody to normal mink IgG or albumin, or with normal rabbit serum. Antiserums were prepared and characterized as previously described(4) and were specific for albumin or IgG when checked by immunoelectrophoresis. Sufficient rabbit antibody was added to precipitate all the mink IgG or albumin present in the serum. Large flocculent precipitates were removed by low-speed centrifugation and then discarded. The clear supernatants were titrated for infectivity in normal mink of the genotype Aa using the characteristic rise in IgG by 65 days as determined by serum protein electrophoresis. A

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