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 floccullent 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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single pool of infectious serum was used in 2 experiments. Different rabbit anti-mink IgG and normal rabbit serums were used in 2 experiments, and a single rabbit anti-mink albumin was used only in the 2nd experiment. Fifty percent endpoints of infectivity were determined(7). Fluorescent antibody studies were done as previously described(4) except that frozen sections of kidney were washed in saline prior to fixation.

Results. The tabulation of the 2 sets of titrations is shown in Table I. Since the results of the 2 titrations were similar, they were combined for the calculation of ID_{50}. In the presence of normal rabbit serum, the ID_{50} of mink serum was $10^{-4.4}$ while, with the removal of IgG, the infectivity was reduced to an ID_{50} of $10^{-1.8}$, or a reduction of 2.6 logs. The decrease in infectivity was specifically associated with the removal of IgG, and was not primarily due to coprecipitation of the virus with the large flocculent precipitates, since treatment with anti-albumin, which resulted in about twice as much precipitate as the treatment with anti-IgG, reduced infectivity only 1.0 log.

Since circulating virus-IgG complexes were shown in these experiments, the possibility of localization of such complexes in the glomeruli of AD affected mink was re-investigated, although we previously reported essentially negative results for such localization(4). If frozen sections of kidney from the mink with virus-IgG complexes were washed with saline for 5 minutes prior to fixation to remove non-tissue bound protein, mink IgG could be demonstrated in the glomeruli by the fluorescent antibody technique. The IgG was present in small amounts in glomerular capillaries and in mesangial areas in a granular pattern, and was absent in normal mink kidney, a result in accord with the findings of Williams and coworkers(8). The glomerular localization of host IgG in AD is much less than that seen in complex-induced glomerulonephritis described by Dixon and coworkers (9), and its relationship to the pathogenesis of the renal lesions of AD remains to be determined.

Discussion. This *in vivo* demonstration of an infectious virus-antibody complex is apparently analogous to the *in vitro* observation of a persistent non-neutralizable virus fraction by Dulbecco and coworkers(10). Mandel(11) showed that this *in vitro* persistent fraction was complexed with antibody. Wallis and Melnick(12) have shown that the persistent fraction is the result of virus aggregation and that antibody presumably does not have access to the necessary sites on the virus to cause neutralization.

Notkins and coworkers(13) have shown that mice infected with the lactic dehydrogenase virus can have neutralizing antibody coexistent with circulating virus, and that the circulating virus exists as a non-neutralized complex with immunoglobulin. However, mice infected with the lactic dehydrogenase virus apparently do not have any obvious disease, except for slight splenomegaly and lymph node enlargement(14), while infected mink have prominent tissue lesions and a frequently fatal disease. Our previous demonstration of IgG-containing complexes in Aleutian disease by analytical ultracenrifugation may be

TABLE I. Titration of Aleutian Disease Virus Contained in Infective Mink Serum After Removal of G Immunoglobulin or Albumin, or in the Presence of Normal Rabbit Serum. ID_{50} indicates serum dilution where 50% of mink were infected.

Dilution of infective mink serum	Number of mink infected/Number tested				
	+ Anti IgG		+ Anti albumin	+ Normal rabbit serum	
	Exp 1	Exp 2	Exp 2	Exp 1	Exp 2
6×10^{-2}	3/5	1/4	3/4	6/6	4/4
10^{-2}	0/6	1/4	3/4	5/6	4/4
10^{-3}	0/6	0/4	3/4	4/6	3/4
10^{-4}	0/6	0/4	2/4	6/6	4/4
10^{-5}	0/6	1/4	0/4	1/6	1/4
10^{-6}	0/6	—	—	0/6	—
ID_{50}	$10^{-1.8}$		$10^{-3.4}$	$10^{-4.4}$	

related to the virus-antibody complexes shown in these experiments(4).

The demonstration of infectious virus-antibody complexes in this common, but severe disease of mink suggests that similar mechanisms should be searched for in other diseases such as mouse mammary tumors(15) in which antibody to the causative agent has not been found by the usual tests.

Summary. Mink affected with Aleutian disease have viremia which persists until death. Removal of G immunoglobulin from infectious serum of affected mink markedly reduces the virus titer. The virus in the serum exists as a complex with immunoglobulin, in which *in vivo* infectivity is still present.

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Muramidase Activity in Leukemic Rats.*† (32540)

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Muramidase, or lysozyme, discovered by Fleming in 1922 in human tears, was later found to be widely distributed in many tissues, including leukocytes(1). Recently Finch *et al* demonstrated markedly elevated muramidase levels in the serum of patients with monocytic and chronic granulocytic leukemia (2). Osserman confirmed these observations and found that large amounts of this enzyme were excreted in the urine of patients with monomyelocytic leukemia(3).

The presence of abnormally large amounts of muramidase in the serum and urine of patients with monocytic and granulocytic leukemia is of considerable interest; further investigations on the properties and functions of this enzyme may contribute important information on the characteristics and behavior of leukemic leukocytes.

In this laboratory studies have been carried out for the past 10 years on leukemia in the rat. Recent investigations have shown that high levels of muramidase activity occur in the serum, urine, ascitic fluid and myelocytic cells of rats with transplanted chloroleukemia.

Materials and methods. Muramidase activity was measured by a modification of the method of Smolelis and Hartsell(4). To 2 cc of lysozyme substrate (Difco) was added 0.2 cc of test serum, urine or homogenate and the optical density read over 90 seconds (ΔOD 540/90 sec). Units can be converted to micrograms of purified egg white lysozyme (Difco). Twenty-four hour urine samples were obtained from either individuals or pools of control and leukemic rats as well as various

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