tistical difference between the slope of the curve for the relationship between oxygen consumption and running velocity for quadrupedal locomotion in the chimpanzee and the slope of the corresponding curve for bipedal locomotion. There was likewise no statistical difference between the slopes of the corresponding curves for the capuchin monkey.

The observed cost of locomotion in the chimpanzee was about 50 percent higher than would be predicted from the relationship between the cost of running and body size for quadrupedal animals. The observed and predicted costs of running in the capuchin monkey were nearly identical. The energy cost of bipedal locomotion in the spider monkey was also close to the predicted value for quadrupedal running (0.41 versus 0.38 ml of oxygen per gram per kilometer).

It is clear, although somewhat unexpected, that a number of primates expend the same amount of energy whether they move on two or on four legs. Thus the cost or efficiency of bipedal versus quadrupedal locomotion probably should not be used in arguments weighing the relative advantages and disadvantages that bipedal locomotion conferred on man.

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7. Wind velocity was matched to tread speed. The air temperature was 22°C, and the relative humidity was less than 30 percent. Room air was pulled past the faces of the animals at between 50 and 150 liters per minute (at standard temperature and pressure). The difference in oxygen concentration between air flowing into and out of their masks was measured with a Beckman model G-2 paramagnetic oxygen analyzer. We used only steady-state oxygen consumption values. We considered a steady state to have been reached when there was less than a 5 percent variation in the oxygen consumption during a 30-minute period. At speeds exceeding 4 km/hour the animals tired, and we used 15- instead of 30-minute periods. We calibrated flowmeters to an accuracy of better than 1 percent, using a Brooks "Vol-u-meter" under pressure gradients identical to those used in our experimental system. The accuracy of the entire system was determined by bleeding known amounts of nitrogen into the face mask at air flows identical to those used in the experiments and determining the dilution of oxygen in the room air. The accuracy was better than ± 2 percent.
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Isolation of Aleutian Mink Disease Virus by Affinity Chromatography

Abstract. *Affinity chromatography was used to isolate the Aleutian disease virus of mink. Dissociation of the immunoadsorbent-virus complex with 0.75 molar sodium chloride and then with a glycine-hydrochloride gradient released infective particles resembling picornaviruses. The elution profile suggests that two different types of virus-antibody complexes are formed, one dissociated by sodium chloride and another that requires glycine-hydrochloride in addition to sodium chloride for release of virus.*

Affinity chromatography has been widely used to isolate dilute antigens, enzymes, haptens, and ligands (*1*). However, viruses have not been isolated by this technique, presumably because of the difficulty in obtaining sufficient quantities of specific antibody. The virus causing Aleutian disease of mink (*2*) and ferrets (*3*) seemed suitable for isolation by this method, because large amounts of antibody (*4*) are produced during the disease, resulting in the excess of gamma globulin (hypergammaglobulinemia) characteristic of Aleutian disease.

Although Aleutian disease was described in 1958, the agent has remained unclassified, and the various strains used in research are uncharacterized. The virus that we passaged and used in this study had a target-size molecular weight of 1.5×10^6 (estimated from inactivation of virus by ionizing radiation) and produced typical infiltrates of lymphocytes and plasmacytes in all soft organs, with a doubling of serum gamma globulin concentration by 30 days after inoculation. The Aleutian disease antibody [found in the immunoglobulin G (IgG) fraction] from serum of chronically infected mink was separated free of virus by ion exchange

Table 1. Evidence of infectivity of eluates from experiments 1 and 2. Ornithine carbamoyltransferase (OCT) was assayed 7 days after inoculation. Lactate dehydrogenase (LDH) was assayed 10 to 21 days after inoculation. The quantitative phytohemagglutination (PHA) test, done weekly from 7 to 28 days after inoculation to evaluate cellular immunity, was scored as stimulation (S) or no response (NR). The IgG values were determined 64 days after inoculation. Plasmacytosis in kidney, liver, and spleen was graded from normal (0) to most severe (++++) when animals were necropsied 64 days after inoculation. A diagnosis of normal (N) or Aleutian disease (AD) was made for each animal. The eluate dilutions used for inoculation are given in parentheses.

Mink	Inoculation	OCT (units)	LDH (units)	IgG (%)	PHA	Lesions	Diagnosis
		Experiment 1					
23	NaCl eluate	125	10,000	22	NR	++	AD
20	NaCl eluate	90	8,000	30	NR	++	AD
29	NaCl eluate (10^{-3})	35	15,000	27	NR	++	AD
19	NaCl eluate (10^{-6})	50	5,000	40	NR	+++	AD
30	NaCl control	15	2,000	12	S	±	N
8	Acid eluate	14,000				+	AD*
9	Acid eluate	150	9,000	34	NR	++++	AD
10	Acid eluate (10^{-3})	1,400	12,000	27	NR	+	AD
7	Acid eluate (10^{-6})	50	6,000	31	NR	+++	AD
27	Acid control	10	1,500	9	S	0	N
24	Pronase eluate	15	1,200	11	NR	0	N
25	Pronase eluate (10^{-3})	1	1,700	8	S	0	N
		Experiment 2					
3	Acid eluate	960	18,000	19	NR	+	AD*
4	Acid eluate	185	14,000	26	NR	†	AD
5	Acid eluate	150	12,000	27	NR	†	AD

* Animal died. † Histologic material not examined.

chromatography on diethylaminoethyl cellulose (*5*). The procedure for preparation of the immunoadsorbent (Sepharose-antibody column) has been described (*6*). In each of three experiments, Sepharose 4B (Pharmacia) was activated by addition of 37.5 mg of CNBr per milliliter of Sepharose suspension. An aqueous solution of CNBr (4 mg/ml) was added to Sepharose 4B suspended in two volumes of 0.05*M* bicarbonate buffer, *p*H 9.0. The volumes of Sepharose 4B were 150 ml in experiment 1, 500 ml in experiment 2, and 900 ml in experiment 3. The *p*H of the suspension was raised to 11.0, and 2.5*N* NaOH was added intermittently until the *p*H remained stable. This activated mixture was then washed with 20 liters of cold 0.05*M* bicarbonate buffer, *p*H 9.0. The IgG was placed in 200 ml of 0.1*M* bicarbonate buffer, *p*H 8.4, and added to the activated Sepharose 4B (0.5 g of antibody per 100 ml of suspension). The coupling reaction was conducted for 16 hours at 4°C with slow stirring. The unbound IgG was removed by washing the Sepharose 4B in columns (2.5 by 30 cm in experiment 1, 4 by 40 cm in experiment 2, and 7 by 50 cm in experiment 3) with phosphate-buffered isotonic saline, p*H* 7.6, until the absorbance at 280 nm of the effluent was less than 0.005.

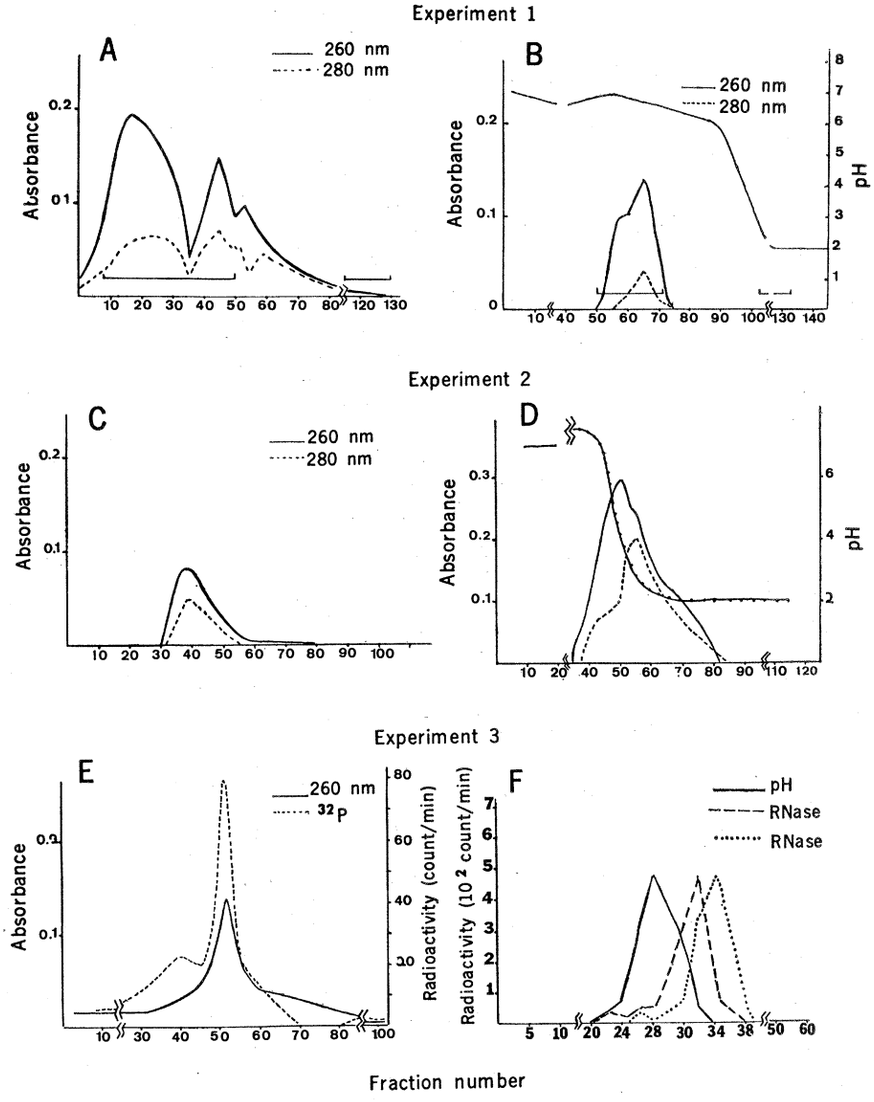

Fig. 1. Elution profiles from Sepharose-antibody columns charged with tissue extracts from mink infected with Aleutian disease. Components eluted with 0.75*M* NaCl in each experiment are shown in (A), (C), and (E). Components eluted by the addition of acid in experiments 1 and 2 are shown in (B) and (D), respectively. The solid bars above the abscissa in (A) and (B) indicate the fractions pooled for determining infectivity in experiment 1. The components eluted by NaCl in experiment 3 were adjusted to *p*H 5.0 in 0.1*M* NaCl and rechromatographed (F) on Bio-Gel agarose-M50 after the following treatments: none (solid line); ribonuclease (*RNase*), 0.02 mg/ml at 25°C for 2 hours (broken line); or ribonuclease, 1.0 mg/ml at 37°C for 2 hours (dotted line).

The infective antigenic material prepared in mink heterozygous for the Aleutian gene, was suspended in 0.04*M* phosphate containing 0.85 percent NaCl (*p*H 7.6) at a ratio of 1 g of tissue per 5 ml of buffer. Tissue homogenates were prepared from liver (experiment 1), spleen (experiment 2), and liver, spleen, lymph nodes, kidney, lung, and cleaned small intestine (experiment 3). In each case, the tissues were harvested 7 days after the mink was inoculated with 1×10^6 infectious doses of virus. In experiment 3, the mink received daily injections of [^{32}P]orthophosphate in phosphate-buffered isotonic saline, 0.5 ml (0.5 mc) per day starting 2 days after virus infection and continuing until 2.5 mc was given.

Extracts were prepared by processing minced tissues in a blender for 10 minutes in cold phosphate-buffered saline, *p*H 7.6, and then subjecting them to six freeze-thaw cycles. These suspensions were centrifuged twice at 15,000*g* for 40 minutes at 4°C to remove insoluble material. The extracts were held frozen, and were centrifuged again before the reaction with immunoadsorbent. The volumes of infective extract percolated through the columns were 30 ml in experiment 1, 300 ml in experiment 2, and 400 ml in experiment 3. The antigen-containing columns were then washed until the absorbance at 280 nm of the effluent was less than 0.01.

Chromatographic elution of the virus (Fig. 1) was started with 0.75*M* NaCl, and fractions of 5 ml (experiment 1) and 10 ml (experiments 2 and 3) were collected until absorbance at 260 nm of the eluates was less than 0.01. Further elution was effected with 0.2*M* glycine-HCl in 0.75*M* NaCl in experiment 1 and 0.4*M* glycine-HCl in 0.75*M* NaCl in experiment 2; the *p*H range was 6.5 to 2.0. Acid elution in experiment 3 was done by adding 200-ml portions of 0.1*M* acetic acid–sodium acetate buffer in 0.75*M* NaCl to achieve stepwise decreases of 0.5 *p*H units from *p*H 5.5 to 3.0, followed by 200 ml of 1.0*M* acetic acid.

In experiment 1 (Fig. 1A), material absorbing at 260 to 280 nm (fractions 9 to 50) was pooled and concentrated for inoculation into mink. Fractions 100 to 130, eluted after the peaks of ultraviolet-adsorbing material, were also

concentrated and tested for infectivity, as was the material eluted from the column after the immunoadsorbent was digested with Pronase. In general, elution profiles obtained in experiments 1 and 2 were similar. However, a direct comparison of the two elution profiles cannot be made because of differences in column sizes, acid eluants, and organs used to prepare tissue extracts. Nevertheless, material absorbing at 260 nm was eluted from both columns with NaCl, and additional material was eluted with acid.

In experiment 3, in which the infectious material contained ^{32}P, elution with 0.75*M* NaCl produced the characteristic pattern with a single component as indicated by absorbance and isotope labeling. Elution with acetic acid–sodium acetate produced a broad range of low absorbance not exceeding 0.065 at 260 nm. However, these fractions were concentrated and found to contain radioactivity.

In experiment 1, the fractions eluted by NaCl and by acid were infective, whereas the fractions eluted after the peaks were not infective. The criteria we used for virus transmission were the rapid increase in gamma globulin concentration, a three- to fivefold increase in spleen weight, and development of infiltrates of lymphocytes and plasmacytes in kidney, liver, and spleen. Frequently, the earliest indication of virus infection was a three- to fivefold increase in serum ornithine carbamoyltransferase 6 to 7 days after inoculation (*7*). The clinical findings that established the infectivity are summarized in Table 1. The lesions in mink inoculated with the isolated agent were similar to those seen in naturally infected mink (*2*). In experiment 2, only the acid eluate was tested, and it was found to be infective.

The salt and acid eluates were resolved by counterelectrophoresis (Abbott Scientific Products), with Aleutian disease hypergammaglobulinemic serum in the antiserum wells, for 1 hour at 5 volt/cm in barbital buffer (*p*H 8.2, 0.05 ionic strength). The zones between the antigen and antiserum well were sectioned for electron microscopy. This material was fixed in 4 percent glutaraldehyde followed by 1 percent OsO_4, dehydrated in acetone, and embedded in Epon (*8*). The grids were stained with a solution containing 1 percent lead ion as a mixture of acetate and citrate (*9*).

The particles shown in Fig. 2 resemble icosahedral picornaviruses and are about 200 Å in diameter. In general, the virus particles eluted from the column with NaCl have the same appearance as those eluted with a glycine-HCl gradient. If the viruses in the eluates are similar, the chromatographic character of the immunoadsorbent would indicate at least two ranges of antibody affinity. In the acid eluate, some of the particles appear to have undergone dissociation of the capsid protomers despite NaCl concentrations usually sufficient to prevent degradation (*10*). This may be like the reaction leading to the formation of 14*S* subunit capsids followed by release of RNA, as seen with mouse encephalomyelitis (ME) virus at *p*H 5.7 in the presence of 0.1*M* chloride ion (*11*). The RNA concentration in the acid eluate of experiment 2 was 0.5 μg/ml measured by the orcinol method (*12*). When the NaCl eluate of experiment 3 was subjected to ribonuclease digestion, a shift of ^{32}P to forms of lower molecular weight was shown on sieve chromatography (Fig. 1F).

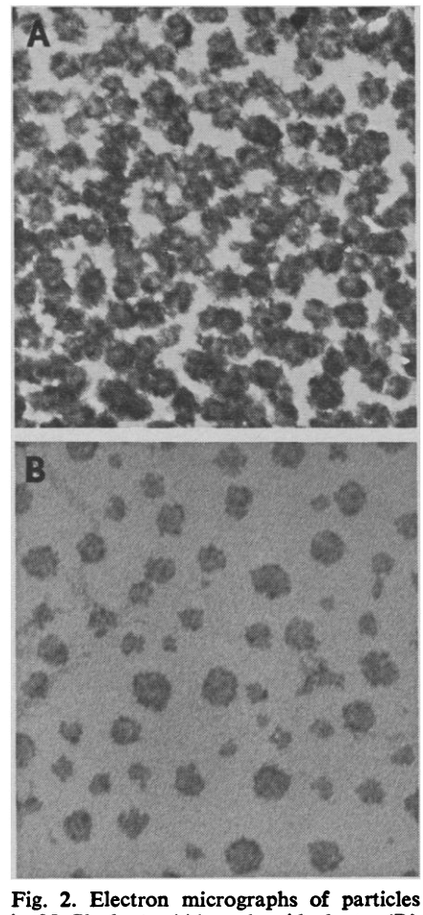

Fig. 2. Electron micrographs of particles in NaCl eluate (A) and acid eluate (B) from experiment 1 (× 133,000).

The lowest theoretical size requirements for spherical animal viruses containing a single strand of RNA in an icosahedral shell are a maximum diameter of 142.6 Å with a capsid layer 25 Å thick (*13*). It was suggested (*13*) that these viruses would be visualized in ultrathin sections as crystalline arrays of electron-opaque particles. The morphologic characteristics of the virus we isolated by affinity chromatography, along with the apparent dissociation of the particle in dilute acid and the demonstration of RNA, suggest that it may be a member of the closely related cardioviruses and enteroviruses. These features are consistent with the early observation where crystalline arrays of viruses were seen in endothelial cells of mink with Aleutian disease (*14*).

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