in other body cells. An illustration of such a difference between cells is given by the unusual ability of those of the ileum and kidney to absorb chloride against concentration gradients. While this process may not involve control of chloride transfer directly, it is associated with metabolic processes that can be inhibited by added chemical compounds (11,12).

The experiments reported here do not suggest the presence of any metabolic processes controlling the exchange of chloride between erythrocytes and plasma, but they do not eliminate this possibility. They are consistent with the hypothesis that chloride enters red cells by passive diffusion. Approximate calculations show that the thickness of the plasma layer surrounding each erythrocyte in whole blood is about one micron, and that the red cell surface area in the 10 ml samples of blood used totals approximately 6 m^2, so that ample opportunity for rapid diffusion exists. However, because the actual rate of chloride transfer could not be measured, and because all possible metabolic systems were not inhibited, the results are not conclusive.

Summary. The rate of transfer of chloride into and out of human erythrocytes was estimated *in vitro* with Cl^{36} and found to be at least 600 to 1300 times greater than that of sodium and potassium respectively. Incubation at low temperature, the conversion of hemoglobin to methemoglobin, and the addition of metabolic poisons, were all without detectable effect on the rate of transfer. These data are compatible with the hypothesis that chloride enters erythrocytes by passive diffusion, but they do not eliminate the possibility that metabolic processes responsible for Cl transfer may exist which are capable of transporting chloride into and out of the red cell at a very rapid rate.

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A Pneumotropic Virus Isolated from C_3H Mice Carrying the Bittner Milk Agent. (20044)

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In a preliminary report(1), account was given of a virus isolated from C_3H mice known to carry the Bittner Milk Agent. This virus kills young suckling mice although inoculation of older suckling and of adult mice results in no obvious disease. Pathologic findings, as fully described elsewhere(2) are limited largely to the lungs regardless how the virus is inoculated and are different from those encountered in other types of mouse pneumonitis known to us. They consist primarily of swelling and proliferation of endothelial cells lining small blood vessels. The nuclei are swollen and contain inclusion bodies staining

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positively by the nuclear Feulgen procedure.

As this pneumotropic virus has thus far shown no relationship to other viruses, it is regarded as a hitherto undescribed agent, provisionally referred to as K-virus. Whether it is the Bittner Milk Agent or associated with it in any way has not been determined. Experiments have been set up, however, in conjunction with Dr. Howard B. Andervont, in an effort to answer this problem. Regardless of what relationships K-virus may prove to have on further study, its unique properties make it a subject of interest. The present communication describes further isolations of K-virus, production of specific immune serum, differentiation of K-virus from other viruses by neutralization test, titrations of virus in ill mice, and other properties not previously described.

Isolations of virus. Five isolations of K-virus in a total of 22 attempts made from female C_3H mice of a strain known to carry the Bittner Milk Agent,[†] have all caused illness on first passage in Swiss mice inoculated at one to 3 days of age. No blind passages have been resorted to. The first isolation, referred to as Lot 1, was from pooled liver, spleen, and tumors of 2 non-pregnant, non-lactating C_3H mice(1). Four other attempts to isolate virus from C_3H mice bearing tumors were wholly unsuccessful. The remaining 4 isolations (Lots 43, 47, 61, and 63) were from lactating mice, with litters of from 14 to 18 days of age which were removed the night before the mothers were sacrificed. Liver, spleen, and kidneys were pooled in each case and a separate pool made of mammary gland tissue. Such pools were emulsified in 10% meat infusion broth and inoculated intracerebrally and intraperitoneally in doses of 0.03 cc and 0.05 cc respectively. Illness first appeared within 9 to 12 days. In 2 of the mother mice, K-virus was isolated from mammary gland tissue as well as from liver, spleen and kidneys. All of the mice from which K-virus was originally isolated appeared well and had healthy young. K-virus, however, was present in the liver, spleen, and kidney pool of one mother in a titer of 10^{-4} and in the mammary tissue of the same mouse in a titer of 10^{-1} when these pools were assayed by intracerebral inoculation of 3-day-old mice. Of 5 C_3H mice 6 months of age nursing their first litters, K-virus was isolated from 3. Lot 47 virus was isolated from a mouse having her second litter. Twelve attempts to isolate K-virus from mothers with their third or fourth litters were unsuccessful. In the course of 15 passages with Lot 1 virus and 8 passages of Lot 43 virus no change in the pathogenicity of K-virus has been noted. The 5 viruses isolated appear to be similar in pathogenic effects and, as described below, were neutralized by the same antisera.

The disease picture in mice infected with K-virus remained the same in original as in later passages, regardless of route of inoculation. Inoculated mice grew normally and remained well-nourished until they became ill more or less abruptly. Even when moribund, they had milk in their stomachs. The principal manifestation of illness was labored "chugging" respiration, afflicted mice usually dying within 5 hours of its appearance. About 50% of young suckling mice had gross pulmonary consolidation, accompanied in some with an increase of pleural fluid. On histologic section, however, lungs of ill mice all presented a striking pathology(2). Altogether, 60 unsuccessful attempts to isolate virus have been made. A wide variety of mice have been tried including male and female C_3H, C-strain, and hybrid mice; all known to carry the *Bittner* Milk Agent; C_3H mice known to be free of the agent, and in addition Swiss mice, using pools of liver and spleen in most instances and tumor tissue or blood alone in others.

Susceptibility of mice and routes of inoculation. K-virus is infectious for suckling mice by a variety of routes including intranasal, subcutaneous, intraperitoneal, intracranial, and by stomach tube. Of these, only the last 2 have been employed at all routinely. The stomach tube was a 22-gauge needle with an extra blunt, slightly curved tip(3). In one experiment titrations of a pool of liver, spleen, and kidney by 3 different routes of inoculation

[†] Mice used for isolation of K-virus were kindly supplied by Dr. Howard B. Andervont, National Cancer Institute, National Institutes of Health, Bethesda, Md.

were compared. Approximate titers were 10^{-4} by stomach tube, 10^{-7} by intraperitoneal, and 10^{-8} by intracerebral inoculation. The latter route was the most sensitive in spite of the fact that the inoculum used was .03 ml, as compared with .05 ml for intraperitoneal inoculation and 0.1 ml for the stomach tube; these amounts being those routinely employed. In another experiment Lot 43 virus was given to successively older mice both intracerebrally and intraperitoneally in an attempt to determine at what age susceptibility to apparent infection was lost. Resistance first definitely appeared in mice 12 days of age, but it was only in mice 18 days of age that resistance was complete, as no illness or deaths occurred in this group. Two mice survived in the 8-day group, but this was somewhat unusual as survivors have not been encountered in other experiments in which mice of this age were used. Although they never appeared ill, K-virus was readily recovered from a pool of liver, spleen, and kidney made from this latter group of mice 24 days after they had been inoculated.

Distribution of virus in infected mice. To determine the distribution of K-virus in diseased mice, a pool of liver and spleen from first passage of Lot 43 virus was passed to 3-day mice intraperitoneally. Five pools of infective material were harvested from these second passage mice when they were moribund 8 days later. These pools were of blood, lungs, brains, intestinal contents, and finally of liver, spleen, and kidneys combined. Intestinal contents were collected by excising and chilling lower intestinal tract at 4°C, then pressing out the contents. Each pool was frozen in dry ice, thawed, then centrifuged at 4000 rpm for ½ hour prior to making serial dilutions for titration in brains of 3-day-old mice. Deaths occurring within 5 days were considered non-specific. All titrations were discontinued in 18 days although an occasional mouse inoculated with higher dilutions of virus died after this time. Continued spot checks indicated that mice became moribund while well-nourished, that they had a "chugging" respiration, and that at least 50% had gross pulmonary consolidation, these features being characteristic of infection with K-virus. Highest concentrations of virus (10^{-8}) occurred in lungs and in the pool of liver, spleen, and kidneys. Brains titered 10^{-5} and intestinal contents 10^{-6}. Blood from the original pool titered 10^{-4}, but due to loss of material, the titration had to be continued with blood from other mice similarly inoculated. This blood titered 10^{-7}. Bladder urine from a sick mouse produced typical illness in 3 of 6 mice inoculated intracerebrally with 0.02 ml. Subsequent experiments have shown that lungs as well as pools of liver, spleen, and kidneys from infected mice will not infrequently have an intracerebral titer of 10^{-9}.

To determine the rate of growth of K-virus nearly a million LD_{50} doses of virus were given to 3-day-old mice by intracerebral inoculation. Lungs and pools of liver, spleen, and kidneys of small groups of these mice were harvested one, 4, and 6 days later, as well as on the 7th day when they were ill and dying. Within 24 hours of inoculation, lungs had an infectivity titer of 10^{-2} and the liver, spleen, and kidney pool of 10^{-3} on titration in brains of suckling mice. Virus titers rose steadily and were maximal from tissues of those animals moribund on the 7th day, at which time tissues from both pools titrated 10^{-9}. Results on the 4th day indicated that growth of virus took place somewhat more rapidly in the lungs.

Neutralization tests. Good antisera to Lot 1 and Lot 43 viruses were produced in rabbits when pooled liver, spleen, kidney, lungs, and brains of infected mice, treated by differential centrifugation, were used as antigen. Ten per cent tissue suspensions were centrifuged at 4,500 rpm for ½ hour and the supernate recentrifuged at 15,000 rpm for one hour. The final sediment, suspended 1:10 in saline, was injected intravenously into 1800 g rabbits, 3 doses of 1-2 cc each being given at weekly intervals. Rabbits were bled 7 to 14 days after the last injection. In neutralization tests, sera were inactivated at 56°C for ½ hour and final mixtures contained equal amounts of serum and virus dilutions which were incubated not longer than ½ hour. Neutralization tests were carried out by intracerebral inoculation of 3-day-old Swiss mice. Rabbit serum prepared against Lot 1 virus neutralized 100 LD_{50} doses of K-virus in a

TABLE I. Results of Neutralization Tests in Which Different Lots of K-Virus Were Run against A) Specific K-Virus Immune Rabbit Sera and B) Antisera Prepared against Other Viral Pathogens of Mice.

	Sera	Time in days when all mice dead	K-virus lot used	Virus dilutions 1:20	10^{-2}	10^{-3}
A	Normal rabbit	8	Lot 47	ND	ND	7/7
	Lot 1 immune		"	0/9	0/8	0/6
	Normal rabbit	10	43	ND	ND	9/9
	Lot 1 immune		"	0/9	0/8	0/9
	Normal rabbit	10	I	ND	ND	9/9
	Lot 1 immune		"	ND	0/8	0/8
	Normal rabbit		43	ND	ND	8/8
	Lot 43 immune		"	0/8	ND	ND
B	NDV	14	43			8/8
	LCM	9	"			8/8
	PVM	12	"	ND	ND	8/8
	Coxsackie A*	10	"			9/9
	Coxsackie B†	11	"			9/9

* Pool of mouse antisera representing 10 types in Group A.
† " " " " " 4 " " " B.
ND = Not done.

dilution of 1:20. Serum prepared against the more virulent Lot 43 virus, however, fully neutralized an equal amount of virus in a dilution of 1:320. All viruses isolated in dilutions of 1:20 were neutralized by the same K-virus hyperimmune rabbit sera (Table I). Normal pre-inoculation rabbit serum failed to protect against the same virus preparations diluted 10^{-3}. A variety of other sera, which served as controls and failed to neutralize K-virus are divisible into 3 groups. The first group consisted of specific immune sera‡ prepared against other viral agents of mice which might in any way be confused with K-virus (Table I). These sera include pneumonia virus of mice (PVM),§ lymphocytic choriomeningitis (LCM), Newcastle disease (NDV), a pool of mouse antisera representing 10 types of Group A Coxsackie viruses and finally a pool representing antisera to 4 types of Group B Coxsackie viruses.‖ A second group of sera were from normal adult mice of C_3H strain known to carry and of others known to be free of the Bittner Milk Agent, and from regular Swiss and C57 (black) mice. Human sera made a final group which included gamma globulin and sera from 2 of us (L.K. and H.W.M.) who have had laboratory exposure to K-virus. None of the sera in any of these 3 groups neutralized K-virus in final dilution of 10^{-3}, which represented 100 to 1000 LD_{50} doses of virus in these tests. Although naturally occurring antibodies have not been encountered in adult mice, continuing studies indicate that they may develop naturally in older suckling C_3H mice which never show signs of illness.

Physical properties. As discussed in the preliminary report(1), K-virus is resistant to freezing and thawing, to exposure to room temperature for 16 days and to penicillin and streptomycin and it passes readily through a Selas 03 bacteria tight filter. K-virus also resists 30 minutes exposure to 20% ether.

Discussion. Evidence presented above suggests that K-virus is an undescribed pathogen of mice. Its behavior in killing suckling but failing to cause illness or lesions in adult mice is not, to our knowledge, characteristic of other known mouse viruses. Coxsackie viruses

‡ As neutralizing sera against the Psittacosis-Lymphogranuloma group are not readily available, a complement-fixation test with K-virus immune rabbit serum was kindly run by Dr. M. R. Hilleman of the Army Medical Service Graduate School. Control and diagnostic antigens both gave a titer of 1:20, indicating that relationship of K-virus to the Psittacosis group is unlikely.

§ Kindly supplied by Dr. Frank L. Horsfall, Hospital of the Rockefeller Institute for Medical Research, New York City.

‖ Kindly supplied by Dr. R. J. Huebner from the Laboratory of Infectious Diseases, National Microbiological Institute, Bethesda, Md.

behave in somewhat similar fashion but are not regarded as of murine origin. So far there is no evidence that K-virus belongs to this group, as it does not produce any sign of neuro-muscular changes nor do Coxsackie viruses lead to pulmonary consolidation. Furthermore, Coxsackie antisera from 14 different types in Group A and Group B have failed to neutralize K-virus. A special effort has been made to determine whether K-virus might be related to the Psittacosis-Lymphogranuloma group. There is no relationship on pathologic grounds, as LCL bodies do not appear in K-virus infected tissue. Centrifugation experiments indicate that K-virus is probably smaller than any member of the psittacosis group. Furthermore, complement fixing antibodies for the psittacosis-LGV group have not been found in K-virus immune serum. Antisera to other viruses infecting mice such as PVM, LCM, and NDV fail to neutralize K-virus, which resembles none of them in the type of disease produced. On results of serologic tests, disease pattern, and on the type of endothelial proliferation seen on histologic section of infected lungs(2), it would appear that K-virus is a previously undescribed agent. Our interest has centered largely in its ability to produce acute illness. A question of importance, however, is whether latent infection with K-virus can, in later life of its murine host, lead to mammary-adenocarcinoma.

Longer term experiments essential to answering this question have been kindly set up by Dr. Howard K. Andervont. At the present time, in absence of proof from definitive experiments, the authors do not claim that K-virus is identical with the Bittner Milk Factor. It is hoped that further investigations will throw more light on the pathogenesis, mode of spread, immunology and relationships of K-virus infections in mice.

Summary. 1. Five isolations of a virus which causes a fatal pneumonitis of suckling mice by various routes of inoculation have been made from C_3H mice known to carry the Bittner Milk Agent. 2. Older suckling mice are increasingly resistant to this virus and no apparent illness has been produced by inoculation of adult mice. 3. Specific neutralizing antisera have been produced in rabbits. 4. Due to its unique behavior and failure to show serologic or other relationships to known mouse viruses, K-virus has been regarded as an hitherto undescribed agent. 5. Long term experiments have been set up to determine, if possible, whether K-virus is identical with or related to the Bittner Milk Agent.

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Occurrence of Generalized Infection of Enteric Origin in Mice Poisoned with Nitrogen Mustard.* (20045)

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The toxic effects of the nitrogen mustards on experimental animals include a number which resemble those produced by ionizing radiation(1-8). No observations seem to have been made, however, on the occurrence in animals poisoned with nitrogen mustard of spontaneous systemic infections such as have been found after exposure to moderate doses of total body x-radiation. For example: Miller, Hammond and Tompkins(9), described a high incidence of bacteremia in mice irradiated with 450 or 600 r during the second

* This investigation was carried out under a contract between the U. S. Atomic Energy Commission and the University of Chicago.