serums effected a retarding of tumor development to a rate below that observed with the agent in saline solution, and ultimately a smaller number of tumors was produced.

Conclusions. The milk factor of mammary mouse carcinoma is highly antigenic and stimulates the formation of antibodies in both rabbits and rats. The mouse cancer antiserum neutralizes and renders inactive mouse cancer centrifugates. Equally effective antiserums are prepared from spontaneous and from transplant tumors. Normal rabbit and rat serums have a slight effect in inactivating the mouse cancer virus. The finding that antiserum against normal mouse tissue does not have any neutralizing or adverse effect on the milk agent indicates that the agent is a virus of exogenous origin.

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Cell-Blockade in Canine Distemper.*

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The interference phenomenon of animal viruses was first observed by Hoskins[1] and by Magrassi[2] independently. Magrassi observed an interference between nonencephalitic and encephalitic herpes viruses in rabbits, and Hoskins reported an interference between neurotropic and pantropic yellow fever viruses in monkeys. Subsequent investigations by Findlay and MacCallum,[3] Dalldorf,[4] Jungeblut and Sanders,[5] Ziegler, Lavin and Horsfall,[6] the Henles,[7] and others have shown that the cell-blockade, or interference phenomenon, can occur in many animal viruses under experimental conditions.

We have conducted experimental studies of simultaneous infections of young foxes with virulent distemper virus and a distemper virus modified by ferret passage, and found that the phenomenon of interference, or cell-blockade, can be demonstrated for these viruses. Foxes inoculated *intranasally* with virulent distemper virus can be protected from fatal infection by subsequent inoculation of the ferret-passage distemper virus, which is of low virulence for foxes and dogs. In foxes injected *intramuscularly* with the virulent distemper virus, the course of the infection is also blocked by the introduction of the modified virus either 3 days before, or at the same time that, the virulent distemper virus is given. However, if the interval elapsing between the intramuscular injection of the virulent virus and the subsequent inoculation of distemperoid virus is as long as 3 days, there is very little interference with the course of the virulent infection.

Materials and Methods. The animals inoculated were red fox pups 10 to 16 weeks old. In any one experiment the foxes were very nearly the same age. The virulent distemper virus was of dog origin, and had been passed experimentally through foxes until it acquired an extremely high virulence for that animal. The ferret-passage virus was the distemperoid virus described by Green.[8]

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1 Hoskins, M., *Am. J. Trop. Med.*, 1935, **15**, 675.

2 Magrassi, F., *Z. f. Hyg. u. Infektionskr.*, 1935, **117**, 501, 573.

3 Findlay, G. M., and MacCallum, F. O., *J. Path. and Bact.*, 1937, **44**, 405.

4 Dalldorf, G., *J. Imm.*, 1939, **37**, 245.

5 Jungeblut, C. W., and Sanders, M., *J. Exp. Med.*, 1942, **76**, 127.

6 Ziegler, J. E., Jr., Lavin, G. I., and Horsfall, F. L., *J. Exp. Med.*, 1944, **79**, 379.

7 Henle, W., and Henle, G., *Am. J. Med. Sci.*, 1944, **207**, 705.

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TABLE I.
Interference in Foxes between Virulent Distemper Virus Inoculated intranasally and Distemperoid Virus Inoculated Intramuscularly.
(Exp. 1.)

Days after injection of distemper virus	Group 1 a 10 foxes inj. with virulent distemper virus (Controls) Sick	Deaths	Group 2-a 10 foxes inj. simultaneously with virulent distemper virus and distemperoid virus‡ Sick	Group 3-a 10 foxes inj. with distemperoid virus 3 days after virulent distemper virus‡ Sick	Group 4-a 9 foxes† inj. with distemperoid virus 12 days after virulent distemper virus‡ Sick
16	1/10*		2/10		
17	1/10		2/10		
18	1/10		2/10		
19	2/10		2/10		1/9
20	3/10		2/10		1/9
21	4/10		3/10		2/9
22	4/10		3/10		2/9
23	6/10		5/10	2/10	4/9
24	6/10		5/10	2/10	4/9
25	6/9	1	5/10	2/10	4/9
26	6/9		5/10	2/10	4/9
27	6/9		2/10	2/10	3/9
28	6/8	1	2/10	2/10	3/9
29	7/7	1	2/10	2/10	3/9
30	6/6	1	2/10	2/10	3/9
31	2/2	4	0/10	0/10	1/9
32	2/2				0/9
33	2/2				
34	2/2				
35	1/1	1			
36		1			

* The denominator indicates the number of foxes living, and the numerator the number sick on any given day.
† One fox in this group had a broken leg and was killed.
‡ No deaths.

The virulent virus was injected in a dosage of 100 mg and the distemperoid virus in a dosage of 200 mg. All animals were observed daily for symptoms of distemper. When deaths occurred, the disease was identified by the presence of cytoplasmic inclusions in the epithelial cells of the bladder or the trachea.

Interference following intranasal inoculation with virulent virus. Forty red fox pups were divided into groups of 10. Group 1-a, which received virulent distemper virus only, was inoculated intranasally. Animals in this group exhibited symptoms of distemper by the 16th day, and all died of distemper between the 25th and 36th days. The other 3 groups received the same virus intranasally but were given distemperoid virus in addition. Group 2-a received simultaneous inoculations of the two viruses. Two foxes showed symptoms on the 16th day, and by the 23rd day 5 showed mild signs of the disease. All animals had recovered by the 31st day. Group 3-a was given the distemperoid virus 3 days after injection of the virulent virus. Only 2 animals in this group displayed symptoms, which were discernible on the 23rd day. Both were normal by the 31st day. Group 4-a was given distemperoid virus 12 days after receiving the virulent virus. Mild symptoms of distemper were shown by one animal in this group on the 19th day and by 4 animals on the 23rd day. All had recovered by the 32nd day. Details regarding the occurrence of symptoms, deaths, and recoveries are shown in Table I.

These results demonstrate a very decisive interference, or blocking effect, of distemperoid virus on the course of a virulent distemper infection. They show also that a virulent infection corresponding to the natural

TABLE II.
Results of Intramuscular Inoculations of Foxes with Virulent Distemper Virus and Distemperoid Virus.
(Exp. 2).

Days after inj. of virulent distemper virus	Group 1-b 10 foxes inj. with virulent distemper virus (Controls) Sick	Deaths	Group 2-b 10 foxes inj. with distemperoid virus 3 days before virulent distemper virus	Group 3-b 10 foxes inj. simultaneously with virulent distemper virus and distemperoid virus Sick	Deaths	Group 4-b 10 foxes inj. with distemperoid virus 3 days after virulent distemper virus Sick	Deaths
12			Normal				
13	1/10		"			1/10	
14	3/9	1	"			all	1
15	5/9		"			"	2
16	5/9		"			"	3
17	all	1	"			"	2
18	"	1	"	2/10			2
19	"		"	2/10			
20	"	1	"	2/10			
21	"	2	"	1/10	1		
22	"	1	"	0			
23	"	1	"	0			
24	"	1	"	0			
25			"	0			
26			"	0			
27			"	0			
28			"	0			
29			"	0			
30		1	"	0			

disease can be blocked off if distemperoid virus is injected during the incubation period or in the early stage of the generalized infection.

Interference following intramuscular injection of virulent virus. In our second experiment 40 young foxes, in groups of 10, were injected intramuscularly with virulent distemper virus. All 4 groups received the virulent distemper virus at the same time, and 3 of the groups were subsequently given intramuscular injections of distemperoid virus at varying intervals.

Group 1-b was injected with virulent distemper virus only. On the 13th day after the inoculations, the animals began to show symptoms. By the 17th day all the animals were sick, and at the end of 30 days all had died of typical distemper. Group 2-b received distemperoid virus 3 days before the inoculation of virulent distemper virus. These animals remained well, showing no symptoms whatsoever of distemper. Group 3-b was inoculated with virulent distemper virus and distemperoid virus simultaneously. Two animals exhibited symptoms on the 18th day after inoculation. One of them died on the 21st day, while the other recovered. The rest of the foxes in this group showed no symptoms of distemper. Group 4-b received distemperoid virus 3 days after the virulent distemper virus. All the animals showed symptoms by the 14th day, and all died of typical distemper by the 18th day. Details of the occurrence of symptoms, deaths, and recoveries are shown in Table II.

In the third experiment 40 young foxes were also used in groups of 10. The control group received only virulent distemper virus. The other 3 groups were inoculated intramuscularly, first with virulent distemper virus and then with distemperoid virus, which was given to the respective groups 3, 8, and 12 days later.

Group 1-c, the controls, began to show symptoms on the 14th day after the injection of virulent virus. All the animals died of distemper by the 29th day. Group 2-c, which received the distemperoid virus 3 days after the virulent virus, began to exhibit symptoms of distemper on the 12th day. By the 36th day 8 animals had died and 2 had re-

TABLE III.
Results of Intramuscular Inoculations of Foxes with Virulent Distemper Virus and Distemperoid Virus.
(Exp. 3.)

Days after inj. of distemper virus	Group 1-c 10 foxes inj. with virulent distemper virus (Controls) Sick	Deaths	Group 2-c 10 foxes inj. with distemperoid virus 3 days after virulent distemper virus Sick	Deaths	Group 3-c 10 foxes inj. with distemperoid virus 8 days after virulent distemper virus Sick	Deaths	Group 4-c 9 foxes* inj. with distemperoid virus 12 days after virulent distemper virus Sick	Deaths
12			4/10				2/9	
13			5/10				2/8	1
14	4/10		8/10		4/10		3/7	1
15	6/10		9/10		5/10		3/7	
16	all	2	all	3	5/10		3/7	
17	"		"		6/10		all	2
18	"		"		6/10		"	1
19	"	1	"		5/9	1	"	2
20	"	3	"	2	5/9		"	
21	"	1	"	1	4/7	2	"	
23	"		"		4/6	1	"	1
24	"		"		4/6			1
25	"	1	"		all	1		
26	"	1	"		"			
27	"		"		"	1		
28	"		"		"	1		
29		1	"		"			
30			"		"	1		
31			"	1	"	1		
36			0/2	1	0/1			
Total No. deaths		10		8		9		9

* One fox in this group had a broken leg and was killed.

covered. Group 3-c received the distemperoid virus 8 days after the virulent virus. Symptoms first appeared on the 14th day. Nine animals were dead by the 31st day, and 1 had recovered. Group 4-c, inoculated with the distemperoid virus 12 days after the inoculation of virulent virus, began to show symptoms on the 12th day. All animals in this group were dead by the 24th day. Details of the symptoms, deaths, and recoveries are shown in Table III.

The first experiment on the intramuscular injection of virulent virus demonstrated that the distemperoid virus completely protected foxes when it was inoculated before the virulent distemper virus and gave almost complete protection when the inoculations were simultaneous. Both of the experiments on intramuscular injections included a group inoculated with distemperoid virus 3 days after the virulent virus was given. In both cases the controls died within approximately the same length of time. Some differences were observed in the 3-day groups. In Experiment 2 all the animals died but in Experiment 3 only 8 of 10 animals died. The recovery of 2 animals appeared due to protection, since foxes seldom recover once they have exhibited symptoms of virulent distemper. In the second intramuscular experiment little or no protection was apparent in the 8-day group and no protection in the 12-day group. A comparison of the results obtained from intranasal and intramuscular inoculations of virulent distemper virus is shown in Table IV.

Discussion. Our results seem to show that when the virulent distemper virus is inoculated *intranasally,* an injection of the modified virus given at the same time or 3 to 12 days later, so interferes with, or blocks off, the virulent infection that severe symptoms do not develop and recovery is rapid and complete.

TABLE IV.
Comparison of Intramuscular and Intranasal Inoculations of Foxes with Virulent Distemper Virus in Interference Experiments.*

Inocula	40 foxes inj. intramuscularly			40 foxes inj. intramusc.			40 foxes inj. intranasally		
	No. injected	No. sick	No. dead	No. injected	No. sick	No. dead	No. injected	No. sick	No. dead
Virulent distemper virus only	10	10	10	10	10	10	10	10	10
Distemperoid virus 3 days before distemper virus	10	0	0						
Distemperoid virus and distemper virus simultaneously	10	2	1				10	5	0
Distemperoid virus 3 days after distemper virus	10	10	10	10	10	8	10	2	0
Distemperoid virus 8 days after distemper virus				10	10	9			
Distemperoid virus 12 days after distemper virus				9†	9	9	9†	4	0

* All distemperoid virus injected intramuscularly.
† One fox had a broken leg and was killed.

The results obtained in the case of *intramuscular* inoculation of the virulent virus are similar, provided the modified virus is given before, or even at the same time as, the virulent virus. A striking difference, however, is observed in the number of recoveries of treated foxes when the modified virus is given after intramuscular injection of virulent virus. In this case, little or no interference follows the injection of the modified virus.

It appears that protection occurred when the modified virus was given an opportunity to reach tissue cells ahead of the virulent virus. On the other hand, if the virulent virus was able to invade a majority of susceptible cells before the modified virus, no protection occurred. In the intramuscular inoculations the virulent virus, when given first, seems to have immediately entered the blood stream as free virus and to have been distributed rapidly to large numbers of tissue cells. However, in the intranasal inoculations, the virulent virus appears to have invaded the animal body slowly, perhaps principally through neighboring tissue cells. At least, it did not seem to have become free virus in the blood stream and to have invaded large numbers of tissue cells ahead of the later-injected modified virus. The mechanism appears to be a reciprocal cell-block. Either the modified virus will be blocked by the virulent virus, if the virulent virus has already invaded the susceptible cells; or the modified virus, if it gets to the tissue cells first, will block the effect of the virulent virus on those cells. All our experimental results point to a mechanism of cell-blockade rather than to interference by one virus directly with the other.

Summary. A modified ferret-passage distemper virus exhibits an interference, or cell-blockade, phenomenon with respect to a virulent distemper virus in foxes. If the virulent virus is inoculated intranasally, as occurs in a natural infection, interference occurs when the modified virus is inoculated at the same time as, or after, the virulent virus. If the virulent virus is inoculated intramuscularly, the virulent infection can be blocked off by the modified virus only if the modified virus is given before, or at the same time as, the virulent virus. After an intramuscular injection of a virulent virus, any effect of the modified virus is in turn blocked off. The results appear to be determined by the virus that seeds the most tissue cells first. In the case of intranasal inoculation, the distemperoid virus seems to have a definite therapeutic effect during the incubation period and in the stage of early symptoms.