4. Bucher, O., *Schweiz. med. Wochschr.*, 1940, v70, 910.

5. Okado, H., *Folia Pharmacol. Japon.*, 1934, v18, 208; *Chem. Abstr.*, 1934, v28, 7276.

6. Sanjo, K., *Folia Pharmacol. Japon.*, 1934, v19, 151; *Chem. Abstr.*, 1935, v29, 3038.

7. Bräm, A., *Acta Anat.*, 1951, v13, 385.

8. Fell, H. B., Allsopp, C. B., *Brit. J. Exp. Path.*, 1946, v27, 305.

9. King, H., Ludford, R. J., *J. Chem. Soc. (London)*, 1950, v1950, 2086.

10. Perry, V. P., Evans, V. J., Earle, W. R., Hyatt, G. W., Bedell, W. C., *Am J. Hyg.*, 1956, v63, 52.

11. Earle, W. R., Highhouse, F., *J. Nat. Cancer Inst.*, 1954, v14, 841.

12. Frost, D. V., Spruth, H. C., in *Symposium on Medicated Feeds,* Med. Encyclopedia, Inc., New York, 1956, p136.

13. Healy, G. M., Fisher, D. C., Parker, R. C., *Canad. J. Biochem.*, 1954, v32, 319.

14. Webb, J. M., Levy, H. B., *J. Biol. Chem.*, 1955, v213, 107.

15. Kavanagh, F., in *Advances in Applied Microbiology,* W. W. Umbreit, Ed., Academic Press, New York, 1960, v2, p65.

16. Eagle, H., Doak, G. O., *Pharmacol. Rev.*, 1951, v3, 107.

17. Loy, H. W., Schiaffino, S. S., Savchuck, W. B., *Anal. Chem.*, 1961, v33, in press.

Received September 26, 1960. P.S.E.B.M., 1960, v105.

A Study of the Relationship Between Canine Distemper and Measles in the Dog.* (26171)

JAMES H. GILLESPIE AND DAVID T. KARZON†

Veterinary Virus Research Institute, Cornell University, Ithaca, and Dept. of Pediatrics and Dept. of Bacteriology and Immunology, University of Buffalo School of Medicine, Buffalo, N. Y.

Evidence has been presented for(1-3) and against(4,5) the suggestion that the viruses of human measles and canine distemper are antigenically related. This report describes the results of inoculation of dogs with live measles virus and subsequent challenge with virulent canine distemper virus. Despite the minimal canine distemper antibody response which followed the measles inoculation, all these dogs resisted challenge with distemper virus.

Materials and methods. The Edmonston strain of measles virus(6) was obtained from Dr. John F. Enders, after 28 transfers in human kidney tissue culture. Two pools of live measles virus were used. Pool 1 had been passaged 3 times in rhesus monkey kidney tissue culture and had an infectivity titer of $10^{4.0}$/0.1 ml in that system. Pool 2 was in the 6th tissue culture passage and titered $10^{4.5}$/0.1 ml. Control material consisted of uninoculated monkey kidney tissue culture fluid derived from the same batch of cells in which the measles virus was produced.

The Snyder Hill strain of distemper virus, maintained by serial intracerebral transfer in dogs, was previously reported(7) to produce convulsions and/or death in dogs with regularity, when given intracerebrally. This same strain, when administered as spleen suspension by the intravenous route, produced the classical disease described by Dunkin and Laidlaw(8).

The dogs used in these studies came from a purebred colony of beagles, reared and maintained in isolation by the Veterinary Virus Research Inst. The 19 dogs represented 6 different litters, ranging in age from 5 to 24 weeks at time of measles injection. Each litter was placed together in a separate isolation unit for observation a few days prior to inoculation.

Three groups of dogs were inoculated with measles virus or control tissue culture fluid, then challenged with distemper virus, the time and route of challenge varying with each group. Group A (Tables I and II) included 9 dogs, consisting of 3 separate experiments with litters of 3 dogs each. In Group A, 2 dogs of each litter were injected intravenously

* Supported in part by a grant from The National Foundation.

† Markle Scholar in Medical Science.

TABLE I. Protective Effect of Live Measles Virus Inoculation against Subsequent Canine Distemper Virus Challenge in Dogs.

Group	No. of dogs	Inoculation material	Clinical response	Distemper challenge Time (days)	Distemper challenge Route	Effects of challenge Fever	Convulsions	Died
A	6	Measles	None	24–28	Intrav.	0/6*	0/6	0/6
	3	Tissue culture control	"	24–28	"	3/3	0/3	1/3
B	2	Measles	"	84	"	0/2	0/2	0/2
	2	Tissue culture control	"	84	"	2/2	0/2	0/2
C	3	Measles	"	45	Intracer.	2/3†	0/3	0/3
	3	Tissue culture control	"	45	"	3/3	3/3	2/3

* Fraction = No. positive/No. tested. † Fever lasted only 2 days.

with 1 ml of live measles virus. The remaining dog was injected intravenously with 1 ml of control tissue culture fluid. Twenty-four to 28 days after injection, each dog was injected intravenously with 1 ml of a 10% suspension of spleen containing Snyder Hill virus strain. Group B consisted of 2 dogs inoculated with measles virus and 2 with tissue culture control material. The distemper virus challenge was delayed until 84 days after inoculation. In Group C, 2 litters of 4 and 2, respectively, were used as split litters kept together in the same unit. Three dogs were injected intravenously with 1 ml of measles virus. The other 3 were injected intravenously with 1 ml of control tissue culture fluid. Forty-five days later, each dog was injected intracerebrally with 0.25 ml of a 20% brain suspension of Snyder Hill virus.

In all experiments, the dogs inoculated with tissue culture control material were maintained in the same isolation unit as their litter mates inoculated with measles virus. Dogs in Groups A and B were given measles virus pool 1, and those in Group C were given pool 2. Serum was obtained from the dogs prior to each injection and 28 days after challenge with distemper virus, if still living. Temperatures were recorded and animals observed for signs of illness daily. The abdomen was shaved on some of the dogs, so that a rash could be detected more readily.

The egg-adapted Onderstepoort strain of distemper virus was used in the distemper neutralization test. After inactivation at 56°C for 15 minutes, each serum was diluted in phosphate buffered saline, pH 7.2. Each serum dilution was mixed with an equal volume of virus that contained 300 EID_{50} of Onderstepoort virus. The mixtures were incubated at 6°C for 2 hours. A group of 5 fertile eggs that had been incubated for 7-8 days was inoculated with 0.2 ml of the mixture on the chorioallantoic membrane, by the method of Gorham(9). The eggs were examined for lesions after further incubation of 6-7 days.

In the measles neutralization test, approximately 100 $TCID_{50}$ of measles virus was incubated with serum dilutions at room temperature for 1 hour. The mixture was inoculated into monkey kidney tissue culture and the endpoint read after 7 days' incubation. Measles complement-fixation tests were performed using untreated virus grown in monkey kidney tissue culture as antigen.

Results. All dogs given measles virus intravenously remained well and developed no rash. Twenty-four to 28 days after measles injection, the measles neutralizing titers of 6 dogs in Group A ranged from 64 to greater than 256 (Table II). Despite the substantial measles neutralizing antibody response, there was a minimal distemper response. The distemper neutralizing titers of 4 dogs were less than 2.5 and the other 2 had titers of 5 and 7, respectively. The measles neutralizing titers of 2 dogs in Group B, 84 days after measles injection, were 64 and 128, while distemper titers were 5 and 12. Measles comple-

TABLE II. Neutralization Response of Dogs to Measles Virus and Canine Distemper Virus.

Group	Inoculation material	Dog No.	Neutralization titers* — Measles: Pre-measles inoc.	Measles: Pre-distemper challenge	Measles: Post-challenge	Canine distemper: Pre-measles inoc.	Canine distemper: Pre-distemper challenge	Canine distemper: Post-challenge
A	Measles	753	<4	128	32	<2.5	<2.5	1,100
		754	<4	128	1024	<2.5	<2.5	10,000
		728	<4	>256	256	<2.5	<2.5	500
		731	<4	64	64	<2.5	<2.5	400
		771	<4	128	128	<2.5	7	300,000
		772	<4	128	128	<2.5	5	8,000
	Tissue culture control	752	<4	<4	trace	<2.5	<2.5	55
		729	<4	<4	<4	<2.5	<2.5	15,000
		770	<4	<4	died	<2.5	<2.5	died
B	Measles	768	<4	128	>1024	<2.5	12	300,000
		769	<4	64	256	<2.5	5	4,000
	Tissue culture control	766	<4	<4	<4	<2.5	<2.5	30,000
		767	<4	<4	<4	<2.5	<2.5	300,000
C	Measles	786	<4	32	128	<2.5	28	3,000,000
		790	<4	N.D.	256	<2.5	28	300,000
		787	<4	128	32	<2.5	30	60
	Tissue culture control	788	<4	<4	died	<2.5	<2.5	died
		789	<4	<4	<4	<2.5	<2.5	100,000
		791	<4	N.D.	died	<2.5	<2.5	died

* Reciprocal of 50% serum neutralization endpoint. N.D. = not done.

ment-fixing antibody rise paralleled the neutralizing response in all cases. The 5 unit contact dogs in Groups A and B, given control tissue culture fluid, remained well. They had no demonstrable measles or distemper antibodies after 24 or 84 days.

When given Snyder Hill virus intravenously 24-84 days later, the 8 dogs inoculated with measles virus (Groups A and B) showed no signs of illness. Twenty-eight days later, their measles antibody titers ranged from 32 to greater than 1024, and distemper antibody titers ranged from 400 to 300,000. The 5 unit control dogs that were similarly injected showed typical signs of distemper infection, with a diphasic temperature curve and catarrhal signs, and 1 died. The 4 survivors had distemper antibody titers ranging from 55 to 300,000 twenty-eight days later. Dog No. 752 with the titer of 55 had a more severe clinical illness than the other survivors. Two months after challenge, when complete recovery had taken place, its serum titer was 2250. Injection of Snyder Hill virus into the unit control dogs produced no measles neutralizing antibodies, or only a trace.

The Group C dogs given measles virus developed measles neutralizing antibody titers of 32 and 128 and distemper neutralizing antibody titers of 28 to 30, on the 45th day. Following intracerebral challenge with Snyder Hill strain, 1 dog (No. 787) showed no signs of illness, whereas the other 2 dogs (Nos. 786 and 790) had a definite febrile reaction 2 days after inoculation, for 2 consecutive days, with no other observable signs. These dogs developed distemper antibody very rapidly and to high titer. Eight days after intracerebral inoculation, the distemper neutralizing antibody titers were 25,000 and 250,000, and at 28 days, 300,000 and 3,000,000, respectively. In contrast, the non-febrile dog had a neutralizing titer of 60 at the 8th and 28th days. The 3 Group C unit contact dogs had no neutralizing antibodies to distemper virus or measles virus at the time of intracerebral distemper challenge. These dogs developed characteristic central nervous system manifestations and 2 died at 7 and 8 days post-inoculation. The survivor had a distemper neutralizing titer of 7 at 8 days and 100,000 at 28 days after challenge.

Discussion. The challenge tests demonstrate that live measles virus given intravenously protects dogs against virulent distem-

per virus given 24-84 days later. Control dogs failed to develop neutralizing and complement-fixing antibody to measles virus and were presumably not infected, though they were maintained in close contact with dogs inoculated with measles virus. Furthermore, they became ill when challenged with distemper virus.

Our data support the concept that these 2 viruses have an antigenic relationship. Of the 11 dogs inoculated with measles virus, 7 developed low but definite distemper neutralizing titers, ranging from 5 to 30. However, the possibility that interference may be operative in the protection afforded by measles virus is difficult to eliminate entirely. The dependence of interference upon continued association of the interfering agent with the host cell(10) makes this unlikely, in view of the long period (84 days) over which the protection was demonstrable. The antibody response to live measles virus in the dog suggests that the virus replicates in that host. Following a single intravenous inoculation of measles virus, high titers of both neutralizing and complement-fixing antibodies are produced in the dog, quantitatively resembling the response to measles infection in man or monkey. It has been reported(11) that measles virus was propagated serially in puppies, producing mild illness with viremia, fever and rash. For these studies, virus obtained directly from blood and pharyngeal washings from children in the acute stage of measles was used, as compared to the tissue culture adapted Edmonston strain used here.

Studies by Warren *et al.*(3) agree closely with the present experiments. These authors demonstrated production of measles neutralizing and complement-fixing antibodies in dogs, following inoculation with live measles virus grown in human heart tissue culture. Despite the fact that relatively low levels of canine distemper virus neutralizing antibody resulted from measles immunization, a partial protection against intraperitoneal challenge with virulent Snyder Hill strain of distemper virus was observed by these authors. On the other hand, it was found that the ferret was resistant to infection by measles virus, as judged by failure to produce measles complement-fixing antibody in that host. Furthermore, inoculation of ferrets with live measles virus did not protect against distemper challenge. Cabasso *et al.*(5) similarly failed to protect ferrets against distemper by hyperimmunization with measles virus-adjuvant mixture.

The rapid and high titered canine distemper neutralizing antibody response following intracerebral challenge in 2 dogs of Group C (Nos. 786 and 790) may be interpreted as an anamnestic response. The dogs had brief febrile responses and developed titers of 25,000 and 250,000 on the 8th day following canine distemper virus administration. Such levels are usually not attained following primary infection until 3 to 4 weeks have elapsed. This brief febrile response and anamnestic reaction has been observed recently in distemper immunity studies in dogs given a series of 2 or 3 injections of inactivated distemper virus at two week intervals, followed by intravenous injection of virulent distemper virus 3 months later. The third dog in Group C (No. 787) had no illness and no increase in titer, suggesting that sufficient protection was present at time of intracerebral inoculation to interfere with production of illness and antibody increase. Similar results have been observed in studies of the immune mechanism in distemper(12,13), *i.e.*, a low antibody titer (20-80) may in some individuals result in an apparent complete resistance to distemper virus replication, and consequently no illness or increase in neutralizing antibody titer occurs.

These studies strengthen the case for an antigenic relationship and emphasize the need for virus challenge as well as serologic studies in establishing the immunological relationship of viruses.

Summary. Dogs inoculated with live measles virus readily developed neutralizing and complement-fixing antibodies to measles. The dogs showed no signs of illness and did not spread the infection to unit contact litter mates. Following measles inoculation, 7 of 11 dogs developed low titers of canine distemper neutralizing antibody. All measles-inoculated dogs, including those with no demonstrable canine distemper neutralizing an-

tibody, were protected against intravenous or intracerebral challenge with virulent canine distemper virus, while the contact controls, not receiving measles virus, became ill, and some died. The dogs which had received distemper virus alone developed distemper neutralizing antibody, but developed no measles neutralizing antibody.

1. Adams, J. M., Imagawa, D. T., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 240.
2. Carlstrom, G., *The Lancet*, 1957, v273, 344.
3. Warren, J., Nadel, M. K., Slater, E., Millian, S. J., *Am. J. Vet. Res.*, 1960, v21, 111.
4. Cabasso, V. J., Kiser, K. H., Stebbins, M. R., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 227.
5. Cabasso, V. J., Avampato, J. E., Kiser, K. H., Stebbins, M. R., *Bact. Proc.*, 1960, 106.
6. Enders, J. F., Peebles, T. C., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 277.
7. Gillespie, J. H., Rickard, C. G., *Am. J. Vet. Res.*, 1956, v17, 103.
8. Dunkin, G. W., Laidlaw, P. P., *J. Comp. Path. and Therap.*, 1926, v39, 213.
9. Gorham, J. R., *Am. J. Vet. Res.*, 1957, v18, 691.
10. Schlesinger, R. W., in *Viral and Rickettsial Infections of Man*, 3rd edition, J. B. Lippincott Co., 1959, 145.
11. Riasantzeva, N. E., *J. Microb., Epid. and Immunobiol.*, 1956, v27, 22.
12. Gillespie, J. H., *Cornell Vet.*, 1960, v50, 514.
13. Robson, D. S., Gillespie, J. H., Baker, J. A., *Cornell Vet.*, 1960, v50, 503.

Received September 29, 1960. P.S.E.B.M., 1960, v105.

The Effect of Niacinamide on Cerebral Circulation. (26172)

T. F. HUANG AND C. C. CHAO (Introduced by C. McC. Brooks)
Department of Physiology and Surgery, Medical College, National Taiwan University, Taipei, Taiwan

It has been reported previously(1,2) that niacinamide has no significant effect on cerebral blood flow when given in doses of 50 to 100 mg intravenously. Large doses of the drug (3-5 g, i.v.) have been shown to increase cardiac output in the dog to a remarkable degree(3) and it was thought that there might be an associated increase in cerebral blood flow. Consequently the effects of small and large doses of niacinamide on cerebral circulation were determined to test this hypothesis.

Method. Dogs were anesthetized by pentobarbital 40 mg/kg i.v. Cerebral blood flow was measured by the N_2O method described by Kety and Schmidt(4-6). The sagittal sinus was exposed and punctured by a needle (15 gauge) and connected by polyethylene tubing with a 3-way stopcock, a manometer (1 mm I.D., 45 cm height) and a heparinized sampling syringe(6,7).

As we had no ready made $N_2O(15\%)$-O_2 (21%)-$N_2(64\%)$ mixture we mixed O_2 and N_2O in the ratio of 60 to 40 by a flowmeter. The O_2 content of the mixture was analyzed by a Scholander gas analyzer(8) and it was found that the gases mixed successfully in the desired ratio.

Control blood samples were obtained 15 min. after puncture of the sinus. After another 15 min interval, the drug was injected intravenously in 20 ml of water and 5 to 15 min later blood samples were taken again. The samples were analyzed in a Van Slyke-Neill manometric apparatus by the method of Orcutt and Waters(9). Mean arterial blood pressure (MABP) readings were taken from the femoral artery. O_2 consumption of the brain ($CMRO_2$) was determined by multiplying arteriovenous O_2 difference and cerebral blood flow (CBF). The data were treated by t-test(10).

Results. The effect of large doses of niacinamide (350 mg/kg) on cerebral circulation in 7 dogs 5-15 min after injection is shown in Table I. CBF/100 g/min increased significantly from 39.4 $\pm$ 7.2 (mean $\pm$ s.e.) to 56.2 $\pm$ 12.0 ml ($P<.01$), and $CMRO_2$/100 g/min. from 2.71 $\pm$ 0.85 to 4.18 $\pm$ 1.47 ml ($P<.05$), while MABP decreased from 139.3 $\pm$ 22.3 to 95.0 $\pm$ 16.6 mm Hg ($P<.001$)