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Received May 3, 1954. P.S.E.B.M., 1954, v86.

Intradermal Test for Determining Resistance to Infectious Canine Hepatitis Virus.* (21043)

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Studies on infectious canine hepatitis virus (ICH) are limited, in part, because of a) the inability to use a convenient small laboratory animal and b) complement-fixation tests are not always a reliable index of past infection. Many resistant dogs may show a negative complement-fixation test. The costly and elaborate procedure for raising disease-free animals is impractical for most laboratories. Street dogs are barred from routine use because of natural infection and resulting immunity.

In the course of clinical and experimental studies with ICH virus, it was found that the intradermal injection of apparently normal dogs with lymphoid tissue from an infected animal caused a marked reaction in some cases. The relationship of this reaction to resistance to ICH virus was, therefore, investigated.

Materials and methods. Virus strains. Two strains of infectious canine hepatitis virus were used in the study. One strain, designated 9868, was obtained from Dr. H. R. Cox and the other, 21V33, from Dr. C. J. York. Both strains were passed through at least 2 serial passages in dogs before use. Inocula used were 30% suspensions of infective liver. Intravenous injections were used and dogs killed on the third post-inoculation day by an overdose of sodium pentabarbital. Several dogs were used for each passage and one chosen that demonstrated a sharp temperature rise and simultaneous clinical symptoms of the disease. The liver was harvested under sterile conditions and placed in a previously weighed Waring blendor flask. The flask and liver were weighed and the weight of the liver alone determined. Sufficient buffered saline (pH 7.2) was added to make a 30% suspension of liver and the mixture homogenized for 3 minutes. The homogenized material was distributed in 2 ml amounts to sterile ampoules, sealed, shell frozen and stored in a dry ice chamber. Prior to intravenous injection, an additional 1 ml of sterile saline was added to the thawed suspension. *Street dogs* obtained from a stock supply were used without regard to age, size or sex. The great variation in animals led to the assumption that this sampling would represent a cross section of an average urban canine population. One hundred and twenty-four dogs were used in the series. Dogs were maintained in community pens except for challenge experiments, when they were placed in individual cages. *Complement-fixation tests.* A liver antigen was used in all tests. This was prepared from the liver of a dog experimentally infected with strain 9868 (Lederle). The liver was homogenized with sufficient buffered saline to make a 30% suspension. The mixture was centrifuged at 2000 r.p.m. for 15 minutes. The supernatant was distributed to 1 liter round-bottom flasks and shell frozen in a dry ice-alcohol bath. After immediate thawing, the mixture was recentrifuged at 2000 r.p.m. for 30 minutes. The resulting supernatant was distributed in 50 ml amounts, formalized (0.4%) and shell frozen. Lyophilization was performed after storage for 24-72 hours at −20°C. The dried material was ground with a mortar and pestle and stored in brown bottles at 4°C. This material

* Aided in part by a grant from the Hendrick's Research Fund.

was resuspended at a ratio of 100 mg of dry material to 1 ml of saline immediately before use. Complement-titrations were done in the presence of antigen and antigen titrations were done against known positive sera. Approximately 2 units of antigen were then used in subsequent tests. Blood samples were obtained at the same time skin tests were performed, serum separated and stored at –20°C until the tests were done. Sera were diluted 1-4 and inactivated at 57°C for 30 minutes prior to use. Positive and negative serums were included in each test as controls. Titers of 1-8 and above were considered positive.

Skin test antigens. Cervical lymph nodes were used for the preparation of skin test antigens. Dogs in the acute phases of infectious hepatitis were killed. The submandibular nodes were removed and examined for evidence of lymphadenopathy. Only glands that were enlarged and edematous were used for antigen production. The node was stripped of fat, weighed and placed in a stainless steel blendor. Sufficient saline was added to make a 1:10 suspension. The mixture was homogenized for 5 minutes and the material allowed to stand for 5 minutes. The decanted fluid was used as an antigen after formalizing to 0.2%. One-tenth milliliter was injected intradermally as a test dose. Reactions were read 24 and 48 hours after injection. Definite raised indurated areas at the site of injection with or without surrounding erythema were considered positive and slight or absent indurations classified negative.

TABLE I. Comparison of Skin-Test Reactions and Complement-Fixation Reactions for Infectious Canine Hepatitis Antibodies.

No.	Skin reaction		C-F positive*
	Positive	Negative	
18	12	6	2/10
10	6	4	1/6
7	1	6	0/1
3	3	0	0/3
6	3	3	1/3
2	1	1	0/1
2	0	2	—
3	2	1	1/2
5	3	2	1/3
11	6	5	1/5
7	3	4	0/2
17	5	12	1/5
Total 91	45	46	8/41

* No. positive/No. showing positive skin reactions.

TABLE II. Challenge of 35 Dogs Showing Positive and Negative Skin Reactions with ICH Virus.

No.	Skin reaction	Method of challenge	Response (Positive/No. challenged)
16	Positive	I.V.*	1/16
12	Negative	I.V.	11/12
5	"	I.O.	5/5
2	Doubtful	I.V.	1/2

* I.V. = intravenous; I.O. = intraocular.

Results. A total of 91 animals were examined by the intradermal skin test. Eighty-two of these animals were also examined for complement-fixing antibodies. Of the dogs showing a negative skin test, all but one were negative in the complement-fixation test.

As seen in Table I, only a few animals showing positive skin reactions were also positive in the complement-fixation test. Eight of 41 dogs with positive skin tests or 19.5% had positive serologic reactions. Although the formation is not listed, dogs that had been experimentally infected with virulent virus developed complement-fixing antibodies.

Table II shows the results of challenge of both negative and positive reactors with virulent infectious canine hepatitis virus. Seventeen negative reactors were challenged. Of this group, one animal reacted doubtfully and 16 developed infectious hepatitis. In a group of 16 dogs with positive reactions, 14 were resistant to experimental infection. An additional animal with a doubtful reaction was positive to challenge and another was negative.

The effect of prophylactic doses of infectious canine hepatitis antiserum on the subsequent skin test is shown in Table III. Twelve animals were given serum before the skin test and 10 showed positive reactions. In 59 animals not receiving serum, only 28 were positive to the intradermal test. Passive immunization apparently results in rendering the animal sensitive to skin test antigen. The subsequent natural disease ratio is also shown. The only conclusion that can be assumed from the latter is that animals showing a positive ICH skin test reaction probably carry specific antibodies for other common diseases of the dog as leptospirosis, canine distemper and broncho

TABLE III. Relationship of Serum Prophylaxis to Infectious Canine Hepatitis Skin Test and Subsequent Incidence of Disease.

No.	Serum admin.	Skin test		Subsequent disease ratio	
		Positive	Negative	Pos. skin	Neg. skin
12	Before skin test	10	2	2/10	1/2
59	No serum	28	31	8/28	19/31

pneumonia. Incidence of these infections in negative contact animals indicates that exposure was common.

Discussion. Infectious canine hepatitis (ICH) is well known as a common disease of the dog and fox, but other species are not susceptible either naturally or experimentally (1-3). Basically, the agent is an interesting subject for virus study because of its resistance to adaptation to other species and high specificity for endothelial structures(4). Also, it is of growing importance as a natural pathogen of the canine(5).

There is insufficient evidence at present to consider an interdermal test as a possible routine diagnostic measure for infectious canine hepatitis. Extensive field trials will be necessary to evaluate its efficiency fully. It is probable in cases of question as regards prophylactic infectious canine hepatitis serum therapy, that all animals showing a negative or questionable reaction should be considered susceptible to the disease. Positive reactors in all probability are immune, providing recent serum prophylaxis has not been given.

The test may remove the necessity of challenging individuals in a litter to determine the immune status before using them for research on infectious hepatitis. The usual procedure presently followed is to challenge one member of a litter and assume the status of the remaining puppies according to the challenge result.

The present complement-fixation test is inadequate as a diagnostic procedure. Apparently, many naturally recovered animals carry insufficient titers for recognition by the test. Also, the complexity of the antigen preparation and lack of standardization lessens its value and effectiveness as a routine diagnostic test. The intradermal test, if as effective as preliminary study indicates, though not perfect, would far surpass the complement-fixation test as a routine in both experimental and clinical work. Future studies are contemplated in order to evaluate the anamnestic effect of the skin test on complement-fixing antibodies.

Summary. An intradermal test for detecting resistance to infectious canine hepatitis is described. Sixteen of 17 animals showing negative tests were susceptible to the disease. Fourteen of 16 positive reactions were resistant to experimental challenge. The test apparently has a much greater sensitivity than the complement-fixation test.

The technical assistance of Mr. Sam Martin in carrying out many details associated with this work is appreciated.

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Received April 21, 1954. P.S.E.B.M., 1954, v86.