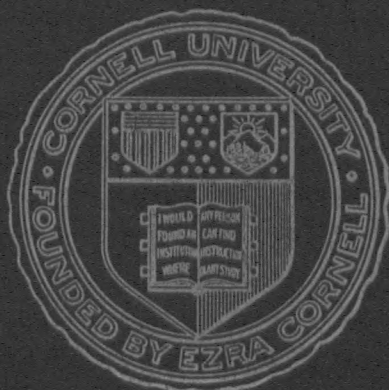


THE INSTITUTE REPORT



From the
VETERINARY VIRUS RESEARCH INSTITUTE
Cornell University, Ithaca, New York

September, 1967

Volume 17

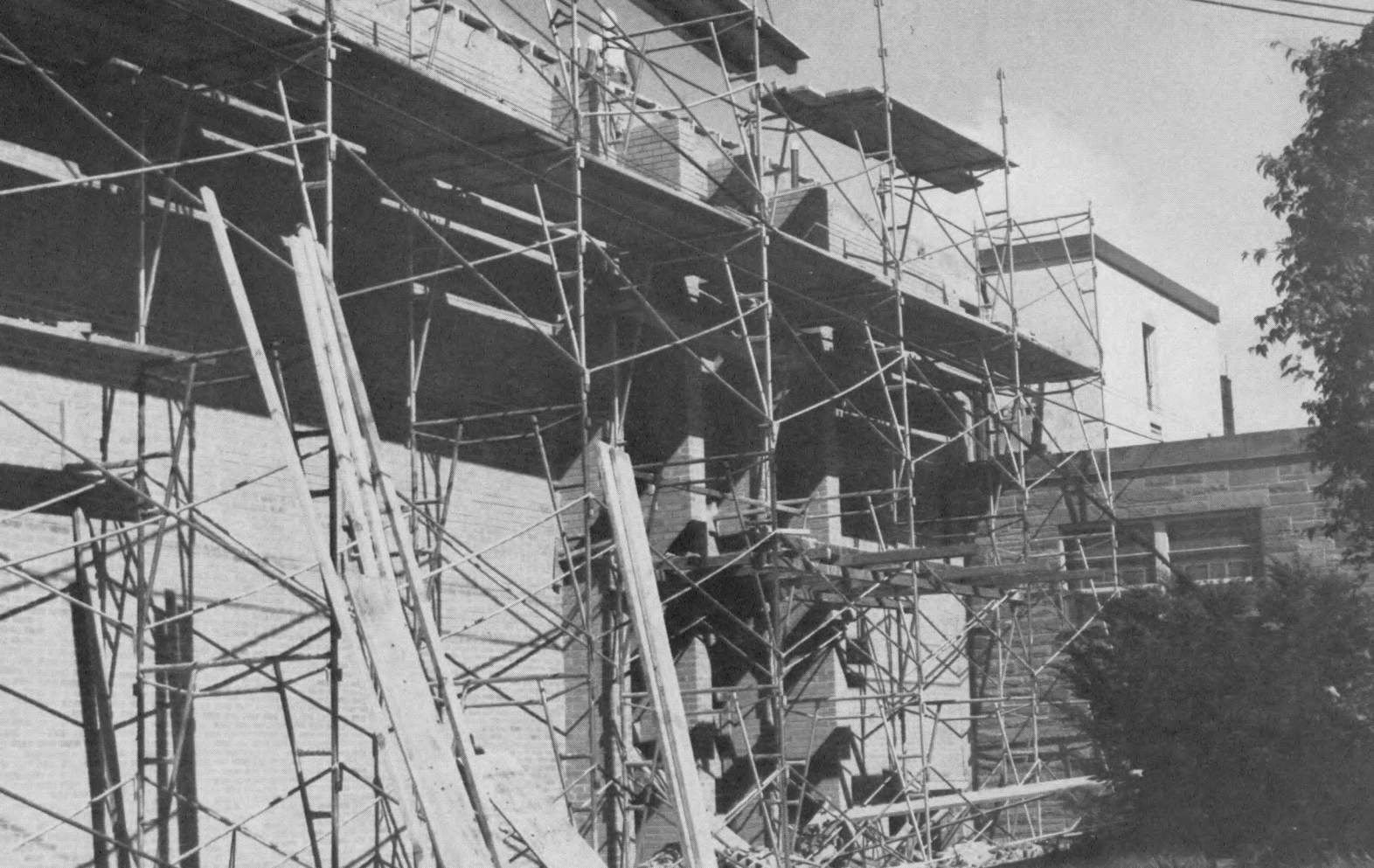
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REPORT OF THE DIRECTOR

TO THE FOUNDERS AND SUPPORTERS OF THE CORNELL
RESEARCH LABORATORY FOR DISEASES OF DOGS

AT the close of each fiscal year at the University, our annual report is prepared. It is written specifically for all of those persons whose interest and contributions make possible the research being carried out in the Laboratory. Their names are listed in the Acknowledgments section. Each one is sent a copy of this report as a means of expressing our appreciation.

Facilities

By November 1, 1967, a new wing of the Veterinary Virus Research Institute should be completed. If all goes well, work in its laboratories should begin before the New Year. The new building is being provided by funds given to Cornell University specifically for this purpose and by funds from the National Institutes of Health. The frontispiece shows the roof being put in place. Designer of the new building is Mr. James D. Parkes of the Cornell Planning Office, Mr. George N. Woikow is the architect, and Stewart and Bennett, Inc. are the contractors.

Increased space now makes possible addition of new members to the professional staff, better technical services, and convenient placing of much-needed new equipment.

With approval of Cornell University fiscal authorities, the Cornell Research Laboratory for Diseases of Dogs now has a budget of \$150,000 annually, an increase from the past \$120,000. This seems a very small amount indeed for dogs in a country that spends more than ten billion dollars for medical and biological sciences.

With better facilities made possible by the new building, we hope that increased biochemical research will aid in understanding the nature of dysplastic hips in dogs, as well as other problems. We hope also now to be able to investigate parasites of dogs, beginning with heartworm and coccidia, by application of new methods of research that have proven so successful with viruses and bacteria.

Additions to Staff

At the time the Cornell Research Laboratory for Diseases of Dogs was founded in 1950, all staff positions of necessity were temporary ones, and, as a consequence, we lost many good workers who trained here but were compelled to move elsewhere to positions with more security. Permanent positions on the University faculty must be approved by the Board of Trustees, and all such positions must have guaranteed funds available in advance in order to insure sufficient salary over the years. At the present time, this amounts to some \$300,000 for each position. Fortunately, we have been able to provide two such permanent positions for the Cornell Research Laboratory for Diseases of Dogs, the Caspary Professorship of Nutrition and the John M. Olin Professorship of Virology. Eventually, we hope that more professorships of this caliber can be made available.

Recently appointed as Assistant Professor of Virology, Dr. Max J. G. Appel received his Ph.D. degree in Virology from Cornell in January, 1967. His thesis problem concerned a detailed study of distemper, which will be described more fully later in this report. By the use of immunofluorescent techniques, for the first time understanding was obtained as to where the virus of distemper travelled in the body and why some dogs survived the disease while others died.

Appointed for September 1, 1967, as Assistant Professor of Parasitology, Dr. Helen Lee received her Ph.D. degree in Parasitology from Cornell in June, 1967. Her thesis problem concerned coccidiosis. For a long time veterinarians have suspected that interaction occurred between coccidia and infectious diseases, especially distemper. Such interaction could mean the death of dogs that otherwise might survive. Almost nothing is known at the present time about coccidiosis in dogs, although, because of their great economic value many studies have been made in poultry.

Not yet appointed, an Assistant Professor of Biochemistry also is being considered.

Research Findings

Pathogenesis of Canine Distemper Virus.

Distemper is an air-borne virus. The first exposure to this disease usually occurs if a dog inhales virus through the nasal passages and into the lungs.

The fluorescent antibody technique was used for sequential studies on spread and distribution of canine distemper virus. Virus did not grow originally in the lungs. Cells called macrophages, whose duty is to engulf inhaled noxious materials, take the virus to adjacent lymph nodes. Virus replication was found initially for between 6 and 9 days in lymphatic tissues. Mononuclear cells containing virus were found in connective tissue before virus was detected in epithelial cells. Later, epithelial surface structures, including glands, appeared highly infected.

Virus spreads by way of the blood stream to all parts of the body, especially back to the lungs, where it multiplies rapidly. During this period of great viral activity, the dog itself may appear perfectly normal, although an elevation of temperature occurs, as well as reduction of white blood cells. Classical signs of illness begin about 14 to 16 days after initial exposure.

Presence of virus, clinical symptoms, and mortality are related to time and level of antibody formation. If an individual dog is able to produce antibody by the 9th day, there is survival. If antibody cannot be produced until the 12th day, the dog usually dies. Many dogs that do survive have been so severely affected that their health is impaired for long afterward.

Studies on the Effect of Antilymphocyte Serum in Relation to Virus Persistence and Immune Tolerance.

Anti-dog-lymphocyte serum prepared in rabbits was studied for immunosuppressive action and effect of possible virus persistence in a system similar to immune tolerance.

Comparative Studies of Interferon Production in Tissue Cultures Induced by Virulent and Attenuated Viruses.

The plaque reduction method was used as an indicator system for interferon. Interferon was prepared from canine distemper virus infected tissue culture fluids and tested to determine whether attenuated canine distemper virus was a more potent interferon inducer than virulent virus.

A New Method for Demonstration and Assay of Virulent Distemper Virus.

Until this project began there was no way in which distemper virus could be demonstrated and measured in the laboratory. A new method of tissue culture was developed which now makes this possible. Ability to demonstrate and measure virulent distemper virus is necessary for understanding immunity to distemper and pathogenesis of distemper virus.

Genetics of Antibody Production.

Under the guidance of Professor F. B. Hutt, and with the cooperation of the Norwich Pharmacal Company, whose extensive Beagle colony is being utilized in this study, determinations are being made as to whether there may be a basis in genetics for ability of an individual dog rapidly and consistently to produce antibodies. The fact has become apparent that about one-third of dogs immunized against distemper seem unable to maintain a satisfactory titer from year to year. Not only do certain breeds seem to be poorer producers of antibody than others, but individuals within the breed also seem to vary in ability to produce antibodies. In preliminary studies, matings have been made to produce litters of puppies that may respond with a high titer of antibodies to vaccination with distemper, while others perhaps will produce low titers. Further analyses of the genetics of antibody production are being planned.

Heterotypic Virus Protection.

The natural protection given puppies by colostrum from immune mothers has been of importance in attempts to immunize puppies against disease because colostrum prevents successful vaccination. Distemper is so prevalent that nearly every puppy is exposed, and each day a puppy remains unprotected may mean life or death. Now at last, whether or not the mother is immune and no matter how high or how low her titer of antibodies may be, the newborn puppy can be protected temporarily against distemper by the use of heterotypic measles vaccine, always provided, of course, that the vaccine is properly made, shipped, stored, and administered to an otherwise healthy puppy that has not already been exposed. Heterotypic protection will last until the puppy is old enough to have lost all maternal antibodies and can commence having routine inoculations with distemper vaccine. Heterotypic measles vaccine for puppies is not designed to take the place of distemper vaccine, but rather to enable a puppy to survive until it can be immunized successfully with distemper vaccine.

We might repeat here, no vaccine that we have tested gives permanent immunity. For continued protection through life, there must be reinforcement from time to time by additional vaccinations, just as the Public Health Service demands smallpox vaccination within three years for all travellers entering the U. S.

Canine Brucellosis.

This recently-recognized infection of dogs, found thus far primarily in Beagles, was studied intensively during the past year. The causal bacterium, initially isolated and identified at the Cornell Research Laboratory for Diseases of Dogs, closely resembled other members of the *Genus Brucella*. *Brucella canis* was suggested as a name for the canine abortion agent, following a meeting of brucellosis research workers who had an opportunity to study the organism in detail.

The disease now has been identified in 34 states, including Alaska. Other breeds in addition to Beagles have been found infected, but, fortunately, *Brucella canis* does not seem to spread readily to human beings. Serological studies are made periodically of all laboratory personnel who come in close contact with the organism. For over a year no titer against the disease has been found in the persons studying it.

Common signs of infection in dogs have been abortion, sterility, and epididymitis. Bacteremia for periods exceeding 10 months appeared common. An agglutination test has been developed, evaluated, and now has been offered diagnostically in several laboratories. Studies have continued on the pathogenesis, pathology (in cooperation with the Departments of Large Animal Medicine, Obstetrics and Surgery, and Pathology), and possible development of prophylactic procedures. At the present time there is no adequate treatment or bacterin. Control consists of serological tests and the removal of infected animals.

Serology of Canine Herpesvirus.

Canine herpesvirus-antibody complexes have been found to be unstable. Kinetic studies of the neutralization reaction indicated that dissociation of virus-antibody complexes was suppressed by the addition of complement. A similar effect was achieved by addition of anti-canine globulin after the first stage of neutralization had occurred. Treatment of purified virus preparations with materials that damaged but did not destroy the outer envelope of the virus resulted in typical first-order kinetic neutralization curves. It thus appeared that the envelope principally was responsible for the "poor neutralizability" of this virus. Based on these findings, a neutralization test protocol was developed.

Canine herpesvirus does not infect man, but is similar to the herpesvirus of human beings, which is known to be able to persist in the cells and to recrudescence as fever blisters or cold sores, or, under some conditions, to grow in the eye. The virus is widespread in dogs, but whether or not it can persist has yet to be determined. It can occur in the genital tract of dogs.

Viral Pathogenesis and Chronic Effects of Persistence.

Studies on the factors that affect viral susceptibility and persistence have been continued. Canine herpesvirus was used as a model infection. Alterations in ambient temperature profoundly affected the growth of virus in tissues, and appeared to play a dominant role in determining the course of infectious processes. Elevated temperatures favored survival of neonatal pups, whereas a lower body temperature resulted in unrestricted viral growth and death. Normally resistant dogs older than two weeks of age seemed susceptible if their body temperature became lower. Puppies must be kept warm and not allowed to become chilled.

Picodnaviruses.

A new group of very small viruses is just beginning to be recognized by virologists. The first virus to be recognized in this group is that of feline panleucopenia, usually a fatal infectious disease of cats that may often reach epidemic proportions. Dr. Croghan, on assignment from the Agricultural Research Service, chose this interesting virus and has developed as his Ph.D. thesis much information concerning it. Ultrafiltration studies showed that the virus passed easily through 50 mu millipore filters. Genetron treatment was used to purify infected cell culture fluids which had been centrifuged at 100,000 X G for one hour. Electron microscopy showed particles of 20-25 mu size. What appeared to be a viral coat was removed by treatment with ribonuclease, and this allowed observation on the capsomere structure. The best model was a 12 capsomere icosahedron. From the fact that this was a small DNA virus, and all current isolates of this type and size have been associated with tumors, testing for oncogenicity has been started. Pathogenesis of the virus has been conducted by use of fluorescent antibody on frozen sections.

In rats and mice, similar viruses have been isolated from tumorous tissues. During the past year, while on sabbatical leave at the Institut für Mikrobiologie und Infektionskrankheiten der Tiere, Ludwig Maximilians

Universitat in Munich, Dr. Sheffy found a similar organism closely associated with hog cholera virus. Now that we have developed background information on this group of viruses, surveys of dogs with tumors will be made.

Miscellaneous.

In addition to the preceding studies briefly described, numerous preliminary studies are made and the development of various laboratory tests constantly are underway. Serological tests, studies of blood and antibody for various viruses, bacteriological methods, and other procedures are always subject to improvement. Change in temperatures during tests, salt and pH balances, and similar techniques are considered constantly. Routes to administer each vaccine for maximum results are combined with other studies, and immunization is studied and evaluated continuously.

IN APPRECIATION

We are appreciative of all supporters whose interest and financial help make possible the research program of the Cornell Research Laboratory for Diseases of Dogs. In addition, we would like to thank the following for their generous contributions which made possible the new building addition: Mr. Richard King Mellon, Mr. John M. Olin, Mr. and Mrs. Spencer T. Olin, Mr. and Mrs. A. Wells Peck.

Also we are grateful to those numerous individual dog owners and veterinarians, 399 in number, who have made special gifts towards equipping the new addition to the laboratory. Space has not permitted a special listing of their names.

We are also grateful to those individual dog owners who have made special gifts towards equipping the new addition: Mr. George A. Anderson, Mr. and Mrs. Gaylord Donnelley, Mr. and Mrs. E. Roland Harriman, Mr. Carl Holmes, Mrs. William S. Ladd, Dr. Samuel Nesbit, Miss Joan Pierce, Mr. Edgar M. Queeny, Mrs. Marion duPont Scott, Mr. and Mrs. Bruce Smallridge, Mrs. Esther duPont Thouron, and Mr. Robert Winthrop.

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COOPERATING STAFF

(These doctors of veterinary medicine, physicians and scientists in various parts of the United States are contributing much time and effort, without compensation, in order to help secure necessary information on which to base programs for control of infectious diseases.)

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PUBLICATIONS FROM THE VETERINARY VIRUS
RESEARCH INSTITUTE

Publications for the first ten years were listed in the Institute Report for 1960. Those for each following year were listed in its annual report. Papers published during the past year include the following:

- (199) APPEL, MAX J. G.: Studies on the Pathogenesis of Canine Distemper. Thesis, Cornell University, 1967.
- (200) _____: Jones, O. R.: Use of Alveolar Macrophages for Cultivation of Canine Distemper Virus. Submitted to Proc. Soc. Expt. Biol. & Med., July, 1967.
- (201) BAKER, JAMES A.: Possibilities for Prevention of Disease in the Future. Submitted to the Amer. Jour. Vet. Res., July, 1967.
- (202) CARMICHAEL, L. E.: Infectious Canine Abortions. Hounds and Hunting, 1967, 64, 14-18.
- (203) _____: Characteristics of a Newly Recognized Brucella Species Responsible for Canine Abortions. To be submitted to Cornell Vet., 1967.
- (204) _____: Kenney, R. M.: Infectious Canine Abortions Caused by a Newly Recognized Species of *Brucella*. To be submitted to Path. Vet., 1967.
- (205) CROGHAN, D. L.: Present Standard of Biologics for Canines. 16th Gaines Veterinary Symposium, October, 1966, 33-36.
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- (207) HUTT, FREDERICK B.: Genetic Selection to Reduce the Incidence of Hip Dysplasia in Dogs. Submitted to the Amer. Jour. Vet. Res., June, 1967.
- (208) KAHRS, R. F.: Determination of a 95 Percent Antigenic Dose for Bovine Virus Diarrhea Vaccine. Amer. Jour. Vet. Res., 1966, 27, 1551-1554.
- (209) _____: Robson, D. S., and Baker, J. A.: Epidemiological Considerations for the Control of Bovine Virus Diarrhea. Proc. U. S. Livestock Sanit. Assoc., 1966, 70, 145-153.
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- (211) SHEFFY, B. E.: Mayr, A., Bachmann, P. A., and Siegl, G.: Electron Optical and Buoyant Density Studies of Hog Cholera Virus. Arch. ges. Virusforsch., July, 1967.
- (212) VOLENEC, F. J.: Sheffy, B. E., and Baker, J. A.: Heterotypic Hog Cholera Protection in Swine: An analysis of the Response. Proc. U. S. Livestock Sanit. Assoc., 1966, 70, 295-301.

ACKNOWLEDGMENTS

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To JOHN C. NEFF in sincere appreciation of your foresight in helping establish the Distemper Evaluation Laboratory as a unit in the Cornell Research Laboratory for Diseases of Dogs.

From the time of its founding your continuous support of the Laboratory and interest in its work have been of the greatest value to the advancement of research. Presented this 24th day of August, 1966.



James A. Baker

*Director
Cornell Research Laboratory
for Diseases of Dogs*



Dr. James A. Baker, left, Director of the Veterinary Virus Research Institute, presents on the behalf of Cornell University, a certificate of appreciation to Mr. John C. Neff at a dinner given in his honor.

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Individuals	62%
Companies	18%
Dog Clubs (Including AKC)	11%
Veterinarians	9%

Perhaps there should be some discussion in the various dog clubs and breed clubs as to whether or not their own dogs have ever benefitted personally from the knowledge made possible by work in the Cornell Research Laboratory for Diseases of Dogs.

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