CDC RABIES CONTROL STUDIES

Ernest S. Tierkel

Rabies control studies are receiving special emphasis on the program of the Veterinary Division recently transferred to CDC. (CDC Bulletin, Oct., Nov., Dec. 1947, page 31.) Work in the rabies laboratory is designed to make a critical evaluation of laboratory methods used in the diagnosis of rabies. Experiments are being conducted on the efficiency, duration, and dosage of all types of canine rabies vaccines. Studies are being made in the field on methods of immunization, other methods of control, reporting of the disease and statistical evaluation. Efforts are made to disseminate educational material to professional and non-professional groups.

Rabies is one of the important diseases of animals that can be transmitted to man. The number of reported cases has increased alarmingly in the last several years. Work in the rabies laboratory is directed toward ultimate eradication of rabies from the United States.

STUDIES ON METHODS OF LABORATORY DIAGNOSIS

Adequate diagnostic methods are a basic prerequisite to public health attack against any disease. Rabies may be demonstrated in the laboratory by observing Negri bodies (virus inclusion bodies) in brain tissue or by producing the disease in experimental animals by injecting infected material. Efforts are being made to evaluate procedures in common use in various laboratories.

This article is abridged from a paper entitled "Inauguration of Rabies Control Studies by the U. S. Public Health Service" presented before the Section on Sanitary Science of the American Veterinary Medical Association at the 84th annual convention, August 18 - 21, 1947, at Cincinnati, Ohio.

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DIRECT MICROSCOPICAL EXAMINATION OF SUSPECTED MATERIAL

Three methods are recognized for preparation of brain tissue to be stained and examined microscopically, 1) the touch-impression method, 2) the rolling technique, 3) the spread-smear method.

In the touch-impression method, small cut sections of Ammon's horn, cerebral cortex, and cerebellum are placed on clean blotting paper. The slide is touched against the cut surface of the section and pressed gently downward with just enough pressure to create a slight spread of the exposed surface of the tissue against the slide. Depending on size of the section, three to four impressions can be made on one slide. Preliminary observations indicated that this method is satisfactory. A maximum amount of nerve tissue is concentrated in a small area and the nerve cell and glial structure are preserved. Since most of the histological structure is intact, the chance of finding Negri bodies is good. This technique is preferred for research work where detailed observations must be made.

The rolling technique consists of cutting a piece of brain tissue about the size of a fresh garden pea and rolling it gently over the entire surface of one side of the slide with a toothpick or wooden applicator. Here there is very little damage to nerve cell structure and a wide area of the slide is covered. The concentration of tissue material is rather sparse, however, and there is greater possibility of missing Negri bodies when only few are present.

When the spread-smear technique is employed a small section of brain tissue is placed on one end of the slide and the section of tissue is crushed with another slide. Then the tissue is drawn across the length of the first slide with the slide used for crushing. This results in a fairly homogeneous spread of tissue in a thin film, covering about three-quarters of the slide. There is sometimes a copious concentration of tissue if the section used is too large. In this preparation, an extensive area must be examined and there is often damage to the neurone and glial structure. In spite of these objections, this is the method of choice in most diagnostic laboratories.

Various stains are being used in attempting to find the best for differentiating Negri bodies. Stains to be tested or proposed for evaluation include Sellers' differential stain, Johnson's modification of Sellers', Williams' basic fuchsin-methylene blue, Mann's methyl blue-eosin, Mallory's tissue stain, Wolbach's modification of Giemsa, eosin-methylene blue, hematoxylin and eosin, and others. Tissue fixatives include air dried fixation, methyl alcohol, acetic-Zenker's solution, and formalin.

Only a few staining techniques have been tried thus far. Of those tested, Sellers' stain is the most rapid and easily handled. Methyl alcohol is the stain solvent. This enables fixing and staining to be done in one operation. With Sellers' stain Negri bodies are observed in a magenta or heliotrope to pink-red color with dark blue to black basophilic inner granules. All parts of the nerve cell stain blue and the glial structure and interstitial tissue stain pink.

DIAGNOSIS OF RABIES BY ANIMAL INOCULATION

Negri bodies cannot always be demonstrated in brains of animals dying of rabies. Hence it is important to do animal inoculations with Negri-negative brains. In several laboratories it has been found that 10 to 12 percent of brains shown to be positive by mouse inoculation were missed by direct smear microscopical examination.

It is strongly recommended that laboratories furnishing rabies diagnostic services be equipped to do animal inoculations of Negri-negative brain tissues. The procedure is simple and inexpensive. The animal of choice is the white mouse. These animals are inexpensive and easily handled. Intracerebral inoculation of a suspension of infected brain material will produce typical and constant symptoms after an incubation period varying from five to eleven days. Production of Negri bodies is consistent.

The test is performed by injecting 0.03 ml. of a saline suspension of emulsified

1 BRAIN FROM A SUSPECTED RABID ANIMAL.

2 THE HEMISPHERE IS OPENED WITH A LONGITUDINAL CUT, EXPOSING AMMON'S HORN.

A LATERAL SECTION IS CUT FROM AMMON'S HORN, PLACED ON SLIDE.

3

THE TISSUE IS SPREAD WITH ANOTHER SLIDE.

4

5 AFTER DRYING, THE FILM IS STAINED BY IMMERSING MOMENTARILY IN SELLERS' STAIN.

6 EXCESS STAIN IS REMOVED BY WASHING SLIDE IN RUNNING WATER. WHEN DRY, THE FILM IS READY FOR MICROSCOPICAL EXAMINATION FOR NEGRI BODIES.

SMEAR SPREAD TECHNIQUE

FOR PREPARING FILMS OF DOG BRAINS

Rabies is perpetuated by transmission from dog to dog. Man is an accidental host.

pooled material of brain tissue from Ammon's horn, cerebral cortex, and cerebellum into each of three white mice. Daily observations of mice are made for 14 days and symptoms recorded. Microscopical examination should be made for Negri bodies on each mouse that dies. If there are no deaths at the end of 14 days, the mice should be sacrificed and an attempt made to demonstrate Negri bodies in the brains.

Techniques of the test are illustrated in the "Idea Exchange" of this issue on pages 30 and 31.

Experiments are being conducted in an effort to find a bactericidal agent, which will not affect rabies virus, against contaminating bacteria in decomposed brains. This is necessary since mouse inoculation tests cannot be performed with contaminated material until bacteria are inactivated.

OTHER DIAGNOSTIC METHODS

Efforts to devise other means of diagnosing rabies have proved unsatisfactory. The histopathological picture of rabies infections, such as inflammatory and degenerative changes in the brain and nervous tissue, is not sufficiently specific to be of practical diagnostic value. Serologic tests have also been unsatisfactory, although specific complement-fixing antibodies have been demonstrated in rabid animals. Apparently they are not present in sufficient quantities for the application of a dependable complement-fixation test.

STUDIES ON CANINE RABIES VACCINE

Studies are underway to investigate the potency of various prophylactic canine rabies vaccines. Specific objectives are (1) to determine the duration of immunity conferred by the available commercial phenolized vaccines, (2) to refine the system of dosage with these vaccines in order to obtain optimum immunity response, (3) to test the safety and relative antigenicity of newly developed live-virus vaccines, such as egg embryo vaccines, and (4) to test the efficacy of new experimental killed-virus vaccines in which the virus has been inactivated with chemical substances or ultra violet irradiation.

In connection with these studies, different strains of fixed and street virus must be standardized to determine their value for use as challenge viruses and their adaptability for use as vaccines following treatment.

VIRUS-SERUM NEUTRALIZATION TESTS

One of the most important aids in vaccine potency experiments is the serum-neutralization test. In this test a standard fixed virus of known infectivity titre is mixed with the serum of an animal. After varying periods of incubation and refrigeration the mixture is inoculated into mice. In this way alteration in infectivity of the virus caused by presence of virus neutralizing substance in the serum is determined. Since the titre, dilution, and dosage of the virus is known, the minimum lethal dose (MLD) of the virus can be calculated. For example, if a given mixture of 100 minimum lethal doses of rabies virus and unknown serum is inoculated intracerebrally into four mice and a rabies mortality of four deaths out of four mice results, it may be assumed that the serum tested shows no evidence of presence of virus-neutralizing substance against 100 MLDs of rabies virus. If a similar test is made using the serum of another animal, and there is no mortality in the four mice, it is evident that a sufficient amount of neutralizing antibodies was present in the serum of this animal to offer complete protection against 100 MLDs of fixed rabies virus. Intermediate results are interpreted as gradations in the amount of virus neutralizing substance present in the blood of the animal tested.

The neutralization test was of inestimable value in the selection of animals for vaccine potency experiments. It was necessary to use animals which were comparable with regard to susceptibility to infection and immunity response to vaccination. Animals were first selected which were similar with regard to their neutralizing antibody reactions; they were then subgrouped to be as comparable as possible with regard to age.

Effects of various types of experimental vaccines in producing virus neutralizing substance in the sera of the dogs is being studied. Neutralization tests are being conducted at 20-day intervals after vaccination before the challenge inoculation of rabies virus is made. Preliminary results indicate that neutralizing antibodies, against 100 MLDs of virus, were present in all of the dogs 20 days after vaccination. Ninety-six percent were completely protected, 2 percent were protected significantly but not completely, and only 2 percent were protected but slightly. Eighty-two percent of blood specimens obtained before vaccination contained little or no protecting neutralizing substance. Subsequent studies will be designed to compare various vaccines as to quantity and dufation of neutralizing antibodies.

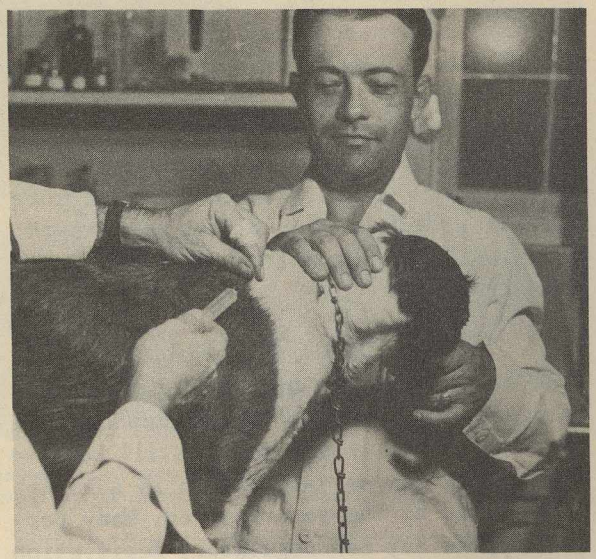
It has not been proved definitely that presence of rabies virus neutralizing substance in the blood-serum is an indication of true immunity against infection. The virus-serum neutralization test is, however, the most satisfactory means of determining relative susceptibility to infection and of measuring immune response to vaccination.

EPIDEMIOLOGIC STUDIES

Epidemiologic studies on rabies incidence and control during 1946 are being conducted in Alabama, with the cooperation of the Alabama State Department of Health. The items being considered are:

1. Number of animal rabies cases reported
2. Number of human rabies cases reported
3. Number of canine prophylactic vaccinations given
4. Number of human vaccine treatments administered
5. Number of stray dogs impounded
6. Number of stray dogs destroyed
7. Facilities for collecting and impounding of strays
8. Estimated dog population of county
9. Facilities for laboratory diagnosis of rabies

When accumulation of information is completed, a critical evaluation of existing control programs will be made. It is hoped that intensive study of present programs will provide information upon which recommendations for improvement can be made. When plans for operation are



Local veterinarians can supply effective vaccine against canine rabies.

interested, influential, and respected citizens of the community. The ideal Rabies Advisory Committee includes the chairman of the local board of health, a veterinarian, a representative of the local kennel and sportsman's clubs, one or more dog owners, a local judge, and officers of various civic organizations. A committee of this type should hold meetings, gather latest information on rabies control, formulate educational campaigns, and disseminate the result of their findings to the public. By the prestige of the members, confidence of the citizens of the community is obtained.

TRAINING AID

To assist in meeting the need for educational material on rabies control an educational filmstrip on this subject has been produced in cooperation with the Production Division of CDC. The film has been reviewed in this Bulletin. (CDC Bulletin – July, August, September 1947, p. 34-35).

NATIONAL RABIES PROGRAMS

PHILADELPHIA CONFERENCE ON RABIES

Because of its nation-wide importance, a conference on rabies was held at Philadelphia on April 9, 1947. The purpose of the conference was to bring together for discussion on a country-wide basis, all national agencies concerned with the problem. Agencies represented were the American Medical Association, American Public Health Association, American Veterinary Medical Association, American Animal Hospital Association, Bureau of Animal Industry of the U. S. Department of Agriculture, and U. S. Public Health Service. Three major points were emphasized by the conference:

(1) Rabies in the United States is of sufficient national importance to make desirable the participation of the federal government in coordinating efforts of states in control programs.

(2) Rabies in animals should be made a reportable disease. Information obtained should be analyzed properly and distributed to all concerned.

(3) In order that control be facilitated, attention must be directed toward the following:

1) Provision of adequate diagnostic facilities.

2) Control of animals capable of transmitting rabies.

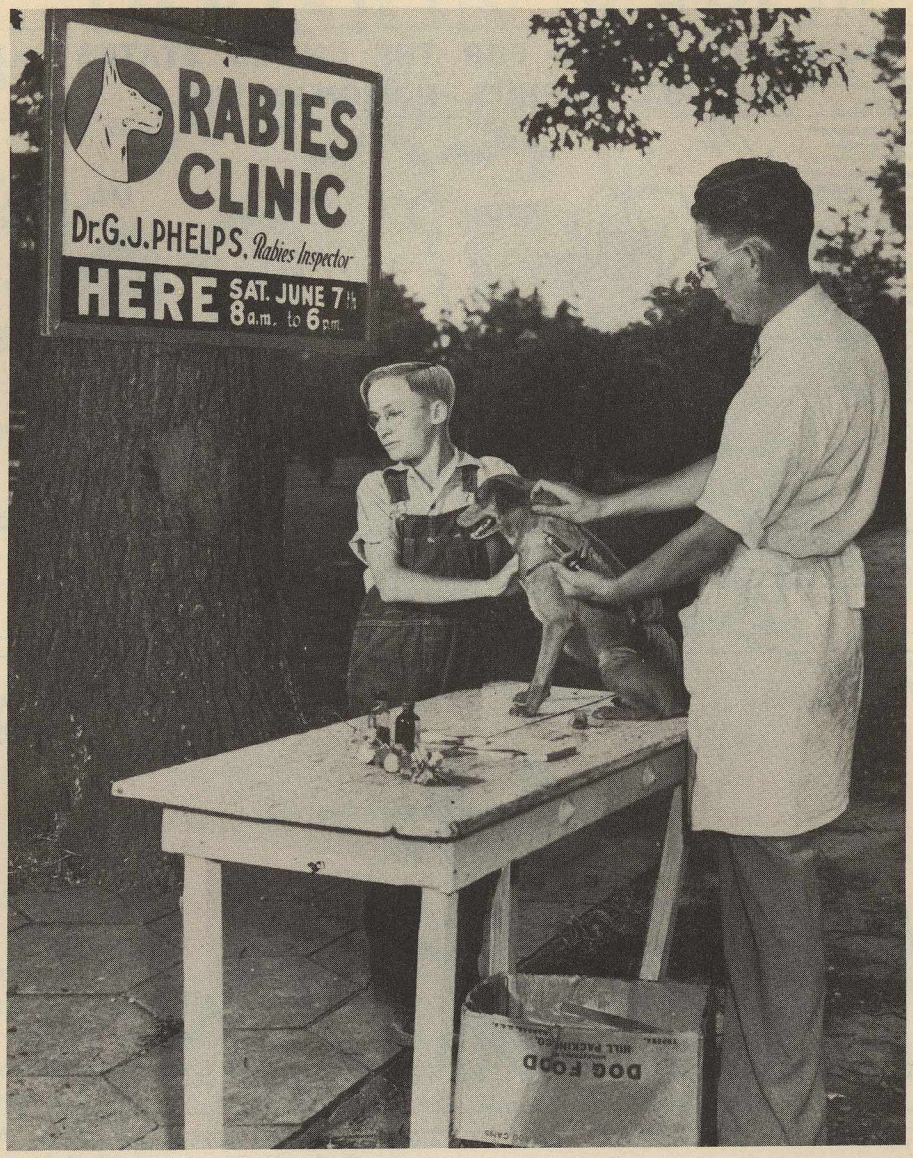
3) Mass immunization of susceptible animals, particularly dogs.

Recommendations of the conference were submitted for appropriate action to the official representatives of the agencies in attendance.

PROPOSAL FOR A FEDERAL RABIES CONTROL COMMISSION

Many communities have very effective rabies control programs; in many instances neighboring communities have weak programs. Often epidemics recur in rabies-free areas. In some cases, states operate a type of control program which conflicts with that in neighboring states. Since rabies cannot be confined by political boundaries, effective control can be done only on an interstate basis with a properly authorized national agency assuming responsibility of coordination.

It is hoped that the U.S.P.H.S. can aid in formulating a Federal Rabies Control Commission, composed of representatives



Dog owners cooperate "willingly" with dog vaccination campaigns.

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of the Service of the U. S. Bureau of Animal Industry, and of the U. S. Fish and Wildlife Service. Such a commission would insure uniform practices of rabies control based on proven scientific information. The commission would assist specifically in several important ways:

(1) In the distribution of the latest accepted diagnostic procedures to the States.

(2) By instituting an accurate system of reporting.

(3) By keeping local control authorities informed of the most effective immunization techniques.

(4) By formulating standard licensing and dog control ordinances.

(5) By preparing and distributing educational material.