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EXPERT COMMITTEE ON
RABIES

Fourth Report

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EXPERT COMMITTEE ON RABIES

14-19 December 1959

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EXPERT COMMITTEE ON RABIES

Fourth Report

1. INTRODUCTION

The Expert Committee on Rabies met in Geneva from 14 to 19 December 1959. Dr N. Veeraraghavan was elected Chairman, Dr H. Koprowski, Vice-Chairman, and Dr K. Habel, Rapporteur.

Considerable research has been carried out since the meeting of the Expert Committee on Rabies in 1956, much of it along the lines recommended in the third report.¹ Collaborative research efforts of members of the WHO Expert Panel on Rabies were continued and results of these efforts will be considered later in this report (section 6.5). The new knowledge in rabies acquired as a result of research efforts during the past three years (summarized in section 2) necessitated a careful re-appraisal and modification of recommendations made in the third report.

The Committee decided to prepare its report as a self-contained document which would minimize the need to cross-refer to previous reports. It therefore took the liberty of incorporating verbatim into the present report parts of the first three reports,² and made modifications of previous recommendations where, in the opinion of the Committee, newer knowledge warranted such modifications. Since a majority of the members of the present Committee served on the previous three Committees, it was not felt necessary to indicate editorially in the present report verbatim incorporations or modifications of previous reports. The Committee, however, wishes to acknowledge its deep appreciation to all members of the Expert Panel on Rabies and other rabies research workers and control officials for their collaboration, which has greatly simplified the task of preparing the present report.

2. SUMMARY OF RECENT ADVANCES

2.1 Vaccines

(a) An inactivated virus vaccine prepared from infected duck embryo has been developed which is almost free of the factor responsible for post-vaccine allergic encephalitis (section 6.1).

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1957, **121**

² *Wld Hlth Org. techn. Rep. Ser.*, 1950, **28**; 1954, **82**; 1957, **121**

(b) It has been demonstrated that phenolized as well as irradiated vaccine may be freeze-dried with preservation of its antigenicity and increased stability on storage (section 4.1 and Annex 3).

(c) A new batch of international reference vaccine has been made available (section 4.2.3).

2.2 Antirabies serum

(a) Methods of production and concentration of antibody have been improved (section 5).

(b) An international standard serum has been established.¹

2.3 Diagnosis

A highly specific, rapid method of diagnosis has become available with the successful application of the fluorescent antibody technique to rabies (section 3.2).

2.4 Prevention of rabies in man

(a) Additional field experience has confirmed the fact that combined serum and vaccine treatment gives the best results in post-exposure treatment (section 6.5).

(b) Amended schedules of post-exposure treatment have been developed to reduce the interference between serum and vaccine in the combined treatment (sections 6.3 and 6.5).

(c) The demonstration of efficiency of the antibody response to booster doses of vaccine has led to the development of a recommended procedure for pre-exposure immunization in special population groups (sections 6.4 and 6.5).

2.5 Pathogenesis

Studies of susceptibility and salivary gland tropism of rabies virus in certain wild vectors have opened the way to explaining some aspects of the ecology of the disease (section 7.8).

2.6 Tissue culture of virus

Preliminary results of growth of rabies virus in several tissue culture systems offer promising possibilities for future use of this technique in basic quantitative studies of the virus, in diagnosis, and even as a possible source of virus for vaccine production.

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1956, 108, 11.

2.7 Results of WHO questionnaire

More clear-cut information concerning the present world situation in rabies is now possible and there is evidence of progress in the application of modern methods (section 8.1).

3. DIAGNOSIS

The microscopic examination of brain tissue for Negri bodies, isolation of rabies virus from tissue specimens and, where necessary, the confirmatory serum-virus neutralization test are still the most important techniques in the laboratory diagnosis of rabies. However, new advances have been made in the application of other methods for use in rabies diagnosis, examples of which are the fluorescent antibody and complement-fixation tests. The Committee stresses that the latter methods are valuable tools to be added to the rabies diagnostic armamentarium and are not meant, at this time, to supplant existing techniques.

3.1 Complement-fixation test

Some progress has been made in investigations to adapt the complement-fixation test for routine use in the diagnosis of rabies. In this test, the antigen is prepared from the tissue specimen submitted to the laboratory. Control antigens (positive and negative) should be prepared from tissues of the same animal species. Control negative and positive sera should be prepared in guinea-pigs. The Committee feels that this test can be used appropriately in laboratories with sufficient experience. It must be pointed out, however, that although a positive test should be an accurate indication of the presence of the infection, a negative test does not preclude the possibility of rabies and should be tested further by accepted techniques.

3.2 Fluorescent antibody test

The most recent advance in the diagnosis of rabies has been the successful experimental use of the fluorescent antibody technique. This test is based upon the microscopic examination of tissue specimens for specific fluorescent staining when the tissue is placed in contact with antirabies serum which has been "tagged" (or "labelled") by the addition of a fluorescent dye. This fluorescence is visual evidence of specific antigen-antibody reaction. The details of the test are available in the literature.^{1, 2} The

¹ McQueen, J. L., Lewis, A. L. & Schneider, N. J. (1960) Rabies diagnosis by fluorescent antibody, *Amer. J. publ. Hlth* (in press)

² Goldwasser, R. A., Kissling, R. E., Carski, T. R. & Hosty, T. S. (1959) *Bull. Wld Hlth Org.*, **20**, 579

investigations of this diagnostic method have revealed that this test, when properly executed, can establish a highly specific diagnosis on test specimens within a few hours, and that there is a high degree of correlation between the fluorescent antibody and the mouse inoculation test.

The Committee wishes to encourage rabies diagnostic laboratories to develop proficiency in carrying out this test in order that further comparative studies can be done with this and other diagnostic tests. However, it should be pointed out that exacting standards of performance, equipment and reagents are necessary and these are related to adequate training and proficiency of the diagnostician as well as to the quality of reagents and equipment employed.

4. VACCINES

4.1 Vaccines available

The following vaccines are available for immunization of man and animals :

VACCINES AVAILABLE FOR IMMUNIZATION OF MAN AND ANIMALS

Vaccine	Strain of virus	Tissue used for preparation of vaccine	For use in :	Potency test
Live virus : LEP *	Flury, 40-60 egg passage	Chicken embryo	Dog	Guinea-pig
K*	Kelev	Chicken embryo	Dog and cattle	Guinea-pig, mouse †
HEP *	Flury, above 180th egg passage	Chicken embryo	Cattle, cat, dog and man	Guinea-pig, mouse †
Nervous tissue *, **	Fixed	Central nervous system	Man, dog, cattle and other animals	NIH, Habel
Inactivated : Duck *	Fixed virus	Duck embryo	Man	NIH, Habel
Nervous tissue *, **	Fixed virus	Central nervous system	Man, dog, cattle and other animals	NIH, Habel

* Available in freeze-dried form.

** Available in liquid form.

† See section 4.2.1.

For the production of any vaccine the Committee recommends that a particular strain of virus¹ should be used which, through experience in the laboratory and in the field, has given satisfactory results. High infectivity

¹ Available from WHO on request.

titres are essential for successful production of live-virus vaccines of avian origin as well as other vaccines. Detailed information on the methods of production of the different types of vaccine is available in *Laboratory techniques in rabies*¹ and from other sources.

The Committee notes the advance made in the successful freeze-drying of phenolized nervous tissue vaccine (see Annex 3). It wishes to stress at the same time that high standards should be applied to the technique of freeze-drying, particularly when applied to the living virus vaccines, since this can be an important source of difficulty in obtaining potent vaccines. It is expected that proper application of freeze-drying methods will improve stability of vaccines for field use.

4.2 Safety and potency tests

The Committee feels that in addition to the established safety and identity tests² the vaccines should be tested for presence of virus pathogens other than rabies by inoculation into animal species in which the vaccine was produced.

The Committee wishes to re-emphasize the importance of potency-testing each batch of vaccine. Because of the problems of stability, samples of vaccine should be recalled from distributing points for re-testing at various time intervals after the original test.

4.2.1 *Live virus vaccines*

The guinea-pig test, as described in the monograph, *Laboratory techniques in rabies*,¹ has been applied on an extensive scale to chicken embryo vaccines prepared with Flury and Kelev strains of virus and has proved its great usefulness as a routine potency test. A useful modification of this test was introduced through the use of fixed virus for challenge purposes. Correlation of the two challenge methods—fixed and street virus—has been established by parallel tests on field samples of the chicken embryo vaccines. Thus either type of challenge virus can be used with equal efficacy.

In addition to the guinea-pig potency test, another test became available for HEP Flury and Kelev viruses because of their apathogenicity for certain strains of adult mice when injected intracerebrally. Subsequent challenge of these mice with either street or fixed virus indicated their resistance and the antigenic potency of the vaccine. The actual test consists of the intracerebral inoculation into mice of 0.03 ml of 10^{-1} through 10^{-5} dilutions of the vaccine, and 14 days later, challenging the mice intracerebrally with

¹ *Laboratory techniques in rabies*, 1954, Geneva (*World Health Organization: Monograph Series*, No. 23)

² Using antirabies serum for live virus vaccines in a serum neutralization test.

sufficient fixed or street virus to kill all the control mice. Mice receiving up to 10^{-3} dilution of the vaccine should be protected. Comparison of the results of this test with those of the standard guinea-pig potency test and results in the field should continue to be investigated.

Potency tests for live virus nervous tissue vaccine are the same as for inactivated virus vaccines, which will be considered next.

4.2.2 *Inactivated virus vaccines*

For those laboratories which choose to perform the NIH test as described in *Laboratory techniques in rabies*,¹ the Rabies Reference Vaccine is available from the Statens Seruminstitut, Copenhagen.² It is highly desirable that this and other potency tests be standardized as much as possible in order that results be consistently reproducible and that there be valid bases of comparison from one test to another.

4.2.3 *International reference vaccine*

Since 1957, WHO has made available a reference vaccine for any laboratory wishing to check the potency of its own product. This vaccine, known as Reference Vaccine 155D, was an ultraviolet-light-inactivated, dried product supplied by the National Institutes of Health, United States of America. Because the supply of this lot of vaccine was limited and its subsequent use rather extensive, a new lot was required. The National Institutes of Health have kindly supplied Reference Vaccine 164, which has been accepted by the Committee and will be made available through WHO.

A review of the suggested use of this reference vaccine follows. National laboratories are urged to prepare their own stock of a reference vaccine which, after comparison with the Reference Vaccine 164, could be used to supply routine production laboratories within a country. This is made the more easily practicable by the recent development of a method of freeze-drying phenolized vaccine (section 4.1 and Annex 3). Ideally, the reference vaccine prepared by each country should be used in the potency test of every production lot of vaccine by the NIH potency test technique (described in *Laboratory techniques in rabies*³), and it is hoped that national

¹ *Laboratory techniques in rabies*, 1954, Geneva (*World Health Organization : Monograph Series*, No. 23)

² Department of Biological Standardization, Statens Seruminstitut, Copenhagen, Denmark (custodians of WHO international biological standards)

³ *Laboratory techniques in rabies*, 1954, Geneva (*World Health Organization : Monograph series*, No. 23). As the antigenic value of Reference Vaccine 164 is higher than that of the previous reference vaccine (lot 155D), a test vaccine having a relative antigenic value of 0.3 compared to Reference Vaccine 164 can be passed as suitable. The minimum acceptable antigenic value of a test vaccine in relation to Reference Vaccine 155D was 0.6.

laboratories will strive to use it in this manner. Where this is not possible, reasonable amounts of the International Reference Vaccine will be supplied to countries wishing to test the comparative potency of a large production batch of their own vaccine.

5. ANTIRABIES SERUM

Potent antirabies serum has been produced in horses, donkeys and mules, using different methods of hyper-immunization of animals and purification and concentration of the resulting serum. It is suggested that the animal tissue used for immunizing the animals should be of different species from that used for preparation of vaccine for human use. The recent development of production of gamma-globulin obtained from antirabies horse serum is noted with satisfaction by the Committee.^{1, 2, 3}

5.1 Potency test

The test animal. Normal mice of either sex weighing 10-14 g each are used. For each serum sample, at least 36 mice should be provided (six dilutions, a minimum of six mice per dilution), an equal number for the International Standard Antirabies Serum (or its national equivalent), and 24 mice for virus titration.

The challenge virus. Any strain of virus of known virulence may be used. The CVS (standard challenge virus) strain of fixed virus, as described in the monograph, *Laboratory techniques in rabies* (page 117), is very useful for this purpose.

*The International Standard for Antirabies Serum.*⁴ This is a horse serum, without preservative, supplied in dried form in sealed ampoules. The mean content per ampoule is 86.6 mg with a standard deviation of 4.3 mg. The International Unit for antirabies serum has been defined as the activity contained in 1 mg of the International Standard,⁵ and after reconstitution of the dried serum with 1 ml of distilled water, the International Standard

¹ Michalenok, Z. V. (1957) *Probl. Virol.*, **2**, 52

² Selimov, M. A., Durasova, M. N., Rogozina, E. N., Ratgauz, V. G. & Maiorova, I. I. (1957) *Ž. Mikrobiol. (Mosk.)*, **28**, No. 7, 952

³ Selimov, M. A., Kovalevskii, M. F. & Semenova, E. V. (1957) *Ž. Mikrobiol. (Mosk.)*, **28**, No. 9, 1252

⁴ Available to national laboratories on request to the Department of Biological Standardization, Statens Serum Institut, Copenhagen, Denmark (custodians of WHO international biological standards).

⁵ *Wld Hlth Org. techn. Rep. Ser.*, 1956, **108**, 11

Serum will hold approximately 80 International Units per ml. After reconstitution, the serum remains stable for at least two years if kept sterile and under refrigeration at 4°C, or in the frozen state.

Procedure. Six serial twofold dilutions, from 1 : 50 through 1 : 1600 of both the serum under test and the Standard are prepared in distilled water containing 2% normal horse serum.

One volume of challenge virus suspension is added to one volume of each serum dilution (making *final* serum dilutions of 1 : 100 through 1 : 3200). The amount of virus suspension should be such that each mouse receives between 20 and 1000 LD₅₀, preferably about 100 LD₅₀.

The mixtures are incubated in a water-bath at 37°C for one hour, and 0.03 ml quantities are injected intracerebrally into mice (at least six mice per dilution).

In order to determine the number of LD₅₀ of virus actually used, the virus dilution used in the test is mixed with an equal quantity of 2% normal horse serum and the mixture incubated with other virus-sera test mixtures for one hour at 37°C. Following incubation serial tenfold dilutions are made and injected into mice *after* inoculations have been completed with the mixtures containing the sera under test.

The mice are observed for 14 days after injection. Mice dying before the fifth day after inoculation with CVS virus are eliminated from the test; all mice dying between the fifth and fourteenth days, after showing signs of rabies (paralysis, convulsions), are considered to have died of rabies. Mice still living on the fourteenth day but showing signs of rabies are counted as deaths from rabies. The LD₅₀ of the test virus may be calculated according to the method of Reed & Muench (1938).¹

Note. The assay should be performed with serum before the addition of any chemical preservatives or after removal of the preservatives (for example, by dialysis against saline).

A serum shall pass the test for sufficient therapeutic potency if, in a single comparative assay, it is revealed to be equal to or better than the International Standard Serum. In case a serum fails the test, two more similar tests may be carried out. If the serum proves equal to or better than the International Standard Serum in both these additional tests, it shall pass. The outcome is "equal" or "better" if the total survivor fraction (survivors/total number of mice) for the serum under test is equal to or larger than that for the standard.

¹ Reed, L. J. & Muench, H. (1938) *Amer. J. Hyg.*, **27**, 493

6. PREVENTION OF RABIES IN MAN

6.1 Summary of developments since the third report¹

Results reported from several institutes have confirmed the effectiveness of the combined serum and vaccine treatment of exposed human beings, and the Committee wishes to emphasize that this is definitely the best method now available.

The phenomenon of interference with the antibody response to vaccine by antirabies serum has been further demonstrated and possible means for avoiding it have been developed. Slight revisions in the Guide for Post-Exposure Treatment (section 6.3) have been made in view of these findings.

The development of an inactivated virus avian embryo vaccine (duck embryo) shown in experimental work to contain only minimal amounts of the factor or factors responsible for allergic encephalitis as compared with nervous tissue vaccines similarly tested should help to reduce post-vaccinal nervous system reactions, and it has been shown that antihistamines can reduce the incidence of serum sickness following the use of serum.

Attempts to reduce the number of doses of vaccine have been unsuccessful. The factors involved in pre-exposure immunization with a subsequent booster dose of vaccine at the time of exposure have been further clarified and recommendations amended.

6.2 Local treatment of wounds

All bite wounds and scratches by animals should receive immediate local treatment. For bite wounds, thorough cleansing with soap or a detergent and flushing of the wound may be supplemented by the judicious use of concentrated nitric acid in puncture wounds where the site permits. Where possible, bite wounds should not be immediately sutured.

In those patients the nature of whose exposure requires the use of serum (see section 6.3) a part of the serum dose should be infiltrated into the tissue beneath the wound, when this is feasible. The value of local application of powdered antirabies gamma-globulin into the bite wound is being studied.

The application of ordinary antiseptics and antibiotics or antitetanus procedures should follow the local treatment recommended above, when indicated.

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1957, 121

6.3 Guide for specific post-exposure treatment

The accompanying guide and explanatory notes represent a slight modification from similar charts published in previous reports of the Committee.

GUIDE FOR SPECIFIC POST-EXPOSURE TREATMENT

Nature of exposure	Biting animal*		Recommended treatment** (in addition to local treatment)
	At time of exposure	During observation period of ten days	
I. No lesion ; indirect contact	Rabid	—	None
II. Licks : (1) unabraded skin	Rabid	—	None
(2) abraded skin, scratches and unabraded or abraded mucosa	(a) healthy	Clinical signs of rabies or proven rabid (laboratory)	Start vaccine at first signs of rabies in the biting animal
	(b) signs suggestive of rabies	Healthy	Start vaccine immediately ; stop treatment if animal is normal on fifth day after exposure
	(c) rabid, escaped, killed or unknown	—	Start vaccine immediately
III. Bites : (1) mild exposure	(a) healthy	Clinical signs of rabies or proven rabid (laboratory)	Start vaccine at first signs of rabies in the biting animal
	(b) signs suggestive of rabies	Healthy	Start vaccine immediately ; stop treatment if animal is normal on fifth day after exposure
	(c) rabid, escaped, killed or unknown	—	Start vaccine immediately
	(d) wild (wolf, jackal, fox, bat, etc.)	—	Serum immediately, followed by a course of vaccine †
(2) severe exposure (multiple, or face, head, finger or neck bites)	(a) healthy	Clinical signs of rabies or proven rabid (laboratory)	Serum immediately ; start vaccine † at first sign of rabies in the biting animal
	(b) signs suggestive of rabies	Healthy	Serum immediately ; followed by vaccine ; vaccine may be stopped if animal is normal on fifth day after exposure
	(c) rabid, escaped, killed or unknown	—	Serum immediately ; followed by vaccine †
	(d) wild (wolf, jackal, fox, bat, etc.)		

* This schedule applies equally whether or not the biting animal has been previously vaccinated.

** See explanatory notes opposite.

† Course of vaccine to be followed by supplemental doses of vaccine of non-nervous tissue if possible, 10 and 20 days after the last usual dose.

6.3.1 *Complications of antirabies treatment*

The Committee points out that reactions to antirabies horse serum, even though the serum is concentrated and purified, do occur to approximately the same degree as with other sera of animal origin. An immediate anaphylactic type of reaction should be avoided by careful questioning about past history of allergy and the routine use of an intradermal or ophthalmic test for sensitivity. In case of a positive sensitivity test, the usual precautions of desensitization should be followed.

The incidence of serum sickness which is encountered following administration of serum can be reduced by the concomitant administration of antihistamine drugs in large, sustaining doses for several days after treatment.

6.3.2 *Nervous-tissue vaccine*

It is known that the incidence of neuroparalytic accidents following a course of nervous-tissue vaccine varies from one country to another. The Committee recognized that it is impossible at present to determine the basis for these apparent differences, but any consideration of the matter must take into account such host factors as the population involved, and the physiological state of the individual, species of animals used in vaccine production, method of inactivation and dosage schedule. The incidence of these complications is high enough in certain areas of the world to indicate the need for further efforts to eliminate them.

When neuroparalytic accidents or premonitory symptoms indicating their development occur, the following possible procedures are suggested, although at the present time, no definitive scientific evidence is available

EXPLANATORY NOTES TO THE GUIDE

For the benefit of physicians who will use this Guide, a detailed history of exact circumstances of exposure is essential to determine the action to be taken (see Annex 2).

The general principles on which the foregoing guide is based are that in mild exposures a course of vaccine following the above-recommended local treatment is sufficient, whereas following severe exposures, and in all cases of unprovoked wild animal bites, antirabies serum together with vaccine should be employed. As with vaccine alone, it is important to start combined serum and vaccine treatment as early as possible after exposure. For the reasons stated in section 6.5 serum should be administered in a single dose (not less than 40 International Units per kg of body-weight) at the start of treatment, followed by a course of not less than 14 daily doses of vaccine. In all cases where serum is followed by a full course of vaccine it is suggested that two supplemental doses of vaccine be administered at 10 and 20 days following the completion of the usual vaccine schedule. Where possible, these supplemental doses should be with a vaccine of non-nervous-tissue origin.

Sensitivity to serum should be tested before serum is used (section 6.3.1).

It is fully recognized that this table is only a guide and in certain situations specific conditions may warrant modifications, e.g., exposure, especially in young children or where a reliable history cannot be obtained, and particularly in areas where rabies is known to be enzootic even though the animal at the time of exposure is considered to be healthy. Such cases may justify treatment immediately in a modified way. Possible modifications would be that, following local treatment of the wound as described above, a single dose of serum or three doses of vaccine at daily intervals, and no further vaccine, be given as long as the animal stays healthy for 10 days following exposure.

Another example of a local situation in which a modified interpretation of these recommendations may be indicated is that of rabies-free areas where frequent exposures to animal bites are encountered. In such localities, adequate laboratory and field experience indicating no infection in the species involved may justify the local health authorities in recommending no specific antirabies treatment.

as to their effectiveness. If, in view of the degree of original exposure, the amount of immunization already obtained is considered to be adequate, all further vaccine administration may be discontinued; if further immunization is indicated, vaccine prepared from non-nervous tissue should be used in lieu of brain-tissue vaccines. In either instance the use of such products as corticosteroids and ACTH should be considered.

Inactivating agents used in the production of killed vaccine should be safe for man and used in a concentration which will produce minimum reaction in the vaccinated individual.

6.3.3 *Vaccines prepared from avian embryos*

Persons known to be sensitive to egg protein should not ordinarily be given vaccines of avian origin without proper precautions. Chicken-embryo and duck-embryo vaccines have shown no deleterious effect in man except for local reactions, including adenopathy.

6.4 Immunization of man against rabies before exposure

Rabies prophylaxis in man has long been a special problem when it involves particular groups of individuals with unusual risks of repeated exposure, such as veterinarians, dog handlers, field naturalists, laboratory workers, etc. In these groups, repeated exposure means repeated treatment, thus increasing the possibilities of severe reactions to the vaccine, especially those involving the central nervous system.

There are now two types of vaccine available which are almost devoid of encephalitogenic properties which may be used for prophylactic immunization of man—namely, the duck-embryo and the HEP chicken-embryo vaccine. It is suggested that the schedule of immunization consisting of three intradermal doses of these vaccines be given five to seven days apart followed by a booster dose administered one or more months (preferably two to six months) after the last dose of vaccine. If the individual continues to work under risk, he should be revaccinated with a similar booster dose every two to three years. However, results obtained in field trials indicate that regardless of the schedule of immunization, antibody response does not occur in all the vaccinated individuals. If a prophylactic immunization is carried out with these vaccines it is essential that a detectable antibody response should be checked on a serum sample obtained one to two months after the completion of vaccination. Booster doses can be repeated until antibody is detectable.

It was also found that a single injection of any potent antirabies vaccine, i.e., chicken-embryo, duck-embryo or nervous tissue vaccine, given to an individual who had had antirabies treatment in the past resulted in a prompt and significant antibody rise. It is therefore recommended that in case of

mild exposure of an individual who has demonstrated an antibody response to antirabies vaccination received in the past, a single booster dose of vaccine be given. In case of severe exposure the Committee feels that the usual post-exposure treatment (section 6.3), consisting of administration of antirabies serum and a full course of vaccine, should be given.

6.5 Results of experimental studies co-ordinated by WHO on the efficacy of antirabies serum and vaccine

Studies on inoculation of different schedules of various vaccines and serum in normal human subjects have been continued. These experiments were devised to answer the following questions :

1. Can the number of doses of vaccine for post-exposure treatment be reduced and still produce an antibody response equivalent to the currently used vaccine schedules and what is the effect of a dose of antiserum on this response ?
2. Does a dose of serum given at the start of a primary course of immunization interfere with the ability of a later booster dose of vaccine to call forth antibody production ?
3. Does a dose of serum given at the time of a booster dose of vaccine in a previously immunized individual interfere with the booster response ?
4. How rapidly are antibodies produced as the result of a booster dose of vaccine in previously immunized persons ?

The results of these studies in approximately 400 individuals show :

1. The antibody response to a reduced number of doses of vaccine is not as good as with the usual schedules and is usually inhibited by a dose of serum given at the start of treatment.
2. Serum at the start of a course of a reduced schedule of primary immunization may sometimes interfere with the ability of the subject to respond to a later booster dose of vaccine.
3. Serum given at the time of a single booster dose of vaccine interferes with the booster effect.
4. Individuals previously immunized with a primary course of vaccine respond well to a booster dose of vaccine at 30, 60, 90 or 120 days following the first inoculation of vaccine¹ and the antibody response to the booster dose appears within eight days. However, not 100% of all such individuals show this booster effect.

¹ This does not exclude individuals, such as those referred to in section 6.4, who have received antirabies vaccine many years previously.

7. CONTROL OF RABIES

7.1 Introduction

The ultimate solution of the problem of rabies depends on the control and eventual elimination of the disease from reservoir and vector animal populations. Experience has shown that this may be accomplished by the establishment of transmission barriers, such as animal vaccination, stray dog elimination and the reduction of excessive numbers of wildlife vectors. Successful use of these techniques will depend on carefully planned and well-executed programmes and the quality of materials used.

7.2 Immunization of dogs

The Committee considers prophylactic vaccination of dogs against rabies to be one of the most important weapons in rabies control and urges countries where infected areas exist to adopt vaccination programmes for their canine population.

One intramuscular inoculation of LEP Flury chicken-embryo vaccine in dogs three months of age or older produces excellent immunity which lasts at least three years. Such proven effectiveness of this vaccine in the laboratory and field resulted when a dose of 3 ml of 33% suspension of infected chicken-embryo tissue was used, and the Committee stresses that this amount of vaccine should be the minimum dose when an effective immunity of three years or more is desired. Other living virus and inactivated virus vaccines (see vaccine table, section 4.1) found to be antigenic by careful laboratory and field studies may be used for immunization of dogs.¹ However, the Committee recommends annual revaccination of dogs inoculated with the latter vaccines, since available data indicating the duration of immunity following their use are limited.

Puppies less than three months old can be vaccinated safely with HEP Flury or Kelev chicken-embryo live-virus vaccine² or with any of the inactivated virus preparations, but not with LEP Flury vaccine. Because of the results of experiments indicating that puppies less than three months old do not develop an immunological response to rabies vaccination as readily as older dogs, it is recommended that a puppy injected with rabies vaccine before three months of age be revaccinated with any potent rabies vaccine as soon as possible after it reaches the age of three months.

The Committee, after careful examination of experimental and field data related to the vaccination of dogs with living attenuated virus, reaffirms

¹ The dosage of inactivated nervous tissue vaccine for both puppies and adult dogs is 5 ml of 20% tissue suspension or its equivalent (e. g., 10 ml of a 10% suspension).

² A dosage of 3 ml of 33% suspension for both HEP Flury and Kelev vaccines may be used in puppies.

the safety of this procedure and considers that such virus used for vaccination purposes does not constitute a danger for man or animals through transmission from saliva of the vaccinated dog. The Committee also found no evidence for the existence of alleged street virus carrier states in dogs immunized with rabies vaccine.

7.3 Immunization of other animals

Experimental and field data on the use of vaccines in other animals are not so extensive as those concerning the dog. The following recommendations should therefore be considered with this limitation in view.

7.3.1 *Cattle*

The chicken-embryo-adapted HEP (Flury) and Kelev strains, or inactivated vaccines, are recommended for pre-exposure vaccination of cattle. HEP Flury strain vaccine for cattle is given intramuscularly into the thigh, using a minimum dosage of 3 ml of a 33% suspension of tissue. Kelev strain vaccine is similarly administered in a dosage of 6 ml of a 60% suspension of tissue. Although a single dose of these vaccines elicits adequate antibody response in a significant number of animals, a second dose of vaccine given 30 days after the first may result in a booster effect and provide more complete protection. For inactivated nervous tissue vaccines, 30 ml of a 33% suspension of tissue, or an equivalent quantity (e. g., 15 ml of 60% suspension), is recommended.

Protection conferred by vaccine in cattle lasts at least one year, but the duration of immunity beyond this period is not known.

Carefully controlled studies have not been made concerning post-exposure treatment of cattle exposed to rabies. Limited observations indicate that 14 daily inoculations of 1.5-2 g wet weight (7.5 ml of 20% tissue concentration) of inactivated nervous tissue vaccine confer protection. Such a schedule, with or without the use of serum, would rarely be economically feasible. Reduced dosage schedules of inoculations spaced several days apart are occasionally practised but the value of such schedules is not known.

7.3.2 *Cats*

Cats may be effectively immunized with either nervous tissue vaccine or HEP (Flury) strain vaccine. LEP (Flury) strain vaccine should not be used in cats. The dosage of vaccine for cats is about one-half the dog doses, i.e., 1.5 ml of 33% tissue suspension of HEP vaccine, or 3 ml of 20% nervous tissue suspension of inactivated vaccine.

7.3.3 *Other species*

Insufficient data are available to make recommendations with respect to the efficacy of vaccines in other species of animals. Here a word of caution is necessary. Species may vary widely as regards susceptibility to the various strains of rabies virus. For example, LEP Flury vaccine has been widely used with safety in adult dogs, but has been shown to be hazardous for foxes, skunks and cattle. The Committee, therefore, strongly recommends that living antirabies vaccines should not be used in any species of animal without previously determining the safety as well as the efficacy of the vaccine for the particular species in question.

7.4 **Field use of canine vaccination**

Since approximately one month is required for canine vaccines of either type to elicit a maximum level of immunity, restrictive measures (leashing, confinement) for dogs which are involved during an epizootic may be lifted 30 days following vaccination.

With regard to methods which will rid an area of enzootic or epizootic canine rabies, no significant degree of success can be expected unless there is a well-organized intensified programme of mass immunization in addition to stray dog elimination. Mass immunization programmes involve the establishment and operation of temporary clinic sites strategically located throughout the problem area, as well as a substantial zone surrounding the geographical focus of infection. The vaccination of dogs privately by veterinary practitioners is an important adjunct and should be encouraged. The programme should be directed towards the swift reduction of susceptible animals, and this can be achieved by the immunization of at least 70% of the entire dog population of the area in the shortest possible period.

Canine immunization should be made an integral part of all long-range rabies control programmes and, as a sound public health procedure, dog owners should be encouraged to have their pets vaccinated as soon as possible after they are three months of age.

The Committee again stresses that all vaccines used for immunization should have previously passed an adequate potency test (section 4.2).

In enzootically infected areas and in rabies-free areas faced by the constant danger of introduction of the disease, continued programmes of vaccination should be adopted which would at least provide for the annual vaccination of new dogs and for the revaccination of all dogs every three years with LEP Flury vaccine, or annually with other vaccines.

7.5 **Elimination of stray dogs**

Vaccination procedures will not reach the stray or ownerless dog, which remains a potential threat in the transmission of rabies in many countries.

An efficiently conducted programme requires the operation of a local pound or humane animal shelter where stray animals may be kept for a few days and, if unclaimed at the end of that period, destroyed humanely. The practice of making stray impounded animals available for adoption as pets should be suspended during outbreaks of rabies in a community. Collection of strays should be carried out by teams of dog wardens and assistants in properly equipped trucks. Personnel responsible for this activity should be properly qualified and in-service training courses should be offered to them in such subjects as care and management, basic principles of animal disease control, first-aid, etc. Licensing or registration of all dogs is often a valuable adjunct. If properly enforced, it separates out the ownerless strays, helps to defray the expense of control activities and may serve as the basis for a reasonably accurate dog census.

7.6 Handling of dogs and cats bitten by rabid animals

The Committee strongly urges that unvaccinated dogs and cats bitten by a known rabid animal should be immediately destroyed. If the owner is unwilling to destroy the exposed animal, strict isolation of the animal in a kennel for six months should be enforced.

If the animal has been previously vaccinated within three years with Flury strain vaccine (or within one year with other vaccines), revaccination and restraint (leashing and confinement) for 30 days should be carried out.

It is important to develop means for rapid, routine investigation of persons and animals bitten by rabid animals so that immediate steps can be taken for the prevention of rabies in the exposed person and so that the bitten animal can be removed as a potential link in the chain of transmission.

7.7 Administrative aspects of rabies control

Experience has shown that the efficient organization of a rabies control programme is best accomplished by means of a central authority headed by a public health officer, preferably a veterinarian who has full executive power and who devotes his full time to this work. The development of a state- or province-wide programme under a public health veterinarian can ensure effective uniformity of control practices among the local jurisdictions. In his capacity as co-ordinator of the state or provincial programme, he should be responsible for the following activities to ensure effective programme operation :

(a) working closely with committees of medical and veterinary associations in dissemination of information on current control procedures ;

(b) enlisting support of livestock organizations, animal protection societies, kennel and sportsmen's clubs ;

(c) arranging regular exchange of information on state of infection and control with neighbouring states ;

(d) collecting and analysing human and animal morbidity data on animal bites, rabies exposures, rabies cases, vaccinations and post-vaccinal side-reactions ;

(e) developing and improving the reporting system, in close collaboration with the diagnostic laboratory on interpretation of findings, and keeping informed of the geographical movement of infection throughout the state ;

(f) improving methods for shipment of specimens to laboratories and methods for collection, impoundment and destruction of stray dogs ;

(g) conducting training courses and research projects in various phases of rabies control ;

(h) promoting a continual and energetic publicity campaign.

7.7.1 *International transfer of dogs and cats*

The following measures should be taken when animals are imported from countries where rabies is known to exist.

A rabies-infected area can be considered one where an indigenously acquired rabies infection has been confirmed in man or animals at any time during a previous two-year period.

1. Countries now free of rabies should continue either to prohibit the importation of dogs and cats, or to subject them to a prolonged period of quarantine, preferably six months, at the port of entry.

2. In countries free of rabies but where prolonged quarantine measures cannot be invoked, 3 (a) and 3 (b) below may be applied. This recommendation should not be construed, however, as discouraging more stringent measures, such as longer quarantine or restraint periods upon entry, where applicable.

3. Where strict quarantine measures are impracticable, as, for instance, in countries with extensive land borders and with rabies already present in domestic or wild animals, the following is recommended :

(a) animals should be vaccinated more than one month before, but within 36 months of, departure with LEP vaccine, or within 12 months with other vaccines. Certificates signed by the appropriate veterinary authorities in the country of origin should accompany each animal (see Annex 1). Where any doubt exists with respect to the potency of the vaccine used in the animal's country of origin, the animal should be considered unvaccinated ;

(b) unvaccinated animals should be vaccinated upon arrival and quarantined for not less than 45 days, or where quarantine measures are

impossible to apply, the animal should be kept under surveillance for a similar period and not allowed to run at large.

7.8 Wildlife rabies

Wildlife rabies has become the major practical problem in certain areas and continues to be the underlying reservoir for maintaining the disease in many parts of the world.

Recent studies in wild animal rabies have afforded new information on the natural behaviour of the disease in wild life. For instance, in the USA, foxes and skunks are the principal sylvatic vectors of rabies, and most of the submaxillary salivary glands of naturally infected foxes have been shown to contain rabies virus. In no instance have salivary glands been found infected without concurrent infection of the central nervous system, indicating that foxes are not capable of transmitting the disease as symptomless carriers. Their transmitting potential, however, is great, as evidenced by the fact that experimentally infected foxes were shown to experience relatively long periods of illness during which time the saliva may be positive for rabies virus.

Search for rabies virus in large surveys of small wild rodents in highly enzootic and epizootic rabies areas has revealed no evidence of infection, confirming previous preliminary reports that these species do not serve as important reservoirs of the disease in nature.

Programmes for population reduction of proven vector species continue to be the only available method for sylvatic rabies control. The choice of techniques for carrying out these programmes depends upon various local conditions. For many species, poisoning is far more efficient and certainly more economical than trapping. This method, however, must be applied with extreme caution, particularly in areas of dense human and domestic animal populations. Use of poisons has proved more feasible with the small vector species (e.g., thallium sulfate for mongooses and strychnine-treated eggs for skunks) where it has been possible to devise protected bait stations, eliminating the danger of accidental poisoning of children and domestic animals. Gassing of dens is applicable on a seasonal basis for short periods of time when family groups of vector animals are together, and can be used as an adjunct to the other methods, such as trapping and poisoning. Trapping, although not as efficient as and more expensive than the use of poisoned baits, is still the method of choice in most areas because it can be used more safely than poisons. It should be emphasized that, for maximum effectiveness, a programme utilizing these methods should be directed by professionally trained predator-control specialists.

It should be pointed out that the predator-control methods being devised and tested in the field are by no means directed at the mass

destruction of any of the vector species. It is the aim of these control methods simply to reduce the population level to a threshold which will no longer be able to support an epizootic of rabies.

7.9 Bat rabies

Vampire-bat rabies continues to be a major rabies problem in Mexico and in Central and South America. Attempts have been made to control the disease in these areas by gassing and shooting of vampire bats in their diurnal resting-places. Newer methods of control, such as traps with live bait, have thus far given equivocal results, and are now being further improved and tested.

Although some instances of rabies in frugivorous and insectivorous bats had been reported previously from the vampire-bat rabies areas of Latin America and Trinidad, bat rabies was unknown in all other areas of the world until 1953, when rabies virus was isolated from an insectivorous bat in the United States of America. Since then, over 350 virus isolations have been made in bats from 24 States in widely diverse geographical areas of the United States of America as well as from one province in Canada. These include four species of solitary or tree-living bats and 20 species of colonial or gregarious bats. Additional isolations have been reported from Turkey, Yugoslavia and a suspected instance in Western Germany. All bats involved were of the insectivorous type.

About 100 of the proven instances of bat rabies were reported as episodes involving the biting of human beings. Five human rabies deaths in the United States of America and one in India have been attributed to exposure by insectivorous bats.

Epizootiological investigations in bat rabies have revealed that it is extremely difficult to transmit rabies by inducing known rabid bats with infectious saliva to bite other susceptible animals in the laboratory. In other studies there is suggestive evidence that some species of insectivorous bats may be capable of becoming symptomless carriers. Surveys of selected bat populations have yielded high proportions of virus isolations during periods when die-offs were noted.

No definitive programme can at this time be recommended for control of bat rabies. Methods for ridding human dwellings of bats roosting in them include the use of naphthalene flakes and various fumigant gases. These methods, however, are only temporary and should be followed immediately by bat-proofing of the structure.

Persons should be warned not to pick up or handle sick or strangely behaving bats, and all persons bitten by bats in known infected areas should receive antirabies treatment.

The deliberate transfer of bats and other wild life between widely separated geographical areas should be discouraged.

8. EXCHANGE AND DISSEMINATION OF INFORMATION ON RABIES

8.1 Collection of statistics

The Committee examined the answers returned by some 200 rabies laboratories, institutes and medical and veterinary services in 75 countries¹ to a WHO questionnaire sent out further to the recommendations made in the third report (section 10).²

This excellent response to the questionnaire indicates clearly the willingness of rabies workers and authorities throughout the world to collaborate closely in the fight against this disease.

The Committee notes the work of the Office international des Epizooties and the Food and Agriculture Organization in the collection of animal statistics and that steps have already been taken for close collaboration of these organizations with WHO in the collection and dissemination of statistics on rabies in animals and man. The Committee recommends that such information be distributed periodically to all collaborating institutes.

Annex 2 reproduces with slight modifications the case-record form recommended in the third report,² which has been very useful in the compilation of rabies statistics. The information gained from the careful keeping of such records is of great use not only to the institute itself, but also to national and international authorities dealing with rabies. Many problems of the prevention and treatment of paralytic accidents, serum sickness, and efficacy of post-exposure treatment still remain to be solved and the periodic compilation and analysis by WHO of results obtained in different countries on these problems will be of great value in assessing the effectiveness of measures being employed. The Committee urges, therefore, that the case-record form in Annex 2 or a suitable modification be used in all antirabies treatment centres and that close collaboration be maintained with WHO in this connexion.

8.2 Exchange of information

The Committee recommends that WHO circulate to the collaborating institutes information documents on new developments in rabies so that an exchange of information may be established. The results of work in the

¹ Since the meeting of the Committee more replies to the questionnaire have come in, making a total of 222 from 89 countries and territories.

² *Wld Hlth Org. techn. Rep. Ser.* 1957, 121

rabies field could thus be widely communicated and, where indicated, be co-ordinated by WHO.

8.3 Regional technical seminars

The Committee noted with satisfaction that WHO has continued to hold regional technical seminars similar to the one held in Coonoor, India, in 1952, to provide countries with an opportunity of sending leading rabies workers to learn the latest methods in rabies techniques. A meeting of this type was held in Muguga, Kenya, in July 1955, in collaboration with the Commission for Technical Co-operation in Africa South of the Sahara (CCTA) and was attended by 40 representatives from 22 countries in Africa and the Eastern Mediterranean Region. Another meeting was held in Caracas, Venezuela, in April 1957, and served an equally useful purpose for countries of the Americas. Not only do these meetings provide the latest information on rabies, but they also serve to promote appreciably the adoption of more uniform techniques and principles in important aspects of the disease. This should increase the effectiveness of work carried out in different countries.

In recent years new techniques have been developed (e.g., the fluorescent antibody technique in diagnosis, section 3.2; drying of vaccine, Annex 3), and similar meetings for the future are recommended to bring up to date technical activities concerning rabies in different regions.

8.4 Monograph on laboratory techniques

The publication by WHO of a monograph entitled *Laboratory techniques in rabies*¹ has been a very useful step in providing, in a concise form, practical and advanced standardized laboratory techniques.

The Committee considered that while the monograph as a whole reflects the latest techniques in this field, revision and expansion of certain of its sections are indicated in the light of new knowledge, and it therefore recommends that WHO should give consideration to undertaking the publication of a second edition of this monograph in the future. However, since new techniques are rapidly being developed at the present time, the Committee considers it advisable to wait one or two years until these new techniques have been adequately tested. At that time a new edition of the monograph could incorporate these techniques. In the meantime, information documents prepared by WHO should be made available upon request by rabies workers.

¹ *Laboratory techniques in rabies*, 1954, Geneva (World Health Organization : Monograph Series, No. 23)

9. FUTURE RESEARCH

9.1 Possible modification of post-exposure treatment of man

(a) Local treatment of bite wounds: further experimental investigations are necessary to clarify the type of local treatment to be recommended. Experimental and field evaluation of the efficacy of dried antirabies gamma-globulin applied in the bite wound should be continued.

(b) Serum-vaccine treatment:

- (1) Can the dose of serum be reduced without loss of efficacy?
- (2) Can non-nervous-tissue vaccines be used intradermally with better antibody response than with subcutaneous administration?

9.2 Vaccines

(a) Further tests of the stability of freeze-dried phenolized vaccine are indicated.

(b) Efforts should be made towards the development of highly antigenic non-nervous tissue vaccines capable of effective immunization with reduced schedules.

9.3 Serum

The production of concentrated prophylactic serum of human origin might offer certain advantages and this possibility should be investigated.

9.4 Inapparent infections

Further evidence based on stricter criteria are required to establish the existence of inapparent rabies infections in nature and to determine if a "silent" carrier state is possible in animals other than bats.

9.5 Ecology and pathogenesis

More information is needed on the relative susceptibility of different animal species to rabies, and quantitative evidence is needed concerning the presence of virus in their salivary glands. Studies on pathogenesis and immunity mechanisms are also recommended to clarify basic questions in these fields.

9.6 Bat rabies

Ecological and physiological studies of bats should be encouraged and pathogenetic studies of rabies in various species are needed. Countries

which have not yet made surveys of their bat population for the existence of rabies infection should do so.

9.7 Tissue culture

Recent encouraging developments in this area warrant special efforts to develop a tissue culture system which would be sensitive to demonstration of cytopathic effect of rabies virus and to support virus growth to high titre. Such a system would make quantitative work with rabies virus more exact and furnish material for many basic virological investigations. Growth of rabies virus in non-tumour tissue for possible use in preparing a non-nervous tissue vaccine for use in human beings is needed.

9.8 Research meetings

In view of rapid advances now being made on basic problems in rabies research, the Committee strongly recommends that WHO arrange for periodic meetings of rabies research workers.

Annex 1

SUGGESTED INTERNATIONAL VETERINARY CERTIFICATE OF HEALTH AND RABIES VACCINATION FOR DOGS AND CATS

This is to certify that the following dog/cat has undergone veterinary examination on (date) and has been found to be free of signs of communicable disease; and further that the said dog/cat has been vaccinated against rabies on (date)

Breed Colour Sex Age Weight

Vaccination No. Country of origin and }
countries visited during }
previous year (give dates) }

Type of vaccine Manufacturer Lot No.
(phenol-inactivated,
chicken-embryo, etc.)

Dose Route of administration

Owner of animal Address

(Signed)
Veterinarian

Address

Date

Annex 2

SUGGESTED CASE RECORD FOR HUMAN RABIES EXPOSURE

Case No. Referred by

A. Person bitten

Name Date of bite

Age Geographical locality of biting episode

Sex Site(s) of bite on the body

Home address

Nature of bite:

Single Mild

Multiple Moderate

Severe

Local wound treatment

Treatment:

*Vaccine**Serum*

Size or quantity of individual dose Dose

Route of administration Date administered

Dates administered Animal source of serum

Type of vaccine (phenol or UV inactivated, etc.) Results of sensitivity test: Positive Negative

Manufacturer and lot No. Manufacturer and lot No.

Previous rabies vaccine treatment? Previous serum treatment?

Date Type Date Type

Were there complications of treatment? If so, specify treatment of undesirable sequelae and outcome

Status of exposed person after 6 months:

Alive

Died of rabies

Died of other causes

Unknown

Date of death

B. Biting animal

Kind of animal

Description :

Breed Age Sex Weight

Animal vaccinated against rabies ?

Type of vaccine Date

Outcome :

Under observation Killed Escaped

Outcome during days :

Signs of rabies Healthy Died without signs
of rabies

Results of laboratory examination :

Positive Negative

Negri bodies Animal inoculation Other (fluorescent
antibody, etc.) **Annex 3****PREPARATION OF FREEZE-DRIED PHENOLIZED ANTIRABIES
VACCINE**

Considerable experience has been gained in the USSR laboratories on the preparation of a freeze-dried phenolized antirabies vaccine.

This method has been applied in the USSR to Semple and Fermi vaccines in a 20% emulsion. To the 20% vaccine a volume of drying medium is added to give a final concentration of 0.5% gelatine and 7.5% glucose or saccharose and 10% tissue suspension in M/400 phosphate buffer, pH 7.2. The preparation is dispensed in volumes of 1.5 or 3 ml into 10-ml ampoules, frozen at -70°C and dried in a vacuum-drying apparatus, with a vacuum of not less than 100 microns, at room temperature for 24-72 hours. The vaccine should never be allowed to thaw during the drying process. The residual moisture content of the dried antirabies vaccine must not exceed 2% and preferably should be less than 1%.

The vaccine, diluted before use with the initial volume of physiological salt solution, will contain 10% brain suspension, which corresponds to a dose of 3 ml of liquid 5% vaccine of the Semple or Fermi type.

The lyophilized phenolized vaccine is tested in the usual way for potency, safety, etc.

It is recommended that laboratories producing freeze-dried phenolized vaccine for the first time should carry out potency tests of the dried preparation before and after incubation in a water-bath at 37°C for seven days to establish stability.