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**WHO EXPERT COMMITTEE ON
RABIES**

Fifth Report

	Page
1. Introduction.	3
2. Advances in research.	4
3. Pathogenesis.	7
4. Diagnosis	8
5. Vaccines	10
6. Prevention of rabies in man	16
7. Control of rabies in animals	21
8. Wildlife rabies.	27
9. Exchange and dissemination of information on rabies.	29
10. Future research	31
Annex 1. Guide for post-exposure treatment.	33
Annex 2. Suggested international veterinary certificate of health and rabies vaccination for dogs and cats.	36
Annex 3. Suggested case record for human rabies exposure.	37

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WHO EXPERT COMMITTEE ON RABIES

Fifth Report

1. INTRODUCTION

The WHO Expert Committee on Rabies met in Geneva from 1 to 7 June 1965. The meeting was opened by Dr P. Dorolle, Deputy Director-General of WHO on behalf of the Director-General. He referred to notable recent advances in rabies research and the need for their early application in combating the disease in the field and in the treatment of exposed persons. Dr H. Koprowski was elected Chairman, Dr M. Selimov Vice-Chairman, and Dr K. Habel Rapporteur.

Since the last WHO Expert Committee on Rabies met in December 1959, two scientific advisory research groups have been convened by WHO, one in 1961 and one 1963, to pursue the recommendations for research made in the fourth report of the Committee. These interim meetings have helped considerably to accelerate collaborative research on many aspects of rabies, especially in regard to problems of pathogenesis, basic studies on the rabies virus, tissue culture techniques, diagnostic procedures, vaccine development, improved therapeutic schedules of post-exposure treatment, and the local treatment of wounds. The results of this research and other studies carried out in different laboratories throughout the world were reviewed by the Committee. It also examined and approved manuscripts prepared for the second edition of the WHO monograph *Laboratory Techniques in Rabies*. It is hoped that the new edition will be as useful to laboratory workers in rabies as was the first edition published in 1954.

Two additional collaborative activities carried out under WHO auspices since publication of the fourth report of the Committee deserve special mention. The first was a study on serum-vaccine schedules for man, the fourth in a series the results of which will be submitted for publication in the *Bulletin of the World Health Organization*. The second was a training course and symposium on rabies held in Moscow in June 1964 and attended by 21 rabies workers from 20 countries.

As in the case of the fourth report, the Committee decided to prepare its report as a self-contained document and incorporated verbatim those parts of the previous report that do not require change. Where new information has accrued the text has been modified accordingly.

2. ADVANCES IN RESEARCH

2.1 Nature of the virus particle

In various laboratories, electron microscope examination of animal brains and tissue cultures infected with rabies virus has revealed the presence of virus particles (virions) which are localized by ferritin-tagged specific gamma-globulins but are not observed if the virus has been neutralized by antirabies serum. These properties strongly suggest the specific nature of the particles.

The particles appear as short or elongated rod-like structures lined by a double membrane and often show a central canalicular helix. Whereas the diameter of the virion is fairly constant at 60-80 μ , its length may vary in the elongated forms from 120 μ to over 300 μ . The shorter forms usually display a rounded shape at one end which gives them a "bullet-like" appearance. In tissue cultures, at least, the longer forms seem to be associated with street-virus strains and the short forms with the fixed strains of virus.

On material partly purified by column adsorption or by centrifugation the same morphological aspects are observed. Some particles show disintegration images in which the content of the particle is seen oozing out of the blunt-end of the rod-like shape. Others demonstrate the uncoiling of a central filament which appears to be a fragment of a nucleoprotein helix.

Studies on the multiplication of rabies virus in tissue culture show that rabies antigen appears in the perinuclear cytoplasm approximately 15 hours after infection of the cell. Later, the virus appears throughout the cytoplasm and virus particles can be seen separating from the cell wall as described for myxoviruses. At the time when tissue culture fluids contain the maximum amount of virus, the cytoplasm of infected cells shows partial autolysis with severe metabolic disturbances and some cells appear to be almost filled with virus particles.

The morphological appearance of the rabies virus, together with the RNA-like nature of its nucleoprotein component suggests a relationship with the myxoviruses, with which it shares a number of common characters, although there are also some differences. Further studies are needed, however, before a definite classification can be made of rabies virus.

2.2 Nature of the inclusion bodies

Following infection with rabies virus, inclusion bodies are often formed both *in vitro* in tissue culture and *in vivo* in the brains of infected animals. The inclusions found in tissue culture tend to be morphologically homogeneous and have been shown to contain protein and RNA. Inclusions

found in the brains of infected animals, on the other hand, are often composed of a less homogeneous matrix in which a number of granules are embedded. Evidence has been presented which indicates that this matrix also contains protein and RNA and that the behaviour of the RNA to cytochemical stains is similar to that of the RNA found in the inclusion bodies *in vitro*. The granules contain RNA which differs in its cytochemical reactions from the RNA in the matrix substance.

All inclusion bodies have been shown to contain rabies antigen, but electron microscope studies have failed to show morphological forms identifiable as complete rabies virus particles. There is some evidence that inclusion bodies are formed as a result of defence mechanisms of the cell or of abortive viral replication.

2.3 Antigenic structure

Some progress has been made in determining the antigenic structure of rabies virus, including demonstration by the complement fixation test of a complex soluble antigen in virus-free material obtained by high-speed centrifugation. The gel-diffusion technique, complement-fixation, cross-protection and serum-neutralization tests have shown slight antigenic differences when laboratory strains of rabies virus of different origin were compared. Strains of virus isolated from naturally infected animals from different parts of the world may vary as regards pathogenicity for experimental animals, but are very similar when studied by the cross-protection test.

2.4 Purification

Concentration and partial purification of virus of tissue-culture origin has been accomplished by precipitation with heavy metals and high-speed centrifugation. Preliminary experiments aimed at determining the density of viral particles using cesium chloride and sucrose-gradient centrifugation have not yielded definitive results.

Virus of mouse-brain and tissue-culture origin has been partially purified with good virus recovery by passage through ECTEOLA¹ chromatography columns.

2.5 Interference and interferon

Experiments carried out to determine whether infection with rabies virus leads to the production of interferon and whether rabies virus is

¹ A cellulose ion-exchange adsorbent (containing epichlorohydrin and triethanolamine) used in the chromatography of proteins and nucleic acids.

susceptible to interferon have given negative results in some laboratories and positive results in others. The discrepancies are probably due to quantitative factors: not only is the proportion of culture cells simultaneously infected with rabies virus often low and therefore less likely to produce detectable amounts of interferon but tests for the effect of interferon on rabies virus cannot be sensitive in the absence of a plaquing technique for virus. There is no reason to expect that rabies virus should be uniquely negative in respect to interferon when evidence suggests that all other viruses are sensitive. Attempts to prevent rabies in experimental animals by treatment with active interferon preparations have been negative.

Interference has been demonstrated both *in vitro* and *in vivo* in experiments in which inapparent rabies infection has reduced the ability of a second challenge virus to multiply efficiently.

2.6 Tissue culture

Since the publication of the fourth report of the Committee, substantial progress has been made in studies of rabies virus in tissue culture, both street and fixed strains of virus having been successfully propagated in a variety of tissue-culture systems. Although it was originally believed that rabies virus would grow only in nervous tissue, it has now been demonstrated that it can be adapted to primary as well as established lines of many cell types from a variety of species.¹ Even when virus multiplies readily, however, a cytopathic effect is not generally observed nor does the presence of viral antigen necessarily interfere with cell division. Virus multiplication in tissue culture can be demonstrated most easily and rapidly by the fluorescent-antibody staining technique.

Methods of adaptation have been developed which have permitted modifications of virus properties and demonstration of different types of chronic infection; propagation of the virus in tissue culture has made it possible to demonstrate some of its physical and chemical properties; while electron microscopy has permitted demonstration of the morphology and cycle of development.

A serum-neutralization test using tissue culture techniques in conjunction with immunofluorescence has been developed and may be used to supplement the mouse test. Tissue-culture techniques cannot yet be routinely used for diagnostic purposes. Virus propagated in tissue culture has proved a potent antigen for the immunization of animals when used either in the attenuated or inactivated state. There is promise that similar preparations may be obtained for human use.

¹ For details see: *Laboratory techniques in rabies*, 2nd ed., Geneva, 1966 (*World Health Organization: Monograph Series*, No. 23) (in press).

2.7 Fourth collaborative study of serum-vaccine schedules in man ¹

The results of three previous studies on this subject were considered in previous reports of the Committee and have been published in the *Bulletin of the World Health Organization*. The fourth study was carried out in 50 human volunteers and was designed to evaluate further the interfering effect of serum when given with vaccine and to determine whether booster inoculations of vaccine could offset the blocking of antibody production.

The results indicated that :

(1) The antibody response to a course of 12 to 14 inoculations of Semple-type vaccine was markedly inhibited by a dose of serum given on the same day that vaccine treatment was started. This confirms results obtained in previous studies.

(2) Booster doses of vaccine given 10 and 20 days following the last dose of vaccine overcame the suppressive effect of serum given on the same day that vaccine treatment was started.

(3) Antibody response to booster doses was demonstrable within three days after injection. It is concluded, therefore, that booster doses of vaccine are essential whenever serum is given with vaccine (see section 6.4).

3. PATHOGENESIS

There is unequivocal evidence that in experimental animals rabies virus customarily passes from the site of exposure to the central nervous system via peripheral nerves. Exceptions include young animals of highly susceptible species, such as the hamster, and animals whose resistance has been altered, for example by intracerebral trauma or shock. Pathogenesis in man is believed to be essentially by neural transmission. Blood-borne infection in nature is probably the exception rather than the rule. Air-borne infection from bats is possible under certain conditions but the pathogenesis is obscure.

It is thought that in most instances virus tends to invade nervous tissue within a relatively short time after exposure. Although virus multiplication at the site of introduction is possible, it is not necessary for the initiation of infection. There is increasing evidence that in certain species virus may multiply in tissues such as the salivary gland, kidney, pancreas, adrenals, brown fat and, in some species, muscle and lung. Strains of virus have been encountered in nature that are more pathogenic in suckling mice than in mature mice when injected intracerebrally.

¹ The Committee acknowledges with appreciation the co-operation of Dr Ch. Sérié, formerly Director of the Pasteur Institute, Addis Ababa, Ethiopia, where the study was carried out.

There is clear evidence that virus may move centrifugally from the central nervous system via the peripheral nerves. It is believed that the salivary gland customarily becomes infected in this manner. However, the role of extraneural spread requires further clarification.

Pathogenesis in bats remains obscure, although the spread of virus from the site of injection to the central nervous system has been demonstrated.

4. DIAGNOSIS

4.1 General considerations

In the hands of competent well-trained technicians, using satisfactory equipment and reagents, the fluorescent-antibody test is the best single test currently available for the rapid diagnosis of rabies. However, microscopic examination of brain tissue for Negri bodies, isolation of rabies virus from tissue specimens and, where necessary, the confirmatory serum-virus neutralization test remain important techniques in the laboratory diagnosis of rabies. They are particularly useful, under certain conditions, as supplements to the fluorescent-antibody test. The complement-fixation and gel-diffusion tests cannot at present be considered useful diagnostic tests. Tissue-culture methods for the direct isolation of virus and for serum-virus neutralization tests remain experimental.

4.2 Detection of virus and antigen

4.2.1 *Fluorescent-antibody test (direct staining)*

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. Fluorescence is visual evidence of specific antigen-antibody reaction. The details of this test as well as of the indirect test are described in the second edition of the WHO monograph *Laboratory Techniques in Rabies* (in press). It has been shown that this test can establish a highly specific diagnosis on test and field specimens within a few hours, and that there is a high degree of correlation between the results of the fluorescent-antibody test and those of the mouse-inoculation test.

Several laboratories that have used the fluorescent-antibody test extensively have reported positive results both in brain tissue and in salivary gland materials that gave negative results in mouse-inoculation tests, thus demonstrating that rabies antigen may be detected in the absence of infectious virus. On the other hand, negative results with fluorescent staining have been obtained in a very few cases where mouse-inoculation indicated the

presence of rabies virus. Some laboratories have also reported difficulties in the use of the fluorescent-antibody technique in badly decomposed animal brains and in salivary-gland specimens.

It is therefore strongly recommended that laboratories should continue to use the diagnostic tests in which they are already proficient until convinced of the reliability of the fluorescent-antibody test in their own hands.

4.2.2 *Biological test*

The intracerebral inoculation of mice coupled with the microscopic examination of brain tissue for Negri bodies is still one of the most useful tests in the laboratory diagnosis of rabies and should be used whenever humans have been bitten by suspect animals and the fluorescent-antibody test is negative. Some strains of street virus are more pathogenic for suckling mice than for adult mice when inoculated intracerebrally.

Certain tissue-culture systems have been shown to be susceptible to infection with street virus. Usually, however, extensive multiplication of virus can be obtained only after adaptation to the cell system used. In such cultures, rabies antigen can be detected by fluorescent-antibody staining as early as 14 hours after inoculation. Although existing tissue-culture systems are not suitable for the rapid diagnosis of rabies infection, it is recommended that new methods and cell systems be sought which might be suitable for the primary isolation of street virus *in vitro*. The neutralization test should be used to identify the virus isolate whenever the biological or other tests give doubtful results.

4.3 Evaluation of the immune state

4.3.1 *Neutralization test*

This test performed in mice is very useful for the detection and quantitative assay of antibody in the individual organism. It has been reported to be more sensitive for the detection of neutralizing antibody in suckling mice inoculated intracerebrally than in adult mice inoculated intracerebrally. It should be pointed out that protection tests in experimental animals have shown that some immunized animals may resist challenge despite the lack of detectable neutralizing antibodies in the blood.

Some laboratories have reported the use of a rapid neutralization test in tissue-culture systems using fluorescent-antibody techniques to detect infection. These methods show promise and warrant further investigation.

4.3.2 *Indirect staining*

Indirect fluorescent-antibody staining has been successfully employed for the detection and quantitative assay of peripherally circulating anti-

body. It is of course necessary to use a species-specific labelled antiglobulin for each species of serum under test. One advantage of this technique is that it is possible to obtain final results within a few hours. However, the correlation between the titre of antibody as determined by fluorescent staining and the degree of immunity in the species tested as measured by the results of protection or neutralization tests has not yet been fully determined.

5. VACCINES

5.1 Vaccines available

The vaccines listed in the following table are of established value and are available for immunization of both man and animals. They are discussed in sections 5.2.1 and 5.3.1. Vaccines under development for use in man are discussed in section 5.2.2; those under development or in limited use for the immunization of animals are discussed in section 5.3.2.

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 ^a	Flury, 40-60 egg passage	Chicken embryo	Dog	Guinea-pig
K ^a	Kelev, 60-70 egg passage	Chicken embryo	Dog and cattle	Guinea-pig, mouse
HEP ^a	Flury, above 180th egg passage	Chicken embryo	Cattle, cat and dog	Guinea-pig, mouse
Nervous tissue	Fixed	Central nervous system	Man, dog, cattle and other animals	NIH, Habel
Inactivated : Duck	Fixed virus	Duck embryo	Man	HIH, Habel
Nervous tissue	Fixed virus	Central nervous system	Man, dog, cattle and other animals	NIH, Habel

^a These vaccine strains should not be passaged in mice or other animals at any time.

5.2 Vaccines for immunization of man

5.2.1 Vaccines of established value (see table above)

Many different techniques have been used and continue to be used in the production of vaccines for immunization of man. Vaccines have been of two main types : those containing live attenuated virus and those containing

virus that has been inactivated by any one of a variety of agents. The trend over the last 50 years has been toward inactivated vaccines, because they lend themselves more easily to centralized production, they keep better under field conditions, especially if lyophilized, and they provide increased assurance of safety. Until recent years, the sole source of virus for vaccine production has been infected brain tissue — mostly goat, rabbit or sheep — and nervous tissue vaccines are still the most extensively used. The most common types of brain-tissue vaccine in use today are the Semple type, using virus completely inactivated by incubation at 37°C in the presence of phenol, and the Fermi type, incubated at 22°C in the presence of phenol and still containing residual infectious virus. More recently, virus grown in duck embryos and inactivated by beta-propiolactone has been widely used in the USA. In all instances, the Pasteur or similar strains of fixed virus have been used for production purposes.

Duck-embryo vaccine was developed in an attempt to eliminate those factors in brain-tissue vaccines responsible for post-vaccinal complications of the central nervous system. Evidence today indicates that although this hazard has been reduced it has not been completely eliminated. Other new vaccines now in established use have been developed with the same goal. The fact that the "paralytic factor" is not present in the nervous tissue of many species at birth but appears at some later stage of maturation prompted the production of vaccines from the brains of suckling animals. Thus, there has been limited use of a suckling-mouse-brain vaccine in which the virus is inactivated by ultraviolet irradiation and of a suckling-rat-brain vaccine prepared by the Fermi method. Besides being apparently free from the "paralytic factor", vaccines prepared from the brain of suckling animals have the added advantage of high potencies because of higher virus content in the donor animal. However, they offer some practical problems as regards mass production.

5.2.2 *Vaccines under development*

Several new types of vaccine are under investigation for possible use in the immunization of man, although they have not as yet been tested in field trials.

The attitude of the Committee is that any type of vaccine which passes the required safety and potency tests is eligible for field trial. New vaccines are considered "established" only when they have been found effective and safe after use in a large number of exposed individuals of various ages under field conditions.

The vaccines most extensively studied have been those produced in tissue cultures. They have been found in general to be more antigenic in animals such as the guinea-pig, horse and monkey than vaccines made

from the same strain of virus propagated in animals. Another advantage of tissue-culture vaccines lies in the fact that they contain appreciably smaller amounts of extraneous material than do organ-suspension vaccines (this is true also of a vaccine made in very young chick embryos). Extension of efforts to produce vaccines free from encephalitogenic properties has led to the use of newborn-rabbit-brain tissue as a substrate for virus growth. Vaccine so produced has been found to be antigenic in experimental investigations. Finally, partially purified rabies virus has been used to prepare an experimental vaccine capable of passing the standard potency test.

5.2.3 *Safety*

The Committee welcomed the new approaches aimed at the development of safe and potent human antirabies vaccines produced in tissue culture, since the vaccines at present used for the immunization of man are extremely crude biological products. The amount of protein present in currently used vaccines and responsible for untoward reactions is unquestionably greater than in any tissue-culture preparation.

Recognizing that tissue-culture vaccines may become available in the near future, the Committee recommends that any tissue-culture system for cultivation of rabies virus for production of human vaccine be of known origin, free of any contaminating material, and show no evidence of abnormal growth. Criteria of the presence of abnormal growth include spontaneous morphological transformation upon continuous cultivation *in vitro* of the same type of cells as were used for growth of the rabies virus, presence or development of marked karyological abnormalities, production of tumours in hamsters, etc. The Committee further recommends that WHO take active steps to develop minimum requirements for rabies vaccines. These requirements should specify the criteria to be fulfilled for growth of rabies virus for production of vaccines. Further, as it seems likely that a potent future tissue-culture vaccine may be of the live-virus type, it is recommended that the final product be thoroughly tested for specificity and for the absence of extraneous agents, and that the vaccine virus meet current minimum requirements of attenuation established for live-virus rabies vaccines.

With the advent of tissue-culture vaccines of other viruses for immunization of man, safety requirements have been made more stringent, and the Committee considers that these stricter requirements should now so apply to virus vaccines made from sources other than tissue culture. Obviously, normal healthy animals should be used in vaccine production. Specifically, it is recommended with respect to established human rabies vaccines that they should be free from extraneous agents and that the origin of seed virus used for production of vaccine should be well documented and its identity assured. In addition to the routine use of bacterial

sterility tests, consideration should also be given to the development and use of routine procedures for demonstrating the presence of such contaminants as mycoplasma, toxoplasma, or agents indigenous to the species of animal whose tissue is being used for vaccine production. Presence of these latter agents might be best detected by inoculation of an inactivated vaccine or specifically neutralized live-virus vaccine into the species in which the vaccine was produced, i.e., intracerebral inoculation of sheep-brain vaccine into sheep, etc. Brains of sheep or goats from areas where scrapie is known to be present should not be used for vaccine production.

The Committee considers that claims made that a vaccine contains only inactivated virus should be substantiated by adequate tests. It also considers that virus used for the preparation of such vaccines should be of low virulence when injected parenterally, as in the case of the Pasteur strain. The Committee calls attention to the danger of preparation of human rabies vaccine from strains of street virus and fixed virus of uncertain history and experience.

Since there is some evidence that fixed strains of virus can be lethal for man if injected in high enough concentration, the Committee recommends that vaccines of the Fermi type be assayed to determine the concentration of live rabies virus in the final product. No vaccine of such type should be released by the control authorities for general use if the titre of the rabies virus in weanling mice injected intracerebrally exceeds $10^{2.7}$ LD₅₀ per 0.03 ml.

5.2.4 Potency tests

The importance of establishing the potency of *every* batch of production vaccine before release needs continual emphasis and attention. No production laboratory can *assume* that its product is potent. A variety of types of potency test are now available as described in the second edition of the WHO monograph *Laboratory Techniques in Rabies* (in press). Although both qualitative and quantitative types of test are described quantitative tests should be used wherever possible.

While it can be assumed that a vaccine of proven potency in test animals will also be potent in man, as borne out by past experience, the possibility remains that a new type of vaccine may be of low antigenicity for humans. It is recommended that adequate serum-neutralization antibody responses be demonstrated in man before a newly developed vaccine is used in the field.

The stability of a vaccine is an important consideration affecting potency, since a vaccine must be potent at the time of use and not merely when first produced. It is strongly recommended that all production laboratories measure the potency of their vaccines under field conditions typical of the areas in which they will be used and determine the expiry dates of the

products on the basis on these results. The Committee would again like to emphasize that all types of vaccine, including phenolized vaccine, can now be safely lyophilized.

5.3 Vaccines for immunization of animals

5.3.1 *Vaccines of established value* (see table, section 5.1)

In this context, "established" vaccines are those that have been found safe and effective for pre-exposure use under field conditions in large numbers of animals of various ages and breeds. The table in section 5.1 lists those vaccines whose value has been established by careful experimental tests and extensive use in the field. There is, however, a clear need for better vaccines than those now available for domesticated livestock, notably cattle.

The vaccines discussed in section 5.2.1 are now widely employed in different countries throughout the world. This extensive practical experience must be taken into account before considering their replacement by new types of vaccine.

5.3.2 *Vaccines being developed*

Advances in tissue-culture techniques have stimulated the development of new vaccines for animals. Some of these have already been developed to the point where they have been accepted and licensed for use by certain national authorities. While recognizing the merits of some of these vaccines, the Committee considers that insufficient experimental and field experience has so far accumulated for these vaccines to be considered "of established value". Clearly demonstrable superiority with respect to safety, degree and length of protection conferred, ease of production, and stability under field conditions, should be required of new candidate vaccines. This is particularly true when considering new vaccines for use in dogs, since the vaccines at present available are very effective in these animals.

Vaccines under study or already being used to a limited extent include modified live-virus vaccines prepared on tissue cultures from chicken embryos, hamster kidney, swine kidney and dog kidney, and on ultra-violet-light-inactivated vaccine produced in suckling-mouse brain. Some of these vaccines are discussed in section 7.2.

5.3.3 *Safety tests*

In addition to carrying out the safety tests normally prescribed for rabies vaccines (see the WHO monograph *Laboratory Techniques in Rabies*, second edition (in press)), several precautions should be taken before vaccines are accepted for routine immunization purposes in animals.

1. It is important to determine whether or not the living modified strain used in non-inactivated vaccine is excreted in the salivary glands of the species of animal for which the vaccine is designated. Since the pathogenicity of various modified strains of rabies virus for man is not known, excretion of such a strain in the saliva of the inoculated animal might create a public health hazard.

2. Animals of all age groups, especially animals under three months of age, should be used in experimental tests to determine the safety of live-virus vaccines.

3. Observations should be made to determine possible deleterious effects following administration of live-virus vaccines to pregnant animals.

4. The use of homologous serum in tissue-culture medium for growth of virus should be avoided, in order to prevent possible dispersion of infectious agents that may be present in the serum of donor animals, i.e., bovine serum should not be used in vaccines intended for use in cattle. The same consideration applies to the use of primary cell-culture systems, i.e., dog kidney-tissue culture, for vaccines prepared for use in dogs.

5. The route of inoculation of the vaccine should be clearly defined, e.g., subcutaneous, intramuscular, neck or thigh region, etc.

6. Tests for the absence of contaminants, such as mycoplasma, toxoplasma or other extraneous agents, should include intracerebral inoculation of an inactivated vaccine, or specifically neutralized live-virus vaccine, into the species in which the vaccine was produced. The problem of scrapie is referred to in section 5.2.3.

7. With living modified-virus vaccines, it is important to determine the maximum concentration of virus per dose that meets the safety requirements discussed previously. This maximum should not be exceeded in production lots because ample evidence is now available that pathogenicity is often correlated with the amount of rabies virus inoculated into an animal.

5.3.4 *Potency tests*

Standard potency tests for living and inactivated vaccines and the general principles underlying them are described in the WHO monograph *Laboratory Techniques in Rabies* (second edition in press). These principles and tests should be carefully followed. Several proposals have been made for the use of different criteria for potency tests, such as the infectivity titre of living modified vaccines and the capacity to produce serum-neutralizing antibodies. The Committee emphasizes that such tests cannot be accepted as a substitute for potency tests involving direct challenge of vaccinated animals with rabies virus. Only by the latter procedure is it

possible to obtain firm assurance of specificity and efficacy of protection by a given vaccine.

No vaccine should be approved for use in the field—unless an adequately designed experiment demonstrates a duration of immunity of at least one year in the species of animal for which the vaccine is to be used.

5.4 International reference vaccine

The Committee examined the protocols of tests on a stabilized rabies vaccine proposed for use as an international reference vaccine and recommends its establishment as such. However, it is recommended that periodic checks of stock supplies be made to assure that potency does not fall below the acceptable level.

National laboratories are urged to prepare their own stock of reference vaccine which, after comparison with the International Reference Vaccine, could be used to supply routine production laboratories within a country. 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 as described in the WHO monograph *Laboratory Techniques in Rabies*. It is hoped that all national laboratories will use the reference vaccine in this manner.¹ 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 vaccine.

6. PREVENTION OF RABIES IN MAN

6.1 General considerations

Before any decision can be made regarding treatment of the exposed person, information must be available as to whether the biting animal was rabid. An important preliminary step to obtaining this information may be the confinement and observation of the biting animal. This should be for a period of 10 days, except in the case of bats or unmanageable wild species, and preferably under the supervision of a veterinarian.

Initiation of treatment in exposed persons should never await the results of laboratory diagnosis; in any event, local treatment is usually completed and serum and/or vaccine therapy instituted before the laboratory report is received. A report from a reliable laboratory indicating absence of rabies (see section 4) usually justifies cessation of treatment. However, in

¹ The minimum acceptable antigenic value of a test vaccine in comparison with the reference vaccine will be indicated when reference vaccine is issued.

some circumstances the physician may be justified in continuing or even initiating treatment despite such a report.

The Committee wishes to emphasize the value of the local treatment of wounds infected with rabies virus, including first-aid procedures and possibly the use of high-titre antirabies sera or their globulin fractions, both applied topically and infiltrated around the wound.

The Committee expressed the conviction that the combined administration of antirabies serum and vaccine, together with local treatment, provides the best possible prophylaxis of rabies in an exposed person. It is therefore strongly recommended that this procedure be adopted in all cases of severe exposure. It is not intended to suggest, however, that its use is not justified in all genuine cases of exposure, even of a mild nature. On the other hand, it must be recognized that long-term experience has shown the use of a potent vaccine alone to be quite effective in mild exposures. It is important to realize also that deleterious reactions are possible following the use of either antirabies serum or antirabies vaccine and that the combined treatment is more expensive.

The blocking by antirabies sera of the active antibody response to vaccine has been confirmed. In view of this and other findings, slight revisions have been made to the *Guide for Post-Exposure Treatment* published in the fourth report of the Committee (see Annex 1).

Improvements in the safety and effectiveness of rabies vaccines continue to be made. On the evidence of studies to date, vaccines of tissue-culture origin for human use appear feasible and offer potential advantages over existing products. However, vaccines of nervous-tissue or avian-embryo origin will of necessity be the vaccines available, at least during the immediate future. Clinical trials aimed at reducing the number of doses (usually between 14 and 21) of existing vaccines have been unsuccessful. Factors involved in pre-exposure immunization and the advisability of a subsequent booster dose at the time of exposure or to maintain a satisfactory level of immunity have been further evaluated and the relevant recommendations made in the fourth report have been amended.

6.2 Local treatment of wounds

Prompt and adequate treatment of all bite wounds and scratches possibly contaminated with rabies virus is of paramount importance. When medical assistance is not promptly available, immediate first-aid procedures are recommended, including the flushing and washing of the wound with soap and water, detergent or other harmless fluids. Treatment by the physician should, where indicated, include adequate cleansing of the wound, thorough flushing and scrubbing with 20% soap solution and/or the application of a quaternary ammonium compound or other substance of proven lethal effect on rabies virus (see Annex 1). Although judicious use of

concentrated nitric acid in puncture wounds has its advocates, there is no evidence that this product is more effective than quaternary ammonium compounds or 20% liquid soap solution. Where possible bite wounds should not be immediately sutured.

High-titre antirabies serum and its globulin fractions in liquid or powdered form used topically have been shown to be highly effective in preventing rabies in experimental animals. Their use should be considered in all cases of human exposure. Sensitivity to serum should be determined prior to its use. In patients the nature of whose exposure merits use of antirabies serum (see section 6.4), a part of the serum dose should be infiltrated into the tissue around the wound whenever feasible.

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

6.3 Vaccine

Practice varies concerning the volume of vaccine per dose and the number of doses recommended in a given situation. In general, the equivalent of at least 2 ml of a 5% tissue emulsion should be given subcutaneously for 14 consecutive days. Many laboratories use 20 to 30 doses in severe exposures. The Committee felt so strongly concerning the effect of booster doses in producing and maintaining high levels of serum-neutralizing antibodies that it now recommends booster doses at 10 days and at 20 or more days following the last daily dose in *all* cases. This is especially important if antirabies serum has been used, in order to overcome the interference effect (see section 6.4).

6.4 Antirabies serum

Antirabies serum and its globulin fractions are of proven effectiveness in preventing rabies. Potent antirabies serum has been produced in horses, mules, donkeys and other animals using different methods of hyperimmunization and of purifying and concentrating the resulting serum. It is suggested that the antigen used for immunizing the animals should be of tissue-culture origin or from animals of a species different from that used for the preparation of vaccine for human use. The species used to prepare antigen for the production of antirabies serum should be stated on the label of the product when dispensed.

It is recommended that antirabies serum be given as promptly as possible after exposure. However, serum should be administered in all cases where this is practicable, irrespective of the interval between exposure and treatment. Serum should be administered intramuscularly in a single dose of not less than 40 International Units per kilogram of body

weight and followed by a course of not less than 14 doses of vaccine. Because the administration of serum interferes with the production of measurable antibody induced by vaccine, booster injections of vaccine are recommended at 10 days, and at 20 days or longer after completion of the vaccine schedule.

6.5 Guide for post-exposure treatment

The *Guide for Post-Exposure Treatment* published in the fourth report of the Committee was slightly revised to take account of recent findings, especially the confirmation that antirabies serum blocks the active antibody response to vaccine. In addition, instructions were added for the local treatment of wounds involving possible exposure to rabies in accordance with the principles laid down in section 6.2. The revised *Guide* will be found in Annex 1 to the report (page 33).

6.6 Pre-exposure immunization

Persons who run an unusually high risk of repeated exposures, such as laboratory staff working with rabies virus, veterinarians, dog handlers, field naturalists, etc., should be protected by pre-exposure immunization. Such immunization may consist of a short course of 2 to 3 injections of a potent antirabies vaccine, preferably of a non-nervous-tissue type, at one month intervals, followed by a booster injection of vaccine 6 months later. The presence of serum-neutralizing antibodies in vaccinated individuals should be ascertained on a serum sample drawn one month after the booster injection. If negative, booster doses should be repeated until antibodies become demonstrable. Further booster injections should be administered at intervals of one to three years as long as the exposed person remains at risk.

Sufficient information is not available to permit firm recommendations as to the best procedure to follow when an immunized person who has demonstrated an antibody response in the past is exposed to rabies. The following recommendations are based on immunological principles: one booster dose of vaccine in the case of mild exposure or 5 daily doses of vaccine followed by a booster dose twenty days later for severe exposures.

6.7 Complications of antirabies treatment

6.7.1 Antirabies serum

Reactions to antirabies horse serum, even though the serum is concentrated and purified, occur with approximately the same frequency as with other sera of animal origin. Careful questioning about a past history of allergy and the routine use of an intradermal or ophthalmic test for

sensitivity will avoid the risk of an immediate anaphylactic type of reaction. If the sensitivity test should be positive, the usual precautions of desensitization should be followed and, if possible, serum produced in another species should be used.

Serum sickness occurs in approximately 15-25% of persons given heterologous serum of horse origin and is less frequent in those below 15 years of age. Incidence can be reduced by the administration of a large dose of an antihistamine drug with the serum and further sustaining doses for several days afterwards. Persons from families with a history of connective-tissue disease, such as generalized chronic arthritis or lupus, may require special consideration. In persons sensitive to horse serum it is considered advisable to use serum produced in other species.

The Committee notes with interest the possibility that rabies immune human gamma-globulin may become available for the prevention of rabies.

6.7.2 *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 of these apparent differences, but any consideration must take into account such host factors as the population involved, physiological state of the individual, species of animals used in vaccine production, method of inactivation of vaccine, and dosage schedule. The incidence of these complications is sufficiently high in certain areas of the world to justify further efforts to eliminate the factors responsible.

When signs of neuroparalytic accidents develop, the following possible procedures are suggested, although scientific evidence of their effectiveness is not available. If, in view of the degree of original exposure, the amount of immunization already obtained is considered adequate, further vaccine administration may be discontinued; if further immunization is indicated, vaccine prepared from non-nervous tissue should be used in place 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 that will produce minimum reaction in the vaccinated individual.

6.7.3 *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. Local reactions, including lymph adenopathy, commonly occur when duck-embryo vaccine is used, and neuroparalytic or other severe generalized reactions may also occur, but less frequently than with nervous-tissue vaccines.

7. CONTROL OF RABIES IN ANIMALS

7.1 Introduction

Resolution of the rabies problem in man and animals depends on the control and eventual elimination of the disease from reservoir and vector populations in nature. Practical success depends on the carrying out of carefully planned, well-executed programmes and the utilization of effective vaccines and control procedures for the common vector species. The task has been made more complex in many areas by the realization that rabies exists in many species in which control or eradication is difficult, e.g., the bat, fox, skunk or mongoose.

7.2 Specific immunization

7.2.1 *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.

The intramuscular administration of LEP Flury vaccine produced in developing chicken embryos¹ remains the prophylactic procedure of choice in dogs three months of age or older; it produces immunity lasting at least three years. The effectiveness of this vaccine was proved in controlled experiments when a dose of 3 ml of a 33% suspension of infected chicken embryo was used; it is therefore felt that this amount of vaccine should be the minimum dose if an effective immunity of three years is desired. There is evidence from controlled experiments that HEP Flury vaccine produced in developing chicken embryos¹ protects dogs for 2 years. Other living-virus and inactivated-virus vaccines (see vaccine table, section 5.1) found to be antigenic by careful laboratory and field studies may be used for immunization of dogs.² However, the Committee recommends annual revaccination of dogs inoculated with the latter vaccines, since only limited data are at present available indicating that they may confer immunity of longer duration.

Puppies less than three months old can be vaccinated safely with HEP Flury or Kelev chicken-embryo live-virus vaccine^{1, 3} or with any of the inactivated virus preparations, but not with LEP Flury vaccine¹ prepared

¹ It should be pointed out that these vaccines are prepared from whole chicken embryos and should be distinguished from vaccines made from infected chicken-embryo tissue cultures.

² The dosage of inactivated nervous-tissue vaccine for both puppies and adult dogs is 5 ml of 20% tissue suspension or its equivalent.

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

in developing chicken embryos. Experimental evidence indicates that puppies less than three months old may not develop an immunological response to rabies vaccination as readily as older dogs; it is therefore recommended that a puppy inoculated 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.

After careful examination of the experimental and field data relating to the vaccination of dogs with LEP vaccine prepared in developing chicken embryos,¹ the Committee reaffirms 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 the alleged street-virus carrier state in exposed dogs immunized with rabies vaccine.

Modified live-virus vaccines produced in a variety of tissue-culture systems for dogs are at present in the developmental stage (see section 5.2.2) and show promise of successful prophylactic use. In preliminary reports available to date, there is no information on duration of immunity beyond one year following experimental use of these vaccines. It is emphasized that adequately controlled experiments on longer duration of immunity in dogs are needed before consideration can be given to the use of these vaccines to replace established vaccines that induce immunity of known duration.

7.2.2 *Cats*

Cats may be effectively immunized with either nervous-tissue vaccine or the HEP (Flury) strain vaccine produced in developing chicken embryos. Some vaccines of tissue-culture origin have been shown to be safe and effective, but as with other types of vaccine evidence is not available concerning the duration of immunity induced. The dosage of vaccines for cats is about one-half the dog dose, i.e., 1.5 ml of HEP vaccine¹ or 3 ml of a 20% nervous-tissue suspension of inactivated vaccine. Cats should be revaccinated annually.

LEP (Flury) strain vaccine¹ should not be used in cats, since it may in some cases prove pathogenic.

7.2.3 *Cattle*

Rabies in cattle remains a serious economic problem in certain areas of the world, notably in Latin America. Such circumstances offer unique opportunities for the evaluation of vaccines under development.

The HEP Flury and Kelev strains of modified live virus produced in developing chicken embryos,¹ or inactivated vaccines, are recommended

¹ See footnote 1, page 21.

for pre-exposure vaccination of cattle. HEP Flury vaccine is given intramuscularly into the thigh, using a minimum dosage of 5 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 a demonstrable antibody response in most vaccinated cattle, a second dose of vaccine given 30 days after the first may result in a booster effect and enhance protection. For inactivated nervous-tissue vaccines, 30 ml of a 33% suspension of tissue, or an approximately equivalent quantity (e.g., 15 ml of a 60% suspension), is recommended. Booster injections are advisable.

Although the HEP Flury strain¹ elicits an antibody response, problems continue to be reported widely in Latin America, when this product is used under field conditions. Not only are repeated injections of vaccine required, but vaccination failures are frequently reported. It is not known whether vaccination failures are due to lack of vaccine potency, instability of vaccine under field conditions, or faulty inoculation technique. A more effective vaccine for use in cattle is still needed.

No carefully controlled studies have yet been made concerning post-exposure treatment of cattle. Such studies should be undertaken as soon as possible.

Since, excluding occupational hazard, rabies in cattle is primarily an economic rather than a public health problem, greater latitude is possible in evaluating developmental vaccines than in other species, such as the dog. Carefully controlled and evaluated field trials are to be encouraged as, for example, with the ERA strain of modified live virus produced in swine-kidney tissue culture, which has shown good results in preliminary animal experiments.

In view of the economic importance of rabies in cattle and the need for more information on the relative merits of different vaccines, the Committee recommends that carefully designed experiments be undertaken by appropriate national and international authorities to elucidate this problem.

All vaccines for cattle should meet the safety requirements discussed in section 5.3.3.

7.2.4 *Other species*

Insufficient data are available to permit recommendations to be made with respect to the efficacy of vaccines in other species of animals. Caution is necessary, since species may vary widely as regards susceptibility to the various strains of rabies virus. For example, LEP Flury vaccine produced in chicken embryos has been widely used with safety in adult dogs, but has been shown to be hazardous for foxes, skunks and cattle. The Committee

¹ See footnote 1, page 21.

strongly recommends, therefore, 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. Vaccines containing inactivated virus may be safely used in most if not all species, although the dosage required and the degree and duration of immunity elicited are largely unknown.

7.3 General control procedures

7.3.1 *Mass immunization of dogs and cats*

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 during an epizootic may be lifted 30 days following vaccination.

With regard to methods to 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 in 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 5.2.4).

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.

Since rabid cats may present a serious problem, cat owners are encouraged to have these pets immunized. Furthermore, there is no reason why cats should not be included in canine mass immunization programmes if deemed practicable. There is no evidence, however, that rabies persists among cats in areas where canine and wildlife rabies have been eliminated.

7.3.2 *Elimination of stray dogs*

The stray or ownerless dog remains a threat in the transmission of rabies and therefore an efficiently conducted programme for the elimination

of these animals is necessary. Such a programme requires the operation of a local pound or animal shelter in which strays may be temporarily held, and if unclaimed at the end of a short period, destroyed. The practice of making stray impounded animals available for adoption as pets, should be suspended during outbreaks of rabies in a community. Prior to release, rabies vaccination and registration should be carried out. Personnel responsible for the programme must be properly equipped and trained in the care and management of animals, the basic principles of animal disease control and first-aid procedures.

7.3.3 *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 the support of livestock organizations, animal protection societies, kennel clubs 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 and interpretation of findings, in close collaboration with the diagnostic laboratory, and providing information on 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.

7.3.4 *Handling of dogs and cats bitten by rabid animals*

The Committee strongly urges that non-vaccinated dogs, cats and other pets bitten by a known rabid animal should be immediately des-

troyed. If the owner is unwilling to have this done, the animal should be vaccinated and placed in strict isolation for four months or longer.

If the animal has been previously vaccinated within three years with the LEP Flury strain of vaccine,¹ within 2 years with the HEP strain of vaccine,¹ or within one year with other vaccines,² revaccination and restraint (leashing and confinement) for 30 days should be carried out.

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

7.3.5 *International transfer of dogs and cats*

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.

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

(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 four or more months, at the port of entry. If the quarantine period is only four months, leashing and surveillance for an additional two months are recommended.

(2) In countries free of rabies but where prolonged quarantine measures cannot be invoked, measures 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 measures are recommended :

(*a*) Dogs should be vaccinated more than one month but less than 36 months before departure with LEP vaccine,¹ within 1-24 months with HEP vaccine,¹ or within 1-12 months with other vaccines.² Cats should be vaccinated more than one month but less than 12 months before departure (see section 7.2.2). Certificates signed by the appropriate veterinary authorities in the country of origin should accompany each animal (see Annex 2). Where doubt exists with respect to the

¹ See footnote 1, page 21.

² See footnote 2, page 21.

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.

8. WILDLIFE RABIES

8.1 Terrestrial animals

Rabies exists in two epidemiological forms: (a) the urban type propagated principally in dogs; and (b) wildlife rabies, particularly in foxes, jackals, wolves, coyotes, skunks, mongooses (*Herpestes* spp. and *Cynictis penicillata*), weasels and bats.

The current widespread epizootic of rabies in wildlife in Europe, Asia, Africa, North and South America is an example of the cyclical character of diseases derived from wildlife. About 100 years ago there was a similar world-wide outbreak of rabies. Wildlife rabies is transmitted to domestic animals more often than *vice versa*. Rabies recurs in particularly well defined areas in countries in which the disease is enzootic. It has been observed that outbreaks of rabies in wildlife develop most often in such enzootic foci when the epidemic wildlife vector species has become unusually abundant. It is the sporadic interepidemic cases of rabies in wildlife that must be investigated carefully if ways are to be found to prevent outbreaks of wildlife rabies.

The search for rabies virus in surveys of small wild rodents in enzootic rabies areas has revealed no incidence of rabies virus 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 wildlife rabies control. The choice of techniques for carrying out these programmes depends upon local conditions. For many species, poisoning and gassing is far more efficient and certainly more economical than trapping and shooting. 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 thus minimizing or 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 it can be used as an adjunct to

the other methods, such as trapping, poisoning and shooting. Trapping, although more expensive than the use of poisoned baits and not as efficient, is still the method of choice in most areas because it is safer than poisoning. It should be emphasized that, for maximum effectiveness, a programme utilizing these methods should be directed by professionally trained predator-control specialists.

Studies are in progress aimed at finding feasible means for interrupting the reproduction cycle of wild carnivores as a possible method for controlling population densities. It should be pointed out that the predator-control methods at present under development or being tested in the field are not directed at the extinction of the vector species but are designed to reduce population levels to a threshold that will not support an epizootic of rabies.

8.2 Bats

Vampire-bat rabies continues to be a major problem in Latin America. Since the vampire bat feeds only on blood, this animal has become abundant in tropical regions where large cattle ranches furnish a ready source for the blood meal. The natural dynamics of vampire-bat-transmitted rabies is unique in that transmission occurs as a part of the normal feeding habits of this bat. Affected cattle exhibit a characteristic paralytic disease syndrome. Although the vampire bat of parts of Latin America normally dies of the rabies infection, there is experimental evidence that some animals are symptomless carriers and can transmit rabies over a period of several months. Vampire-bat rabies is the major cause of death in cattle of Latin America and has proved a major obstacle to the expansion of its agricultural economy. There have been 160 human rabies deaths attributed to vampire-bat bites reported from 6 countries since the Trinidad outbreak of 1929-1935.

Mass vaccination of cattle has been used as the principal weapon in control. Attempts have also been made to control the disease by the gassing and shooting of vampire bats in their diurnal resting places and by the use of trapping devices. Success of such measures in reducing vampire bat populations depends upon their application in a well organized community effort.

Rabies in insectivorous bats, first discovered in the USA in 1953, has continued to be reported and is distributed ubiquitously throughout North America. Five cases of bat-transmitted rabies in man have been reported in the USA, the last death occurring five years ago. In contrast to vampire-bat rabies in Latin America, natural transmission of rabies from insectivorous bats to terrestrial animals by biting has not been observed to date, except in man. It has been extremely difficult in the laboratory to transmit rabies by inducing known rabid bats with infectious saliva to bite other

susceptible animals. Two persons are reported to have developed rabies in the absence of known exposure to biting bats after entering a cave harbouring millions of bats. Coyotes and foxes held for one week or longer in bat-proof and arthropod-proof cages in this cave also contracted rabies thus demonstrating non-bite transmission of rabies. It should be emphasized that these observations were made only in bat caves of south-western USA where vast numbers of infected bats have been found. There is no reason to believe that this phenomenon is likely to occur outside such cave environments. Although a few cases of bat rabies have been reported from Europe (Yugoslavia and Turkey) and a human rabies death attributed to bat exposure occurred in India, bat rabies has not become a problem in the eastern hemisphere. Surveys of bats for rabies during the past five years in Europe, Asia and Africa have yielded negative results. Control work is largely limited to warning the public not to pick up or handle sick or strangely behaving bats.

9. EXCHANGE AND DISSEMINATION OF INFORMATION ON RABIES

9.1 Collection of statistics

The Committee noted with satisfaction that the yearly world survey of rabies had been regularly carried out and distributed by WHO since 1959 and that the interest and participation of rabies workers, institutes and medical and veterinary services has been steadily increasing. Ninety-seven countries and territories participated in the fifth survey as against 89 in the first. The replies not only indicate increasing application of measures recommended by the Committee in combating the disease but also the adoption of recommended procedures in the laboratory and in the field. This useful activity should be continued and developed further to focus attention on specific problems. For example, the collection of more detailed information concerning vaccine failures will in due course contribute to a better understanding of this important problem. However, the simplicity of the present questionnaire should be maintained in order to hold the interest of participating countries. The Committee noted also the joint and individual efforts of the Food and Agriculture Organization of the United Nations, the International Office of Epizootics, and WHO in the collection and dissemination of statistics on rabies in animals and man.

A logical extension of the foregoing activities would be to collect and disseminate information on trends in the progress or regression of rabies in regions in which it is endemic. This might involve planned surveillance in infected areas with special attention to wildlife studies. Such a collabo-

rative surveillance programme might be started in a selected region and extended to other areas or regions after preliminary difficulties in regard to methodology had been solved. The Committee urges countries in endemic regions that have adequate facilities to collaborate with WHO in the development of a rabies surveillance programme.

The case-record form recommended by the Committee, which has proved very useful in the compilation of rabies statistics, is reproduced in Annex 3. The information gained from the careful keeping of such records is of great use not only to the recording institute but also to national and international authorities concerned with rabies. Since many problems regarding the prevention and treatment of paralytic accidents, serum sickness, and the efficacy of post-exposure treatment remain to be solved, the periodic compilation and analysis by WHO of results obtained in different countries will be of great value in assessing the effectiveness of the measures employed. The Committee urges, therefore, that the case-record form reproduced in Annex 3, or a suitable modification, be used in all antirabies treatment centres and that close collaboration be maintained with WHO.

9.2 Exchange of information

The Committee noted that information documents on new developments in rabies have been distributed to rabies workers and institutes as recommended in the fourth report.¹ Rabies workers responsible for rabies control are urged to send their reports promptly to WHO so that any new introduction or reappearance of rabies in their country and other developments can be communicated widely.

9.3 Regional and inter-regional technical seminars

The Committee noted with satisfaction that WHO has continued to hold seminars on rabies at which leading rabies workers from various countries discuss problems of rabies control and learn the newest laboratory techniques and procedures. The symposium held in Moscow in 1964 and attended by participants from 20 countries included instruction in these techniques as recommended in the fourth report.¹ This useful activity should be continued. It would also be useful to hold discussion and training programmes applicable to the surveillance and control of rabies in wildlife.

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1960, 201.

10. FUTURE RESEARCH

10.1 Basic studies

The following studies are recommended :

(a) investigation of the possible use of interference between infection with rabies virus and infection with other viruses as a tool in basic studies of rabies virus, especially in tissue culture ;

(b) the application of various biochemical and biophysical methods in an effort to purify and concentrate rabies virus of brain and tissue-culture origin ;

(c) experiments to examine the quantitative correlation between virus-neutralizing antibody titres in mice inoculated intracerebrally or intraperitoneally and virus-neutralizing antibody titres in tissue-culture preparations ;

(d) comparison of the efficiencies of intracerebral inoculation of adult and suckling mice and inoculation of tissue cultures for primary virus isolation from diagnostic and field specimens ;

(e) the application of all possible procedures for increasing virus yield in tissue cultures ;

(f) definitive studies on various types of specific antigens and the corresponding antibodies produced in rabies virus infection, using a spectrum of tests and taking into account the time between infection and appearance of the antibodies.

(g) further studies on pathogenesis, with special emphasis on the possible role of inapparent infection and the carrier state in animals.

10.2 Immunization procedures and treatment

The following lines of research are suggested :

(a) investigations on tissue-culture vaccines in man and animals (these should be given high priority) ;

(b) further investigation of nerve-blocking drugs as preventive measures ;

(c) the relative efficacy of homologous versus heterologous antirabies serum in serum prophylaxis and the factors involved ;

(d) properties of virus strains isolated from serum-vaccine failures ;

(e) the effect of maternal antibody on the immunizing capacity of puppies and young animals of other species ;

(f) the pre-exposure immunization of laboratory workers, which offers a special opportunity to compare the antibody-producing ability of various vaccines ;

(g) the value of chemotherapeutic or chemoprophylactic substances possibly effective against rabies virus.

10.3 Ecology and control

The following research is required :

(a) a thorough investigation of interepidemic sporadic cases of rabies for virus-host relationship, distribution of virus in organs, etc. ;

(b) evaluation of anti-fertility agents to determine their effectiveness in controlling vector populations ;

(c) study of possible immunization procedures for wild animal species.

10.4 Future meetings

The special research groups that met in 1961 and 1963 were of great importance in forwarding collaborative investigations on specific aspects of rabies. The Committee therefore recommends that WHO give consideration to convening meetings of similar research groups periodically during the next few years.

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Annex 1**GUIDE FOR POST-EXPOSURE TREATMENT ***

The recommendations given overleaf are intended only as a guide. It is recognized that in special situations modifications of the procedures laid down may be warranted. Such special situations include exposure of young children and other circumstances where a reliable history cannot be obtained, particularly in areas where rabies is known to be enzootic even though the animal is considered to be healthy at the time of exposure. Such cases justify immediate treatment, but of a modified nature, for example local treatment of the wound as described overleaf followed by administration of a single dose of serum or three doses of vaccine daily; provided that the animal stays healthy for 10 days following exposure, no further vaccine need be given. Modification of the recommended procedures would also be indicated in a rabies-free area where animal bites are frequently encountered. In areas where rabies is endemic, adequate laboratory and field experience indicating no infection in the species involved may justify local health authorities in recommending no specific antirabies treatment.

* It is strongly recommended that this guide be reproduced only in its entirety.

A. Local Treatment of Wounds Involving Possible Exposure to Rabies

(1) **Recommended in all exposures**

(a) *First-aid treatment*

Immediate washing and flushing with soap and water, detergent or water alone (recommended procedure in all bite wounds including those unrelated to possible exposure to rabies).

(b) *Treatment by or under direction of a physician*

- (i) Adequate cleansing of the wound.
- (ii) Thorough treatment with 20% soap solution and/or the application of a quaternary ammonium compound or other substance of proven lethal effect on the rabies virus.¹
- (iii) Topical application of antirabies serum or its liquid or powdered globulin preparation (optional).
- (iv) Administration, where indicated, of antitetanus procedures and of antibiotics and drugs to control infections other than rabies.
- (v) Suturing of wound not advised.

(2) **Additional local treatment for severe exposures only**

- (a) Topical application of antirabies serum or its liquid or powdered globulin preparation.
- (b) Infiltration of antirabies serum around the wound.

¹ Where soap has been used to clean wounds, all traces of it should be removed before the application of quaternary ammonium compounds because soap neutralizes the activity of such compounds.

Benzalkonium chloride, in a 1% concentration, has been demonstrated to be effective in the local treatment of wounds in guinea pigs infected with rabies virus. It should be noted that at this concentration quaternary ammonium compounds may exert a deleterious effect on tissues.

Compounds that have been demonstrated to have a specific lethal effect on rabies virus *in vitro* (different assay systems in mice) include the following :

Quaternary ammonium compounds

- 0.1% (1 : 1000) benzalkonium chloride = mixture of alkylbenzyltrimethylammonium chlorides
- 0.1% (1 : 1000) cetrimonium bromide = hexadecyltrimethylammonium bromide
- 1.0% (1 : 100) Hyamine 2389 = mixture containing 40% of methyl dodecylbenzyltrimethylammonium chloride and 10% of methyl dodecylxyllylene bis(trimethylammonium chloride)
- 1.0% (1 : 100) methyl benzethonium chloride = benzyl dimethyl[2-(2-[p-(1,1,3,3-tetramethylbutyl)tolyl]oxy)ethoxy]ethyl ammonium chloride
- 1.0% (1 : 100) benzethonium chloride = benzyl dimethyl[2-(2-[p-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy)ethyl] ammonium chloride
- 1.0% (1 : 100) SKF 11831 = p-phenylphenacylhexamethylenetetrammonium bromide.

Other substances

43-70% ethanol ; tincture of thiomersal ; tincture of iodine and up to 0.01% (1 : 10000) aqueous solutions of iodine ; 1% to 2% soap solutions.

B. Specific Systemic Treatment

Nature of exposure	Status of biting animal (irrespective of whether vaccinated or not)		Recommended treatment
	At time of exposure	During observation period of ten days	
I. No lesions ; indirect contact	Rabid	—	None

<p>II. Licks :</p> <p>(1) unabraded skin</p> <p>(2) abraded skin, scratches and unabraded or abraded mucosa</p>	<p>Rabid</p> <p>(a) healthy</p> <p>(b) signs suggestive of rabies</p> <p>(c) rabid, escaped, killed or unknown</p>	<p>—</p> <p>Clinical signs of rabies or proven rabid (laboratory)</p> <p>Healthy</p> <p>—</p>	<p>None</p> <p>Start vaccine ¹ at first signs of rabies in the biting animal</p> <p>Start vaccine ¹ immediately; stop treatment if animal is normal on fifth day after exposure</p> <p>Start vaccine ¹ immediately</p>
<p>III. Bites :</p> <p>(1) mild exposure</p> <p>(2) severe exposure (multiple, or face, head, finger or neck bites)</p>	<p>(a) healthy</p> <p>(b) signs suggestive of rabies</p> <p>(c) rabid, escaped, killed or unknown</p> <p>(d) wild (wolf, jackal, fox, bat, etc.)</p> <p>(a) healthy</p> <p>(b) signs suggestive of rabies</p> <p>(c) rabid, escaped, killed or unknown</p> <p>(d) wild (wolf, jackal, pariah dog, fox, bat, etc.)</p>	<p>Clinical signs of rabies or proven rabid (laboratory)</p> <p>Healthy</p> <p>—</p> <p>—</p> <p>Clinical signs of rabies or proven rabid (laboratory)</p> <p>Healthy</p>	<p>Start vaccine ^{1, 2} at first signs of rabies in the biting animal</p> <p>Start vaccine ¹ immediately; stop treatment if animal is normal on fifth day after exposure</p> <p>Start vaccine ^{1, 2} immediately</p> <p>Serum ² immediately, followed by a course of vaccine ¹</p> <p>Serum ² immediately; start vaccine ¹ at first sign of rabies in the biting animal</p> <p>Serum ² immediately, followed by vaccine; vaccine may be stopped if animal is normal on fifth day after exposure</p> <p>Serum ² immediately, followed by vaccine ¹</p>

¹ Practice varies concerning the volume of vaccine per dose and the number of doses recommended in a given situation. In general, the equivalent of at least 2 ml of a 5% tissue emulsion should be given subcutaneously daily for 14 consecutive days. Many laboratories use 20 to 30 doses in severe exposures. To ensure the production and maintenance of high levels of serum-neutralizing antibodies, booster doses should be given at 10 days and at 20 or more days following the last daily dose of vaccine in all cases. This is especially important if antirabies serum has been used, in order to overcome the interference effect.

² In all severe exposures and in all cases of unprovoked wild animal bites, antirabies serum or its globulin fractions together with vaccine should be employed. This is considered by the Committee as the best specific treatment available for the post-exposure prophylaxis of rabies in man. Although experience indicates that vaccine alone is sufficient for mild exposures, there is no doubt that here also the combined serum-vaccine treatment will give the best protection. However, both the serum and the vaccine can cause deleterious reactions. Moreover, the combined therapy is more expensive; its use in mild exposures is therefore considered optional. As with vaccine alone, it is important to start combined serum and vaccine treatment as early as possible after exposure, but serum should still be used no matter what the time interval. Serum should be given in a single dose (40 IU per kg of body weight) and the first dose of vaccine inoculated at the same time. Sensitivity to the serum must be determined before its administration.

Annex 2

**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 as declared by owner (give dates) { _____

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

Dose _____ Route of administration _____

Owner of animal _____ Address _____

(Signed) _____

Veterinarian

Address _____

Date _____

Annex 3

SUGGESTED CASE RECORD FOR HUMAN RABIES EXPOSURE

Case No. _____ Referred by _____

Person bitten

Name _____ Date of bite _____

Age _____ Geographical locality of biting episode _____

Sex _____

Home address _____ Site(s) of bite on the body _____

Nature of bite : _____

Single	<input type="checkbox"/>	Mild	<input type="checkbox"/>
Multiple	<input type="checkbox"/>	Moderate	<input type="checkbox"/>
		Severe	<input type="checkbox"/>

Other persons bitten by the same animal, if any _____

Treatment

Local wound treatment _____

<i>Vaccine</i>	<i>Serum</i>
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 <input type="checkbox"/> Negative <input type="checkbox"/>
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	<input type="checkbox"/>	
Died of rabies	<input type="checkbox"/>	Date of death _____
Died of other causes	<input type="checkbox"/>	
Unknown	<input type="checkbox"/>	

Status of other persons bitten by the same animal if known _____

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 :

Results of laboratory examination :

Signs of rabies

Positive Negative

Healthy Negri bodies Died without signs
of rabies Animal inoculation Other (fluorescent
antibody, etc.)