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**EXPERT COMMITTEE ON
RABIES**

Third Report

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WORLD HEALTH ORGANIZATION

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EXPERT COMMITTEE ON RABIES

Third Session

Paris, 26 November - 1 December 1956

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EXPERT COMMITTEE ON RABIES

Third Report *

1. INTRODUCTION

The Expert Committee on Rabies held its third session at the Institut Pasteur, Paris, from 26 November to 1 December 1956. Dr P. Lépine was elected Chairman, Dr A. Kemron, Vice-Chairman, and Dr H. Koprowski, Rapporteur.

This meeting was devoted to the consideration of advances made in the field of rabies since the second session of the Committee in September 1953. Some of the recommendations in the second report¹ of the Committee have been modified in the light of these advances, and the present report should therefore be read in conjunction with it.

During the three years which intervened between the second and third sessions of the Committee, a considerable amount of research, co-ordinated by WHO, was carried out on various aspects of rabies which had been considered previously by the Committee as requiring attention. This applies especially to studies centred on possible modifications of pre- and post-exposure treatment of humans, the use of vaccines in animals, and problems of field control, particularly in connexion with wildlife. Much of this material was still unpublished at the time of the present session and was submitted to the Committee in the form of working documents.²

1.1 Regional meetings

The Committee noted with satisfaction that WHO had continued to hold regional meetings similar to the one held in Coonoor, India, in 1952, to provide countries with an opportunity of sending leading rabies workers

* The Executive Board, at its nineteenth session, adopted the following resolution :
The Executive Board

1. NOTES the third report of the Expert Committee on Rabies ;
2. THANKS the members of the Committee for their work ; and
3. AUTHORIZES publication of the report.

(Resolution EB19.R15, *Off. Rec. Wld Hlth Org.*, 1957, 76, 5)

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1954, 82

² The information contained in many of these working documents will be published in the *Bulletin of the World Health Organization*.

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 is scheduled for Latin-American countries; it will be held in Caracas, in April 1957, and should serve an equally useful purpose for countries of this region. Not only do these meetings provide the latest information on rabies, but they 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.

1.2 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 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 near future.

2. RESULTS OF INVESTIGATIONS ON THE EFFICACY OF ANTIRABIES SERUM AND VACCINE

2.1 Relationship between virus neutralizing antibodies and protection against rabies

The Committee recognizes the fact that the evaluation of any clinical and field procedure can be as accurate only as the supporting laboratory tests at present available. The presence of specific neutralizing antibodies against rabies is determined by intracerebral inoculation of mice with serum-virus mixtures following a procedure described in the WHO monograph (page 69). This test is not as exact as the procedure used with other virus diseases, e.g., poliomyelitis, Newcastle disease, influenza, etc., where more quantitative types of tests are available.

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

Having these reservations in view, the Committee considered that, in experimental animals, actual challenge of an animal supposedly immune is the best index of its resistance. The Committee takes cognizance of the fact that in some species a vaccinated animal may resist overwhelming challenge inoculation with virulent virus in spite of the absence of demonstrable specific antibodies in its blood. It is well recognized by workers in the field of rabies that resistance to actual infection following vaccine prophylaxis is based on other immunological factors, as well as the presence of antibodies in the blood. The Committee considers, however, that immunity in man, other than that assessed by actual post-exposure field trial (see section 2.2), can be estimated at present only through the presence of neutralizing antibodies in his blood. The Committee realises that work should be continued in order to increase the sensitivity of the rabies neutralization test, and recommends that any other tests which may give a better picture of actual resistance should be investigated (see sections 11.2, 11.3 and 11.4, page 24).

2.2 Field trial in severely exposed individuals in Iran

The Pasteur Institute of Iran, Teheran, was recommended by the Committee at its first session in 1950 as the place to organize a trial for demonstrating the value of antirabies serum associated with phenolized vaccine treatment.¹

This recommendation was made on the grounds that in a stringent critical study of its statistics the Institute had demonstrated that the mortality-rate among individuals severely bitten in the head or neck (always most numerous in persons attacked by wolves) remained very considerable after treatment with vaccine only (reaching 40%), whereas mortality among cases treated at the Institute for bites on the limbs or trunk, even if very deep, or for slight wounds of the head or neck, was very low and comparable to the observations made in other institutes.

At its second session in 1953, the Committee considered that although the results of supplementary serum treatment obtained in three years had been encouraging they involved too few individuals to be statistically significant.² In 1954, the expected opportunity occurred. Twenty-nine people bitten by the same rabid wolf, 18 of whom had very severe wounds in the head and neck, arrived in Teheran less than 36 hours after having been bitten. The patients were divided into different groups; some received treatment with phenolized vaccine alone, while the others

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1950, **28**, 8 (section 7)

² *Wld Hlth Org. techn. Rep. Ser.*, 1954, **82**, 8

received the same treatment associated with one or several serum injections.

The results of these trials were published in the *Bulletin of the World Health Organization*.¹ Of 5 subjects bitten on the head and receiving treatment with vaccine only, 3 died of rabies. Of 13 subjects with the same type of bite receiving one or more injections of serum associated with vaccine treatment, 1 only contracted rabies.

The study was supplemented by a number of laboratory tests : isolation and identification of the virus in the wolf brain and in the brain of the subjects who died of rabies, and systematic research of the antibodies in the serum obtained from periodical bleedings of all the subjects. These results were also published in the WHO *Bulletin*.²

In addition to the striking trial mentioned above, several opportunities permitting further evaluation of this combined treatment have since occurred in Iran. The results presented at the present meeting have confirmed those reported above, and they are considered by the Committee as being conclusive.

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

2.3.1 Serum used with vaccine

2.3.1.1 Experiments in laboratory animals. The superiority of treatment with one dose of serum, followed by a course of vaccine, over either serum or vaccine alone was again demonstrated by means of a peripheral challenge of street virus in guinea-pigs.

Investigations were carried out to elucidate a phenomenon for which there was suggestive evidence in the results of the first WHO-co-ordinated serum-virus neutralization tests on non-exposed persons receiving antirabies serum and varying doses of vaccine.³ This phenomenon consisted of an interfering effect of passively administered antibodies on the vaccine antigen, resulting in a lack of active antibody response to the vaccine after the second week following the start of immunization which had become apparent in persons treated with serum and a short course of vaccine (7 daily doses). Experiments in mice confirmed this interfering effect and demonstrated that the serum not only suppressed late active antibody production but also reduced the subsequent immunity of animals to intracerebral challenge with virus.

¹ Baltazard, M. & Bahmanyar, M. (1955) *Bull. Org. mond. Santé*, **13**, 747

² Habel, K. & Koprowski, H. (1955) *Bull. Wld Hlth Org.*, **13**, 773

³ See : Atanasiu, P. et al. (1956) *Bull. Wld Hlth Org.*, **14**, 593.

2.3.1.2 *Inoculation of different schedules of vaccine and serum in normal human subjects.* These experiments were devised for three purposes :

(1) The first was to determine whether, as had been reported by some workers, reduced schedules of vaccine immunization in man would give antibody responses comparable to those obtained by the daily dosage schedule used at present.

(2) The second was to determine whether 3 doses of HEP (high egg passage) Flury vaccine (see section 6.1, page 12) given intradermally to man produced an adequate antibody response.

(3) The third was to find the effect of one or two doses of serum on the response to these vaccine schedules.

The results of these experiments may be summarized as follows (see section 2.1, page 4) :

(a) Three doses of phenolized vaccine at 5-day intervals gave an antibody response, but it was less quantitatively than that following 14 daily doses of the same vaccine.

(b) One or two doses of serum almost completely suppressed demonstrable active antibody response to three doses of phenolized vaccine given 5 days apart.

(c) One dose of serum reduced, but did not completely suppress, active antibody response to 14 daily doses of phenolized vaccine, whereas two doses of serum given on the first and fifth days of treatment did suppress the antigenicity of this vaccine schedule.

(d) Three intradermal doses of HEP Flury vaccine given 5 days apart gave a barely demonstrable level of antibody response, but the individuals inoculated in this way responded promptly and efficiently in producing antibodies to a later booster dose of the same vaccine (see section 3, page 8).

(e) Serum in one or two doses completely suppressed antibody response to 3 doses of HEP Flury vaccine, and also prevented the preparation of the individuals to respond to a later booster dose of this vaccine.

2.3.1.3 *Experimental investigations of effectiveness of reduced vaccine schedules.* Experiments in laboratory animals, where not only antibody response but actual immunity to virus challenge could be tested, were carried out in parallel with the experiments in normal human subjects reported above. Studies in mice where intracerebral challenge was administered 14 days after the start of the vaccine schedules indicated that reduced schedules involving several early doses followed by 1 or more booster doses after the tenth day gave immunity comparable to that obtained with 12 daily doses. However, in guinea-pig experiments with a peripheral

challenge by street virus given 7 days after the start of treatment, 3 doses of vaccine 3 days apart gave an immunity which was quantitatively less than that given by an equal quantity of vaccine administered in daily doses.

2.3.1.4 *Conclusions.* If passive antibodies are maintained for too long a period by repeated doses of serum, or if less than 14 doses of vaccine are given even after a single dose of serum, there is definite interference by the serum with the antigenicity of the vaccine. Therefore, it is recommended that in the combined serum-vaccine prophylaxis of severely exposed individuals a single dose of serum be followed by 14 or more doses of vaccine.

At the present time, there is insufficient evidence to warrant a reduction in the usual daily dosage vaccine schedule.

3. 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, postal and other delivery men, 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.

In this connexion, the Committee took into consideration results of immunization of non-exposed adult individuals; in an adequate number of subjects it has been found that a single dose of chicken-embryo vaccine given many years after previous immunization resulted in a prompt and significant antibody rise. Furthermore, there is good evidence that 3 intradermal doses of chicken-embryo vaccine (HEP, see section 6.1, page 12) given 5 days apart prepare the individuals to respond, at a later time, to such a booster dose given several months after the preparatory doses. There is reason to believe that from the immunological standpoint a similar preparation and booster effect may be expected from any potent rabies vaccine.

On the basis of the rapidity and the degree of antibody response to a booster dose of vaccine following such a preparation, as well as of factors discussed in section 2.1 (page 4), it is suggested that on subsequent exposures a booster dose be given in the case of mild or moderate exposure, and, in the case of severe exposure, a second booster dose, one week later.

4. COMPLICATIONS OF ANTIRABIES TREATMENT

4.1 Serum

The Committee recognizes 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 equine origin. An immediate anaphylactic type of reaction should be avoided by 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 percentage incidence of the delayed-type reactions (serum sickness) has varied from 0 to 20. These should be treated according to usual practice.

4.2 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 recognizes 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 factors as the ethnic origin of the population involved, species of animals used in vaccine production, 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 a neuroparalytic accident or premonitory symptom indicating its development occur, the following possible procedures are suggested, although at the present time no definitive scientific evidence is available 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, avian-embryo vaccines should be used in lieu of brain-tissue vaccines. In either instance the use of such products as cortisone, ACTH and antihistamines should be considered.

4.3 Vaccine prepared from avian embryos

Experimental evidence indicates that neuroparalytic accidents do not occur after administration of vaccine prepared from avian embryos. Limited experience in humans confirms this observation.

Apart from local erythema, no deleterious effect of the vaccine has been noted in man. Persons known to be sensitive to egg protein should not be given this vaccine unless it is necessary, and then only with proper precautions.

GUIDE FOR SPECIFIC POST-EXPOSURE TREATMENT

Nature of exposure	Status of biting animal		Recommended treatment (in addition to local treatment) *		
	At time of exposure	During observation period of 10 days			
I. No lesion; indirect contact only	rabid	—	none *		
II. Licks :					
(1) unabraded skin	rabid	—	none *		
(2) abraded skin, scratches and unabraded or abraded mucosa	(a) healthy	healthy	none *		
	(b) healthy	clinical signs of rabies or proven rabid (laboratory)	start vaccine at first signs of rabies in animal *		
	(c) signs suggestive of rabies	healthy	start vaccine immediately ; stop treatment if animal is normal on 5th day after exposure *		
	(d) rabid, escaped, killed, or unknown	—	start vaccine immediately *		
III. Bites :					
(1) mild exposure	(a) healthy	healthy	none *		
	(b) healthy	clinical signs of rabies or proven rabid (laboratory)	start vaccine at first signs of rabies *		
	(c) signs suggestive of rabies	healthy	start vaccine immediately ; stop treatment if animal is normal on 5th day after exposure *		
	(d) rabid, escaped, killed, or unknown	}	—	start vaccine immediately *	
	(e) wild (wolf, jackal, fox, bat, etc.)				
	(2) severe exposure (multiple, or face, head or neck bites)	(a) healthy	healthy	serum immediately ; no vaccine as long as animal remains normal	
		(b) healthy	clinical signs of rabies or proven rabid (laboratory)	serum immediately ; start vaccine at first sign of rabies	
		(c) signs suggestive of rabies	healthy	serum immediately, followed by vaccine ; vaccine may be stopped if animal is normal on 5th day after exposure	
		(d) rabid, escaped, killed, or unknown	}	—	serum immediately, followed by vaccine
		(e) wild (wolf, jackal, fox, bat, etc.)			

* See explanatory notes on opposite page.

Note: The above schedule applies equally whether or not the biting animal has been previously vaccinated.

5. RECOMMENDATIONS FOR POST-EXPOSURE TREATMENT OF MAN

5.1 Local treatment of wounds

The Committee examined sufficient experimental data to enable it to make the following recommendations. Immediate treatment of all bite wounds inflicted by animals, especially those suspected of being rabid, should consist of thorough cleansing with soap or a detergent solution. Such treatment should be followed, where the site permits, by the use of concentrated nitric acid. This may have to be introduced carefully into the depths of puncture wounds. It is recommended not to suture immediately because there is evidence that the closure of a wound is a contributing factor to the development of rabies. There is now sufficient experimental evidence to show that the infiltration of antirabies serum into the tissue beneath the wound, when this is feasible, is effective in the prevention of rabies. The Committee considers this local use of antirabies serum very useful, regardless of the systemic treatment after exposure.

The application of ordinary antiseptics and the local or parenteral use of antibiotics have no prophylactic value against the rabies virus, but may follow the local treatment recommended above to combat bacterial infections.

5.2 Guide for specific post-exposure treatment

The accompanying guide and explanatory notes represent a slight modification of a similar chart contained in the second report of the Committee.¹

EXPLANATORY NOTES TO THE GUIDE

The great importance of local treatment is re-emphasized and the ideal local treatment includes infiltration with antirabies serum together with thorough cleansing of the wound and the application of nitric acid where the site permits (see section 5.1, page 11). The dose of serum used in local infiltration will be dictated chiefly by the site of the bite. However, where possible, not less than 5 ml should be used.

The general principles on which the accompanying guide is based are that in mild exposures a course of vaccine following the above recommended local treatment is sufficient, whereas following severe exposures the full systemic dosage of antirabies serum together with vaccine should be employed. In such systemic use of serum, although it is recognized that the earlier the treatment is started the better, there is no time limit as to when serum can and should be given. For the reasons stated in section 2.3 (page 6), serum should be administered in a single dose (0.5 ml per kg of body-weight) at the start of treatment, followed by a course of not less than 14 daily doses of vaccine.

Sensitivity to serum should be tested before serum is used.

It is fully recognized that in certain situations specific conditions may warrant modifications of the accompanying guide, 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. One possible modification is that, following local treatment of the wound as described above, two or three doses of vaccine be given at daily intervals and no further doses as long as the animal stays healthy for 10 days following exposure.

It should be emphasized at this point that this table is only a guide and that certain circumstances may justify its modification.

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1954, **82**, 12

6. EXPERIMENTAL INVESTIGATIONS ON THE IMMUNIZATION OF ANIMALS AGAINST RABIES

6.1 Types of vaccine¹

Inactivated vaccine : Any vaccine containing non-living virus, whether made from nervous tissue (brain) or other tissues, such as avian embryo.

*Chicken-embryo vaccine*² : LEP (low egg passage) Flury—40th to 50th egg passage of the *Flury* strain ; HEP (high egg passage) Flury—187th to 210th egg passage of the *Flury* strain ; Kelev—99th to 101st egg passage of the *Kelev* strain.

6.2 Immunization of puppies

At the time of the second session of the Committee it was impossible to make firm recommendations on the basis of available data. New information now makes it possible to make recommendations in this connexion with greater confidence.

Recent studies conclusively prove that puppies less than 3 months of age differ significantly from adult dogs as regards their response to pre-exposure immunization, in that a substantial proportion are incapable of responding to antigen regardless of whether they are vaccinated with nervous-tissue, LEP Flury, or HEP Flury vaccine.

There is evidence that young puppies under three months of age will not only fail to respond satisfactorily to antigen, but that an occasional animal vaccinated with LEP Flury strain will succumb to the vaccine virus. This has not been observed with HEP Flury strain in limited numbers of puppies, even when given the vaccine intracerebrally. Deaths attributed to LEP Flury vaccine have never been observed in dogs more than 11 weeks of age.

6.3 Salivary tropism

The possibility that rabies virus may be excreted in the saliva of vaccinated animals has been carefully examined. The Committee considers that there is no well-documented evidence indicating that vaccine virus

¹ As referred to in this report.

² A living modified virus vaccine cultivated in chicken embryos. The strains of virus, and the respective egg passages mentioned, can be obtained by national laboratories on request to the Secretary, Expert Panel on Rabies, World Health Organization, Palais des Nations, Geneva, Switzerland.

appears in the saliva or salivary glands of vaccinated animals, or that vaccination so modifies the resistance of an animal that subclinical infection associated with the salivary excretion of virus follows infection with street virus. On the contrary, results of recent carefully controlled laboratory and field studies indicate that this does not occur.

6.4 Immunization of cats

A recent laboratory study indicates that nervous-tissue vaccine and both the LEP and HEP Flury strains will significantly protect adult cats against severe challenge with street virus. In this experiment, when challenge inoculation was given 70 days after immunization, protection afforded by nervous-tissue vaccine was significantly greater than that induced by either LEP or HEP Flury vaccine.

There is limited evidence suggesting that kittens may also be protected by the three vaccines. Occasionally, a kitten less than four months of age will succumb to LEP Flury vaccine.

6.5 Vaccine studies in other species

Species may vary widely as regards susceptibility to street virus. Cattle, foxes, and skunks, for example, are more susceptible than cats. This is also true as regards susceptibility to modified live virus vaccine. For example, LEP Flury vaccine has been widely used with safety in adult dogs but has been shown to be hazardous for inoculated foxes, skunks, and cattle. It is imperative, therefore, that safety and efficacy should be carefully evaluated before vaccine recommendations are made for new species.

7. RABIES CONTROL IN ANIMALS

This section summarizes the Committee's recommendations on rabies control in animals, including, where appropriate, recommendations made in the first and second reports.

7.1 Control procedures

"Experience has shown that the efficient organization of a rabies-control programme in an infected area 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. A system of weekly reports of rabies cases should be instituted to enable the officer to keep abreast of the problem. He should enlist the support

of all local groups directly or indirectly concerned with rabies, such as public-health authorities, veterinary and medical practitioners, livestock organizations, animal protection societies, etc. These groups can provide material assistance to the rabies-control officer by publicizing the programme and otherwise informing the general public whose co-operation must be obtained before specific measures can be successfully applied. If possible, an antirabies campaign should be co-ordinated on a national basis, or at least in adjacent infected areas.

“ The committee recommends that the following specific measures be applied in affected regions :

- (1) Registration, licensing, and taxation of dogs
- (2) Elimination of stray animals
- (3) Restraint of dogs while the control campaign is under way
- (4) Mass vaccination of dogs free of charge
- (5) Provision of adequate facilities for diagnosis
- (6) Reduction in number of wildlife species where these are a reservoir of the disease
- (7) A continual and energetic publicity campaign.”¹

The three basic principles of an operational programme are elimination of stray dogs, canine vaccination, and control of wildlife vector populations.

7.2 Elimination of stray dogs

“ It has been found that registration or licensing of dogs is an important adjunct to a successful control programme. If properly enforced, this measure rids the area of ownerless stray dogs and assures a reasonable dog census. An efficiently conducted programme requires the operation of a local pound or humane shelter where stray animals may be kept for a few days, and if unclaimed at the end of that period they should be humanely destroyed. Collection of strays should be carried out by teams of dog wardens and assistants in properly equipped trucks.”²

7.3 Recommendations for the pre-exposure immunization of animals (see also section 6, page 12)

7.3.1 Dogs

Additional field experience confirms the laboratory observations that LEP Flury chicken-embryo vaccine produces excellent immunity in dogs for at least three years following a single intramuscular inoculation (posterior thigh muscles). A single inoculation of the Kelev strain vaccine confers good protection in dogs, but the duration of immunity beyond one year has not yet been ascertained.

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1950, **28**, 13

² *Wld Hlth Org. techn. Rep. Ser.*, 1954, **82**, 13

In areas where chicken-embryo vaccine is not available or is impractical, the use of a single dose of nervous-tissue vaccine is recommended. Potency-tested nervous-tissue vaccine confers good immunity for one year, and there is still significant protection three years after vaccination by the intramuscular route.

The Committee urges that all dogs three months of age and over be vaccinated; the vaccination of dogs less than three months of age is not recommended.¹

“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, the committee feels that 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.”^{2, 3}

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 nervous-tissue or Kelev strain vaccine that has been properly stored.

7.3.2 Cats

Cats may be effectively immunized with either nervous-tissue vaccine or chicken-embryo vaccine (see section 6.4, page 13). The duration of

¹ In rabies-infected areas puppies under three months of age should be kept indoors or restrained. Where it is important that puppies under three months of age be vaccinated, inactivated vaccine is recommended, with revaccination after three months of age with any type of vaccine.

² *Wld Hlth Org. techn. Rep. Ser.*, 1954, **82**, 14

³ The passages within square brackets represent modifications of the recommendations, introduced by the Committee at this session.

immunity is unknown. The vaccination of cats less than six months of age is not recommended.

Since the individual rabid cat may present a serious problem, cat owners are encouraged to have their animals vaccinated for their own protection. There is no evidence, however, that rabies persists among cats in areas where canine and wildlife rabies have been eliminated.

7.3.3 *Cattle*

The chicken-embryo adapted HEP (Flury) and Kelev strains are recommended for pre-exposure vaccination of cattle. Although a single dose of the vaccines¹ 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. Cattle vaccinated with one of the strains (HEP Flury) were found to be resistant to challenge inoculation with street virus given one year after immunization.

Inactivated vaccines may be used. In one study, a single dose of 30 ml of 33% suspension of inactivated nervous-tissue protected cattle challenged one year after vaccination, whereas a smaller dose (15 ml) of vaccine did not protect.

7.3.4 *Other species*

Insufficient data are available to make recommendations with respect to the efficacy of vaccines in other species of animals. The Committee strongly recommends that living antirabies vaccines should not be used in any species of animals without previously determining the safety as well as efficacy of the vaccine for the particular species in question (see section 6.5, page 13).

7.4 **Handling of animals 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, the following alternatives are recommended :

- (1) Strict isolation of the animal in a kennel for six months.
- (2) If no previous vaccination has been given within a period of three years with LEP Flury vaccine, or within one year with nervous-tissue or

¹ 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.

Kelev vaccine, administer post-exposure treatment and confine in a kennel for three months. Post-exposure treatment may consist of the administration of antirabies serum (0.5 ml per kg of body-weight) followed by from 1 to 3 doses of chicken-embryo vaccine within the next seven days, or 14 injections of nervous-tissue vaccine.

If the animal has been previously vaccinated within one year with nervous-tissue or Kelev strain vaccine, or within three years with Flury strain vaccine, revaccinate and restrain (leashing and confinement) for 30 days.

7.5 International transfer of dogs and cats

The Committee considers it useful to repeat with slight modifications the general recommendations on the international transfer of dogs and cats made in the second report,¹ without attempting to cover the many different situations faced by countries throughout the world. The following measures should be taken when animals are imported from countries where rabies is known to exist:²

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, such as in countries with extensive land borders and with rabies already present in domestic or wild animals, the following is recommended:

(a) Animals (dogs and cats³) should be vaccinated more than 1 month but within 12 months before departure with inactivated vaccine or Kelev strain vaccine, or within 36 months before departure with Flury strain vaccine (see sections 6.2, 6.4, 7.3.1, 7.3.2, pages 12, 13, 14, 15), the vaccine having previously passed satisfactory potency tests. Certificates signed by the appropriate veterinary authorities in the country

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1954, **82**, 16

² A rabies-infected country can be considered as one where rabies has been confirmed in man or animals at any time during a previous two-year period.

³ The Committee has no data on the duration of immunity conferred by vaccines in cats (see section 6.4, page 13).

of origin should accompany each animal (see Annex 1, page 27, for suggested form of certificate). 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 as 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.

7.6 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. 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 in the smaller vector species (e.g., thallium sulfate in mongooses) 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 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 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 extinction 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.

Vampire-bat rabies continues to be a major rabies problem in Mexico and in Central and South America. Some progress has been noted in controlling the disease in these areas by eradication schemes which employ dynamiting, gassing, and shooting of vampire-bats in their diurnal resting-places. Newer methods of control, such as traps with live bait, are now being tested in the field.

The isolation of rabies virus from an insectivorous bat in the USA in 1953 has aroused further interest in this aspect of wildlife rabies. Since then, 150 cases in insectivorous bats have been reported from 15 geographically diverse States of the USA, as well as some, recently, from Yugoslavia. These include 4 species of tree-living or solitary bats and 10 species of colonial or cave-dwelling bats.

There is no direct evidence to date of natural transmission of rabies from insectivorous bats to man or lower animals. Despite this, the public-health significance of these findings cannot be overlooked since there have been at least 15 instances in which proven rabid bats have bitten human beings. Approximately half of these attacks were unprovoked. Circumstantial evidence has incriminated insectivorous bats as the source of infection in two human rabies deaths.

7.7 International Sanitary Convention

The Committee noted the draft for an International Sanitary Convention for the General Prophylaxis of Rabies as prepared by the Office international des Epizooties. The Committee concerned itself only with the technical provisions of the draft. Certain of these draft provisions are at variance with the recommendations made by this Committee, and the Director-General of WHO may wish to bring the Committee's recommendations to the attention of the Office international des Epizooties.

7.8 WHO-sponsored field trial and demonstration of rabies control using chicken-embryo vaccines in dogs in Israel and Malaya

A progress report on this subject was included in the second report of the Committee¹ and detailed accounts of this work have been published.² After 1953, WHO no longer participated actively in the efforts of these countries apart from supplying technical advice when requested. In reports submitted to the present Committee on results obtained since 1953, the Committee notes the excellent control and virtual eradication of rabies in Malaya and the continued energetic vaccination programme coupled with rigorous control of stray animals. In Israel, after 1953 a recrudescence occurred which was attributed to the unfortunate relaxation, due to other disease-control priorities, of the vaccination campaign and stray-dog control, particularly the latter. Relatively few vaccinated dogs contracted

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1954, **82**, 20

² Kaplan, M. M., Goor, Y. & Tierkel, E. S. (1954) *Bull. Wld Hlth Org.*, **10**, 743 ; Wells, C. W. (1954) *Bull. Wld Hlth Org.*, **10**, 731

rabies in comparison with unvaccinated dogs. The Committee considers that while the value of vaccination has been effectively demonstrated, stray-dog control remains an essential component of all rabies campaigns.

8. POTENCY TESTS AND INTERNATIONAL STANDARDS FOR ANTIRABIES VACCINES AND SERUM

8.1 Potency tests for vaccines

8.1.1 *Nervous-tissue vaccines*

In previous reports, the Committee considered the problems arising out of the necessity of performing *some* test for potency on *each* batch of vaccine. Experience continues to confirm this necessity, and the Committee again invites the attention of national laboratories to the useful service provided by WHO in sending, on request,¹ standard virus strains, and a reference vaccine (see below), and in providing for the occasional check testing in an experienced laboratory of a particular sample of vaccine.

It is highly desirable that a potency test be standardized as much as possible in order that tests be repeatable and that there be some valid bases of comparison from one test to another. The Habel-type potency tests² have been particularly useful in this connexion, and work during the past few years on this problem has resulted in further advances being made, especially in connexion with a reference vaccine which could be included with each test for vaccine potency.

The Committee notes that approximately 1200 ampoules of Rabies Reference Vaccine (155 D) have been made available to WHO for international use by the National Institutes of Health, Bethesda, Md., USA. This stock of ampoules should be adequate for several years if used in the manner recommended by the Committee and described in Annex 2 (page 27).

8.1.2 *Chicken-embryo vaccine*

The guinea-pig test, as described in the WHO monograph *Laboratory Techniques in Rabies* (page 128), has been applied on an extensive scale during the past few years and has proved its great usefulness for routine

¹ Requests should be addressed to the Secretary, Expert Panel on Rabies, World Health Organization, Palais des Nations, Geneva, Switzerland.

² World Health Organization (1954) *Laboratory techniques in rabies*, Geneva, pp. 112, 116 (*World Health Organization: Monograph Series*, No. 23)

purposes in laboratories producing modified virus vaccines in chicken embryos. Further substantiation has been achieved of the correlation between this potency test and parallel tests of the vaccines in larger animals (dogs, cattle) subjected to challenge with street virus.

In addition to the guinea-pig potency test, another test became available for HEP Flury virus because of the apathogenicity of this strain for 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 sufficient fixed or street virus to kill all the control mice. Mice receiving up to 10^{-3} dilution of the vaccine should be protected. Laboratory evidence indicates that this type of potency test, in spite of using the same site of inoculation for the vaccine and challenge virus, is based on actual immunization of animals and not on an interference phenomenon. Again it is stressed, however, that further work is necessary before this modified test can be accepted as a valid potency test, and the Committee recommends that additional work co-ordinated by WHO be undertaken in this connexion.

Some laboratories have experienced difficulties with the handling of street virus required by the guinea-pig potency test. Annex 3 (page 28) describes a method for preserving salivary gland suspensions of street virus in dried form which should be useful in overcoming this type of difficulty. Research is now under way to determine whether fixed virus can be substituted for street virus in the performance of the test, but there is still insufficient data available concerning this substitution to make a recommendation at this time.

8.1.3 *Other vaccines*

New vaccines not falling under the previously discussed categories are being produced, such as inactivated vaccines prepared from infected duck embryos. The same potency test recommended for inactivated vaccines (section 8.1.1) can be used.

8.1.4 *Stability*

Because of the different climatic conditions found throughout the world, it is of the utmost importance for every country to determine the stability of the vaccines produced and used there. Where facilities for adequate and continuous refrigeration are available, properly produced nervous-tissue vaccines should remain stable for several months. Expiration dating, however, should be based on results of a series of potency tests for duration of antigenicity. Where there is a decentralization of vaccine

treatment centres, and vaccines may not be properly refrigerated during transit or storage, it is especially important to determine by means of potency tests the maximum permissible expiratory date for the vaccines. When such a date is reached, it is best to destroy the vaccine if unused, rather than perform another potency test for the purpose of extending the expiratory date, because experience has shown that vaccine suspensions may unpredictably and suddenly lose their potency upon prolonged standing.

Phenolized vaccine which has inadvertently been frozen should be discarded.

Since the efficacy of living chicken-embryo vaccines depends upon a critical amount of live virus being present at the time of inoculation, such vaccines should be kept dried and refrigerated until used ; after reconstitution they should be used immediately.

8.2 Potency test and therapeutic dosages for serum

A potency test for serum was described in the WHO monograph *Laboratory Techniques in Rabies* (page 139) and in the second report of the Committee.¹ Considerable work has since been accomplished on the designation of the International Standard for Antirabies Serum. As a result of this work, modifications are recommended in the potency test applied to routine production batches of serum, and the revised potency test is described in detail in Annex 4 (page 29).

The accomplishment of an actual field trial of antirabies serum in man severely exposed to rabies (see section 2.2, page 5), and an analysis of the serum employed, has given valuable information that has made it possible for the Committee to make the following recommendations on acceptable potency and therapeutic dosage of serum :

(1) A serum shall pass the test for sufficient therapeutic potency if, in a single comparative assay, it is equal or better than the International Standard Serum in the recommended potency test (Annex 4).

(2) In case a serum fails the test, two more similar tests may be carried out. If the serum is equal to or better than the International Standard Serum in both these additional tests, it shall pass.

The Expert Committee on Biological Standardization in its ninth report defined the International Unit as the activity contained in 1 mg of the International Standard.² After restoration with 1 ml of water, the

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1954, **82**, 25

² *Wld Hlth Org. techn. Rep. Ser.*, 1956, **108**, 11

reconstituted International Standard Serum will have approximately 80 International Units per ml. Therefore, an acceptable therapeutic serum should contain a minimum of 80 International Units per ml.

The dosage recommended for therapeutic purposes is 0.5 ml (or approximately 40 International Units) per kg of body-weight. While this recommendation is made on the basis of animal experimentation and actual field trial in human beings to date, the Committee recommends that further collaborative assays be carried out on the relative potency of the International Standard Serum and the serum actually used in any future field trials in human beings. The results of these assays should be distributed to members of the Committee to determine whether any modifications to the present recommendation might be advisable.

9. DIAGNOSIS

The Committee feels that there is as yet insufficient evidence for the validity or superiority of proposed diagnostic methods, such as complement-fixation and paper electrophoresis, and therefore they cannot be considered as substitutes for the established histopathological and biological methods currently in use, as summarized in the WHO monograph (Part I, pages 15-74).

10. RABIES REPORTS AND RECORDS

The necessity of prompt reporting of rabies within a country for national control purposes has been stressed previously by the Committee. For international purposes it is important that information of the current status of rabies in countries throughout the world be made available. The Committee strongly recommends that such information be supplied to WHO periodically, preferably every 6 months, so that this information would be available for Member States.

In the past, rabies statistics have often been difficult to interpret because of gaps of information on various aspects of the disease in places where post-exposure treatment is given. The Committee recognizes that detailed information forms are often used, but these frequently defeat the purpose for which they are intended because of reluctance to fill out all the details requested in such forms. A simplified form is therefore suggested in Annex 5 (page 31) which covers the principal items in connexion with

suspected rabies exposure in human beings. The Committee recommends the use of this, or of a similar, form to rabies authorities.

11. FUTURE RESEARCH

11.1 Human prophylaxis

The Committee suggests that investigations should be continued on the efficacy of *reduced* vaccine schedules with special emphasis on booster doses given at varying intervals after the first injection, and the effect of serum on this treatment. These investigations should include parallel experiments in: (a) normal animals, (b) animals after exposure to rabies virus, and (c) non-exposed human subjects.

11.2 Tissue culture

After considering the results obtained with other viral agents grown in tissue culture, the Committee considers that efforts should be continued to find a tissue culture system applicable to rabies virus production and serological tests.

11.3 Antibody determination

Further evaluation of more sensitive methods of antibody demonstration which have been reported is recommended. Search for other reliable serological procedures should be continued.

11.4 Immunity and resistance

The Committee recognizes the need for further basic research concerning the mechanisms of resistance to rabies infection. This should include not only resistance artificially induced, but also so-called "natural" resistance.

11.5 Stability and potency tests for vaccines and sera

As referred to in section 8 (page 20), research should be carried out on the stability of vaccines produced and used in different areas of the world, and on modifications of potency tests for vaccines and sera.

11.6 Factors causing complications of antirabies treatment

As pointed out in section 4 (page 9), the advances made in specific prophylaxis of man and animals are complicated by significant side reactions. The Committee considers that the complications of specific immunization are of sufficient importance to warrant investigations from all possible approaches, including search for effective substitutes devoid of side reactions as well as the purification of existing products.

11.7 Effect of corticosteroids and ACTH upon antibody development

Since corticosteroids and ACTH may be used in complications of human antirabies treatment and because of possible antibody-depressing activity of these compounds, the Committee feels that their effect upon the development of antibodies against rabies following a course of specific treatment should be investigated.

11.8 Therapy

Because of the inevitably fatal outcome of rabies infection of man, investigations of any possible method of therapy should be encouraged.

11.9 Studies on protective treatment of man following severe exposure

In areas where the local situation lends itself to the evaluation of the efficacy of prophylaxis after severe exposure, further investigations should be carried out, aimed at increasing the already demonstrated effectiveness of combined serum-vaccine treatment.

11.10 Wildlife rabies

As previously recommended by the Committee, ecological studies should be continued, to elucidate the role of the wild animals involved in any area where sylvatic rabies is a problem. Such studies should include laboratory investigations on susceptibility to infection, pathogenesis and immunological response. At the present time, this is especially indicated in such species as foxes, skunks, jackals and bats.

Because of the special ecological situation involved in rabies infection in bats, investigations even in countries free of rabies should be directed towards determining the presence of rabies in this species.

Research to improve the effectiveness of control of various vector species is of practical importance.

11.11 Diagnostic techniques

Research work should be continued on more rapid and specific methods for the diagnosis of rabies.

11.12 Duration of immunity studies with vaccines in animals

Continuation of studies on the effectiveness and duration of immunity conferred by different vaccines in animals should be encouraged.

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**INTERNATIONAL REFERENCE VACCINE**

The need has often been expressed for an international reference vaccine which could be used for comparison, from the standpoint of potency, with a locally produced vaccine. Such a reference vaccine has been used successfully on a national scale for several years in the USA where the National Institutes of Health, Bethesda, Md., supply ampoules of a reference vaccine which are used in potency tests of each batch of vaccine in production laboratories.¹ The quantity of ampoules of Reference

¹ The use of this reference vaccine in potency tests is described in detail in: World Health Organization (1954) *Laboratory techniques in rabies*, Geneva, p. 117 (*World Health Organization: Monograph Series*, No. 23).

Vaccine 155 D, kindly made available to WHO by the National Institutes of Health, is limited, but nevertheless may serve a very useful purpose internationally.

Because of the limited quantity of this Reference Vaccine available for international distribution,¹ it would be desirable for national laboratories to prepare their own stock of a reference vaccine which, after comparison with the Reference Vaccine 155 D, could be used to supply routine production laboratories within a country. Reference Vaccine 155 D, however, is an ultraviolet light-inactivated dried product, and the production of such a vaccine can be undertaken at the present time only in relatively few countries. Therefore, where such an undertaking is not feasible, Reference Vaccine 155 D will be supplied in reasonable quantities to countries wishing to use this vaccine for routine potency tests for large batches of vaccine.

As pointed out in section 8.1.4 (page 21), it is highly advisable that the stability of rabies vaccines produced and used under particular local conditions be ascertained. Reference Vaccine 155 D would be very useful for this and other purposes, such as checking the validity and comparability of the potency test used in a laboratory with those used in other laboratories.

Annex 3

PREPARATION OF FREEZE-DRIED CHALLENGE VIRUS

The following procedure has been successfully used at the Pasteur Institute of southern India, at Coonoor :

Immediately after a dog dies of rabies following intramuscular inoculation with a strain of street virus, the submaxillary glands are dissected out aseptically. The fibrous tissue is dissected away as much as possible. The gland tissue is weighed and finely minced with scissors. It is then ground into a smooth paste in a mortar using neutral glass powder as abrasive. The mortar is kept chilled continuously by placing it in a tray containing ice. A quantity of ice-cold inactivated 50% rabbit serum in distilled water containing 5% glucose, calculated to give a 10% final suspension of tissue, is slowly added, continuing the grinding all the time. The suspension is then centrifuged at about 1000 r.p.m. for 5 minutes. The glass powder settles down at the bottom of the centrifuge tube and

¹ Available to national laboratories on request to the Department of Biological Standardization, Statens Seruminstitut, Copenhagen, Denmark (custodians of WHO international biological standards).

the coarse particles of fibrous tissue settle over the layer of glass powder. The supernatant is pipetted out and measured quantities are distributed into ampoules and freeze-dried without delay. By this method, uniform suspensions are obtained which on repeated titration intracerebrally or peripherally give regular and reproducible results over long periods (longer than 18 months).

Annex 4

MINIMUM REQUIREMENTS FOR POTENCY OF ANTIRABIES SERUM *

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 (6 dilutions, a minimum of 6 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 rabies virus of known virulence may be used. The CVS (standard challenge virus) strain of fixed virus, as described in the WHO monograph on rabies (page 117), is very useful for this purpose.

*The International Standard for Antirabies Serum*¹

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,² and after reconstitution of the dried serum with 1 ml of distilled water the International Standard Serum will hold approximately 80 International Units per ml. After reconstitution, the serum remains stable for at least

* A revision of the minimum requirements as given in Annex 2 of the second report of the Expert Committee on Rabies (*Wld Hlth Org. techn. Rep. Ser.*, 1954, **82**, 25) and in the WHO monograph entitled *Laboratory techniques in rabies* (section 13, pages 117-124).

¹ Available to national laboratories on request to the Department of Biological Standardization, Statens Seruminstitut, Copenhagen, Denmark (custodians of WHO international biological standards).

² See *Wld Hlth Org. techn. Rep. Ser.*, 1956, **108**, 11.

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 6 mice per dilution).

A control titration of the virus suspension is made simultaneously to determine the number of LD₅₀ actually used in the test. The virus suspensions are mixed with equal quantities of 2% normal horse serum, incubated and inoculated 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 5th day after inoculation with CVS virus are eliminated from the test ; all mice dying between the 5th and 14th day, after showing signs of rabies (paralysis, convulsions), are considered to have died of rabies. Mice still living on the 14th 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.

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 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 or better than the International Standard Serum in both these additional tests, it shall pass (see section 8.2, page 22). 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.

Annex 5

SUGGESTED CASE RECORD FOR HUMAN RABIES EXPOSURE

Case No. _____ Referred by _____

A. Person bitten

Name _____ Age _____

Address _____ Sex _____

Date of bite _____

Nature of bite: _____ Site(s) of bite _____

Single Mild
 Multiple Moderate
 Severe

Local wound treatment _____

Treatment:

<i>Vaccine</i>	<i>Serum</i>
Individual dose _____	Dose _____
Date begun _____	Date administered _____
Date ended _____	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? _____ Date _____ Type _____

Treatment complications (syndrome, treatment, final outcome):

Status of exposed person after 6 months:

Alive
 Died of rabies
 Died of other causes Date of death _____

B. Biting animal

Kind of animal

Description:

Breed..... Age..... Sex..... Weight.....

Animal vaccinated against rabies?

Type of vaccine..... Date.....

Disposition of biting animal:

Under observation Killed

Outcome during days

Results of laboratory examination:

Signs of rabies

Positive Negative

Healthy Negri bodies Escaped Animal inoculation