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## CURRENT PROGRESS

### Immunization Against Rabies

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The methods used for both pre-exposure and post-exposure immunization against rabies were studied. In pre-exposure immunization duck embryo vaccine should be used. In post-exposure immunization either duck embryo or Semple-type vaccine appears to be effective in stimulating antibody production. Both vaccines may cause neurological sequelae. A dose of vaccine should be given 20-50 days after completion of the primary course of vaccination. Immune serum should be used in all severe exposures especially of the head and neck, and in individuals in whom the commencement of vaccination has been unduly delayed. In individuals who have been previously vaccinated reinforcing doses have been found to be effective even as long as 20 years after the primary vaccination. A tissue culture vaccine has been developed and is about to undergo field trials.

**R**ABIES in Canada presents a small but real hazard to man. For those in daily contact with animals, wild or domestic, it is an appreciable one. In rural areas requests to the practitioner for advice and prophylaxis are not uncommon.

Rabies is prevalent among animals in most parts of Canada except the Maritime Provinces and Newfoundland. From April 1964 to March 1965, the Health of Animals Branch of the Canadian Department of Agriculture reported 1655 cases in wild and domestic animals. The distribution was as follows: Quebec, 236; Ontario, 1355; Manitoba, Saskatchewan, British Columbia and the North West Territories combined, 84. Ontario

Les auteurs ont étudié les méthodes utilisées pour l'immunisation contre la rage, tant avant qu'après morsure. Pour l'immunisation avant morsure, on emploiera le vaccin préparé sur embryon de canard. Pour l'immunisation après morsure, l'un ou l'autre des types de vaccins, embryon de canard ou Semple, est efficace et stimule la production d'anticorps. Les deux vaccins peuvent entraîner des séquelles neurologiques. On devra donner une dose de vaccin de 20 à 25 jours après la fin de la série primaire de vaccination. Le sérum immun devra être employé dans tous les cas graves, surtout si la morsure est située à la tête et au cou et en général pour les individus chez lesquels on a indûment retardé le début de la vaccination. Chez les sujets qui ont déjà été vaccinés, des doses de consolidation se sont révélées efficaces, même dans un délai de 20 ans après la première vaccination. Un vaccin sur culture de tissus a été mis au point et est à la veille de subir les épreuves cliniques de masse.

thus accounted for 80% of all cases reported in Canada during that 12-month period.<sup>1</sup> Rabies first appeared in the Province of Ontario about 1953, spreading southwards along the Ottawa Valley and through the Muskoka District to involve all Southern Ontario except the southwestern area of the province from London to Windsor.

After an initial peak of 2485 cases reported in 1958 the incidence fell sharply to 300 in 1960, but since that year there has been a gradual increase to the present annual level of about 1400. Over the past 10 years rabies has been confirmed in 9200 animals in Ontario. Of these, 53% have been wild animals, chiefly fox and skunk, 32% farm animals, mostly cattle, and 15% dogs and cats.<sup>2</sup>

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From 1926 to 1964 there were 18 recorded deaths of humans from rabies in Canada.<sup>3</sup> Between 1950 and 1958 there were none; in 1959 a child and a young adult died of rabies in Ontario; and in 1961 an aged man died of the disease in Alberta.<sup>4</sup> Post-exposure immunization is an accepted practice in Canada, yet it carries its own hazards. The physician, when confronted with a patient known or suspected of having been exposed to the disease, must weigh these hazards against the risk of rabies itself. The recommendations of the World Health Organization Expert Committee on Rabies, contained in the World Health Organization Technical Report No. 321 of 1966, provide a detailed guide for the management of patients exposed to rabies (Appendix A, B, C).

#### HISTORICAL BACKGROUND

In 1885, following his classical studies on fowl cholera, swine erysipelas and anthrax vaccines, Pasteur turned to the problem of rabies. Why he should have chosen this disease is uncertain but it may well have been, as Bulloch<sup>5</sup> remarks, because of the hold that rabies had on public opinion rather than its importance as a cause of death, a situation that still exists.

Pasteur succeeded in developing a method of prophylaxis which in essence has lasted for 80 years to the present time. Rabies is unique in that the incubation period in man is usually long enough for vaccines to induce antibodies in the blood before the disease becomes clinically manifest. Pasteur used suspensions of the spinal cords of rabbits infected with rabies, either killing or attenuating the virus by drying the cords for varying periods. Daily injections of the vaccine were given, the virulence being progressively increased. His first case, a 9-year-old boy, began a series of 13 daily injections on July 8, 1885, after having been severely bitten by a known rabid dog 60 hours previously. He survived to live an expected life span.

#### Modern Rabies Vaccines

Modern vaccines are prepared either from an emulsion of virus-infected rabbit brain and cord in which the virus has been inactivated by phenol (Semple), or from virus grown in duck egg embryo and inactivated by beta-propiolactone. At present a tissue culture vaccine is being developed by the Connaught Medical Research Laboratories in Toronto. This will undergo field trials soon.

The use of Semple vaccine is well established but encephalitis and myelitis presumably associated with the concomitant introduction into the

body of heterologous brain substance are serious and rather frequent sequelae. The reported incidence varies considerably.<sup>6</sup> During the past 10 years some 9000 14-dose courses of Semple-type vaccine have been issued by the Ontario Department of Health and six cases of neuroparalytic sequelae have been reported. In the first three years wastage was estimated at 12%, in the next seven years at 3-5%. Thus neuroparalytic sequelae occurred in one patient of every 1400 actually vaccinated. All of these patients recovered.<sup>2</sup> Many patients do, however, complain of unpleasant if less dramatic side effects. One of the present writers (R.S.M.) can personally vouch for the occurrence of profound psychological disturbances, including depression and a quite uncharacteristic suicidal tendency, during a course of Semple vaccine. Gibbs *et al.*<sup>7</sup> studied the electroencephalographs of 69 patients receiving Semple vaccine and found that 10 developed abnormal rhythms. Duck embryo vaccine produced no such changes in any of 22 subjects in the same study. Experience in Ontario with the duck embryo vaccine is limited. It has to be purchased from commercial firms, whereas a Semple-type vaccine is distributed free by the Provincial Department of Health. Also the duck embryo vaccine has been in use for only a few years. Four cases of neurologic reaction have been reported following the use of duck embryo vaccine since 1961, one of which had a doubtful association with the vaccine. All of these patients recovered.<sup>8</sup> Administration of duck embryo vaccine to two nurses was discontinued by Gardner and Robson<sup>9</sup> because nausea, drowsiness and depression developed. The antibody response is comparable following the administration of either type of vaccine, antibodies appearing seven to 10 days after the first dose of a 14-day course,<sup>10</sup> and in comparable titre.<sup>11</sup>

#### Elective (Pre-exposure) Immunization

It is obviously desirable that individuals such as veterinarians who are working with potentially rabid animals should be immunized. The W.H.O. Expert Committee on Rabies<sup>12</sup> recommends a prophylactic course, consisting of two or three doses of a potent anti-rabies vaccine, preferably of non-nervous-tissue type, at intervals of one month and followed by a booster dose six months later. The presence of serum-neutralizing antibodies should be ascertained 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 recommendation 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 followed by a booster dose 20 days later for severe exposures.<sup>12</sup> It must, however, be emphasized that neither pre-exposure nor post-exposure immunization courses of rabies vaccine are effective in stimulating adequate antibody response in all persons. J. H. Richardson found one individual, a chronic alcoholic, who failed altogether to develop an antibody response.<sup>13</sup> There is considerable individual variation in the titre reached after the first revaccination, and it is therefore *desirable that the antibody level be checked* about one month after the last dose. In persons who developed antibodies after a course of vaccine administered as long as 20 years previously, it has been demonstrated that a booster dose of vaccine will restore the antibodies to an effective level.<sup>11, 18</sup> Unfortunately, facilities for determining antibody levels are limited, and so far as the writers are aware, laboratories in Canada are not equipped to make such determinations routinely.

#### *The Incubation Period of Rabies*

Rabies is unique in that except in severe bites about the head and neck the incubation period is long enough for massive doses of vaccine to produce an antibody response judged sufficient to protect an exposed individual.

After leg bites it is about 60 days, after arm bites about 40 days, and after head bites about 30 days. Unusually severe bites to the head and the introduction of large amounts of virus tend to shorten the incubation period, even to as little as six days. Rarely it may be as long as 100 days and a booster dose may be desirable after initial vaccination.

The recommendations of the World Health Organization<sup>12</sup> provide a detailed guide to the management of patients exposed to rabies. In practice, however, problems do arise which require further consideration. Patients who have been indirectly exposed to a rabid animal or contaminated by saliva on apparently unbroken skin usually present themselves in a state of marked anxiety. Sometimes the animal has escaped or has been so mutilated as to preclude examination of the head. If a domestic animal is involved and is still alive and well, it is placed under observation for 14 days. With the exception of bats, animals may

be infectious through their saliva for a period of four to five days before the onset of clinical symptoms until death, a total period of up to 14 days. No treatment of the patient is required if the animal remains well during that period. If it develops symptoms of rabies, immunization of the patient should be started at once. The attending veterinarian or the local government veterinarian should be notified. The latter may be contacted through the nearest office of the Health of Animals Branch, Department of Agriculture. The animal is allowed to die, which usually occurs within a week, and the head is submitted to the Animal Diseases Research Institute, Hull, P.Q. This is usually done by the government veterinarian. A report on the presence of the characteristic Negri bodies will be available within a few hours of receipt of the specimen, as will the result of examination for rabies antigen by the sensitive fluorescent-antibody technique. If these tests are negative, animal passage is carried out; positive results are available within 15 days but a negative report requires about 30 days.<sup>14</sup> If the animal has been prematurely killed, the brain should also be submitted for examination, since only in this way can it be established whether or not it had rabies. Very rarely the incubation period in animals may last, as in man, for several months.

We believe that the traditional reluctance to employ rabies vaccine when exposure has not been well established should be reconsidered, since the risk of rabies, however small, with the resulting peace of mind for both patient and doctor, outweighs the small inconvenience and danger of the vaccination. We do not believe that it is desirable to withhold immunization pending receipt of a laboratory report on the brain of a suspected animal, and we do not hesitate to immunize those who have been exposed to animals not available for observation or examination. The risk of death from rabies vaccination with either Semple or duck embryo vaccine appears very small. The risk of developing rabies may also be small,<sup>7</sup> but death is the only outcome if it is contracted.

The efficacy of rabies vaccination has been studied in the field by Sabeti and his colleagues<sup>16</sup> at the Pasteur Institute, Teheran. Rabies is endemic among wolves in Iran, and persons who are bitten are taken to the Institute for treatment. The results of many years' work, in cooperation with the World Health Organization, have recently been summarized.<sup>15</sup> For 13 years before 1949 the classical method of vaccination using phenolized (Semple) vaccine was followed with what Sabeti calls disastrous results.

Of 167 vulnerable patients treated, 44 died of rabies. Immune serum was then used in addition to vaccination in order to provide an early high titre of antibody before the patient responded to the antigenic stimulus. Of the next 85 patients treated in this way only three died. Because of a possible antagonism between serum and vaccine a standard therapy was evolved, and since 1957 antiserum has been given when the patient is first seen and the first dose of vaccine 24 hours later. Under this regimen neither the disease nor death occurred among 117 patients. Finally in 1960 a booster dose of vaccine was instituted, given 20 to 50 days after completion of the initial course of vaccination in order to maintain a high level of antibody and to avoid the occasional death after a long incubation period.<sup>16</sup>

The W.H.O. Expert Committee on Rabies recommends "serum immediately, followed by vaccine" for all severe exposures through multiple bites or bites to face, head, finger or neck, but for mild exposures only when a wild animal is involved. Otherwise vaccine only is recommended for mild exposures. Immune rabies serum is available commercially in Canada.\* It is of equine origin, and the usual precautions against anaphylaxis must be taken. The W.H.O. Expert Committee also recommends booster doses of vaccine (of non-nervous origin) at 10 and 20 days after the last usual dose.

In the United States, veterinary associations and the Red Cross Society are reported to be co-operating to provide a source of immune rabies serum of human origin.<sup>17</sup> Veterinarians and others who receive pre-exposure immunization with booster doses are suitable donors. If human serum is to be available in Canada, it seems probable that the initiative for collecting it should come from that department of government concerned with rabies immunization and from those medical practitioners working in the field of rabies prophylaxis.

\*Available from: Lederle Laboratories, 5550 Royal Mount Avenue, Town of Mount Royal, Montreal 16, Quebec.

Appendix (From W.H.O. Technical Report Series No. 321. 1966):

#### 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.<sup>1</sup>

<sup>1</sup>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.

#### SUMMARY

Both duck embryo and Semple vaccines appear to be effective and relatively safe agents for stimulating antibody response against rabies in humans.

Individuals whose occupation renders them liable to contact with rabid animals should receive pre-exposure immunization.

Duck embryo vaccine is the preferred immunizing agent for pre-exposure immunization.

Little or no hesitation should be entertained in immunizing individuals whose exposure to rabies is doubtful or minimal. The immunization program may be stopped if subsequent laboratory examinations are negative.

Immune serum (equine) is indicated after severe exposure, delay in initiating treatment, or in the event of bites on the head and face.

There is a need in Canada for laboratory facilities for rabies antibody titration and for a source of immune human serum.

#### ADDENDUM

We have recently learned<sup>19</sup> that a rabies antibody titration service has been instituted. This is being carried out by the Connaught Medical Research Laboratories, which will perform titrations on request for a fee of \$10.00 each.

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- (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.

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

<sup>1</sup>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.

<sup>2</sup>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.

C. GUIDE FOR POST-EXPOSURE TREATMENT

The recommendations 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 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 rabies-free areas 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.