Potency of Commercial Rabies Vaccine Used in Man

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RABIES vaccines for use in man must meet minimum requirements of the Division of Biologics Standards, National Institutes of Health, Public Health Service (*1*). Prior to September 1, 1953, vaccines were evaluated for potency by the Habel method (*2*), a test still widely used throughout the world and preferred in this country for rabies vaccine containing inactivated virus for use in animals. Since September 1, 1953, however, vaccines for man have been evaluated in the United States by the NIH test against standard reference vaccine supplied by the National Institutes of Health. This test is used also in certain other countries with the reference vaccine provided by NIH and distributed by the World Health Organization (*3*).

Tests in a laboratory of the New York State Department of Health indicate that reference vaccine lot 164, supplied by NIH from October 5, 1960, to May 2, 1961, afforded little protection in mice according to the Habel method. All lots of commercial rabies vaccine of nervous-tissue origin sampled passed the Habel test. Samples of duck embryo vaccine either failed both the NIH and Habel tests or passed the latter marginally.

Methods and Procedures

All vaccines were purchased from wholesale druggists or obtained from local health departments, stored at 4° C. until used, and tested prior to the expiration date. Manufacturers were designated by letter. Companies A, B, and C were the only companies from which nervous-tissue vaccine could be obtained, and company D was the sole producer of duck embryo vaccine. Company A has since discontinued production of vaccine.

Vaccine potency tests by the Habel method were made according to his procedures (*2*); those by the NIH test, as outlined by the Division of Biologics Standards of the National Institutes of Health (*1*). Unless otherwise specified, 5- to 6-week-old albino mice of the Albany Swiss strain were used for all tests. Mice were selected by stratified random sampling according to age, weight, and sex, if both males and females were used. Standard challenge virus (CVS25) was supplied by NIH, and infective mouse brain suspensions, stored at −56° C., served as the source of virus. Unless otherwise required, distilled water containing 2 percent normal horse serum was the diluent in all experiments. The standard reference rabies vaccine lots 164 and 167 were furnished by NIH.

Experiments

Preliminary tests in mice indicated that the standard reference vaccine lot 164, received from NIH in March 1961, possessed little protective activity. In five tests for potency by the Habel method, no appreciable evidence was obtained that the reference vaccine had value in protecting mice against challenge with rabies virus (table 1). Failure to protect presumably reflects vaccine instability and loss of potency after the reference vaccine was standardized and first distributed. The results of preliminary studies were presented to the Division of Biologics Standards on April 25, 1961, and lot 164 was withdrawn from use in May 1961.

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Representative samples of both duck embryo and nervous-tissue vaccines were then tested in 4- to 5-week-old non-Swiss mice of the Albany standard strain. Since the NIH test was impractical without a satisfactory reference vaccine, the Habel method was used. To meet the requirements of the Habel test, a vaccine must protect mice against not less than 1,000 LD_{50} of virus when challenged intracerebrally. In some tests the vaccinated mice were challenged with doses of virus ranging from 10^{-1} to 10^{-5} inclusive; in others the challenge dose ranged from 10^{-2} to 10^{-6}. Vaccines of nervous-tissue origin tended to give higher values than those of duck embryo origin, but none protected against 1,000 LD_{50} of virus.

Awareness that the strain of test mice used might be important (*4*) led to tests of five different vaccines in mice of the Albany standard, Albany Swiss, and Swiss Webster strains (table 2). The 50 percent effective dose (ED_{50}) of virus for each test was estimated according to the Reed-Muench method, as is customary in the Habel test, and also by the more reliable moving-average interpolation (MAI) method of Thompson (*5*). The Reed-Muench method has been shown by Thompson (*5,6*) to be based on erroneous principles and to be capable of

Table 1. Results of Habel tests of NIH rabies reference vaccine lot 164

Vaccine	Response of test mice, by virus dose (number reacting/number inoculated)							
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Vial 1	6/6	10/10	10/10	10/10	8/10			
Controls				10/10	10/10	10/10	4/8	0/10
Vial 2	5/5	9/9	8/8	9/10	10/10			
Controls				10/10	10/10	8/9	4/10	1/9
Vial 3		10/10	10/10	10/10	10/10	8/10	1/10	
Controls				10/10	10/10	7/10	2/10	1/10
Vial 4		10/10	10/10	8/10	9/10	5/10	1/10	
Controls				6/6	3/10	6/10	1/10	0/10
Vial 5		10/10	10/10	9/10	10/10	9/10	8/10	
Controls				9/9	10/10	10/10	8/10	1/10

Table 2. Influence of strain and age (weight) of mice used on results obtained in testing rabies vaccine by the Habel method [1]

Vaccine No.	Strain and weight of test mice									
	Albany standard				Albany Swiss, 16–26 gm.		Swiss Webster, 16–26 gm.		NIH standard Swiss, 16–26 gm.	
	11–15 gm.		16–26 gm.							
	R-M	MAI	R-M	MAI	R-M	MAI	R-M	MAI	R-M	MAI
1			17	13	433	473	46	53		
2			27	33	294	200	16	12		
3			285	215	1,549	1,995	804	611		
4			87	80	412	266	283	465		
5			877	465	966	555	1,122	1,178		
6, test 1	19	24	95	88	286	562			33	178
6, test 2	35	31	100	100	286	562			78	1,000
7			71	61	108	161			57	440
Reference vaccine lot 167					19,680	3,162			1,219	2,158

[1] R-M—Reed-Muench method; MAI—moving-average interpolation method. Each value is a ratio of the ED_{50} estimate for the inoculated animal to that for the control.

yielding unreliable estimates of the ED_{50}. As an index of protection, the ratio of the ED_{50} estimate for the inoculated animal to that for the control was used (table 2). Albany Swiss mice had a higher index in all five comparisons than the Albany standard strain mice according to either the Reed-Muench or the MAI estimates. They also had a higher index in four of the five tests according to the Reed-Muench method and in three of the five according to the MAI method than the Swiss Webster strain mice. All mice in these tests weighed 16–26 gm., which corresponds approximately to an age of 5–6 weeks.

A sixth vaccine was tested in duplicate with Albany standard, Albany Swiss, and NIH standard Swiss mice weighing 16–26 gm., and with Albany standard mice weighing 11–15 gm., which corresponds approximately to an age of 4–5 weeks. A seventh vaccine was tested with mice of these strains weighing 16–26 gm. These tests extended the series of comparisons between Albany Swiss and Albany standard mice of 16–26 gm. to eight, in all of which the Swiss mice yielded the greater index. In each of the two tests comparing the Albany standard mice of 11–15 gm., with three strains of mice weighing 16–26 gm., the lighter mice gave the lowest index according to both Reed-Muench and MAI estimates.

Reference vaccine lot 167, supplied by NIH in July 1961, was likewise tested, but only with Albany Swiss and NIH standard Swiss mice (table 2). With the two tests on vaccine 6 and the one on vaccine 7, this test provided four comparisons between these two strains of mice. The Albany Swiss mice yielded the greater index in all four tests according to the Reed-Muench method but in only two of the four according to MAI estimates.

In all these comparisons the difference is statistically significant according to the sign test (*7*) only for the Albany Swiss versus the Albany standard strain mice weighing 16–26 gm. The probability that the indices for each of the eight comparisons of these strains would differ in the same direction is $2^{-7}=1/128$. Since this does not exceed 0.01, the observed direction of differences is highly significant statistically.

On the basis of these observations, all subsequent tests were conducted with 5- to 6-week-old Albany Swiss or NIH standard Swiss strains. (The NIH mice were supplied by Dr. Karl Habel, chief, Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases.) Samples of vaccine representing 7 lots of Semple-type nervous-tissue vaccine of rabbit origin and 18 lots of vaccine of duck origin were tested by the Habel method. One lot of duck embryo vaccine was tested twice, using samples obtained from different sources. Vaccinated animals received challenge virus doses ranging from 10^{-1} to 10^{-5}, and control animals received doses ranging from 10^{-4} to 10^{-8}, inclusive. The results of all tests are given in table 3.

Table 3. Results of Habel tests of commercial rabies vaccines of duck embryo and nervous-tissue origin

Manufacturer and lot No.	Months before expiration date	ED_{50} protection	
		Reed-Muench	Moving-average interpolation
	Duck embryo vaccines		
D 737244	1	540	403
D 737246	1	948	1, 042
D 738156	8	1, 403	1, 059
D 738873	2	344	141
D 738876 [1]	1	3, 811	----------
D 738880	12	271	126
D 740650 [1]	1	185	126
D 740652	8	857	631
D 740660	3	369	79
D 740666	13	3, 365	541
D 740669	2	1, 167	438
D 740672 [1]	5	79	46
D 742080	7	219	213
D 742082	7	920	611
D 742086 [1]	14	255	40
D 742087	14	4, 446	6, 839
D 742088	14	22	8
D 742088	14	813	596
D 742091	15	232	233
	Nervous-tissue vaccines		
A 256A	1	4, 046	[2] >15, 850
A 258A	1	1, 135	155
A 259A	3	[2]>19, 320	[2] >15, 850
B 5205	1	8, 710	[2] >15, 850
C 212194	2	3, 945	15, 850
C 212195	3	1, 503	1, 585
C 212196	3	[2]>19, 320	[2] >15, 850

[1] Test mice were NIH standard Swiss strain.
[2] Protection beyond what could be evaluated.

By the Reed-Muench method of estimating the ED_{50}, as is customary in the Habel test (*3*), all seven lots of nervous-tissue vaccine passed, with values ranging from 1,135 to more than 19,320; the median was 4,046. By the more reliable moving-average interpolation method, one lot of vaccine, with a value of 155, failed. The ED_{50} of the other vaccines ranged from 1,585 to more than 15,850. The median for all vaccines exceeded 15,850. End points were not determined for four vaccines.

Whereas all the nervous-tissue vaccines passed the Habel test when evaluated according to the Reed-Muench method, 14 (73.7 percent) of the 19 samples of the duck embryo vaccine failed to pass when similarly evaluated. Values for the individual vaccines ranged from 22 to a maximum of 4,446; the median was 540. Of the 18 vaccines evaluated by the moving-average interpolation method, 15 (83.3 percent) failed, the values ranging from 8 to 6,839; the median was 318. Data obtained with one vaccine were not suitable for estimation by the MAI method.

Six lots of duck embryo vaccine were also evaluated for potency by the NIH method with reference vaccine lot 167 (table 4). This reference vaccine gave Habel test values of 54,450, 50,230, and 19,680 respectively when tested on three occasions in 5- to 6-week-old Albany Swiss mice, and 1,219 in NIH Swiss mice of similar age. None of the vaccines met the NIH requirement that the antigenic value of a vaccine under test should be at least 0.66 that of the reference vaccine. Vaccines of nervous-tissue origin were not tested by this method.

The effect of increasing the tissue concentration of duck embryo vaccines was next studied in the hope of increasing vaccine potency. Two lots of vaccine, 742088 and 742091, were tested

Table 4. Results of NIH tests of duck embryo vaccines using reference vaccine lot 167

Amount of tissue (mg.)	Response of test mice (number reacting/number inoculated)						
	Vaccine lot No.						Reference vaccine lot 167
	738873	740666	740669	742087	742088	742660	
20.0	2/16	2/16	5/16	6/16	0/16	2/16	0/16
4.0	11/16	7/16	12/16	11/16	10/16	12/16	6/16
0.80	14/16	15/16	16/16	15/16	16/16	15/16	14/16
0.16	15/16	16/16	16/16	16/16	15/16	16/16	15/16
Antigenic value [1]	0. 41	0. 64	0. 24	0. 26	0. 46	0. 32	----------

[1] Obtained by dividing the ED_{50} of the reference vaccine by the ED_{50} of the test vaccine according to the Reed-Muench method.

Table 5. Effect of increasing tissue concentration on protective activity of commercial rabies vaccines of duck embryo origin

Vaccine lot No. and tissue concentration (percent)	Response of test mice, by virus dose (number reacting/number inoculated)								ED_{50} protection (Reed-Muench method)
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	
742088:									
0.5	9/10	9/10	7/10	4/10	1/10	--------	--------	--------	813
1.0	2/10	3/10	5/10	6/10	0/10	--------	--------	--------	33, 650
Controls	--------	--------	--------	10/10	10/10	8/10	1/10	0/9	----------
742091:									
0.5	8/9	7/10	7/10	3/10	1/10	--------	--------	--------	232
1.0	5/10	4/10	0/10	1/10	0/10	--------	--------	--------	15, 140
Controls	--------	--------	--------	10/10	9/10	3/10	0/10	0/10	----------

by the Habel method with both the recommended dose of 0.25 ml. of a 0.5 percent tissue emulsion and a like volume containing 1.0 percent tissue. The results indicate that protection was markedly increased by doubling the tissue concentration (table 5). Whereas both vaccines failed to pass the test when animals were inoculated with the standard tissue suspensions, protection appeared to be increased 41.4-fold for one vaccine lot and 65.3-fold for the other when the tissue concentration was increased and the data were evaluated by the Reed-Muench method. The response of test animals given the higher tissue concentrations of vaccine 742088 simulated that reported by Habel (*8*) to be most commonly observed with vaccines of intermediate potency.

Discussion

Present-day commercial rabies vaccines for human use have definite limitations; deaths from rabies continue to occur in vaccinated persons and 14 or more daily injections of vaccine must be recommended for those under treatment for the prevention of rabies. In these respects, there has been little progress since the days of Pasteur.

Duck embryo vaccine, with its comparatively low content of nervous tissue, is a marked advance over nervous-tissue vaccines in regard to freedom from neurological complications (*9,10*). This product is currently preferred by most practicing physicians and health officers, both for postexposure treatment of persons bitten by rabid animals and for preexposure vaccination of high-risk groups.

The significance of the low values obtained with the duck embryo vaccines tested in this study is difficult to assess since vaccine efficacy in man must of necessity be evaluated principally in clinical trials or by measuring the ability of vaccine to stimulate production of serum neutralizing antibodies. Both methods have limitations. Most clinical or field evaluations lack adequate controls, and the relationship between the presence and titer of demonstrable serum neutralizing antibodies and resistance to rabies can be evaluated only in animals, since challenge experiments are not made in man.

The results of our studies indicate that improvement in antigenicity of certain lots of vaccine is desirable. It should be emphasized that the requirement of the Habel test that vaccine protect against at least 1,000 LD_{50} of virus is minimal; it may be too low. Past experience has shown that Semple-type vaccines of nervous-tissue origin can be so produced and standardized that the minimum is exceeded substantially. On the other hand, duck embryo vaccine has never been required in the United States to meet Habel-test standards, since it was approved for release after the Habel test was replaced by the NIH test in 1953. Our studies indicate that duck embryo vaccines of recent production, obtained through normal commercial channels, not only fail the NIH test but usually fail the Habel test or, at best, pass marginally. Considerable variation apparently exists between the antigenicity of different lots of vaccine; this does not appear to be correlated with the age of the vaccine.

Vaccine standards and testing procedures for rabies vaccines for human use should be critically reviewed and reevaluated. It is not known what effect, if any, the use of substandard reference vaccine has had on the potency of commercial vaccines. It is known, however, that many of the vaccines tested in this study were evaluated against reference vaccines other than lot 164 prior to release. Every effort should be made to assure distribution of stable reference vaccines of satisfactory potency. Where this cannot be done, minimum requirements for vaccines should be established in terms of the amount of tissue required to protect against a fixed number of LD_{50} of virus as tested by the NIH method.

Vaccines such as those tested in this study which failed the Habel test would not be acceptable in those countries now using this method of measuring vaccine potency and would not have met the NIH standards for such products in this country prior to September 1953. Since each firm producing nervous-tissue vaccine containing inactivated virus for use in animals has been required by the Animal Quarantine Division of the United States Department of Agriculture to substantiate the efficacy of its product by the Habel test before licensing, such vaccines presumably would not be approved for use in animals.

The widespread use of hyperimmune antirabies serum further emphasizes the need for rabies vaccines of high antigenicity. Administration of serum, recommended under certain conditions by the World Health Organization's Expert Committee on Rabies (*11*), has been shown to be capable of interfering with production of serum neutralizing antibody by vaccine. It is logical to expect that suppression of antibody production by serum is least when vaccines of high potency are used and greatest with vaccines of low antigenicity.

Evidence has been presented that the protective effect of duck embryo vaccine can be increased substantially by increasing the tissue concentration. If the tissue concentration can be increased without unduly increasing the risk of adverse local or systemic reactions, as seems likely, vaccine potency could be substantially improved; this possibility should be explored further.

The market samples of duck embryo vaccine which did not pass the tests in this study would not have been released had they been tested previously against satisfactory standard reference vaccine. The National Institutes of Health asserts it has corrected this situation. Current production lots of duck embryo vaccine, lot 780485 and higher, contain embryonic tissue equal to a 10 percent wet weight suspension of nervous tissue and have met the minimum requirements of potency of the National Institutes of Health when tested against satisfactory reference vaccine. The use of even greater amounts of tissue is under investigation by the company (personal communication).

Summary and Conclusion

Each of seven lots of Semple-type nervous-tissue vaccine of rabbit origin produced by three companies passed the Habel test when examined in a laboratory of the New York State Department of Health, although considerable variation existed between lots. Thirteen (72.2 percent) of 18 lots of duck embryo tested did not meet minimal Habel-test standards; the others passed marginally. Six lots of duck embryo vaccine evaluated for potency by the NIH method failed to meet minimal standards established by NIH for vaccines containing inactivated virus. The potency of this type of vaccine can be markedly improved by increasing the amount of tissue present.

Lot 164 of reference vaccine, distributed by the National Institutes of Health and the World Health Organization as an aid in evaluating vaccine potency, offered little protection when evaluated by the Habel method in mice. Special efforts are necessary to assure distribution of stable reference vaccines of satisfactory potency.

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