Titration of Smallpox Vaccines from Ten Countries Sent to East Pakistan During the 1958 Smallpox Epidemic *

by S. P. WANG M.D., *Senior Guest Investigator, United States Naval Medical Research Unit No. 2, Taipei, Taiwan; and Chief, Division of Virology, Taiwan Serum Vaccine Laboratory, Shih-lin, Taiwan; and J. THOMAS GRAYSTON M.D., Chief, Divisions of Microbiology and Epidemiology, United States Naval Medical Research Unit No. 2, Taipei, Taiwan; and Assistant Professor of Medicine, School of Medicine, University of Chicago, Chicago, Ill., USA*

During the 1958 smallpox and cholera epidemics in Pakistan, the United States Naval Medical Research Unit No. 2 sent a team of scientists to East Pakistan to advise and assist in laboratory diagnosis and vaccine production. Many countries of the world had sent smallpox vaccine to aid in the control programme undertaken by the Pakistan Government. Samples of many of these vaccines from the various lots received were made available to us during May 1958 by the Pakistan Government for potency testing. This report shows that despite the varied methods of shipping and handling from all over the world the great majority of these vaccines of widely different manufacture showed adequate potency.

Materials and methods. The vaccines that were titrated were collected in Dacca, East Pakistan, presumably from recently received lots. All had been removed from shipping containers, and each lot sampled was being used in the field. A few of the samples tested had been taken into the field and returned to Dacca. Samples were not obtained from all countries shipping vaccine to Pakistan. Some of the samples from partially used boxes or returned from field use did not have identifying numbers and expiration dates. A list of the vaccine samples tested with the information available follows:

<i>Vaccine</i>	<i>Producer</i>
A	Commonwealth Serum Laboratories, Melbourne, Australia. Lymph vaccine in 50% glycerol. Date of issue: 23 April 1958. 100 doses per vial.
B	Connaught Medical Research Laboratories, University of Toronto, Toronto, Canada. Lymph vaccine, glycerinated. Expiration date: 9 August 1958. 1 ml per vial.
C	Behring Werke, Marburg-Lahn, Germany. Lymph vaccine, lyophilized; diluent provided. Date of preparation: 5 May 1958. 100 doses per vial.
D	Landes-Impfanstalt, Düsseldorf, Germany. Lymph vaccine, glycerinated. Expiration date: 21 May 1958. 250 doses per tube.

* From the United States Naval Medical Research Unit No. 2 (NAMRU-2), Taipei, Taiwan, and the Department of Medicine, School of Medicine, University of Chicago, Chicago, Ill., USA. This investigation was supported in part by a contract Nonr-2121 (07) between the Office of Naval Research and the University of Chicago. The opinions and assertions contained herein are those of the authors and are not to be construed as official or as reflecting the views of the Navy Department or the naval service at large.

- E Institut Pasteur, Teheran, Iran. Lymph vaccine, glycerinated. Expiration date unknown. One dose per capillary tube.
- F Kitasato Institute for Infectious Diseases, Tokyo, Japan. Lymph vaccine, glycerinated. Expiration date: 20 July 1958. 10 doses per capillary tube.
- G Institute of Public Health, Dacca, East Pakistan. Lymph vaccine, glycerinated. Date of preparation: 5 March 1958. 100 doses per 2 ml.
- H Department of Health, Manila, Philippines. Lymph vaccine in dried state; diluent provided. Expiration date unknown.
- I Institut sérothérapique et vaccinal suisse, Berne, Switzerland. Lymph vaccine, glycerinated. Expiration date: 20 August 1958. 100 doses per vial.
- J Gamaleia Institute, Moscow, Union of Soviet Socialist Republics. Lymph vaccine, lyophilized; diluent provided. Expiration date: 24 December 1958. 10 doses per ampoule.
- K Lister Institute of Preventive Medicine, Elstree, Herts., United Kingdom of Great Britain and Northern Ireland. Lymph vaccine, lyophilized; diluent provided. Date of preparation: 11 April 1958. 25 doses per tube.
- L Lederle Laboratories Division, American Cynamid Co., New York, N. Y., United States of America. Chick-embryo vaccine, lyophilized; diluent provided. Expiration date unknown. 100 doses per 2 ml.
- M National Drug Co., Philadelphia, Pa., United States of America. Lymph vaccine, glycerinated. Expiration date unknown. One dose per capillary tube.
- N Wyeth Laboratories, Inc., Marietta, Pa., United States of America. Lymph vaccine, glycerinated. Expiration date: 9 August 1958. One dose per capillary tube.

Vaccines E, H and M were received without identifying labels. They are presumed to be from the stated source on the basis of a hand-written note accompanying the material.

All vaccines were transported under refrigeration to Taipei where the titrations were carried out. The chorio-allantoic membrane of the chick embryo was employed for titration of the vaccines. The method used was that of Burnet^a of counting the pock lesions produced on the membrane. Studies of this method have been made recently by Overman & Tamm,^b and Cockburn et al.^c Briefly, the following procedure was adopted.

Dried vaccines were reconstituted according to the instructions of the manufacturer. The liquid form of vaccinia suspension was then diluted as follows: 0.1 ml of each vaccine was mixed with 9.9 ml of diluent to give a 10^{-2} dilution, and tenfold serial dilutions were made through 10^{-7} . The diluent used was McIlvaine's 0.01 M phosphate buffer (pH : 7.4), containing 10% broth, 50 units per ml of penicillin and 2 mg per ml of streptomycin. 0.1 ml of each dilution (10^{-3} through 10^{-7}) was inoculated on the chorio-allantoic membrane of each of five 11-12-day-old eggs. After inoculation, eggs were incubated at 35°C for 44-48 hours and opened to record the number of pocks on the chorio-allantoic membrane. The

^a Burnet, F. M. (1936) *Spec. Rep. Ser. med. Res. Coun. (Lond.)*, No. 220

^b Overman, J. R. & Tamm, I. (1956) *J. Immunol.*, **76**, 228

^c Cockburn, W. C., Cross, R. M., Downie, A. W., Dumbell, K. R., Kaplan, C., McClean, D. & Payne, A. M.-M. (1957) *Bull. Wld Hlth Org.*, **16**, 63

potency is expressed in infectious units per ml of the original vaccinia suspension. This is computed from the mean pock count at the lowest dilution giving discrete, countable pocks.

Results. The results of the infectivity titrations of the 14 samples of vaccine are shown in the accompanying table. There is no connexion between the sequence of the vaccines and the sequence of producers given above. All the vaccines were titrated at one time and repeat titrations were performed on the three vaccines with the lowest infectivity titre along with two high titre vaccines. The repeatability of the titrations on second test was within one dilution tube, which represented less than a tenfold variation from the first test.

INFECTIVITY TITRES OF 14 SMALLPOX VACCINES
TESTED ON CHORIO-ALLANTOIC MEMBRANE
OF CHICK-EMBRYOS

Vaccine number	Infectious units per ml	
	tested on 23 June 1958	tested on 9 July 1958
1	2.6×10^7	2.2×10^7
2	3.3×10^7	
3	6.0×10^7	
4	3.3×10^7	1.1×10^8
5	2.2×10^7	
6	2.8×10^7	
7	1.0×10^8	7.6×10^8
8	1.3×10^7	
9	1.8×10^7	
10	9.0×10^8	9.4×10^8
11	6.4×10^7	
12	4.6×10^8	1.6×10^8
13	3.1×10^7	
14	1.2×10^7	

The studies of Cockburn and co-workers^c have suggested that vaccines which show pock count infectivity titration of 10 million units per ml will prove to be of excellent potency as measured by the percentage of vaccination "takes" produced in susceptible individuals.^d The table shows that 11 of the 14 vaccines showed titres higher than 10 million infectious units per ml. Vaccine No. 10 showed nearly 10 million units and No. 7 vaccine on retest also approached this number. Only vaccine

^d These workers found a theoretical relation of 99% "takes" with vaccine showing pock count infectivity of 1.3×10^7 and 90% "takes" with vaccine showing 2.4×10^8 infectivity units.

sample No. 12 fell significantly below the optimal 10 million units. Since this vaccine had been removed from its original carton there was no way of checking how long it had been in Pakistan or the conditions of shipping and storage.^e

No consistent difference in potency was found between the lyophilized and glycerinated vaccines. The one vaccine of chick-embryo origin showed high-titre infectivity.

Conclusions. With one exception the vaccines tested showed sufficiently high infectivity titres to be considered of adequate potency. Because of the lack of control of the conditions to which the vaccines were subjected prior to infectivity titration, no conclusion can be drawn from these tests concerning the superiority of any particular vaccine or type of vaccine. Within the limitations of the present investigation, a favourable attitude can be taken in emergency situations towards the use of smallpox vaccine produced in a variety of laboratories and shipped by modern methods to distant parts of the world.

^e Another sample of vaccine, prepared by the same producer as sample No. 12, and identical in appearance to sample No. 12, was obtained directly from the producer and designated No. 15. It was titrated on 17 December 1958, along with vaccines Nos. 8 and 12 as controls. Sample No. 15 showed 1.8×10^7 infectious units per ml, while Nos. 8 and 12 showed 2.1×10^7 and 1.5×10^8 infectious units per ml respectively. This test would seem to indicate that the vaccine prepared by the producer of sample No. 12 had excellent potency and leaves unexplained the reason for the poor quality of the material obtained in Dacca and designated No. 12 in the table.