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Report
of a
CONSULTATION ON TRANSFER OF TECHNOLOGY
FOR PRODUCTION OF RABIES VACCINE *

Geneva, 16-17 January 1986

- * Convened by the Rockefeller Foundation in collaboration with the Veterinary Public Health Unit, Division of Communicable Diseases, WHO, Geneva.

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The meeting was opened by Dr. F. Assaad, Director, Division of Communicable Diseases, W.H.O., who emphasized the importance of dealing with the difficult matter of the transfer of technology, in this case rabies vaccine production. This was the third in a series of meetings held by the WHO Consultation on Transfer of Technology for the Production of Rabies Vaccine, the first in October, 1984¹, the second in December, 1984². Dr. Assaad appointed Dr. Scott Halstead as chairman.

Dr. Halstead suggested that each participant identify himself (the list of participants is given in Annex A). A discussion then began about the progress made at three laboratories in adapting rabies virus strains to VERO cells.

Dr. Baer (Atlanta) stated that only limited testing had been done with one strain (Pasteur) on VERO cells at CDC - at the 5th or 6th passage level only low virus titers were obtained.

Dr. Schneider (Tubingen) said that he had adapted 3 strains of virus to VERO cells as follows:

¹ Report of a WHO Consultation on Transfer of Technology for Production of Rabies Vaccine, Geneva, 23-26 October 1984 (Document WHO/Rab.Res./85.21, WHO/Rab./85.197).

² Report of WHO Group of Experts on Rabies Vaccine Production Techniques, Geneva, 20-22 December 1984 (Restricted document - no reference).

1. PV₄ (the same as RV31 from Institut Pasteur) from fetal bovine kidney cells; now gives a titer of 10⁷
2. PV₂ (PV₁₃ Habel BOB) currently gives a titer of 10⁵
3. PV₂ (PVI April 26, 1946) "

Dr. Van Wezel (Bilthoven) said that a variety of viruses had been adapted to VERO cells (ATCC) at the Rijksinstituut voor Volksgezondheid en Milieuhygiene including the Wistar strain originally adapted to human diploid cells, the (PV) RV₃₁ (Pasteur) strain, a Chinese strain, and an Egyptian strain originally adapted to primary goat cells. All these strains grew very well on VERO cells (after adaptation to other tissue culture cells). The Chinese strain was not immunogenic. The strain from Egypt had a high titer but the antigenic values after inactivation were limited.

RV₃₁ gave high titers (10⁶-10⁷/ml) and antigenic values of 3 or above. The original Pasteur strain (seed PV) was adapted to primary canine kidney cells, and after 95 passages was further passed 2-3 times on VERO cells, giving resultant antigenic values of .5-1.0.

Dr. Petricciani (WHO) discussed human vaccines in general, beginning with substrates of primary cells and human diploid cells in the 1960s. Recent movement, beyond the traditional cell types, has raised issues still not resolved, including two major safety questions: the presence of extraneous viruses, especially retroviruses, and the potential, through cellular DNA, to produce damage itself. Polio vaccine on VERO cells is licensed in Belgium and France, and is pending in the U.S. This was based on a reduction of cellular

DNA to the 10-100 picogram level, a level below that even theoretically capable of causing damage. (Also important is whether the cellular DNA is incorporated into the virus itself.) Before vaccine production begins with any cell it is essential to characterize the cells properly, including the establishment of a cell bank.

Dr. Bogel (WHO) questioned the level of 10 picograms as a minimum for DNA (based on polio vaccine). That question will be discussed at a coming WHO meeting, especially what level is necessary to cause an oncogenic event; it may very well be that a higher DNA level is permitted.

Dr. Mitchell (Michigan) then discussed the rhesus diploid rabies vaccine produced at the Michigan Department of Public Health. The virus used is the Kissling CVS strain adapted to hamster kidney cells, passed 13 times on rhesus cells. Inactivation is with Betapropiolactone - aluminum phosphate ($AlPO_4$) is used to concentrate the harvest about 17 times. Lactose and human albumin (1% each) are used for stabilization. The volume that has to be centrifuged after $AlPO_4$ addition is limited since approximately 20% of the fluid can be aspirated (the $AlPO_4$ adsorbed material settles to the bottom). Centrifugation is at 600G. After adsorption and centrifugation the antigen is washed three times with serum-free growth medium. The usual potency of the final product is 3-5 I.U.

Extensive washing of the $AlPO_4$ with distilled water is needed. The vaccine is resuspended in physiological saline. Multiple harvests have not been made with stationary cultures, but with roller bottles up to 4 harvests have been made; repeated harvests would, of course, bring the price down considerably.

Clinical trials with the vaccine have gone on for five years, with over 3,000 people vaccinated; over 99% developed acceptable antibody levels. The vaccine was also effective in simulated post-exposure trials. Field use has begun in Michigan, but in limited numbers. Adverse reactions have been mild to moderate. The vaccine regimen recommended for human use (5 doses) is the same as that for human diploid vaccine; the volume used is 1.0 ml. The vaccine is distributed as a liquid, and is not lyophilized. U.S. licensing is expected in March, 1986.

Dr. Mitchell sees no problems with patenting this vaccine or with transferring the technology of production. Dr. Halstead suggested that the level of technology was "eminently suited" for transfer, the conditions at the Michigan laboratory being quite similar to those in some developing countries. It was also emphasized that developing countries want to have technology transferred, and that that technology should now be for continual cell passage rather than primary cells. Dr. Bogel insisted that the production of rabies vaccine should always go hand in hand with the development of national rabies programs, since an infrastructure must be established and available to distribute the vaccines produced, be they the old type vaccine or those produced with newly transferred technology.

Dr. Petricciani said that an international meeting will be held in November, 1986, to involve WHO and numerous other organizations in the development of a cell bank to hold cells fully characterized at given passage levels, in order to avoid each vaccine manufacturer having to go back and again make dozens of passages of a given cell in vaccine development.

The site visits to the various countries were then discussed.

1) Burma - that country currently uses sheep brain vaccine. Rabies in Burma is not an officially notifiable disease, but is a serious national problem; incomplete reporting indicates that there were 42 deaths in 1983, with 2876 persons treated (one-fourth of the districts reporting). Total vaccine production is approximately 20,000 doses per year. The vaccine is tested by the Habel test. The production building is seriously flawed with impossible working conditions.

The Veterinary Viral Vaccine Development Center of the Veterinary Division, Ministry of Agriculture, has FAO support, with modern equipment and production, and a full-time FAO consultant. No rabies vaccine is produced there, but foot and mouth vaccine, hog cholera vaccine, and several chicken vaccines are. There is also a department of medical research with a full spectrum of biomedical research, including building and equipment maintenance by engineers funded by the Canadian International Development Research Center.

The team (Reculard and Halstead) made the following recommendations:

- a. Appropriate priority should be given to the production of good quality human vaccines in Burma.
- b. Consideration should be given to a coordinated multi-agency - Burmese Government program aimed at a significant upgrading of BPI biological production facilities. At a minimum, this upgrading should include adequate and stable electric power, modern air ventilation, sterile filtered air in production rooms, adequate supplies of steam,

water, gas and other production resources and a responsive equipment maintenance staff. This is an essential prerequisite to a WHO rabies vaccine technology transfer program.

c. In addition, the consultants recommend the following actions:

1. Rabies case reporting and dog bite reporting should be improved.
2. BPI should use the NIH method for potency testing.
3. The National Health Laboratory should provide independent quality control on human and veterinary rabies vaccines using the NIH test.
4. The BPI should immediately begin to produce anti-rabies IgG in horses, permitting modern and efficacious anti-rabies treatment to be available in Burma.

Since Burma has 40,000,000 people it would have an estimated need for 300,000 doses of rabies vaccine annually.

Colombia -

Dr. Raul Londono is Director of VECOL (Veterinaria Colombiana) , an unusual institute which is supported by the Federal Ministry of Agriculture (80%) and the private National Livestock Association (20%). The laboratory produces bacterins, bacterial antigens, pharmaceuticals and viral vaccines; its principal product is foot and mouth disease vaccine, which it produces at a rate of 28,000,000 doses yearly. There are space limitations of the present physical plant, wich is to be enlarged dramatically in 1985 and 1986, at a

cost of 12 million dollars. There are numerous fermentors (up to 2,000 liters, but of various sizes), bottling areas, areas for media preparation, storage areas, cold rooms, maintenance areas, bottle washing areas, etc. in the laboratory, which is impressive in its scope, size, and cleanliness; all the people appear busy and organized.

They have been preparing inactivated rabies vaccine for 3-4 years. The vaccine is prepared on BHK₂₁ cells with the PV strain of virus. Present production is 80 liters per week (equivalent to 80,000 doses); production apparently could be increased another 50% to make 126,000 doses weekly, or 5 million doses per year. The vaccine is currently prepared only in roller bottles. Research is being done in 5 liter fermentors by growing the cells and virus in suspension culture; one or two experimental lots of suspension culture vaccine were prepared in 1985. Research recently began on the growth of BHK cells on microcarriers, using BELCO magnetic stirrer culture vessels. They plan to develop their own fermentors for microcarrier culture.

There is keen interest and enthusiasm in the VERO cell technology transfer. An agreement between Colombian Ministries of Agriculture and Public Health and the Pan American Health Organization (PAHO) designates VECOL as the laboratory to produce certain human diagnostic reagents and products, such as PPD (appendix 1).

In contrast, the rabies vaccine production areas of the Instituto Nacional de Salud (National Institute of Health) (with Dr. Mendoza as head of rabies production) were clean, rather spartan, but ample. Suckling mouse brain vaccine for both human and canine use is produced, approximately one

million doses for dogs and 200,000 for humans; its potency is good, averaging 2.5-4.0 IU/ml. A reduced schedule of 7 doses is used in exposed persons. They have had trouble producing enough vaccine in the last two years because of reduced mouse production (due to problems with feed) and the number of total doses produced has fallen by 50%. Preparation of BHK tissue culture vaccine has been attempted since 1979, but with only limited success; there are 2 laminar flow hoods, one for cell production, one for infecting cells with virus and for harvest, both of which were clean and well kept. Although the Institute produces a number of bacterial antigens and vaccines, none of these are produced in fermentors.

Conclusions and Recommendations made by Drs. Van Wezel and Baer:

It is clear that VECOL has the greatest promise for effective technology transfer. It has vast experience with tissue culture and fermentor technology, has an efficient and enthusiastic staff, both professional and technical, and a modern, clean physical plant (although presently a little cramped). Many of the two-dozen or so fermentors we observed have capacities of many hundreds or thousands of liters. Much training is needed to transfer the technology of human VERO cell rabies vaccine, including:

1. Proper virus growth on VERO cells (both monolayer and in fermentors).
2. Seed cell and seed virus organization.
3. Microcarrier culture.
4. Proper control of microcarrier fermentors.
5. Quality control.

It would make scientific and economic sense to produce one human tissue culture vaccine (rabies) in a first-class laboratory that already produces numerous veterinary vaccines; many of the techniques needed and much equipment and supplies are on hand.

Egypt

There has been some experimental work on tissue culture vaccines, but all rabies vaccines are still of brain origin. Current vaccines are of very poor quality; rabies continues to be a great problem up and down the Nile. Many trained and able scientists have apparently left the Organization for Biological Products and Vaccines, and no international 'plans' begun in the last few decades have been effective.

One person (Dr. Rifki Karamany) is responsible for rabies production (Fermi vaccine) with an able group of 9-10 people assisting him; his laboratory is also responsible for producing Rift Valley Fever vaccine. Experimental lots of VERO cell rabies vaccine have been produced, with potency tests pending at Bilthoven (Dr. Van Wezel).

China

George Baer visited the Wuhan Institute for Biological Products in Wuchang, Hubei Province, China, the week of October 28th, 1985. The Institute is one of the main producers of human rabies vaccine in the country, sharing that responsibility with four other institutes throughout the country. The vaccine is produced on primary hamster kidney cells; the hamsters are raised

on an animal farm near the Institute, in an administrative branch of the Institute. The two large incubator rooms observed were full of 10 liter roller bottles, each with a liter of tissue culture fluid, with a total weekly production of 70-80 liters. The total number of human rabies treatments administered nationwide is approximately 500,000.

The facilities in Wuchang are spartan and old-fashioned, the building having been built in the 1930's (or earlier), but was clean and functional, with central heating and air conditioning. All the supplies, including media and glassware, are produced in China, as well as much of the equipment. The staff that produced the vaccine under the direction of Dr. Lin Fang-tao appeared very dedicated and quite knowledgeable.

They are concerned whether their vaccine is the best for China, especially with newer vaccine technology available. The possibility of producing VERO vaccine was discussed and they are very interested in changing to that technology, pending the availability of an adequate seed virus. The facilities at the Institute would permit them to produce much more vaccine than they do now, especially with a VERO vaccine which would give them one logarithm more virus titer per harvest than they get now with the primary hamster cells. Up to a year ago their vaccine potency was tested by the Habel test in Beijing, with high values (high hundreds of thousands or millions) but they have recently changed to the NIH test with adequate levels although with a lot of variability, probably due to the $AlPO_4$ adjuvant added.

The biggest investment needed at that Institute would be training in VERO cell culture and virus adaptation to cells, although a few pieces of equipment (freezers for cells and virus) might also be needed.

India

Rabies - Rabies is endemic in dogs (and other species) in India. There are an estimated 500,000 human treatments yearly and 20-30,000 human deaths. Semple vaccine is used almost exclusively, 14 daily doses of 5 ml.

Coonoor - George Baer visited the rabies vaccine production facilities at the Pasteur Institute of Southern India in Coonoor on October 18th and 19th. The facilities are clean but old, having been built at the beginning of the century. Experimental lots of VERO cell vaccine have been produced for a number of years by Dr. Prasada Rao, who trained under Dr. George Turner. The production rooms are very small and limit the number of doses that can be produced. The lots appear to have good potency. One big advantage the Indians have is access to the VERO-adapted strain of fixed virus from the Pasteur Institute given to them in 1982 in an agreement made while Turner was in India; they have the permission to use the strain in India only. The facilities, equipment, and supplies, are all made in India, including the tissue culture media and glassware. They have begun working on the matter of residual DNA in their harvests. The group there could produce enough vaccine for the country but not with the limited physical plant now used. A new physical plant is planned for the near future. All of the personnel appeared dedicated and meticulous.

Pune - At the Serum Institute at Pune (a private company) they have begun to produce antitoxins (tetanus) as well as DPT vaccine and the Government of India is now interested in working with private companies; this company is interested in producing inactivated measles, rabies, and polio

vaccines. The building is almost ready now, with equipment ready to be installed, with a enthusiastic and motivated virology group. Some have been trained in basic tissue culture techniques in Bilthoven and others are about to be trained in fermentors and other modern methods in virus vaccine production; all indications are that they will be able to produce any vaccine they undertake. A mixing container of 2,000 liters is being installed for DPT production, 4,000,000 doses yearly can be produced. Funding here appears not to be a problem, and there are also other private firms interested in supporting technology transfer.

Indonesia

Rabies is endemic in Indonesia, especially in Sumatra, West and Middle Java, Kalimantan, and Celebes, mostly transmitted by dogs. Although grossly underreported, approximately 10,000 persons annually are treated; 50-70 human deaths are reported. Monkey brain vaccine production was stopped a few years ago; suckling mouse brain vaccine is now used, mostly imported (at a cost of about \$2 per dose). Bio-Pharma is a quasi-governmental institute, with its own budget, which also produces tetanus, diphtheria and pertussis vaccine. The suckling mouse brain vaccine is tested by the NIH test, but with no external reference vaccine. Experience with tissue culture has just begun, with some expertise already present.

The infrastructure at the institute is good with all necessary equipment present - electricity, steam, gas, etc.

Dr. Halstead mentioned that it is imperative to quickly identify those laboratories that should be brought into this program, since the Rockefeller Foundation is reevaluating the thrust of its activities and wants to increase its international presence; one of the main thrusts will be the transfer of technology. A grant would be given to one particular agency to handle the transfer of this rabies vaccine production technology; the original idea was to transfer only VERO cell technology (raw supernate for veterinary use, the purified material for human use), but the rhesus fetal lung vaccine technology is also of interest now. It would certainly be possible to begin with one type of cell and virus and then change to another, the main purpose is training in cell culture production, both at the bench and the management level.

Since the rhesus fetal lung vaccine from Michigan should be licensed in the U.S. in a month or two, with no apparent restriction to the release of the strain of virus to developing countries, it was the consensus of the group that that strain should be used. The Kissling strain, adapted to VERO cells, would then be used for further VERO passage. It may be advisable, also, to use the rhesus diploid vaccine in those countries which need only limited amounts of vaccine.

Colombia appears to have the strongest infrastructure for vaccine technology transfer. They would be in a position to compare VERO and BHK vaccine for veterinary use. In addition, the Ministry of Health there has agreed to purchase human vaccine from the Ministry of Agriculture (VECOL). Both Indonesia and China are countries which also have interest in this

transfer of technology and which should be considered after Colombia; further visits to those two countries are needed to establish exactly how they fit within the program.

Training - The group to be trained should train in a vaccine production facility, to include cultivation technology packaging and vaccine control. The suggestion was made that a 'marriage' be made between VECOL and the Rijksinstituut laboratory, with training of personnel to begin as soon as possible. Seed virus from Michigan (one passage before production seed) is to be sent to Bilthoven the week of January 20th, with adaptation to VERO cells to be done as soon as possible. A preliminary visit is to be made by Bilthoven staff to VECOL, with possible follow-up visits, to arrange details of the training. A total of four persons should be trained in cell culture techniques, starting in monolayer culture, then advancing to fermentor culture with microcarriers. Training in Bilthoven would probably begin in late summer or early fall; there may be initial training in tissue culture and virus adaptation to tissue culture (and English) at CDC.

Further site visits - countries that should be visited are Cameroun (Rabies vaccine production is to begin after tetanus and measles vaccine production in Garova, with UNIDO having set up technology transfer of vaccine production), Morocco (money has been set aside to improve veterinary vaccines, Dr. Reculard is set to go there soon), Turkey (good veterinary disease surveillance, little disease control), China, Indonesia, Algeria, Brazil, and Mexico. Those people to visit countries in 1986 are:

Morocco - Reculard

- Meslin

Indonesia - Bogel

- Van Wezel, Letchworth

China - Baer

- Brown

Algeria - Lundbeck

Turkey - Mitchell

- Van Wezel

Brazil - Baer

- Van Wezel

Mexico - Baer

The meeting was adjourned at 4:00 p.m.

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