

# Effectiveness of Live or Killed Plague Vaccines in Man

K. F. MEYER, M.D.<sup>1</sup>

*While the safety of the available live plague vaccine EV 76 (Paris) continues to be the subject of further study, the USP formol-killed, virulent Pasteurella pestis (Yersinia pestis) suspension capable of protecting 60% of non-human primates, particularly Hanuman langurs (Presbytis entellus), warrants further clinical tests and field trials. Inoculated in a dosage of  $2 \times 10^9$  killed plague bacilli (1 ml), followed by a booster of 400 million organisms (0.2 ml) in 1-3 months, this vaccine stimulates the appearance of passive mouse-protection antibodies (below an index of 10) and passive haemagglutinins in 60%-65% of human subjects. Recent experiences in Viet-Nam demonstrate that personnel vaccinated with the USP vaccine, although frequently exposed, enjoy almost complete freedom from the disease. One of the 4 known and confirmed cases of bubonic plague in North Americans occurred in an unvaccinated individual. Among individuals inoculated with the USP vaccine, 2 confirmed cases of pneumonic plague and 1 case of asymptomatic pharyngeal plague have been recorded. The incidence of plague in the Republic of Viet-Nam during the past 3 years is estimated at 13 263 cases in a population in part vaccinated with a live plague which exhibited inadequate immunogenic efficacy in experimental tests.*

There is no doubt that in the past prophylactic vaccination against plague with "avirulent" (or better, "attenuated") live vaccine as practised by Otten (1936, 1941), Girard (1936), Girard & Robic (1938, 1942) and Grasset (1942, 1946) in endemic areas has substantially reduced both the infection rates and mortality rates in vaccinated subjects. In the absence of more recent experimental comparisons of such vaccines, it is difficult to decide whether advances have been made in immunization with live plague vaccine. Otten (1933, p. 148) stated that "the problem concerning the protective value of plague vaccines in man is not solved. To approach a solution of the question, human susceptibility to plague infection and response to immunization should be known and on this point reliable information *a priori* is not available. The monkey seems to be the most suitable animal to bring this problem nearer solution". This suggestion prompted the formation of a standardized plan to evaluate any plague vaccine, whether living or killed, first on

rodents, then on non-human primates and finally on human subjects. Two reviews (Meyer, 1953; Meyer et al., 1948) concluded that attenuated plague strains must possess the same antigenic constitution as the virulent isolates and must be proved immunogenically potent both for mice and guinea-pigs.

Of 28 macaques (*Macaca mulatta*), each inoculated with  $1 \times 10^9$  (565 million viable) *Pasteurella pestis* (*Yersinia pestis*) strain EV 76, obtained through the courtesy of Dr G. Girard, 18 out of 28 (64%) of the vaccinated and 4 out of 32 (12.5%) of the controls survived a severe subcutaneous challenge infection of  $2.3 \times 10^9$  virulent *P. pestis* strain 195/P. The serum of 5 vaccinated macaques revealed a mouse-protective antibodies index (MPI) below a value of 10 and none displayed any local inflammatory reactions at the site of the infection while the other 13, with an MPI above 10, exhibited varying degrees of ulceration and involvement of regional lymph nodes, indicating that in this species of non-human primate only a relative immunity was conferred by the EV 76, which might have undergone mutation.

Inoculations of 1 ml of the same suspension that was used for macaques were made in 12 human subjects, being given subcutaneously between the

<sup>1</sup> Director Emeritus, G. W. Hooper Foundation for Medical Research, and Professor Emeritus of Experimental Pathology and Microbiology and Lecturer in the History of Health Sciences, University of California, San Francisco Medical Center, San Francisco, Calif., USA.

scapulae in order to reduce the severity of the reaction. All members of the group, varying in age from 21 to 59 years (average 39 years), experienced marked systemic reactions, namely, general malaise, aching and anorexia. Temperatures varied from 98.6 F to 103.6 F (37°C–39.8°C) at the end of 24 hours and remained at 100°F (37.8°C) in 2 of the 12 men for 126 hours. At least half of the group was incapacitated for as long as 72 hours; 2 were hospitalized for 48 hours. Local reactions were confined to hot, erythematous, moderately indurated, painful areas with axes measuring 5 cm–6 cm. One human subject exhibited a dusky erythematous (carbuncle-like) lesion at the site of inoculation which disappeared in 6 days. The verdict of a group of physicians who appraised this trial was that mass vaccinations with the EV 76 strain would be inadvisable because of the unpleasant side-effects. However, despite these reactions, an antibody response compatible with some degree of immunity was recorded in 4 (33%) of the vaccinated subjects. The MPI (average 9.2) was within the range characteristic for human subjects given a single inoculation of killed vaccine suspension containing  $3.6 \times 10^9$  organisms.

Eight months after the basic inoculation, the antibodies of 9 volunteers who were still available had dropped to a lower level (average MPI of 12). Permission to reinoculate with the live vaccine was refused and booster inoculations of 1 mg of Fraction 1 in saline produced no local or systemic reactions. On the 28th day, the average MPI was 8.6; 3 sera yielded an MPI below 5 plus agglutinins and complement-fixing antibodies. This test indirectly demonstrated that, despite severe local and systemic reactions, the vaccination sensitized only 3 of the 9 men, as indicated by reactions to the booster. Hence, further clinical trials were discontinued.

At a WHO seminar on plague control in the USSR in 1965, the low efficacy of killed vaccines and high immunogenicity of live attenuated vaccines, particularly strain EV 76, was emphasized. During the discussions, directions distributed for the use of freeze-dried vaccine fully confirmed that subcutaneous and intracutaneous inoculations tend to produce unpleasant local and systemic reactions.

Cutaneous inoculation is now preferred because it apparently confers immunity with the least discomfort. The inoculation is performed by abrading the skin with a lancet in 3 areas of approximately 1 cm<sup>2</sup>–1.5 cm<sup>2</sup>, placing a drop of concentrated ( $6 \times 10^9$  organisms) vaccine on each section of

scarified skin and making 8 linear, slightly bleeding scarifications into which the vaccine is rubbed with the lancet. Discussions disclosed that vaccine strains, even in the lyophilized state, may lose their immunogenicity. Repeated passage of the strains in guinea-pigs, particularly in the presence of non-toxic doses of iron salts, is required to restore, and even enhance, the immunogenic properties.

All vaccine strains must immunize guinea-pigs, contain F1 and VW antigens and be uracil-independent for growth, but must not produce pigment, i.e., they must be P-negative, on media containing haemin. These determinants are typical for the EV strain, by far the most immunogenic vaccine strain known to scientists in the USSR. Furthermore, large doses of vaccine are considered necessary to ensure a relatively long non-infectious phase of immunity with persistence of inoculated bacilli in the tissues for more than 3 weeks, followed by the sterile phase.

The most important role in the protection of the animal host or man is played by "immunologically competent cells" that react with the antigens in the first stage of the development of immunity and later serve as a source of other cells capable of synthesizing antibodies or resisting toxins. The cells possess "immunological memory"; having accepted antigen stimulation, they give an immune response after repeated vaccination or infection. Scientists in the USSR have revealed that they do not accept serological evidence for the development of antibodies as a possible indication of immunity, and insist that they have never been able to demonstrate antibodies in guinea-pigs proved to be immune to plague 15–20 days after single doses of vaccine. Therefore, they remain sceptical about deductions on the immunogenicity of killed plague vaccines for man.

In view of these reports, experimental studies were undertaken with live vaccines prepared from different EV isolates and from Fraction 1 of negative attenuated *P. pestis* strain M23.

#### EV SAIGON VACCINE

Through the courtesy of the Walter Reed Army Institute of Research, Washington, D.C., vials of a freeze-dried vaccine, prepared with the EV isolate of the Institut Pasteur, Saigon, became available. The vaccine (EV S) was grown on agar at 28°C and suspended in a stabilizer consisting of 0.2 M phosphate containing 7.5% of sucrose, 0.7% of glutamate and 2.5% of human serum albumin. The contents

of the vials, consisting of dead bacilli and approximately 23%–40% of viable *P. pestis*, were reconstituted with sterile water.

The protective dose for mice varied from 2500 to 2800 viable organisms. A dose of 0.1 ml of vaccine (consisting of 4000 or 40 000 viable *P. pestis* EV S) inoculated intradermally in guinea-pigs produced erythematous areas (1.5 cm diameter) with yellowish pinhead-sized nodules which through eschars converted into scars by the 12th day. Lymphadenopathy was slight but evident. The immune response, measured by haemagglutination, complement-fixation and mouse-protective antibodies, was significant: out of 18 vaccinated guinea-pigs, 8 had an MPI below 10 on the 28th day. Challenged with 1 250 000 virulent *P. pestis* inoculated subcutaneously or 8000 intraperitoneally, 40 000 viable EV S organisms protected 80% of the guinea-pigs against subcutaneous, but only 10% against intraperitoneal, infection.

Groups of Hanuman langurs (*Presbytis entellus*), shown by Chen & Meyer (1965) and Meyer (1968) to be highly susceptible to plague, were vaccinated intradermally with 17 800, 178 000 and 1 780 000 viable EV S organisms and they developed erythematous areas (5 mm×10 mm) with indurated centres (2 mm×2 mm) which persisted for 3 to 4 days. The regional lymph nodes were palpable but not strikingly enlarged. Antibody response was vigorous; survivors had an MPI close to, or below, 5 before challenge. Challenged with an LD<sub>50</sub> of 1000 (232 000) virulent *P. pestis*, only 5 of 11 (45%) survived, while 6 of 11 (55%) died in 7–19 days (controls died in 3–10 days) with gross pathological and bacteriological manifestations of bubonic-septicaemic plague. Only 2 of 5 macaques intradermally vaccinated with a single dose (514 million) of EV S organisms survived intraperitoneal challenge.

A group of 5 macaques similarly vaccinated on the same day, but given a booster of 82 000 viable vaccine organisms, all survived intraperitoneal challenge with 7 million virulent *P. pestis* 195/P, fatal to 4 of 5 controls on the 3rd–5th day. Groups of 8 langurs, each vaccinated with 42 million or 420 million viable EV S organisms, respectively, responded exceptionally well with antibodies; in all but 2, the MPI was below 5 on the 28th day. By the 406th day after basic vaccination, a booster inoculation given to one-half of each group had restored the serological reactions. Finally, on the 85th day after the booster inoculation, or on the 491st day after basic inoculation, the 15 langurs that were still alive

were challenged with virulent *P. pestis*, fatal to all controls, and 10 survived. Of the 10 immune langurs, 6 belonged to a group of 8 given 1 booster inoculation each and 4 belonged to a group of 7 not given a booster.

Despite the heterogeneity of the langurs, the experiments proved that the immunity induced by 42 million live EV S organisms persisted for at least 16 months and that a small booster inoculation enhanced the resistance.

Two clinical trials with EV S organisms on human subjects were disappointing. Subcutaneous vaccination with 420 million viable freeze-dried EV S organisms caused pronounced erythema and some induration for 96 hours. Two men had severe local reactions, leading in one to abscess formation; 6 (24%) had fever and 7 (28%) disclosed slight leucocytosis. All subjects had slight lymphadenopathies. Some individuals found the side-effects distinctly unpleasant but milder than those produced by the 1951 vaccine EV 76. Haemagglutinins in low titre but no complement-fixing or mouse-protective antibodies were evident even after booster inoculations with the same vaccine; the human subjects inadequately sensitized failed to respond by producing mouse-protective antibodies.

Similar trials on human subjects by other investigators demonstrated that the mass vaccination with a subculture of an EV strain possessing all the biochemical, nutritional and marker determinants (see Table 1), apparently invasive and immunogenic for guinea-pigs, mice, langurs and macaques and capable of inducing significant antibody levels, produced unpleasant local and systemic reactions on intracutaneous injection.

In response to booster inoculations with Fraction 1 or killed, alum-precipitated vaccine only 5 of 12 human subjects (41%) developed mouse-protective antibodies believed adequate to confer relative active immunity against plague infection even after the primary inoculation of 420 million organisms. Further studies of the EV S strain have been discontinued until the complex factors responsible for the disparities can be understood.

#### EV 76 MADAGASCAR VACCINE AFTER GUINEA-PIG PASSAGE

A subculture of the EV 76 vaccine strain taken to the USA in 1951 and passaged through guinea-pigs previously inoculated with non-toxic doses of iron salts fulfilled the safety and pathogenicity tests on

TABLE 1  
CHARACTERISTICS OF ATTENUATED *P. PESTIS* RECENTLY STUDIED<sup>a</sup>

Strain	Pigment	F 1	CA dependence	Uracil dependence	Pesticin I	Pesticin II	Fibrin
EV 76 (51 f)	—	+	+	—	+	+	+
EV 76 WR	—	+	+	+	+	+	+
EV 76 (Paris)	—	+	+	—	—	+	—
M23 P-negative	—	—	+	—	+	+	+

<sup>a</sup> According to John D. Marshall, unpublished data, 1969.

guinea-pigs as stipulated by Girard (1963, p. 700), Korobkova (1955, 1956) and others. The 50% protective dose (PD<sub>50</sub>) by the subcutaneous route was 3600 viable organisms for mice and 5 for guinea-pigs; however, the LD<sub>50</sub> for mice by intravenous inoculation was 1300. This passage culture, preserved as a freeze-dried live vaccine known as EV 51f, unexpectedly proved highly pathogenic for African green vervet monkeys (*Cercopithecus aethiops*) and langurs but not for macaques.

Three independent series of experimental clinical trials on vervets of different races or subspecies with various dilutions of the EV 51f vaccine resulted in the following fatalities:

(1) *Ethiopian race*: A total of 3 deaths (18%) in 16 vervets tested with 160 million organisms (1 death), 16 million organisms (1 death) and 1.6 million organisms (1 death); complete immunity in survivors challenged on the 41st day after vaccination.

(2) *Kenya race*: A total of 10 deaths (50%) in 20 vervets, highest mortality from inoculation with 218 million organisms, no deaths from inoculation with 218 viable EV 51f organisms; complete immunity in survivors challenged on the 52nd day after vaccination.

(3) *Cercopithecus aethiops pygerythrus* vaccinated in South Africa by Dr M. Isaacson: A total of 13 deaths (26%) in 50 vervets, highest mortalities (40%) with inoculations of 100 million and 100 000 EV 51f organisms; 7 of the 10 survivors resisted subcutaneous challenge inoculation with 8770 organisms of a local (Lesotho), recently isolated, and highly virulent strain of *P. pestis* 166 days after vaccination.

The "high residual virulence" of the EV 51f strain, as reflected by deaths occurring within 6–28 days following subcutaneous vaccination and

anatomical lesions and bacteriological findings of bubonic-septicaemic plague (non-haemorrhagic lymphadenopathy, liver necroses, splenic tumour with granulomas, pleural effusions, extensive degenerative changes in liver, kidneys and adrenals), was even more strikingly demonstrated in langurs.

In connexion with a trial on 6 langurs, using the single-puncture vaccination method with a bifurcated needle, 2 animals were each punctured with the needle charged with 842 500, 84 250 and 8425 organisms. The animals became ill, 3 with fever, 3 with positive blood cultures on the 5th day. Although the local pinkish nodules (1.5 cm–2.5 cm) at the site of puncture disappeared by the 5th day, the regional lymph nodes enlarged to the size of large peanuts or small cherries and yielded EV 51f organisms as late as the 12th day in 1 animal. Four of the 5 successfully infected langurs died on the 12th, 13th (2) and 17th days with autopsy findings of bubonic-septicaemic plague lesions (slightly haemorrhagic lymph nodes, splenic tumour, abdominal and pleural effusions, liver necroses, adrenal haemorrhages and anaemia). Mouse-protective antibodies were present in the properly vaccinated animal while the second survivor, which failed to develop a local reaction, lymphadenopathy and antibodies, succumbed to plague on challenge.

Similar puncture vaccinations on 4 macaques (successful in only 3) produced no illness, only slight lymphadenopathy and sterile blood cultures but high haemagglutinating antibody levels. The duration of immunity in these 3 animals was not determined.

The passaged EV 76 (51f) attenuated for guinea-pigs, according to all criteria accepted as a safe, live plague vaccine when applied by the cutaneous or subcutaneous route even in small doses (100 organisms in *C. a. pygerythrus*), produces a slightly atypical, but fatal, "vaccination plague" in langurs

and vervets but not in macaques. Highly effective, both with respect to antibody response and immunity to severe infection for 2 but not for 5 months, the EV 76 (51f) is capable of producing severe injuries in two species of non-human primates. It is probably *not* innocuous to man.

M23 NON-PIGMENTED, FRACTION-NEGATIVE STRAIN  
SELECTED FROM THE ORIGINAL ISOLATE  
STUDIED BY BURROWS (1957)

At a Commission on Immunization meeting and at the 69th Annual Meeting of the American Society of Microbiology (Janssen & Surgalla, 1969) it was reported that the pigmented M23 strain, shown to be highly immunogenic for guinea-pigs, protected macaques when intradermally inoculated, with only slight local reactions, against lethal aerosol challenge infections. A request was received for a study of this strain to be made in experimental trials on three species of non-human primates. Intraperitoneal and subcutaneous inoculations of 27 million organisms of a freeze-dried vaccine M23 (WR I) were well tolerated by guinea-pigs. The  $PD_{50}$  was 1300 following intraperitoneal, and 2200 following subcutaneous, vaccination. The  $LD_{50}$  for intravenously inoculated mice is 500.

Four langurs were given 40 million M23 organisms intradermally; 6 vervets and 5 macaques were inoculated, 4 intradermally and 2 subcutaneously, with 38 million organisms. All the langurs died (7th (2), 8th and 19th days), while only 2 of the vervets, both vaccinated by the intradermal route, died on the 7th and 17th days respectively; the macaques were only slightly affected.

Differences in susceptibility of the three non-human primates are again strikingly emphasized. Strain M23 is infective and invasive, but even a dose of 38 million viable *P. pestis*, produced only slight local and systemic reactions in macaques, though enlargement of the regional lymph nodes following intracutaneous vaccination was more pronounced than with the EV 51f strain. None of the vaccinated macaques was clinically ill, rises in temperature were slight and local reactions completely disappeared between the 10th and the 14th days.

Reactions in the Kenya race of vervets were quite different; 2 of 4 inoculated by the intradermal route succumbed to the vaccination. All, including those vaccinated by the subcutaneous route, were clinically ill, lost weight and had diarrhoea; some had slight rises in body temperature and yielded positive blood

cultures up to the 8th day (the last day on which they were tested, since repeated handling further impaired their health). Local lesions in the form of persistent nodules and prominently enlarged lymph nodes far exceeded in size the lesions observed in animals vaccinated with EV 51f. The deaths resembled those seen in virulent plague infections, with the exception that the lymph nodes were never surrounded by oedema or haemorrhages. In 1 vervet an encapsulated chronic pulmonary abscess due to a species of *Pseudomonas* may have weakened the animal but *P. pestis* (M23) was isolated from the heart blood and viscera. The second fatality occurred in an animal which was ill on the 5th day and had diarrhoea on the 8th day (from a latent *Shigella* infection and previously established *Plasmodium gonderi*). Despite careful nursing, it died on the 17th day with lung oedema lesions and parenchymatous changes in the liver, kidney and adrenals. As might be expected, *P. pestis* M23 could no longer be demonstrated in the heart blood, but was present in the spleen and lymph nodes. Recovery of the survivors from the vaccination was very slow.

The outcome of the vaccination of 4 langurs in excellent health when inoculated with M23 by the intradermal route was most disconcerting. By the 5th day they were seriously ill and consequently daily examinations were discontinued. They all had fever and yielded positive blood cultures. Local dermal lesions in the form of purplish, protruding, hard nodules were pronounced, and in 1 animal the nodules showed an abscess core by the 19th day. The regional lymph nodes exceeded in size and induration any of those previously seen in vervets or langurs vaccinated with EV 51f. Anorexia and extreme weakness combined with wasting and diarrhoea preceded the early deaths of 3 animals on the 6th (2) and 7th days. Despite careful nursing, the 4th animal unexpectedly died on the 14th day. Autopsy findings were more severe than those observed in langurs vaccinated with EV 51f; splenic tumour, adrenal haemorrhages and necroses and, in particular, diffuse liver necroses and granuloma of varying degrees of intensity, were all pronounced. Enlargement of the inguinal and iliac lymph nodes without oedema or haemorrhages, but with liquefaction of the central cores was marked, exceeding that induced by EV 51f. The gall bladders were greatly enlarged and filled with thick, tarry, blood-tinged material, barely resembling bile. Whether the over-extension of the gall bladder resulted from malfunction of the inflamed intestinal tract or from the

extensive pathological changes in the liver parenchyma will not be decided until sections have been studied carefully. Marked leucocytosis with a shift to 18%–22% monocytosis, together with the gross anatomical lesions, imply that extensive multiplication of the vaccine strain created toxin injuries.

Nothing is known about the nature of the toxins formed by strain M23, but, by analogy, an endotoxin is suspected. The absence of peritoneal and pleural transudates in fatal M23 vaccination in contrast to EV 51f infections suggests some profound changes in water metabolism as a result of non-specific diarrhoea and the inability, or unwillingness, of the animals to replace the loss by drinking water. The plague infections produced in langurs with strain M23 afford a unique opportunity to clarify the pathophysiology of *P. pestis* infection.

Antibody and immunity studies are in progress. One surprising observation should be recorded: the passive haemagglutination test was positive in dilutions of 1 : 8–1 : 256 in the sera of both species of recovered non-human primates, regardless of the mode of vaccination. Theoretically, the appearance of these antibodies considered specific for Fraction 1 was not expected and remains unexplained.

#### EXPERIMENTAL STUDIES WITH A TRANSPLANT OF EV 76 (PARIS)

Disconcerting experiences in the course of experimental studies of attenuated live plague vaccines on non-human primates logically demanded that the original EV 76 should be tested. Through the courtesy of Dr Girard, a subculture of his EV 76 strain of *P. pestis* maintained at the Institut Pasteur, Paris, was obtained in December 1967. The PD<sub>50</sub> for guinea-pigs vaccinated intracutaneously was 40 viable organisms by intraperitoneal challenge and 33 viable organisms when challenged by the subcutaneous route. Even at a dose of 100 000 organisms, the EV 76 (Paris) strain is *non-pathogenic for mice* by intravenous inoculation, but the PD<sub>50</sub> was 38 viable organisms by intraperitoneal challenge and 2800 viable organisms by the subcutaneous route.

The transplant of the original EV 76 stored in the Institut Pasteur for nearly 35 years has retained all the biochemical determinant characteristics and immunogenic properties for guinea-pigs defined by Dr Girard and others. A vaccination experiment on 4 langurs, 6 vervets and 4 macaques inoculated intradermally (left femur) with 26 800 000 viable EV 76 organisms is only partly completed. Regard-

less of age and weight (8 kg–15 kg), the local reactions observed in the langurs in the form of erythematous indurated areas (5 cm × 5 cm) accompanied by prominently enlarged, firm, but movable, regional lymph nodes (up to 4.5 cm × 2.5 cm), receding very slowly by the 25th day, were accompanied by systemic reactions. Fever, anorexia and loss of aggressiveness were noted in all, and positive blood cultures persisted until the 8th day in 1 young langur. Lymph node biopsies revealed confluent growth of EV 76 (Paris) organisms. Recovery was protracted.

Systemic reactions were less marked in the vervets though slight rises in temperature (up to 40°C) for 1 or 2 days were recorded and positive blood cultures were obtained on the 5th day from 2 animals. Cutaneous inflammatory nodules (1 cm × 1 cm) subsided within 12 days, but the inoculation sites remained visible as eschars or desquamated, hairless areas of skin. However, inguinal lymphadenopathy in the form of hard but movable nodes varying in size from 2 cm to 3 cm in some individuals, accompanied by moderate enlargement of the opposite inguinal nodes, resembled the reactions seen in langurs. On the 5th day, biopsies of the enlarged lymph nodes of 4 animals yielded abundant growths on blood plates. Smears prepared from biopsy aspirates revealed unevenly distributed clusters of typical *P. pestis*. As in the langurs, the enlarged nodes persisted for over 30 days.

Local reactions in the macaques were confined to pinhead pink nodules which were reduced to hairless areas (5 cm × 5 cm) by the 8th day. Regional lymphadenopathy in the form of firm but movable nodes varying in size from 1 cm to 2.5 cm was similar to that observed in this species of non-human primates vaccinated with strain M23. Biopsies of the largest nodes on the 5th day yielded no cultures. The animals in this group were active, but after exercise occasionally exhibited a 1 deg.C rise in temperature. One animal had diarrhoea on the 3rd day and a positive blood culture on the 5th day; it died on the 8th day. The autopsy findings were atypical for plague, apparently complicated by a small *Klebsiella* pneumonic area and non-specific gastroenteritis; notwithstanding, EV (Paris) organisms were isolated from the heart blood, left and right inguinal and enlarged left iliac lymph nodes, spleen, liver and bile.

The serological study with respect to mouse-protective antibodies is still in progress, but haemagglutination and complement-fixation titres (1 : 12

to 1:4096 and 1:4 to 1:32) are available. This experiment is described mainly for the purpose of recording the fact that even the EV 76 strain extensively used in human mass vaccinations is highly invasive and pathogenic for 3 species of non-human primates. On challenge with 343 000 virulent *P. pestis* 195/P organisms the 4 langurs proved solidly immune, showing no local reactions at the site of infection which was fatal to non-vaccinated controls on the 4th day. Finally, it bears repeating that the EV (Paris) isolate acquired increased "virulence" upon passage through guinea-pigs. The passage isolate (EV 76-Pf) has an LD<sub>50</sub> of 3000 upon intravenous inoculation in mice, in contrast to the original subculture, of which 100 000 viable organisms caused no deaths.

The time-honoured supposition that animal tests, when standardized, can serve as a basis for measuring the effectiveness of plague vaccines in man has been disproved by recent observations. The use of highly susceptible non-human primates from India and Africa disclosed that the classical EV 76 strain is still invasive, produces local and systemic reactions similar to those repeatedly seen, but rarely reported, in human subjects, causes gross tissue changes and partially measurable clinical reactions (Rotman, 1945). Findings in langurs indicate the presence of injuries which may not be merely temporary.

Similar studies must be repeated by other investigators and correlation between antibodies and cellular reactions determined before permission is granted to conduct clinical trials on human subjects. Preferably, the cutaneous puncture vaccination methods (single or double), using different concentrations of EV 76 vaccine, should be chosen to test the innocuousness of the vaccine, which must be available in a tested, dependable, freeze-dried state.

Live attenuated plague vaccines probably share with many other live bacterial vaccines properties which may entail risks for various individuals. Some racial differences in reaction have been recorded. For example, Caucasian whites react more severely than Malagasies. Until pilot experiments have proved that unpleasant side-effects may be avoided by smaller doses of live vaccine, the claims that the administration of live vaccine by the conjunctival route or by inhalation confers dependable protection must be accepted with reservations.

*Of the live vaccines tested on langurs and vervets, the original EV 76 strain is probably the least harmful.* Certainly, the EV 51f or the EV 76 (Paris) strains after guinea-pig passage, as well as the M23 isolate,

which are all virulent for mice on intravenous inoculation, are definitely harmful and should never be tried on human subjects, despite the fact that they confer immunity to aerosol infections in macaques. The EV S live vaccine, though immunogenic for guinea-pigs, and partially efficacious in langurs, when administered in the standard dose of 400–500 million viable organisms apparently induces little or no protection in man. It is deficient in antigens and enzymes. One wonders how many deteriorated EV 76 mutants have unknowingly been used in "successful" mass vaccinations.

#### THE PRESENT STATUS OF KILLED PLAGUE VACCINES

On 22 October 1941, the Subcommittee on Tropical Diseases, National Research Council Committee on Medical Research (Meyer & McCoy, 1964), passed the following resolution: "Resolved that, even though the available knowledge does not seem to afford definite evidence of the benefits from the use of plague vaccines, it is advisable to vaccinate with killed plague bacilli of an approved strain all military or naval personnel under serious threat of exposure to bubonic plague".

In March 1942, a commercial biological laboratory was instructed to proceed, under the guidance of the writer, with the preparation of a formol-killed agar-grown suspension of virulent plague bacilli in saline, and to determine at the G. W. Hooper Foundation for Medical Research, University of California, the relative protective potency of the vaccine for mice according to the method of Sokhey & Maurice (1935). Results of studies involving the manufacture of the killed vaccine and various antigenic fractions and their protective potency as measured on mice and guinea-pigs have been reviewed (Meyer et al., 1948; Meyer, 1953).

In search of a prophylactic inoculation against plague, the majority of workers had adhered to the principle that a preparation or antigenic fraction which protects rats, mice and guinea-pigs against experimental plague gives good presumptive evidence of efficacy in human beings. The standard procedures developed by researchers since 1942 and adopted to evaluate killed plague vaccines included assays on mice as well as on guinea-pigs. It was found that mice inoculated in 2 steps with 0.002 ml of USP plague vaccine resisted challenge infection with 2000–4000 virulent plague bacilli, but the prophylactic value was relatively low for guinea-pigs.

When administered in the dose customary for human immunization ( $1.5 \text{ mg} = 3 \times 10^9$  organisms), only 10%–20% of the guinea-pigs survived challenge infection.

Other experiments furnished convincing evidence that the degree of guinea-pig protection is raised with large, repeated doses of antigens or with small doses precipitated with synergists (aluminium potassium sulfate (alum) or aluminium hydroxide, but not calcium phosphate). In some tests, 80%–100% of the guinea-pigs were protected with antigens which in the unprecipitated state conferred resistance on only 10% of the animals.

It was further observed that differences in the efficacy of killed vaccines are complicated by the great heterogeneity of the susceptibility of this animal species generally used in various laboratories. It is the actual mass of aggregate bacterial protein rather than the method of killing (treatment with formaldehyde, acetone at  $-70^\circ\text{C}$ , pure ethanol, potassium sulfate, glycerol, irradiation, etc.) of suspensions made from virulent or attenuated plague isolates that controls the immunogenic effect on guinea-pigs.

A so-called "guinea-pig potency test" similar to the mouse potency test, made by emulsifying the vaccine dilutions in an adjuvant, has been included in the evaluation of killed vaccines. Altogether, 20 USP vaccine lots tested on the inbred Hartley strain of guinea-pigs protected between 60%–70% infected with an  $\text{LD}_{50}$  of 200 000 virulent *P. pestis*. The results of these tests, which irrefutably prove that guinea-pigs can be protected against plague with killed vaccines, have recently been confirmed by other workers (Smith & Packmann, 1966). A non-toxic filtrable vaccine, developed from the attenuated EV 76 strain of *P. pestis*, protected approximately 69% of several hundred guinea-pigs against virulent subcutaneous infection. These animals had been inoculated with 2.0 ml of the preparation in 2-dose schedules. Under identical conditions, the USP plague vaccine conferred immunity upon 43% of the vaccinated guinea-pigs. The filtered vaccine owes its superior immunogenic efficacy over the USP vaccine to a greater content of antigen and to substances of high molecular weight.

There is no need to dwell on the puzzling paradox observed during early studies with the soluble protein Fraction 1 plague antigen, which protected mice but not guinea-pigs. This so-called "immuno-unresponsiveness (or paralysis)" is attributable to a prolonged surface blockade of the membranes of the

reticulo-endothelial cells by large doses (over  $50 \mu\text{g}$ ) of Fraction 1. Mice catabolize the protein faster, eliminate Fraction 1 sooner, and do not become immuno-unresponsive. Very small doses of Fraction 1 ( $5 \mu\text{g}$ ) in adjuvant protect 100% of guinea-pigs against severe plague infection (Spivak et al., 1958; Walker, 1962). In one experiment, inoculations with aqueous Fraction 1 (2 mg) followed by 0.5 mg in adjuvant solidly protected 2 of 6 langurs.

Potency tests of killed vaccines on macaques generally proved unreliable because of the relatively low and variable susceptibility of this species. Clinical trials on human subjects before 1944 established the safety and dosage of the vaccines which could be tolerated with the least amount of discomfort in terms of local and systemic reactions. Methods have been developed to measure indirectly, by serum tests, the response of animals, including man, to plague antigens. The passive mouse-protection test, and later the passive haemagglutination test, provided evidence that killed plague vaccines with proved immunogenic potency for mice and guinea-pigs stimulate the appearance of these antibodies in approximately 50%–60% of human subjects.

At the end of the Second World War, the final report (Meyer & McCoy, 1964) on research to develop a standardized type of killed plague prophylactic ended with the cautious statement: "Although the formalin-killed virulent *P. pestis* suspension proven effective in tests of animals has not yet had extensive field trials, there is no reason to doubt that it enhances the immunity mechanism in the body sufficiently to warrant its use. Inoculation with killed plague vaccines or antigens offers a practical approach to lowering the attack rate in endemic areas." All available official and non-official reports were carefully searched for evidence to support the validity of this statement. The fact that no one in the US Armed Forces contracted plague despite potential exposure in the Mediterranean area and the Orient attested to the efficiency of the co-operative measures taken by the Medical Department of the US Army and civilian public health departments. However, in the British Middle East Force there were 26 cases of bubonic plague, including 5 deaths (19.2%), involving 12 Indians, 6 East Africans, 5 British, 1 Italian, 1 "Cape-coloured" and 1 European Jew.

The efficacy of the formol-killed plague vaccine could not be determined under field conditions since the insecticide (DDT) effective against the vector



and certain microbiological agents that proved useful not only for treatment but, more significantly from the epidemiological standpoint, also for prophylaxis, had been employed simultaneously. However, it is reported that prophylactics prepared in India and China and used in field trials had lowered the attack and mortality rates, though not with any overwhelming success. The preparations offered those inoculated a better chance of recovery when they were treated with sulfonamides or antibiotics, or both, preferably early in the course of the disease. Reports on clinical reactions noted in the course of inoculation were of importance in the development of effective prophylactics. Local and systemic reactions, leading to temporary incapacity to perform their duties, were noted in the vaccinated persons following the second inoculation (1 ml–2 ml) of the killed vaccine. The health authorities of one of the Pacific islands were forced to discontinue the use of the Haffkine vaccine imported from India on account of the severity of vaccination reactions occurring in the civil population. Subsequently, over 4600 persons inoculated with the USP preparation accepted vaccination without complaint.

During the outbreak of plague in Dakar, Senegal, an intensive vaccination campaign with the living attenuated EV strain from Madagascar was undertaken by the French health authorities. The vaccine was manufactured locally at the Institut Pasteur in Dakar. More than 180 000 Africans and 20 000 Europeans were vaccinated. In many Europeans, the injection of 1 ml of this vaccine in the scapular region produced severe systemic and local reactions, with tissue slough at the site of inoculation. Among those vaccinated over a period of 15 days (about 5% of the total cases), the case fatality rate was 66%, in contrast to 85% in uninoculated Africans. This report (Rotman, 1945) for the first time called attention to the harmful local effects of a live plague vaccine generally claimed to be "inoffensive".

After 1945, laboratory research on plague vaccine was continued into *techniques of serological methods*; in particular the following tests were improved and evaluated: (1) the *in vivo* mouse-protection test, (2) the *in vitro* passive haemagglutination test, (3) the complement-fixation test with Fraction 1, (4) the macroscopic agglutination test, (5) the opsonocytaphagic test, and (6) the complement-fixation tests with murine toxin.

Standardized procedures to evaluate plague vaccines were adopted, taking into consideration: (1) manufacture of vaccines, (2) measurements of

bacterial count, nitrogen and Fraction 1 content, (3) toxicity and safety tests, (4) immunogenic efficacy in animals (assays on mice, guinea-pigs and non-human primates), and (5) clinical tests on human subjects (local and systemic reactions and antibody response).

The killed plague vaccine now in use in the USA has been studied by means of standardized procedures, with results best illustrated by an example: The plague vaccine USP consists of an aqueous suspension of virulent *P. pestis* (Indian isolate 195/P) grown on agar medium (low in levels of anti-A and anti-B blood-group substances) in Roux bottles at 37°C for 48 hours. The organisms are harvested in physiological saline and killed by adding formol to an over-all concentration of 0.5%. The standardized suspension is supplied in 20-ml vaccine vials, each ml containing  $2 \times 10^9$  bacilli (average total nitrogen 0.167 mg per  $1 \times 10^9$  organisms in suspension). Assayed in the protection test on uniformly susceptible mice (Sokhey & Maurice, 1935; Habbu, Nimbkar & Jhala, 1968) the mouse-potency ratio between various lots of vaccine and the reference standard has varied from 4 to 10; the shelf stability is at least 2 years. The contents of several lots of randomly chosen vials, inoculated intraperitoneally and subcutaneously into guinea-pigs (inbred Hartley strain) in the human dosage (1 ml =  $2 \times 10^9$  organisms), followed by a booster dose (0.2 ml = 400 million organisms) intramuscularly 1–2 months later, protect 50%–70% when the vaccinated animals are challenged with 40 000 to 500 000 virulent *P. pestis*.

In a group of 9 langurs inoculated by the intramuscular route, the USP vaccine produced no local or systemic reactions. The haemagglutination test performed with their blood sera on the 35th day after basic inoculation demonstrated haemagglutinating antibodies in titres varying from 1: 16 to 1: 128, no complement-fixation titre, and mouse-protective antibodies (average of 11), 3 of which had an MPI of 10 or below. The langurs received intramuscular booster doses of 0.2 ml (400 million) of USP vaccine 86 days after the first inoculation. At the same time, 1 animal not previously vaccinated was inoculated with the booster dose.

The significant antibody titres after the booster at the time of challenge infection, recorded in Table 2, indicate that the immune response expressed in haemagglutinin titres was vigorous, with an MPI below 5 in 4 animals and a moderately good MPI in 5 animals. The langur (SE 280) given only the 0.2 ml of booster had no significant antibodies. On

TABLE 2  
ANTIBODY RESPONSE AND RESISTANCE OF LANGURS FOLLOWING INTRAMUSCULAR  
INOCULATIONS WITH USP KILLED PLAGUE VACCINE<sup>a</sup>

Langur	Antibody titres at time of challenge <sup>b</sup>			Reaction to plague infection				Antibody titres after recovery from plague <sup>b</sup>			
	HA	CF	MPI	Blood culture	Day of death	S/T <sup>c</sup>	Pathology	28th day		66th day	
								HA	CF	HA	CF
SE 242	2 048	0	1	—	—		—	6 500	32	2 048	4
SE 254	4 096	0	3	—	—		—	32 000	16	16 384	4
SE 255	64	0	18	+	6th		Septicaemia	—	—	—	—
SE 279	128	0	13	+	16th	3/5	Metastatic pulmonary plague	—	—	—	—
SE 258	512	0	5	—	—		—	131 000	64	16 384	4
SE 280 (C-0.2)	0	0	15	—	21st		Metastatic pulmonary plague	—	—	—	—
SE 266	4 096	0	00	—	—		—	65 000	32	8 192	0
SE 265	128	0	7	—	—	3/4	—	1 024	8	2 048	2
SE 271	256	0	13	—	35th		Metastatic pulmonary plague	—	—	—	—
SE 272	4 096	0	4	—	—		57th day, axillary abscess++	not bled, 32 768			16
Controls:											
SE 314			16	+	5th		Septicaemia				
SE 292			16	+	6th	0/3	Septicaemia				
SE 286			18	+	5th		Septicaemia				

<sup>a</sup> Primary inoculation = 1 ml; booster inoculation = 0.2 ml on 86th day; challenge infection = 131 000 virulent *P. pestis* on 40th day after booster.

<sup>b</sup> HA = haemagglutinin; CF = complement-fixing; MPI = mouse-protective antibody index.

<sup>c</sup> S/T = survivors/total.

the 40th day after the booster inoculation (125th day after primary inoculation), the vaccinated animals, together with 3 uninoculated langurs as controls, were subcutaneously infected with 131 000 virulent *P. pestis* 195/P.

The controls succumbed to bubonic-septicaemic plague within 5–6 days; 1 langur (SE 255) inoculated with the USP plague vaccine, although given a booster, failed to develop a significant MPI (only 18) and died at the same time as the controls. Altogether, 6 of 9 langurs, with an MPI of 1, 3, 4, 5, 7 and 1 with complete protection (00) prior to challenge, survived. In the animal with an index of 13, an axillary bubo developed which yielded the inoculated strain of *P. pestis* in undiminished virulence on the 57th day after challenge. Three vaccinated langurs with an MPI of 13 (2) and 15 succumbed to septicaemic metastatic pulmonary plague on the 16th, 21st and 35th days after challenge. The protracted disease, seen for the first time in langurs, resembled

“mitigated” or “relapsing” plague in relatively resistant rodents. All langurs surviving plague, with the exception of SE272, exhibited either *no* cutaneous reactions or well-defined fleeting erythemas and slight local indurations at the site of infection.

It is important to emphasize that resistance to plague was indirectly reflected by an MPI which was below 5. None of the langurs with an MPI above 10 survived except the animal with the axillary bubo. The high-titre antibody response following recovery persisted for 2 months; in particular, the appearance of complement-fixing antibodies following infection may have been significant. The clinical trial of the killed USP vaccine administered according to directions adopted by the US Army and civilian personnel resulted in the protection of approximately 60% of vaccinated langurs. Almost identical correlations and immunogenic efficacy have been recorded with other lots or combined vaccine preparations containing  $2 \times 10^9$  formol-killed virulent *P. pestis*.

A clinical trial on 25 human subjects with the same lot of USP vaccine by intramuscular injection in a 2-dose schedule proved informative. Local and systemic reactions were all mild; lymph nodes were palpable in 2 men. Subjects with mild respiratory infections complained of myalgia and malaise, but by the 72nd hour the reactions had disappeared. Passive haemagglutinins in titres varying from 1:8 to 1:256 were noted on the 8th and 16th days in 15 of 25 men. By the 28th day, all but 1 of the subjects had reacted with haemagglutinin titres of 1:16-1:1024. Within 3 months, the titres had declined to lower levels or had disappeared in one-quarter of those vaccinated. Single booster inoculations of 0.2 ml (400 million) of the USP vaccine restored the haemagglutinin titre to original levels.

In view of the observations on langurs, passive MPIs determined *in vivo* warrant special consideration. Indices below 10, and particularly those below 5, are usually found in susceptible non-human primates which resist challenge infection. In the 25 human subjects, 16% of the MPIs were below 10 by the 28th day, with only 4% below 5. Thus a single inoculation of vaccine confers little or no active immunity. A small booster inoculation brought the MPI to below 10 in 65% and to below 5 in 26%. Clinical trials on human subjects with other killed vaccine inoculations in a 2-dose schedule have yielded similar percentages for MPIs which are considered adequate to confer relative immunity in approximately 65% of human subjects (Table 3).

Perhaps only by coincidence, survival results for human subjects inoculated in field trials in Kenya (Smidt, 1929) with a locally prepared Haffkine vaccine (2 ml) represented a 61.8%-65% protection rate, corresponding to survivals of human subjects who developed protective antibodies after 2 antigenic inoculations with the USP plague vaccine. However, exceptions have been observed in individuals inoculated 10-20 years before inclusion in recent clinical trials. Invariably the haemagglutination (titres of 1:16-1:65 000) with an MPI as low as 1, or complete protection of all mice inoculated with the sera, was recorded on the 12th-28th days after revaccination. Killed vaccines of the USP type definitely sensitize human subjects to the extent that small, repeated, booster inoculations activate the immune mechanism.

How can protection, indirectly measured by serum antibodies, be brought to a level of 90% with inoculations which are free from side-effects? Experiments on guinea-pigs with a killed vaccine suspension, adsorbed or precipitated with aluminium

salts, improved the percentages of survivors and increased the MPIs in human subjects. So far, vaccine preparations with alum tend to deteriorate relatively quickly; studies to discover the reasons for this deterioration are in progress. Although local reactions, even with intramuscular inoculation, are only slightly more frequent, general systemic reactions are approximately twice as common as those resulting from the USP vaccine without synergists.

Killed bacterial vaccines with oil adjuvants are, justly, out of favour: they are highly efficacious in guinea-pigs and in non-human primates, but in 1 clinical trial on human subjects, a killed (acetone) vaccine emulsified in "Bristol" (peanut oil and aluminium monosterate) and shown to be highly immunogenic in guinea-pigs, caused severe local reactions upon intramuscular inoculation in 8 of 21 men, followed by abscess formation and prolonged illness requiring surgery in 3.

The USP vaccine, given in doses exceeding  $3 \times 10^9$  killed plague bacilli capable of stimulating an MPI below 5 in as many as 75%, caused unpleasant local reactions of varying incapacitating intensity and occasionally severe systemic reactions with fever, myalgia, headache and even vomiting. During the past 5 years, the killed USP vaccine has been used in mass inoculations, necessitated by the fact that US Army personnel have been operating in plague areas which became highly endemic under war conditions.

Published reports (Cavanaugh et al., 1968) emphasize the alarming frequency of plague in the Republic of Viet-Nam since 1962. Indeed, it is stated that 13 263 or 45% of a total of 29 109 known cases occurring in that territory since 1906 have been reported during the last 5 years. Under existing conditions in Viet-Nam, the only measure for personal protection against plague available to most of the population is vaccination. Data on the efficacy of the living plague vaccine (EV strain) employed by the Government of Viet-Nam are lacking, but a marked reduction of human plague has been noted in areas where intensive vaccination campaigns have been carried out.

US Army personnel vaccinated with USP vaccine enjoy almost complete freedom from plague, despite their frequent exposure. One of the 4 known and confirmed cases of bubonic plague among North Americans occurred in an unvaccinated individual. Two confirmed cases of pneumonic plague and 1 case of asymptomatic pharyngeal plague have been recorded among individuals vaccinated with the USP vaccine. However, it should be noted that

TABLE 3  
LOCAL, SYSTEMIC AND SEROLOGICAL REACTIONS RECORDED IN 25 HUMAN SUBJECTS  
INOCULATED INTRAMUSCULARLY WITH USP PLAGUE VACCINE, AQUEOUS <sup>a</sup>

Subjects	Clinical reactions <sup>b</sup>	Basic inoculation: 28th day <sup>c</sup>			Pre-booster: 84th day <sup>c</sup>		Post-booster: 28th day <sup>c</sup>		
		HA	CF	MPI	HA	CF	HA	CF	MPI
1. Co	0,0,+	128	0	18	0	0	0	0	21
2. Pe	0,0,±	64	0	18	4	0	0	0	7
3. Le	0,0,0	16	0	13	0	0	—	—	—
4. Ga	0,+ ,+	128	0	9	0	0	0	0	17
6. Ba	+ ,0,+	1 024	0	8	32	0	64	0	7
7. Bo	+ ,0,+ (L)	256	0	18	256	0	128	0	10
8. Ci	0,0,+	128	0	13	16	0	0	0	9
9. Ra	0,0,+	128	0	12	8	0	0	0	7
10. Ro	+ ,0,+	64	0	10	64	0	128	0	11
11. Gr	0,0,0	32	0	15	8	0	0	0	15
12. Ro	+ ,0,+	16	0	16	0	0	2 048	0	6
13. Bo	0,0,± (F,M)	512	0	11	128	0	1 024	0	2
16. Av	0,0,±	512	0	16	16	0	256	0	14
17. Po	0,0,+ (L)	256	0	12	8	0	1 024	0	4
18. Pe	0,0,±	64	0	15	4	0	1 024	0	2
19. Mc	0,0,+	128	0	16	64	0	1 024	0	4
21. Pe	0,+ ,±	128	0	15	4	0	512	0	2
22. Bl	0,0,0	128	0	14	16	0	256	0	6
23. Se	0,0,0	512	0	12	8	0	2 048	0	00
24. Ca	0,0,± (F,M)	64	0	4	0	0	0	0	6
25. Ba	+ ,0,+	0	0	17	0	0	0	0	16
27. Ri	0,0,±	32	0	16	8	0	0	0	9
28. Ha	0,0,±	128	0	13	64	0	512	0	12
29. Do	0,0,0	128	0	15	8	0	0	0	11
30. Hu	0,0,+	64	0	—	—	—	—	—	—
Average		185.5		13.5	29		432		8.6
MPI 10 and below				16 %					65 %
MPI 5 and below				4 %					26 %

<sup>a</sup> Basic immunization 10 October 1967 (1 ml), booster inoculation 4 January 1968 (0.2 ml).

<sup>b</sup> 0 = No erythema or oedema, slight tenderness to touch disappearing within 48 hours; or, no temperature elevation, chilly sensations, headache or general systemic symptoms. + (mild reaction) = Erythema (2 cm–4 cm axis), little induration, tenderness persisting for 72–96 hours, ± (mild reaction) = Erythema fading, slight tenderness. L = Lymphadenopathy; F = fever; M = myalgia.

The reactions were observed and recorded after 24, 48 and 72–96 hours. Thus, the symbol 0,0,+ , for example, should be interpreted as follows: 24–48 hours, no reaction; 72–96 hours, slight tenderness.

<sup>c</sup> HA = haemagglutinin; CF = complement-fixing; MPI = mouse-protective antibody index.

US Army personnel are protected, whenever possible, with much higher standards of environmental sanitation than those available to the average Viet-Nameese in the same area.

Observations made by Cavanaugh & Marshall (personal communication), incidentally to serological studies, indicate that haemagglutinin titres after vaccination do not merely persist but may actually rise. Altogether, 18 of a group of 37 vaccinated men had significant haemagglutinin titres, some as high as 1:1024, when they began to collect rodents and fleas; within 2 months, 12 revealed definite rises in these antibodies.

More striking, and even more suggestive, are the findings in a group of 122 men who had repeatedly received vaccine inoculations: 44 had no haemagglutinins when they entered field service in heavily infected territories. When examined 6 months later, only 4 had no serum antibodies, while 118 yielded titres of 1:64-1:4096. One wonders how this booster-dose effect occurred in the absence of re-inoculation. An unorthodox, and purely speculative, theory that unrecognized small-dose infections derived from bites of infected fleas might have been responsible deserves further investigation. In fact, it must be stressed that without more extensive knowledge of the nature of the immune mechanism in individual human subjects, it is difficult to explain why artificial immunization with killed and live plague vaccines apparently provides only partial protection.

A group of expert clinicians familiar with the techniques of lymphocytic blastogenesis and hypersensitivity is using plague antigen inoculations as a model for special immunological studies. This group has observed that whenever a volunteer experienced severe local and systemic reactions to the vaccine, he

exhibited the highest *in vitro* lymphocytic response to Fraction 1. In 9 of 12 human subjects, gross, apparently positive, delayed hypersensitivity to Fraction 1 developed on the 7th day after immunization. Some produced 19S and 7S globulin as early as the 7th day.

In conclusion, the following conservative statement appears to be justified: recent experiences in Viet-Nam indicate that persons vaccinated with a killed plague vaccine enjoy almost complete freedom from the disease, although frequently exposed to infection.

While the safety of the available live plague vaccine EV 76 (Paris) continues to be the subject of further study by other investigators, the planning of carefully controlled field trials to assess the efficacy of two of the most widely used and readily available killed vaccines is urgently recommended. The USP formol-killed virulent *P. pestis* suspension is capable of protecting 60% of non-human primates, particularly langurs. Inoculations of 1 ml ( $2 \times 10^9$  killed plague bacilli) of this vaccine, followed in 1-3 months by a booster of 0.2 ml (400 million killed bacilli), stimulate the appearance of passive mouse protection antibodies (MPI below 10) and haemagglutinins in 60%-65% of human subjects.

Experiments on langurs and vervets have provided evidence that these antibodies may be correlated with the degree of resistance to bubonic plague infection. After comparative tests on guinea-pigs and langurs, supplemented by serological tests on human subjects, the improved Haffkine vaccine preparation should likewise be included in a controlled field trial. Additional experimental studies on non-human primates are required to appraise the efficacy of killed or live vaccines against aerosol (pulmonary) infections.

## RÉSUMÉ

### EFFICACITÉ DES VACCINS ANTIPESTEURS VIVANTS OU TUÉS CHEZ L'HOMME

En l'absence de données expérimentales et comparatives récentes, on ne dispose que d'informations incomplètes pour déterminer si l'emploi des vaccins antipesteux vivants ou tués a pour effet de réduire les taux de morbidité et de mortalité dans les régions d'endémicité pesteuse.

Des essais de vaccination ont été pratiqués, notamment chez le singe, à l'aide de divers isolats de la souche vivante atténuée de *Pasteurella pestis* EV 76 de Girard & Robic possédant les mêmes caractéristiques biochi-

miques et nutritionnelles et les mêmes marqueurs que la souche d'origine. Ces préparations vaccinales entraînent des réactions désagréables, non seulement locales, mais aussi générales en raison de leur pouvoir de diffusion dans l'organisme. Administrés à des cobayes, à des souris, à des entelles (*Presbytis entellus*) particulièrement réceptifs, à des singes verts d'Afrique (*Cercopithecus aethiops* sp.) ou à des macaques (*Macaca mulatta*) peu réceptifs, certains isolats suscitent une production notable d'anticorps et confèrent une protection solide contre

l'infection d'épreuve par voie sous-cutanée. Cependant, certains d'entre eux n'ont qu'un faible pouvoir immunogène. La souche originale EV 76, conservée à l'Institut Pasteur de Paris, et qui n'est pas pathogène pour la souris par voie intraveineuse, satisfait à toutes les exigences en matière d'innocuité et confère, à petites doses, une immunité protectrice de bonne qualité au cobaye. L'inoculation intradermique d'une dose de 26 800 000 germes vivants provoque chez l'entelle et le singe vert une réaction cutanée locale, avec érythème et infiltration, et une hypertrophie ganglionnaire marquée qui ne disparaît que lentement. Ces phénomènes ne sont pas observés chez le macaque. La vaccination entraîne une production importante d'anticorps et confère une protection solide. De tous les vaccins vivants étudiés chez l'entelle et le singe vert, la souche originale EV 76 est probablement le moins nocif.

Après des passages répétés sur des cobayes traités au préalable par des doses non toxiques de sels de fer, une souche EV 76 devient pathogène pour la souris en inoculation intraveineuse (vaccin EV 51 f). Injectée par voie cutanée ou sous-cutanée à des entelles et à des

singes verts, elle provoque des réactions locales et générales graves et cause la mort d'une proportion variable des individus. Etant donné les troubles physiopathologiques observés chez les animaux vaccinés, il est très probable que les souches qui ont subi de tels passages ne sont pas inoffensives pour l'homme.

Le vaccin antipesteux tué actuellement utilisé aux Etats-Unis d'Amérique (vaccin USP) a fait l'objet d'études intensives à l'aide de techniques normalisées. Administré selon les directives adoptées par les autorités militaires et civiles, il confère une protection solide à 60% et une protection partielle à 30% des entelles contre une infection d'épreuve par voie sous-cutanée qui tue les animaux témoins non vaccinés. Les essais cliniques effectués chez l'homme montrent que l'injection intramusculaire de deux doses de vaccin à intervalle de 1 à 3 mois suscite dans 60-65% des cas la production d'anticorps protecteurs et hémagglutinants. Lorsqu'il est administré à des doses suffisantes au cobaye, le vaccin tué fait preuve d'un pouvoir protecteur équivalant à celui des vaccins vivants. L'efficacité du vaccin USP a été largement confirmée sur le terrain au Viet-Nam.

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