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Observations on
The Present State of Plague and Plague Control
in the Soviet Union
(according to data available to 31 October 1960)

Report III

by

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III. Plague Vaccination

1. Killed vaccines

A. AD vaccine

As can be gathered from the publications of several authors, like Minervin, Stupnitskii and Tinker[1,2], Mitin[3], Korobkova and co-workers[4], Korobkova[5] and Osolinker[6] during the period from 1934 to about 1943, when immunization with live vaccines became the standard practice, ample use was made in the Soviet Union of AD plague vaccines (vaccines adenatures), manufactured according to the principles of Zilber[7,8] with the aid of concentrated saccharose solutions so as to prevent a decomposition of the bacterial proteins. The essential findings of these workers which, as indicated, are now of historical rather than of actual interest, may thus be summarized:

Minervin and his colleagues[1,2] experimented on sisels and guinea-pigs with sugar vaccines prepared either from a virulent plague strain or from an avirulent mutant (strain AMP) obtained by Pokrovskaia[9] through dissociation of a virulent plague culture under the action of a specific phage. Comparative tests were also made with vaccines manufactured in the usual manner with saline solution and with live AMP vaccine. Results in sisels immunized 3 times at 5-day intervals with doses of 100 million, 150 million and 250 million respectively of these vaccines and challenged one month after the third vaccination with 10 lethal doses of the virulent strain were as follows:

Kind of vaccine	No. of animals	Died	Mortality Percentage
AMP 'salt' vaccine	14	8	57.0
AMP sugar vaccine	11	2	18.0
AMP live vaccine	11	2	18.0
Virulent salt vaccine	8	5	62.5
Virulent sugar vaccine	12	3	25.5
Controls	12	9	75.0

The mortality percentages in guinea-pigs, also vaccinated 3 times with 500 million, 1 billion and 1.5 billion doses of two of the vaccines under test and challenged with 40 lethal doses of P. pestis were:

Kind of vaccine	No. of animals	Died	Mortality percentage
AMP salt vaccine	14	11	78.6
AMP sugar vaccine	15	7	46.6
Controls	15	14	93.3

Results obtained with the AD vaccines, especially those made with the AMP strain were thus remarkably good.

Summarizing the results of comparative tests with AD vaccines obtained from virulent strains cultivated respectively at 37°C and 28°C, Korobkova and her co-workers[4] recorded that the former vaccine was more effective than that obtained from strains cultivated at 28°C.

In her subsequent report, Korobkova[5] stressed the necessity of sterilizing the AD vaccine without the application of heat so as not to damage the heat-labile Vi antigen of P. pestis supposed by her to exist. Consequently, she added to a concentrated suspension of virulent plague bacilli grown at 37°C a double volume of 80% saccharose solution and let the mixture stand at room temperature for 20-25 days so as to effect sterilization.

Korobkova found that two injections of this vaccine protected 82-100% of white mice against a challenge infection with highly virulent plague bacilli and that protection was also afforded to 50-85% of guinea-pigs which had been given three vaccine doses. A sugar vaccine prepared with the avirulent EV strain was less potent. The addition of 0.05% agar to the sugar vaccines increased their protective power.

B. Other vaccines

Gorokhov[10] reported on tests made in guinea-pigs with 5 different killed plague vaccines, namely, (1) a heat-killed vaccine prepared in the usual manner; (2) a vaccine prepared from a virulent strain by addition of 0.2% formol; (3) a formol-killed vaccine made from an avirulent plague strain obtained by Zhukov-Verezhnikov under the action of bacteriophage; (4) a vaccine obtained by killing the organisms with the aid of glycerol; and (5) a vaccine-lysate obtained with the aid of bacteriophage.

The results of comparative tests with these vaccines were summarized by the author thus:

a) None of the vaccines produced local or general reactions.

b) To judge from agglutination tests with the sera of the animals made 15 days after vaccination, the formol-killed vaccine prepared from virulent plague bacilli was most potent; the glycerol vaccine and the vaccine-lysate stood next in value;

c) Results of challenge tests made (i) 20 days after vaccination with 400 and 2,000 lethal doses of a virulent plague strain (1st series) and (ii) 96 days after vaccination with 10 lethal doses (2nd series) are shown in the following tabulation:

Kind of vaccine	No. of test animals	Survived	% of survival	Average life span of succumbing animals
1st series				
Vaccine-lysate	21	5	23.8	6.9
Glycerol-killed	19	4	21.0	9
Formol-killed (virulent strain)	20	3	15.0	9.2
DTO. (avirulent (strain)	20	1	5.0	6.1
Heat-killed	18	0	0	7.4
Controls	19	0	0	5.6
2nd series				
Vaccine-lysate	11	1	9.1	9.5
Glycerol-killed	9	3	33.34	8
Formol-killed (virulent strain)	11	5	45.5	10.5
Dto. (avirulent (strain)	11	1	9.1	8.8
Heat-killed	11	1	9.1	9.1
Controls	8	0	0	7.2

In the opinion of the author, the outstanding efficacy of the formol-killed vaccine prepared from virulent plague bacilli was possibly due to a greatly diminished decomposition of the bacterial protein and a retarded resorption of the organisms rendered firm under the action of formol.

2. Live plague vaccines

A. Monovalent vaccines

1) Vaccines prepared from phage-treated plague strains. AMP vaccine.

As described by Pokrovskaia[9], she isolated in 1931 an avirulent mutant of P. pestis, briefly designated as the AMP strain, during subcultivation of the 4th generation of a bacteriophage-resistent plague culture originally derived in 1929 from a patient w with bubonic plague.

In order to test the immunogenicity of the AMP mutant, Pokrovskaia immunized adult sisels by the intra-muscular route either with one 4-billion dose of the strain or two or three times with doses totalling 6 billion, respectively 3 billion of the avirulent organisms. The immunized animals were challenged about a month afterwards by the same route with virulent plague bacilli--in the first series of tests with doses killing only 4 out of 7 controls, subsequently with doses sufficient to produce a fatal infection in all the controls. Total results were that out of 41 immunized sisels only 4 (10%) succumbed to plague, whereas the mortality among the controls amounted to 80%.

As added by Pokrovskaia, analogous results were also obtained by Tinker in young sisels, which were vaccinated 3 times with doses of 100 million, 150 million and 250 million of the AMP vaccine and challenged one month afterwards with 10 lethal doses of a virulent plague culture. While the mortality in the controls amounted to 75% (9 deaths among 12 animals), only 2 out of the 11 immunized young sisels (18%) succumbed to plague--a result which, as noted above, was also recorded by Minervin, Tinker and Stupnitski.[1,2]

In a subsequent paper Pokrovskaia,[11] besides summarizing the above recorded findings, reported observations on guinea-pigs immunized three times with the AMP vaccine and challenged one month afterwards with massive doses of virulent plague bacilli. Results were as follows:

	Total tested	Died		Survived	
		No.	Percent	No.	Percent
Vaccinated animals	18	2	11.0	16	89.0
Controls	12	12	100.0	-	-

Experiments were also made in groups of sisels which were immunized at 5 days' interval with 3 doses of the live AMP vaccine (500 million, 1 billion and 1.5 billion) and were afterwards put in contact with manifestly plague-affected animals (3 for each

group) so as to create conditions for the epizootic spread of the infection. Results of these and of comparative tests with animals protected by immunization with killed AD vaccine are shown in the following table:

Groups in contact	No. of animals	Died		Survived	
		No.	Percent	No.	Percent
Immunized with live AMP vaccine	40	4	10.0	36	90.0
Immunized with AD vaccine	40	8	20.0	32	80.0
Controls	40	21	52.5	19	47.5

While emphasizing the good results she had obtained with her live vaccine, Pokrovskaia considered further laboratory studies necessary before practical advantage could be made of this method of plague vaccination.

As will be mentioned in a later section of this review, further use of the AMP vaccine was made by Pokrovskaia and Kaganova[12,13] when making experimental studies on the prevention of pneumonic plague invection.

Zh-V vaccine.

Zhukov-Verezhnikov[14] and Zhukov-Verezhnikov and Khvorostukhina[15] recorded that they had obtained a highly immogenic mutant of P. pestis by twice subjecting broth cultures of an originally typical plague strain to bacteriophage action and also resorting to incubation at high temperatures and addition of small amounts of disinfectants.

No details on the immunogenicity of this modified strain could be found in the available literature. As far as is known, the Zh-V vaccine has never been actually used in plague prophylaxis.

Strain 46-S

As will be discussed later in this review, Korobkova[16] compared the pathogenic and immunizing properties of a variant of P. pestis (46-S) obtained through bacteriophage action with the corresponding characteristics of the EV strain.

2) Vaccines prepared from plague strains which had become avirulent spontaneously.

EV vaccine.

EV vaccine.

As described by Girard and Robic[17] in 1934, the EV strain, originally isolated from a victim to bubonic plague, whose initials were E.V., had become so avirulent after having been subjected to monthly agar subcultures at 16°-20°C for a period of five years that it proved suitable for use as a live plague vaccine.

Evaluations of this claim in the Soviet Union seem to have been made first by Tumanskii[18] in 1938 and, according to Korobkova,[16] by a special commission in the Saratov Anti-Plague Institute appointed in the same year.

Tumanskii[18] summarized the results of tests made in guinea-pigs which had been vaccinated once with the EV strain and were challenged 20 days later with virulent plague bacilli (dose not stated) in the form of the following table:

Number of animals	Immunizing dose	Survived	Died
26	12.5 billion	22	4 (not of plague)
8	500.0 billion	8	0

On account of studies on the keeping quality of the EV vaccine, Tumanskii recommended to use as far as possible freshly prepared lots. Still, he found that live EV vaccine preserved at temperatures of 6°C to 13°C in sealed ampulles had remained viable and had hardly undergone changes in its immunogenic power.

Korobkova,[16] working under the auspices of the above mentioned commission, reported on an exhaustive comparative study of the EV strain and the avirulent variant 46-S, which as previously noted, had been produced under the influence of bacteriophage. She established that the avirulent variant 46-S differed from the EV strain by (a) forming smooth colonies; (b) causing on the 4th and 7th day respectively acidification in glycerol-ad-rhamnose-containing media; (c) showing no invasive properties, the organisms remaining localized at the site of injection and even disappearing from there within 48 hours without leading to abscess formation.

As shown by comparative tests, the 46-S variant, used as monovaccine, produced a less solid immunity than the EV strain: single subcutaneous injections of the latter strain protected all guinea-pigs challenged with virulent plague bacilli after 1 1/2 months and 70-80% of the animals challenged after 8 months, whereas under the same conditions the 46-S variant gave worse results.

However, full success was obtained if either mixtures of the two strains (containing 2 billion of the 46-S variant and only 1 billion of the EV strain) were administered simultaneously or the animals were injected first with the 46-S variant and after 7 days with the diminished dose of the EV strain: in both cases all immunized animals resisted challenge tests with virulent plague bacilli made after 8 months. Korobkova concluded, therefore, that "Combined vaccination with the 46-S and EV strains, made either simultaneously or successively, protects guinea-pigs better than even twice repeated administration of the EV strain alone."

Korobkova thus early recommended the method of bivalent plague vaccination which, as will be discussed below, has now become the standard procedure in the Soviet Union.

In a second article, published in 1940, Korobkova[19] reported on studies to improve the technique of manufacturing the EV vaccine and to prolong its keeping qualities. She recommended in the latter respect that the vaccine, brought ot a standard of 2 billion per ml with normal saline solution, should be immediately filled into ampules and that the latter, after sealing, ought to be kept in the freezing compartment of a refrigerator, preferably after their contents had been frozen. Kept under these optimal conditions, the vaccine remained fully suitable for at least one month or possibly even for 2-3 months, provided that it was used immediately after thawing.

Korobkova's advice was to resort for the manufacture of EV vaccine to cultivation of the strain for 2 days at 27°-28°C. However, this procedure was found unsatisfactory by some of the subsequent workers, recently by Garmazova[20] who, besides recording observations of her own on this point (see below), referred to the following statements in the literature:

(a) As noted earlier in the present review, Korobkova and her colleagues[4] found that the AD vaccine obtained through cultivation of virulent plague bacilli at 37°C was markedly more potent than that, for the manufacture of which an incubation temperature of 28°C had been used: guinea-pigs immunized 3 times with the former vaccine and challenged 2 weeks later with 5 lethal doses of a virulent plague strain showed a survival rate of 39%, whereas only 4% of the animals immunized with the 28° vaccine withstood this challenge.

(b) Zheltenkov and Anokhina (1951)* made comparative tests in white mice 3 or 5 times injected subcutaneously with EV vaccines prepared respectively at 37° and 28°C. When challenged

* This publication was not available in the original.

one month later with 100-1,000 certainly lethal doses of a virulent plague strain, 28.5-100% of the former group of animals survived, whereas the survival rates of the animals immunized with the 28°C vaccine ranged from 11.1 to 66.7%.

(c) Analogous results were obtained by Korobkova (1951)* when comparing the efficacy of EV vaccines obtained through cultivation on (i) a special medium which promoted capsule formation of the plague bacilli and (ii) ordinary media. Doses of one billion of the former vaccine administered subcutaneously to guinea-pigs protected 86.7% of the animals challenged 22 days afterwards with 200 certainly lethal doses of a virulent plague strain, whereas only 60% of the animals protected with the ordinary vaccine resisted infection.

Garmazova herself made comparative tests in white mice and guinea-pigs with EV vaccines obtained through 48 hours' cultivation of the strain (a) at 37°C in an atmosphere containing 15-20% carbon dioxide and (b) on the same agar (pH 7.2) at 20°C in the ordinary atmosphere. In each case the white mice received subcutaneously 10 million doses of the vaccines, the guinea-pigs 1.5 billion doses.

The main conclusions reached by Garmazova were as follows: (a) The cultivation of plague bacilli, including the EV strain, on ordinary media in an atmosphere with an increased CO_2 content at 37°C leads to an increased capsule formation. (b) The EV strain, grown in the presence of CO_2, long preserves this property of increased capsule formation under ordinary conditions. (c) the EV culture grown in an atmosphere with an increased CO_2 content and having large capsules has a higher immunogenicity than the identical culture grown under usual conditions and having insignificant capsules. (d) Single injections of the large-capsulated culture (10 million doses subcutaneously) confer immunity to 75-100% of white mice challenged with 100 or more lethal doses of virulent P. pestis, whereas under the same conditions the variant with small capsules only protects 20-50% of the mice. (e) The immunity conferred by the large-capsulated EV culture is more intense than that of the small-capsulated variant. Single administrations of the former culture (in 10 million doses subcutaneously) protect 54.6-70% of white mice against challenge with 2,500-10,000 DCL, made 41 days after immunization. The small-capsulated variant of EV protects under these conditions on 10-18.2% of the mice. (f) The higher efficacy of the large-capsulated variant was demonstrated also in guinea-pig experiments. This culture, in a dose of 1.5 billion administered subcutaneously, protects 100% of the animals against 100,200 or 500 lethal doses of a virulent plague strain (challenge after 32 or 41 days). Guinea-pigs immunized with EV cultures with small capsules show under these

* This publication was not available in the original.

conditions a survival rate of 60-80%. With challenge doses of 50 DCL administered 4 months after immunization the survival rate in the first group is 90%, in the second 50%.

Following up her initial work, Korobkova[21] reported in 1955 on methods of increasing the efficacy of plague vaccination with the EV strain. As shown by the adjoined table (a), she found that single subcutaneous vaccine doses of 300 million protected only 5 out of 10 guinea-pigs challenged after 45 days with 100 MLD of virulent plague bacilli, but that all animals tested could be protected if they were immunized first with 100 million doses and 21 days later with 200 million doses. Complete success was also obtained when two vaccine doses, totaling 600 million organisms, were given at intervals of either 7 or 21 days. A single dose of 600 million protected 9 out of 10 test animals.

As shown by the following table (b), administration of two 300 million doses, given at intervals of 8 or 21 days, protected all test animals against intranasal infection with P.pestis.

Guinea-pits vaccinated once or twice with doses of 600 million were still highly resistant to challenge by the subcutaneous route three months after vaccination. When challenged 8 months after vaccination, 5 out of 9 once vaccinated animals succumbed to injection with 100 MLD, whereas under the same conditions only one out of 10 twice vaccinated guinea-pigs succumbed.

Revaccination with 200 million doses 8 months after the primary inoculation with possibly the same small dose rendered the animals highly resistant to plague.

Another practically important fact established by Korobkova was that, as shown by the adjoined table (c), cutaneous vaccination of guinea-pigs with the EV vaccine (rubbing in of concentrated suspensions into 2 or 3 scarifications) gave better results than subcutaneous injection of the EV vaccine. Further tests, summarized in table (d) showed that cutaneous as well as subcutaneous administration of the EV vaccine conferred also a satisfactory protection against intranasal or intra-ocular infection.

Table (a) - Importance of the number and intervals of vaccinations for the immunity against plague.

Method and number of vaccinations	Doses (in millions) 1st	2nd	Intervals of vaccinations (days)	No. of challenged animals	Survived
1x subcutaneously	300	-	-	10	5
"	600	-	-	10	9
2x subcutaneously	100	200	7	10	7
"	200	400	7	10	10
"	100	200	21	10	10
"	200	400	21	10	10

Table (b) - Resistance of guinea-pigs against intranasal challenge with 2.5 million organisms 45 days after vaccination.

Method and number of vaccinations	Doses (in millions) 1st	2nd	Intervals of vaccinations (days)	No. of challenged animals	Survived
1x subcutaneously	600	-	-	10	5
2x "	300	300	8	10	10
" "	300	300	21	10	10

Table (c) - Comparative efficacy of subcutaneous and cutaneous vaccination.

Method of immunization	Doses	No. of challenged animals	Succumbed	Survived
Cutaneous	2 drops of a 60 billion suspension	10	0	10
Subcutaneous	600 million	10	1	9

N.B. Challenge tests with 100 MLD were made 40 days after immunization.

Table (d) - Cutaneous vaccination against pneumonic plague.

Method of vaccination	Doses	Method of challenge	No. of test animals	Survived
Cutaneous	2 drops of a 60 billion suspension	Intranasal	10	9
Subcutaneous	1 600 million	Intranasal	10	9
Cutaneous	2 drops of a 60 billion suspension	Into eye	10	10
Subcutaneous	1 600 million	Into eye	10	10
Control	-	Intranasal	5	0
Control	-	Into eye	5	0

N.B. Challenge tests were made 31 days after vaccination with 500,000 organisms.

The conclusions reached by Korobkova were that: 1) Live vaccine made from an immunogenic vaccinal strain is the most valuable preparation for the immunoprophylaxis of plague. 2) The insufficient resistance of part of the individuals vaccinated with live plague vaccine depends not only upon immunological peculiarities of their bodies but also upon inadequacy of the methods of vaccination. 3) Through twice repeated administration of the live vaccine, increase of the vaccinal doses, prolongation of the intervals between the vaccinations one may produce in experimental animals a prolonged and intense immunity, protecting them against manifestations of any form of plague. 4) Cutaneous administration of the live vaccine . . . is more effective than subcutaneous vaccination, renders specific prophylaxis easy, does not produce severe reactions and limits the number of contra-indications against vaccination.

Again dealing with the methods for improving the results of immunization with live plague vaccines, Korobkova[22] stated that (a) repeated passage of the EV strain through the peritoneal cavity of guinea-pigs led to an increase and stabilization of the immunogenic properties of the strain due to a process of selection, through which the organisms adapted to a saprophytic existence on artificial media were killed off; and (b) in order to preserve the

most immunogenic organisms one ought to avoid making cultures from the animals but preserve their spleen (the organ best suited for this purpose) in the freeze-dried state.

Experiments on guinea-pigs, made after the material from such spleens had been kept for three years, showed that subcutaneous doses of 20,000 organisms sufficed to protect all 10 animals tested against challenge with 1,000 MLD of a virulent plague culture which killed 7 out of the 10 animals vaccinated with the original EV strain and all controls.

In a 1956 report Zaplatina[23] recorded the results of experimental studies, in the course of which the immunizing properties of the EV strain were compared with those of five other plague strains which had lost their virulence through prolonged storage at room temperature with infrequent subcultivation (once every 6 months). In contrast to the EV strain, these 5 strains acidified glycerol, one also rhamnose, and did not reduce nitrates to nitrites. With one exception (strain 154), the strains used for comparison with the EV strain were not toxic for white mice inoculated with 100 million and 1,000 million doses.

In order to determine the minimal immunizing doses of the 5 strains in comparison with the EV strain, guinea-pigs and white mice were inoculated with variously sized doses of the avirulent organisms and subsequently challenged with a virulent plague strain (100 MLD). Results of these tests are shown in the following tables:

(i) Guinea-pig experiments

	Designation of strain					
Immunizing dose	EV	114	145	150	151	154
50,000 organisms	3/0	2/1	0/3	3/0	2/1	3/0
500,000 "	3/0	3/0	0/3	3/0	3/0	3/0
500 million "	3/0	3/0	3/0	3/0	3/0	3/0

(ii) White mice experiments

	Designation of strain					
Immunizing dose	EV	114	145	150	151	154
10,000 organisms	4/0	4/0	0/4	3/1	2/2	2/2
100,000 "	4/0	4/0	0/4	1/3	1/3	4/0
10 million "	3/1	3/1	2/2	4/0	4/0	3/1

N.B. In the fractions quoted in these tables the numerator indicates the number of animals which survived, the denominator that of the animals which died.

Further tests on guinea-pigs with immunizing doses of 5,000 organisms gave the results recorded below:

Designation of strains	Survived after challenge	Died
EV	1	3
150	4	0
154	3	1

On account of these observations Zaplatina claimed that the plague strains 150 and 154 were more immunogenic than the EV strain and deserved further investigation. As far as is known, however, no further advantage was taken of these two strains.*

Plague strain 64

As described by Smirnova,[24] the glycerol-acidifying strain 64 of the collection of the Irkutsk Anti-Plague Institute, kept viable through stab subcultivation every 3 months at 28°C and subsequent sotrage at 3-6°C, when tested after 12 years, was found to possess properties qualifying it as a vaccinal strain. Tests made in guinea-pigs and white mice to assess its immunogenicity (challenge with 200 lethal doses of virulent P. pestis 35 days after vaccination) gave the following results:

Species tested	Immunizing dose	Immunizing method	Challenge tests: No. of animals	Challenge tests: Succumbed	Challenge tests: Survived
Guinea-pigs	1 drop	intranasal	5	1	4
"	"	conjunctival	5	1	4
White mice	"	intranasal	15	3	12
"	"	conjunctival	15	5	10

* For the convenience of record further observations on the EV strain will be dealt with in subsequent sections of this report.

Species tested	Immunizing dose	Immunizing method	Challenge tests: No. of animals	Challenge tests: Succumbed	Challenge tests: Survived
Guinea-pigs	1 billion	subcutaneous	13	0	13
"	100 million	"	5	0	5
"	1 million	"	4	0	4
"	100,000	"	14	0	14
"	10,000	"	14	0	14
White mice	1 billion	"	14	0	14

Tests to determine the length of time during which guinea-pigs subcutaneously vaccinated with the strain remained resistant to challenge with 200 MLD gave the following results:

Immunizing dose	Challenge after	No. of animals	Succumbed	Survived
100 million	3 months	9	0	9
1 "	"	10	0	10
100 million	6 months	9	0	9
1 "	"	8	2	6

B. Bivalent plague vaccine

EV-46/S

As discussed above (see page 6) Korobkova[16] obtained fully satisfactory results by the combined use of the EV strain and a variant of the local strain[46] thus being apparently the first Soviet worker suggesting immunization with bivalent plague vaccines.

EV-M/74 vaccine

According to a brief remark made by Zhukov-Verezhnikov[25] in 1945, a combined vaccine, prepared from the EV strain and the plague strain M-74, was studied in the Saratov Institute of Microbiology and Epidemiology (the "Mikrob" Institute). The M/74 strain was obviously a local glycerol-acidifying strain, thus, as postulated by Fadeeva* containing an additional antigen PT, which was absent in the EV and other glycerol-negative strains.

* As quoted by Pilenko[26] Fadeeva recorded the observations made by her in this respect (to which Zhukov-Verezhnikov already referred) in a paper read in 1948.[27]

Vaccine 1-17

Though, as described above, favorable results were obtained in the Soviet Union as well as elsewhere with the EV strain, objections to its use were made by some workers like Novikova and her associates[28] and Mikhaleva[29] who maintained that (a) some subcultures of the EV strain showed a marked loss in immunogenicity so that multiples of the immunizing doses had to be used; and (b) it was desirable to work with a vaccine manufactured not only with a glycerol-negative but also with a glycerol-acidifying strain.

As described by Mikhaleva,[29] in order to find a vaccinal strain of the latter kind, 12 glycerol-acidifying plague cultures of the Irkutsk Anti-Plague Institute were screened with the aid of epidermal challenge tests (made presumably in guinea-pigs) so as to establish a loss of virulence with a concomitant preservation of the immunogenic properties. While four strains proved to have become avirulent, only one of them (No. 17), isolated originally from a victim to pneumonic plague (due to a laboratory infection) and kept in the laboratory for 23 years, exhibited immunogenic properties.

In contrast to the well defined history of the strain 17, no information could be elicited in regard to the origin of the glycerol-negative strain 1 which, according to a hardly creditable remark by Novikova and her associates,[28] had also been isolated in the Soviet Union. To judge from publications by Shmuter and Fedorova,[30] Mikhaleva and associates[31] and Levi and co-workers,[32] the strain showed some tendency for dissociation, leading to the appearance of glycerol-acidifying variants

As summarized by Mikhaleva,[29] tests with the strain 17 gave the following results:

(a) It proved to be a typical strain of the "continental" (i.e. glycerol-acidifying) type, possessing stable cultural and biochemical properties through many generations.

(b) Infection of guinea-pigs with 20 billion doses of the strain proved its complete innocuousness. All attempts to restore its virulence through animal passage failed.

(c) The innocuousness of the strain was confirmed through innoculation of 18 volunteers with different doses and by different routes (subcutaneously, intracutaneously and epidermally [cutaneously]).

(d) If introduced in massive or medium-sized doses subcutaneously into guinea-pigs, the strain 17 showed a better capability of spreading and adapting itself to the organs of the animals than the EV strain.

(e) The minimal immunizing dose of the strain ranged from 1 to 10 million organisms. Such doses prevented death of 50-80% of test guinea-pigs challenged with 200 lethal doses of P. pestis.

(f) The immunity due to vaccination with strain 17 began to become manifest on the fourth day, grew gradually and became sufficiently high by the 10th day after inoculation.

(g) Subcutaneous or cutaneous vaccination of guinea-pigs with single 1 billion doses of the strain produced a solid immunity lasting on a fairly high level for 8-12 months, whereas at that time the immunity produced in guinea-pigs with the EV strain had become considerably lowered.

(h) Guinea-pigs vaccinated with 1 billion doses of strain 17 were capable of resisting challenge with 50,000 lethal doses of a virulent plague strain, which killed all animals vaccinated with the EV strain.

(i) As shown by comparative tests, intracutaneous administration of the vaccine 17 proved best, the epidermal method came next and the subcutaneous method last.*

Studies on the immunogenicity of live vaccines in monkeys showed that a bivalent vaccine produced with strains 17 and 1 had higher immunogenic properties than monovalent vaccines produced with these strains. Details of these observations were as follows:

> "Results of a comparative study of the immunogenicity of live vaccines in monkeys showed that the bivalent vaccine made from plague strains No. 1 and No. 17 had the highest immunogenic properties. Among the monovalent vaccines proved best that made from strain 1 while that from strain 17 showed somewhat lower immunogenic properties.
>
> Slighter immunogenic properites were observed in vaccination with the EV strain . . .
>
> A study of the efficacy of the different routes of vaccination with strain 17 in monkeys showed that the best indices of immunity were produced by the intracutaneous method of vaccination; two times repeated inoculation by this route resulted in a survival of 100%.
>
> Single conjunctival and intranasal vaccinations, notwithstanding the administration of increased doses (15 billion organisms) did not prove highly efficacious (2 monkeys out of 5 survived).
>
> Single subcutaneous and intracutaneous administration of median doses (1.5 billion) gave analogous results (out of 5 monkeys 2 survived). Single cutaneous vaccination was less effective in monkeys than twice repeated inoculations.

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* As quoted by Skalov,[33] the advantages of the intracutaneous inoculation with the vaccine 1-17 had already been considered at a conference held in January 1954 (see Altareva et al[34]).

Twice repeated vaccination markedly increased the efficacy of inoculation with median doses by all routes.

High vaccine doses, given twice intracutaneously, gave worse results than single inoculations with the same dose."

Mikhaleva concluded her article by stating that the vaccinal strain 17 possessed in comparison with the EV strain better immunogenic properties and was capable of producing a long lasting and intense immunity in guinea-pigs and monkeys, which were the animals most sensitive to plague infection. She recommended using this strain for the purposes of plague vaccination, particularly for the manufacture of bivalent vaccines.

Vaccine EV-17

It is interesting to note that while Mikhaleva[29] was in favor of a combination of the strain 1 with the strain 17 for the manufacture of bivalent plague vaccines, Klez and Kolesnik[35,36] published in the same volume of reports two articles in which the EV strain instead of the strain 1 was recommended for this purpose.

In the first of these two articles it is stated that "the morbid picture of the process produced in guinea-pigs by single subcutaneous administrations of bivalent plague vaccine (strain 17 and EV) corresponds in general to the immunisatory process noted in separate investigation of these strains in guinea-pigs

The changes found in the organs of guinea-pigs (injected with the bivalent vaccine) are in degree somewhat in excess of those met with after administration of the strain 17 and still more marked than those seen after administration of the EV strain. Thus there is a more considerable tissue hyperplasia in the regional lymph nodes and hyperplasia of the spleen pulp. Moreover, there was found a proliferation of the reticulo-endothelial elements of the liver, which is almost absent if separate use is made of strain 17."

Postulating that the morbid changes found in the vaccinated test animals were proportional to the degree of the antigenic potency of the strains, Klez and Kolesnik claimed to have demonstrated the superior value of their bivalent vaccine.

Recording in their second paper the results of a study on the innocuousness of the bivalent vaccine EV-17 for guinea-pigs, Klez and Kolesnik[36] came to the conclusion that the changes met with in the organs of the vaccinated animals

"are of a character quite equal with the changes becoming manifest in guinea-pigs as a result of their inoculation with the strain 17 or the strain EV . . . but markedly exceed these in degree. This holds true in the first line of the site of introduction of

the microbes, where following single vaccination with either of the two strains usually it does not come to abscess formation.* The cell infiltration in the lungs is also more abundant than in the case of the usual vaccination.

Finally, hyalinized cells are more numerous in the spleen and in the other organs."

Evaluating their findings, the two authors felt certain that subcutaneous administration of even massive doses of the bivalent vaccine 17-EV was innocuous for guinea-pigs.

Continuing the just described studies, Klez and his associates[37] worked with the strain EV 229, the strain 17 and with a bivalent vaccine prepared by mixing equal parts of suspensions of each of these strains containing 750 million of organisms per 0.5 ml of saline. The results obtained with the three vaccines may thus be summarized:

(a) Tests were made in groups of 10 guinea-pigs, immunized respectively with 1.5 billion doses through subcutaneous injection and challenged 21 days later with 100 lethal doses of a virulent plague strain. All animals protected with the bivalent vaccine survived as compared with a survival rate of 9 in the other two groups.

(b) Experiments in white mice with vaccine doses ranging from 500,000 to 50 million proved rather fatal to the animals, especially in the case of strain 17. Still, challenge tests in the survivors, made 20 days after immunization with 100 lethal doses of a virulent plague strain gave the following results:

	Vaccines		
	EV	17	EV + 17
Challenged	24	11	19
Survived	3	8	13

(c) Results in white rats injected subcutaneously with one billion doses of the three vaccines and challenged by the same route with 20 lethal doses of a virulent plague strain were as follows:

	Vaccines		
	EV	17	EV + 17
Challenged	19	19	18
Survived	8	13	16

* In a footnote to their article Klez and Kolesnik admitted, however, that such abscess formation at the site of vaccination had been observed as a rule by other Soviet workers.

The superior value of the bivalent vaccine was thus confirmed.

It is noteworthy that Klez and colleagues,[38] studying the efficacy of combined vaccination against plague, tularemia and brucellosis (see below) worked with the vaccine 1-17.

Kolesinskaia,[39] studying the phagocytic index of the blood leucocytes in plague-immunized guinea-pigs and rabbits found that

(1) As a result of the inoculation of guinea-pigs with the live plague vaccines EV, 17 and EV + 17, prepared with the aid of submerged cultivation, the phagocytic index of the blood leucocytes reached a maximum 15-20 days after immunization. The index was highest in the animals immunized with the bivalent vaccine.

(2) In vaccinated rabbits the phagocytic index reached a maximum after 16-20 days and became also highest after administration of the bivalent vaccine.

(3) A higher level of phagocytic activity stood evidently in relation to a more solid state of immunity of the animals and led to a greater resistance against plague infection.

(4) After immunization with the bivalent vaccine one could note also an increase of the phagocytic index in respect to virulent plague bacilli.

Making a further study of the blook picture in guinea-pigs immunized with the above mentioned vaccines, Kolesinskaia[40] found that after their administration it came to an increase of the number of leucocytes which was most marked after vaccination with the bivalent vaccine, lowest after that with the EV strain. The number of erythrocytes remained unchanged, but the sedimentation rate was slightly increased after administration of either of the three vaccines.

Immunization Against Pneumonic Plague

The problem to what extent the administration of live plague vaccines protects experimental animals against pneumonic plague has been the object of special studies by the authors quoted below:

Korobkova and Krainova[41] summarized the results they had obtained in this respect in the form of the following tables:

	Number of animals	Method of immunization	Vaccine doses	Time and method of challenge	Challenge dose	Percentage of survival
(a)	20	2x intra-nasally	300 and 600 million EV	1 month intra-nasally	300 million	90.0
	10	"	"	1 month subcutaneously	"	100.0
	20	Subcutaneously then intra-nasally	300 and 300 million EV	1 month intra-nasally	"	95.0
	21	Controls	-	Intra-nasally or subcutaneously	"	0.0
(b)	10	1x subcutaneously	2 billion EV	3 months intra-nasally	25 million	100.0
	10	3x intra-nasally	700 million EV each time	"	"	90.0
	9	Controls	-	Intra-nasally	"	0.0
(c)	10	1x subcutaneously	2 billion EV	2 months intra-nasally	50 million	90.0
	10	2x subcutaneously	2 billion 46/s and 500 million EV	"	"	80.0
	8	Controls	-	Intra-nasally	"	0.0
(d)	12	1x subcutaneously	2 billion EV	46 days by inhalation	250 million in 25 ml	100.0
	9	Controls	-	By inhalation	"	0.0

The main conclusions reached by the two authors were that

(a) As established in a considerable number of animals immunized by various routes and with different vaccine doses, it was possible to confer protection against pneumonic plague infection proving fatal for all controls.

(b) A combined method of immunization (subcutaneously and intranasally) with equally large vaccine doses given at 3 days' interval protected 95% of the guinea-pigs against intranasal infection with a highly virulent plague strain.

(c) Single subcutaneously administered massive doses of the EV strain protected guinea-pigs against nasal infection in 90% and against challenge by inhalation in 100%.

As noted earlier in this review, Korobkova[21] reported in 1955 that cutaneous as well as subcutaneous administration of the EV vaccine conferred to guinea-pigs solid protection against intranasal or conjunctival infection with P. pestis. A 1958 report by Korobkova and Krainova,[42] dealing once more with the methods of immunization against pneumonic plague, has not yet become available to the present reviewer.

In the already quoted article on "the cytological method of studying the mechanism of immunity," Pokrovskaia and Kaganova[12] stated that they tried

> "to activate and increase the capacity of the reticulo-endothelial system in the lungs with the aid of repeated introduction of the live EV and AMP vaccines by inhalation . . . . Altogether, during the period from August 1937 until July 1940 we made 7 series of tests on 200 guinea-pigs with the following results: out of the animals receiving a combined vaccination against pneumonic plague, consisting of subcutaneous inoculation and inhalation, survived challenge 82.8%; out of the guinea-pigs immunized only by the subcutaneous route survived 60%, while almost all controls succumbed."*

The authors concluded, therefore, that "through administration of the live EV and AMP vaccines by inhalation, made in addition to subcutaneous vaccination, one is able to intensify the immunity of the lung tissue and to obviate in this way one of the defects of the human body, the scarcity of histiocytes in the lunbs."

Pokrovskaia and Kaganova added that, as shown by tests on themselves and other persons, the administration of the live plague vaccines by inhalation was harmless for man.

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* As quoted by Skalov,[33] Pokrovskaia and Kaganova[13] also recorded these findings in a monograph appearing in 1947.

In a further contribution entitled "The importance of cytochemical investigations for the study of immunological problems," (quoted for the convenience of record out of chronological order) Pokrovskaia and her associates[43] reported the results of observations on guinea-pigs immunized with normal saline suspensions of 2-days' agar cultures of the following vaccinal strains: EV original; a selected type of EV_{76}; the "transparent" form of EV_{76} obtained under the influence of the isotype P^{32} and the strain AMP-3270.

The guinea-pigs of one group were injected with 1.5 billion doses in 1 ml of saline, 0.1 ml being administered intracutaneously and 0.9 ml subcutaneously. The animals of a second group were immunized both by such injections and by inhalations with the "transparent" type of EV_{76}. Suspensions of this were sprayed with the aid of a pulverizator so that they could reach the mouth, nose and eyes of the animals; moreover they were instilled with a pipette into the nostrils and the conjunctival sac.

Inhalation was first made on the 12th day after the injection and repeated twice at intervals of three days. The doses were (1) 2 billion (4 ml of a 500-million suspension); (2) 4 billion (4 ml of a one-billion suspension) and (3) 8 billion (4 ml of a two-billion suspension). Part of the animals were sacrificed at intervals ranging from 40 minutes to 27 days after immunization and impression films were made from their organs for cytological and cytochemical studies, while the others were used for challenge tests. It was found that pneumonic plague infection of the guinea-pigs receiving one vaccine dose intra- and subcutaneously gave fatal results in 48-62.5%, whereas 82% of the animals subjected to the combined vaccination (injection + 3 inhalations) survived. No details of these experiments are quoted.

In an important article on the efficacy of bivalent plague vaccine in the prevention of pneumonic plague, Skalov,[33] besides briefly referring to the observations in point by Pokrovskaia and Kaganova,[13] also quoted Altareva and her co-workers,[34] Grudenkov[44] and Faibich[45] as follows:

> "Altareva, Antonov, Zhdanov, Korobkova and others (1955), studying the efficacy of the various methods of immunization with the EV vaccine, in one instance worked with the vaccinal strain No. 1, which they utilized for intranasal inoculation in doses of 1 billion and 15 billion. After intranasal challenge with 500,000 organisms all guinea-pigs which had received a vaccine dose of 1 billion succumbed, whereas all 10 animals vaccinated with 15 billion doses survived. Analogous results were obtained if the guinea-pigs were challenged subcutaneously with 200 DCL. . . . Grudenkov (1947)[44] concluded from guinea-pig experiments that immunization of these animals through the lungs renders them immune against pneumonic plague, but obtained analogous results with subcutaneous immunization. Faibich,[45]

immunizing guinea-pigs subcutaneously with dry EV vaccine, produced an analogous immunity against pneumonic and bubonic plague."

As described by Skalov,[33] he studied the degree of immun-immunity against pneumonic plague obtainable in guinea-pigs through administration of the vaccine 1-17* by various routes.

According to the instructions for the control and administration of this vaccine it was used in one billion doses for subcutaneous or intracutaneous inoculation or for rubbing in the material into the thrice scarified skin (cutaneous or epidermal route) thus:

Route of Administration	Volume Used for Suspension of Dose (ml)
Subcutaneous	1.0
Intracutaneous or cutaneous	0.1

Intranasal vaccination was effected under anaesthesia through instillation of 2 billion doses in 0.2 ml saline, this double amount being chosen because, as established by Korobkova and Krainova,[41] during intranasal application a large part of the organisms was passed through the esophagus into the stomach, where the bacilli were killed by the gastric juice.

Results of challenge tests made one month after vaccination were thus summarized by the author:

	Subcutaneous Challenge (1,000 DCL)			Intranasal Challenge (200 million organisms)		
Mode of Vaccination	Total animals	Survived	% Survival	Total animals	Survived	% Survival
Subcutaneous	27	26	96.2	28	21	75.0
Intracutaneous	29	29	100.0	28	25	89.2
Cutaneous	28	28	100.0	28	24	85.3
Intranasal	26	18	69.2	26	18	69.2

Commenting on these findings, the author maintained

"that vaccination by the intracutaneous and cutaneous routes confers to the guinea-pigs a high resistance not only against subcutaneous but also against intranasal challenge with a virulent plague culture.

Considering the results of intranasal vaccination one can not

* The vaccine batches for these experiments were produced under aeration in a reactor and then stored for 1 1/2 - 2 months at 8-10° C. They then contained 22-27% viable organisms.

fail to note that the fundamental inadequacy of this method lies in the impossibility of arriving at an exact dosage of the test material. On account of this we postulated that the intranasal method of vaccination does not lend itself to a determination of the efficacy of immunization through the respiratory routes."

Skalov resorted, therefore, to intratracheal introduction of the vaccine, followed 53 days later by challenge tests by the same route. Results of initial tests of this kind, made in comparison with intracutaneous vaccination are shown below:

Mode of Vaccination (1 billion doses)	No. of animals	Survived	Died	Death after an Average Survival Period of Days
Intratracheal	16	11	4 (?)	9.2
Intracutaneous	16	3	13	8.2
Controls	9	0	9	3.0

Since the vaccine batch used for these tests appeared to possess a low potency, Skalov made a second series of observations with another vaccine lot with the following results:

(a) Subcutaneous Challenge (200 DCL)

Mode of Vaccination (1 billion doses)	No. of Animals	Survived	Died	Death after an Average Survival Period of Days
Intratracheal	6	6	0	-
Intracutaneous	7	6	1	10
Controls	6	0	6	4.6

(b) Intratracheal Challenge (200 DCL)

Mode of Vaccination (1 billion doses)	No. of Animals	Survived	Died	Death after an Average Survival Period of Days
Intratracheal	6	5	1	8
Intracutaneous	7	4	3	7.3
Controls	6	0	6	2.8

Thus the intratracheal mode of vaccination was more effective than the intracutaneous method, protecting the animals not only against subcutaneous challenge, but in five out of six instances against intratracheal challenge. The intracutaneously vaccinated animals were fairly resistant against subcutaneous challenge, but less so against intratracheal challenge.

The author concluded that the results of these two series of tests permitted to postulate that the low efficacy of intranasal vaccination was due mainly to an insufficiency of the vaccine doses reaching the lungs. Therefore, he continued,

> "Probably, in all cases of vaccination through the respiratory organs it is indispensable to introduce into them a sufficient vaccine dose, what apparently can be effected most easily through a fine dispersion of dry vaccine."

Skalov recorded that he had made in this connection the following preliminary tests: (a) Suspensions of 5-billion doses of the vaccine were sprayed for 30 minutes with the aid of a pulverizator on the nose and mouth of guinea-pigs, the eyes of which had been previously covered; (b) the same dose of finely powdered vaccine was dispersed with the aid of a rubber baloon. After one hour the animals were killed with chloroform, and their lungs were used for making suspensions, which were afterwards diluted 1,000 times. Agar cultures showed that the lungs of the guinea-pigs sprayed with the dry vaccine contained 10 times more microbes of the vaccinal strains than the lungs of the animals treated with the vaccine suspensions.

In view of these findings it was essential not to only improve the generally practised methods of plague vaccination but to work out a method of plague immunization by the respiratory route. As will be discussed now, efforts were made in this direction by Aleksandrov, Gefen and their associates in a series of articles which began to appear before the report of Skalov was published.

In their initial article, Aleksandrov and Gefen[46] dealt in a general manner with the "physiological" methods of immunization consisting of the introduction of vaccines or other antigens through the intact mucous membranes. These procedures comprised, besides (a) enteral immunization; (b) enteral immunization with the aid of bacteriophage; (c) intranasal and (d) conjunctival immunization also (e) the method of aerogenous (inhalatory) immunization.

Experimental investigations made by the authors to assess the comparative value of these various procedures showed that the live organisms of the vaccinal strains (as well as killed bacteria and anatoxins), when penetrating through the intact mucous membranes, retained their antigenic and immunogenic properties. In accordance

with the length of time during which the antigens stayed on their surface and their absorptive powers, the mucous membranes could thus be classified in descending order: mucosae of (a) the alveoles and bronchioles; (b) the bronchi and trachea; (c) the conjunctiva; (d) the nose and pharynx; (e) the vagina and (f) the gastro-intestinal tract.

While thus the method of aerogenous immunization appeared to be most effective and its value had been confirmed by experimental observations made in the case of various infections (plague, tularemia, tuberculosis, influenza, typhoid, dysentery and diphtheria), nevertheless no large-scale practical advantage had been taken of aerogenous vaccination. This was due mainly to the lack of vaccinal preparations specially suited for immunization by inhalation and to the technical difficulties of implementing this method in mass vaccination campaigns. Ways and means to overcome these difficulties were studied by the authors.

Proposing in a second article improved methods for aerogenous immunization, Aleksandrov and Gefen[47] laid stress upon the fact that all biological preparations, and especially those endowed with biological activity could be preserved best in the dry state, particularly at low temperature in vacuo. Further advantages of the dry preparations were their light weight and, most important, the possibility of using them for aerogenous immunization in dust form.

For their investigations the two workers prepared such vaccinal dusts from the live anthrax vaccine STI, the plague vaccine 1-17 and from live brucellosis and tularemia vaccines, all actually used in the Soviet Union for subcutaneous, intracutaneous or cutaneous inouculation, and also from some actually used anatoxins, especially tetanus anatoxin.

Referring to the technique of manufacturing these dry vaccines, Aleksandrov and Gefen merely stated that the method used

> "was almost identical for the anthrax, plague, brucellosis, tularemia and tetanus vaccines. The difference was that for growing the anthrax, plague, brucella and tularemia vaccinal strains use was made of special nutrient media and cultivation methods, ensuring a maximal yeild of viable organisms. A second difference was that for each vaccinal strain a special substrate was used for drying."

In order to obtain reliable results, dry vaccines had to be used which gave a good growth if cultivated on selective media in a dilution of not less than 10^{-8}.

The best method of aerogenous vaccination was that of mass "spontaneous" immunization, because it was

"most physiological, deep, complete, causing little trauma and least labor-consuming, permitting to deal with large groups within the shortest time and with small amounts of material and labor."

Individual aerogenous vaccination, on the contrary, offered no great advantages in comparison to the usual methods of vaccination.

As further stated by the two authors, they tested their dry vaccines for a period of five years on experimental animals, in 1957-1958 also on man. As test animals served white mice, guinea-pigs, rabbits, sheep and monkeys. The former three species did not prove suitable for an assessment of the value of "spontaneous" aerogenic immunization, because on account of their shallow respiration as well as the anatomical and physiological peculiarities of their respiratory organs they were able to inhale only minimal amounts of the vaccinal dusts. It was necessary, therefore, to resort in their case to excessively high vaccine doses and long periods of exposure.

Not more than 50 mice, respectively 10 guinea-pigs or 3 rabbits were exposed simultaneously in inhalation chambers with a content of one cubic meter. For the sheep, which were exposed in groups of not more than 10, a chamber with a content of 3 cubic meters was used.

Since the authors furnished some details only in regard to the tests they made with brucellosis and tularemia vaccines, it suffices for the purposes of the present review to quote in somewhat abridged form their general conclusions:

1. The method of aerogenous immunization has much in common with the other physiological methods of vaccination. It combines substantially the effect of the intranasal and enteral methods and to some extent that of the conjunctival method.

2. All statements in the literature as well as our own experimental findings demonstrate the high efficacy of the method of aerogenous immunization, provided that highly potent vaccines are used in massive doses and in a rational manner.

3. The dry vaccines in dust form against brucellosis, tularemia, plague, anthrax and tetanus and the method of mass spontaneous aerogenous immunization were studied experimentally and in groups of people. The results were fully promising, but require confirmation in the laboratory as well as through epidemiological studies in man and studies in domestic animals.

In a further 1958 article, entitled "The reactiogenicity and efficacy of aerogenous vaccination against some zoonoses," Aleksandrov, Gefen and their associates[48] again referred to the subject

of immunization with the plague vaccine 1-17. Experimental observations were made with this in chambers with a content of 0.5-1.5 cubic meters, in which the test animals were exposed for periods of 30-60 minutes to 3-10 g of the vaccinal dust, dispersed with the aid of a pulverizator. Control animals were vaccinated once subcutaneously with the same vaccine. Challenge tests, made 30 days after immunization gave the following results:

> "Guinea-pigs, immunized with the dry plague vaccine, after subcutaneous or aerogenous infection with (virulent) plague bacilli in doses of 20-200 MLD survived in 60-80%. Guinea-pigs immunized subcutaneously with identical doses of the live plague vaccine and challenged by the same routes and with the same doses, survived in 70-100%. The mortality of the non-immunized controls was 100%."

Commenting on these results, the authors once more emphasized that guinea-pigs, as well as rabbits, were rather unsuitable for an assessment of the value of aerogenous immunization.

Aerogenous administrations of the plague vaccine (as well as of the other vaccines under test) to man were made in boxes with a content of 3.8 or 10 cubic meters sr in a tent with an air space of 12 cubic meters. The 54 persons exposed in this manner to the vaccine 1-17 apparently received immunizing doses of 750,000 viable organisms (found effective in animal experiments). A general reaction of a moderate degree was noted in only one person of this group, while local reactions were altogether absent. In 17 controls, who had been vaccinated by the subcutaneous route, local reactions were invariably present and general reactions were noted in 6 instances. Complement fixation tests with plague antigen, made in part of the immunized persons 15 days after vaccination, gave a positive result only after vaccine administration by the aerogenous route but not in individuals protected by subcutaneous injection of the vaccine.

In a series of articles published in 1960, Aleksandrov, Gefen and their associates[49,50,51] (a) dealt in a general manner with the theoretical and experimental premises for working out a method for aerosol vaccination; (b) assessed the value of this method for immunization against diphtheria; and (c) reported once more on experimental studies on aerosol immunization against anthrax, brucellosis, tularemia and plague.

As stated in the last mentioned article,[51] Aleksandrov and his co-workers used for aerosol vaccination of guinea-pigs and rabbits chambers with a content of 1.5-5m^3, for that of sheep and monkeys air spaces of 5-20 cubic meters. The aerosol dusts dispersed contained per gram amounts of viable organisms ranging from some tens to 1,000 -2,500 billion. The time of exposure lasted from 15 to 60 minutes. The inhaled doses (AD)were determined according to the formula C. T. Vt, in which C stood for the number of organisms per liter of

aerosol (assessed by cultivation of saline adsorbates of the aerosols), T for the time of aerosol administration in minutes, and Vt for the respiratory volume of the test animals per minute.

As far as their observations on plague were concerned, the authors restricted themselves to the following general statement:

> "On account of the absence of reliable tests we did not study the immunological efficacy of aerosol immunization with dust plague vaccine and limited ourselves to observations on the specific resistance of the immunized animals to challenge with a virulent culture. Our findings showed that it is possible to produce with the plague dust vaccine an immunity against subcutaneous infection with a virulent P. pestis culture. Thus, after subcutaneous challenge with 20 MLD out of 15 guinea-pigs, subcutaneously immunized with 300 million live organisms, died one and out of 10 guinea-pigs immunized with the aid of the aerosol method, also succumbed one, while all controls died. Still, for a more definite solution of this problem there is need for supplementary studies on larger animals (monkeys). Work of this kind is now in hand."

Results of such further studies have been published by Aleksandrov, Gefen and their co-workers[52] in an article appearing in December, 1960, in which they reported on observations made in 428 guinea-pigs, 60 sheep and 14 monkeys (M. rhesus). The dry vaccines used for the work were prepared from the brucella strain BA-19, Gaiski's tularemia strain 15, the anthrax strains STI-1 and No. 3, and the plague strains 1-17 and EV. At different intervals after immunization a part of the test animals was sacrificed for the purposes of bacteriological examination. The results obtained in this manner with plague vaccination were shown by the authors in the following table: (see page 30)

Commenting upon these findings (and also those made in the case of anthrax immunization), the authors stated that the character and the dynamics of the vaccinal process evolving after administration of anthrax and plague vaccines were quite similar to those described in the case of brucellosis and tularemia vaccination. Still, they added

> "in the case of the dry anthrax and plague vaccines there were a number of peculiarities, particularly in regard to the extraordinarily rapid and intense dispersion of the vaccinal microbes in the body of the animals: generalization in the case of guinea-pigs began already 6 hours after immunization with comparatively small doses, so that a period of a localization of the process was almost always non-existent. The maximum of isolations after aerosol immunization with these vaccines was observed up to 7 days, when a rapid disappearance of the organisms from the body of animals ensued . . . . After immunization with the plague vaccine in dust form the sterile phase of immunity began in guinea-pigs

Species of Animals	Method of immunization	Vaccine Dose	Number of Animals Examined	Time of Examination After Immunization	Number of isolations from: Blood Barenchymatous Organs	Lungs	Regional Lymph nodes[a]	Total
Guinea-pigs	By aerosol	1.5 - 150 million	4	1 hour	-	2	1	3
			4	6 hours	2	2	3	7
			14	1 day	10	6	5	21
			12	7 days	9	6	8	23
			10	15 days	4	3	3	10
			4	20 days	-	-	-	0
			10	30 days	-	-	-	0
Guinea-pigs	Subcutaneous	, 1.5 billion	5	1 day	3	-	5	7(?)
			5	7 days	3	-	5	6(?)
			5	10 days	1	-	3	9(?)
			5	15 days	-	-	-	0
			5	20 days	-	-	-	0
Monkeys	By aerosol	1.5 - 150 million	2	3 days	2	-	2	4
			2	7 days	2	1	1	5[b]
			2	15 days	2	1	2	6[b]

Remarks a) In the case of subcutaneous vaccination also from the site of inoculation.

b) Plague bacilli were also isolated in one instance respectively from the peripheral lymph nodes.

within 15 days. The results obtained through aerosol immunization of monkeys with dry plague vaccine (doses of 100 million) indicated an intensive settling down of the organisms in the lungs, the regional lymph nodes and the parenchymatous organs, beginning from the first to the 15th day (limit of observation) after immunization."

As far as the general dynamics, the length of the non-sterile phase of immunity and the period of generalization of the process were concerned, there existed no fundamental differences between the aerosol and subcutaneous methods of immunization against anthrax or plague. Still, in guinea-pigs the period of generalization was longer after aerosol immunization (15 days) than after subcutaneous immunization (7-10 days). According to Aleksandrov and his co-workers, the explanation for this difference was that in the case of aerosol immunization, as a result of which the organisms penetrated through the conjunctivae, the upper respiratory passages, the bronchi, the lungs and the gastro-intestinal tract, various systems of lymph nodes became involved in the

vaccinal process, whereas the number of lymph nodes becoming involved after subcutaneous or cutaneous inoculation was limited. In the opinion of these authors the involvement of numerous lymph nodes created favorable conditions for the development of a state of immunity.

Combined Vaccinations

Plague and gastro-intestinal infections.

As briefly stated by Kalacheva,[53] the possibility of combining vaccination against plague with immunization against other infectious diseases was first demonstrated by Korobkova (1950-1951), who experimented with a combination of live plague vaccine (evidently the EV vaccine) and the TAB vaccine or the NIISI polyvaccine.*

In order to explore the possibility for combined vaccination against plague and cholera, Korobkova and her associates[55] worked with the live plague vaccine 1-17 and with a polyvalent killed cholera vaccine which were mixed at the ratio of 500 million P. pestis and 1 billion cholera vibrios. The cholera vaccine was given to the test animals (guinea-pigs) 3 times at intervals of 7-8 days in amounts of 1, 1 and 2 billions, the plague vaccine once in an amount of 500 million organisms in combination with the first dose of cholera vaccine. Three weeks after administration of the last cholera inoculation one group of the immunized animals was challenged with a virulent P. pestis strain, the other with cholera vibrios.

The conclusions reached by the author were that

1. It was possible to prepare a dry vaccine from mixtures of live plague vaccine and killed cholera vibrios.

2. The dry combined plague-cholera vaccine was highly immunogenic and protective for the test animals. The immunity developing after single subcutaneous administrations of the combined vaccine corresponded to that produced with the plague and cholera monovaccines. The immunity against plague developed later than that against cholera (10 days as against 5-6 days).

3. Injection of rabbits with the combined vaccine furnished no evidence of an antigenic antagonismus or inhibition. The agglutinin titers in the serum of the animals differed little from those produced by the monovaccines.

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* The original publication of Korobkova as well as a 1958 report by her and others[54] dealing with the same subject have not been available to the present reviewer.
The NIISI poly vaccine contains besides typhoid, dysentery and cholera antigens also tetanus anatoxin.

Plague and tularemia.

The observations made by Kalacheva[53] when studying the efficacy of combined vaccination against plague and tularemia may thus be summarized:

1. As shown by the adjoined table (a), subcutaneous injection of guinea-pigs with mixtures of the live EV plague vaccine and live tularemia vaccine, followed one month later by challenge tests, gave fully satisfactory results in the case of tularemia (survival rate 100%), somewhat worse results in the case of plague (86.6% survivals).

2. According to table (b), better results were obtained through subcutaneous administration of a vaccine composed of the vaccinal plague strains 1 and 17 and Gaiskii's tularemia strain No. 15.

3. As illustrated in the adjoined table (c), excellent results were obtained with cutaneous administration of the combined EV and tularemia vaccine.

4. Finally, as shown by a further series of observations, the survival rate of guinea-pigs, subcutaneously vaccinated with a combined plague and tularemia vaccine and challenged 6 months afterwards, was 50% in the case of plague and 80% in the case of tularemia. Similar results were obtained with the corresponding monovaccines.

If the combined vaccine had been administered by the cutaneous route, the survival rates of the animals challenged six months later were 78% in the case of plague and 100% in that of tularemia. If the corresponding monovaccines were used, the survival rates were 73% in the case of plague and 80% in that of tularemia.

Studying the leucocytic reactions in mice immunized with live combined vaccines against plague and tularemia, Kalacheva[56] came to the conclusion that the combined vaccine conferred a higher degree of protection than plague or tularemia monovaccines. This proved that there was no competition between the two antigens in the body of the animals; on the contrary, sometimes one could observe an intensification of the protective reaction.

A third article by Kalacheva,[57] dealing with the reactions produced in man after combined administration of plague and tularemia vaccines, will receive consideration in a later part of this review.

Results of combined plague and tularemia vaccination (Kalacheva[53])

No. of Animals	Type of Vaccine	Vaccine Dosage	Challenge tests: Strain	Dose (DCL)	Animals Tested	Survived
Table (a) EV + tularemia vaccine (subcutaneous route):						
30	Combined	1 billion P. Pestis + 10,000-100,000 B. tularensis	P. pestis	100	15	13
			B. tular.	1,000	15	15
15	Plague mono-vaccine	1 billion organisms	P. pestis	100	15	13
15	Tularemia monovaccine	10,000 - 100,000 orgs.	B. tular.	1,000	15	15
20	Controls	-	P. pestis	100	10	0
			B. tular.	1,000	10	0
Table (b) Plague vaccine 1-17 + tularemia vaccine (subcutaneous route):						
25	combined	500 million each of strains 1-17 + 10,000 B. tularensis	P.pestis	100	15	14
			B. tular.	100(?)	10	10
10	Bivalent	500 million each of strains 1 and 17	P. pestis	100	10	10
8	Plague monovaccine	500 million of strain 1	P.pestis	100	8	7
8	Plague monovaccine	500 million of strain 17	P.pestis	100	8	5
8	Tularemia monovaccine	10,000 orgs.	B. tular.	1,000	8	8
14	Controls	-	P.pestis	100	7	0
			B. tular.	1,000	7	0

No. of Animals	Type of Vaccine	Vaccine Dosage	Challenge tests: Strain	Dose (DCL)	Animals Tested	Survived
Table (c) EV + tularemia vaccine (cutaneous route):						
10	Combined	2 drops of mixture contain. per ml 100 billion P.pestis + 2 billion B. tularensis	P.pestis	100	5	5
			B. tular.	1,000	5	5
5	Plague monovaccine	2 drops of vaccine contain. per ml 100 billion P. pestis	P.pestis	100	5	5
4	Tularemia monovaccine	2 drops of vaccine contain. per ml 2 billion B. tularensis	B.tular.	1,000	4	3

N.B. Mortality in the 10 control animals was 100%

Plague, tularemia and brucellosis.

In order to assess the efficacy of combined vaccination of guinea-pigs against plague, tularemia and brucellosis, Klez and his associates[38] used (a) the plague strains 1 and 17 in a dosage of 750 million organisms each; (b) Gaiskii's tularemia strain No. 15 in a dosage of 25 million; and (c) the vaccinal brucella strain BA in a dosage of 250 million. These strains were cultivated separately for 2 days and 0.5 ml amounts of each, containing the above mentioned doses were mixed immediately before they were used for subcutaneous injection of the test animals. Challenge tests were made 36 days after immunization with (i) 1,000 DCL of a virulent plague strain; (ii) 1,000 MLD of a virulent tularemia strain and (iii) 2 MLD of a virulent Brucella ovis strain--also by the subcutaneous route. Results were thus tabulated by the authors: (p. 35)

The combined vaccine thus showed an efficacy comparable to that of the monovaccines. The local reactions produced by it were also not different from those following the administration of the monovaccines.

In a further publication on the subject presently under review Pilipenko and his co-workers[58] stressed that subcutaneous inoculation with a combined plague-tularemia-brucellosis vaccine was unsuitable for practical

Group of Animals	Brucellosis Number Tested	Brucellosis Proved Immune	Tularemia Number Tested	Tularemia Survived	Plague Number Tested	Plague Survived
Immunized with the combined vaccine	14	11	14	14	14	12
Immunized with the monovaccines	15	13	15	14	11	6*
Controls	9	0	9	1	10	0

* 4 of the 5 succumbing animals were victims of a non-specific infection.

purposes, inasmuch as it led to the formation of infiltrates at the site of injection which persisted for 12-18 days or even for 24-30 days, and in part of the animals also to local abscess formation. Pilipenko and his associates adopted, therefore, the method of cutaneous (epidermal) vaccination which, while equally effective, was more expedient and produced less marked reactions.

To manufacture the combined vaccine, these workers prepared first suspensions from two days old brucella cultures (strains BA or M), containing 10 billion organisms per ml and successively used these fluids for the suspension of dry plague and tularemia vaccines. Three drops of the mixed vaccine were used for the cutaneous inoculation of guinea-pigs with the aid of the technique adopted for smallpox inoculation.

The cutaneously vaccinated guinea-pigs showed no evidence of a general reaction. The local reaction was comparatively slight and lasted only for 6-14 days. As shown by the following table, the same held true in the case of the administration of tularemia monovaccine, whereas the reactions following plague or brucellosis vaccination were less long lasting.

Kind of Vaccine	Number of Test Animals	Without Local Reactions	Local Reactions Lasting for Days: 2	4	6	8	10	12	14	16	18
Combined	180	1	2	2	25	40	43	15	49	-	1
Tularemia	60	-	2	9	3	17	5	10	14	-	-
Plague	60	11	4	13	24	5	3	-	-	-	-
Brucellosis	60	2	-	24	32	2	-	-	-	-	-

Results of challenge tests made two or six months after administration of the various vaccines with 200 DCL of P. pestis and 10,000 DCL of B. tularensis respectively are shown in the following two tables:

Kind of Vaccine	Months after Vaccination	No. of Animals	Results of Challenge Tests		
			Died	Survived	
				Number	Per cent
A) Plague					
Combined	2	10	1	9	90.0
Plague	2	10	-	10	100.0
Combined	6	12	8	4	33.0
Plague	6	12	5	7	58.0
b) Tularemia					
Combined	2	10	3	7	70.0
Tularemia	2	8	1	7	87.0
Combined	6	12	10	2	17.0
Tularemia	6	12	8	4	33.0

Results of challenge tests with 10 organisms of Brucella abortus are illustrated in the following table:

Kind of Vaccine	Months after vaccination	No. of Animals	General Infection	Regional Infection	Not Inf.	Immune	
						No.	Per Cent
Combined (Br.abortus 19)	2	10	1	1	8	9	90.0
" (Br.abortus M)	2	10	-	1	9	10	100.0
Brucella (Strain 19)	2	9	4	1	4	5	55.0
" (Strain M)	2	4	-	-	4	4	100.0
Combined (Br.abortus 19)	6	12	6	2	4	6	50.0
" (Br.abortus M)	6	10	3	0	7	7	70.0
Brucella (Strain 19)	6	12	9	1	2	3	25.0

The conclusions reached by Pilipenko and his colleagues were:

1. The local reactions in guinea-pigs vaccinated cutaneously with a combined plague-tularemia-brucellosis vaccine are considerably more benign and less long lasting than those in animals subcutaneously injected with the combined vaccine. There was no general reaction.

2. The overwhelming majority of the animals challenged 2 months after cutaneous vaccination with the combined vaccine proved resistant to infection with massive doses of plague or tularemia vacilli and to two doses of brucellae causing a general infection. There was no significant difference in the number of animals immunized respectively with the combined vaccine or the corresponding monovaccines in the case of plague and tularemia; in the case of brucellosis there were more immune animals in the group inoculated with the combined vaccine.

3. Six months after vaccination the number of animals which had lost the immunity to plague and tularemia was about two times greater in the group inoculated with the combined vaccine than in the group immunized with the corresponding monovaccines. No such difference existed in the case of brucellosis.

4. Whether these discrepancies are of regular occurrence or merely fortuitous, needs further study. They do not decrease the preferability of the cutaneous method of combined vaccination to the subcutaneous administration of the three vaccines.

In regard to their last conclusion the authors noted that Klez and others,[59] evaluating the immunity of guinea-pigs 7 months after subcutaneous injection with combined plague-brucellosis-tularemia vaccine found no significant difference between the numbers of animals immune to plague after combined vaccination or after the administration of monovaccines, and only a slight difference in the case of tularemia. A majority of the guinea-pigs immunized with either kind of vaccine had become susceptible to the infection.

However, in an article published in January, 1961 Pilipenko and his associates[59a] again recorded differences in the results obtained respectively with a combined plague-brucellosis-tularemia vaccine and with plague monovaccine in guinea-pigs inoculated by the cutaneous route.

To study this problem, the authors sacrificed couples of the inoculated guinea-pigs after intervals ranging from 3 hours to 90 days and used the various lymph nodes as well as the internal organs of these animals for cultivation on agar plates containing sodium sulfite.

In the case of the guinea-pigs inoculated with the combined vaccine, plague bacilli could be cultivated from animals sacrificed after intervals ranging from 3 hours to 5 days, whereas from the animals inoculated with plague monovaccine positive cultures were obtained for a period of 10 days after vaccination. From the site of infection plague

bacilli could be isolated in the former case only from the animals sacrificed 3 hours after inoculation, whereas the animals sacrificed after inoculation with plague monovaccine proved positive in this respect for a period of up to 5 days.

Besides from the site of inoculation, with one exception growth of P. pestis was obtained only in cultivations from the groin and axillary lymph nodes and occasionally from the para-aortic lymph node. Only in one instance, concerning a guinea-pig inoculated with monovaccine and killed on the 10th day after vaccination, a colony of P.pestis was detected on the plate inoculated with material from the spleen of the animal.

Challenge tests with 2,000 DCL of P.pestis made 7 months after inoculation gave the following results:

Group of Animals	Type of Vaccine	Number of Animals	Results of Challenge Tests		
			Died	Survived	
				Number	Percent
(1)	Combined	11	7	4	36.0
(2)	Plague	11	3	8	73.0
(3)	Controls	10	10	0	0.0

Thus the animals inoculated with the plague monovaccine proved twice as resistant as those immunized with the combined vaccine.

Plague, tularemia, brucellosis and anthrax

Experimental studies on the combined use of live vaccines against plague, tularemia, brucellosis and anthrax were made by Vereninova and her co-workers.[60,61]* Summarizing the results of their first study, these authors[61] stated that it was possible

> "to produce a solid immunity in guinea-pigs inoculated with a mixture of three live vaccines: against plague tularemia and brucellosis. No antagonism of the antigens could be observed, as confirmed by the high survival rate of the animals challenged subcutaneously with plague bacilli (500 DCL) and tularemia bacilli (1,000 DCL) and through absence of a generalized infection after administration of brucellae (2 infective doses). It was also established that after immunization of guinea-pigs with a mixture of four live vaccines--against plague, tularemia, brucellosis and anthrax, the last mentioned antigen had a weak immunizatory effect, as shown by the quite low survival rate among the animals infected with the standard spore virus. Evidently the other components of the combined vaccine exerted an inhibiting action on the anthrax antigen.

*As mentioned by Klez and co-workers,[38] analogous studies had been made previously by Pilipenko and his associates.[62]

Better results were obtained with separate administration of the vaccines: first the anthrax vaccine and 10 days later a mixture of the other three vaccines. This method of vaccination ensured a sufficiently solid immunity against the causative organisms of all four infections."

As recorded in their 1959 study, Vereninova and her associates[61] experimented with 300 guinea-pigs divided into three groups as follows:

Number of Group	Mode of Immunization
(1)	Anthrax vaccine subcutaneously and 10 days later subcutaneous administration of plague-tularemia-brucellosis vaccine.
(2)	Anthrax vaccination as above, followed 10 days later by separate cutaneous inoculations of plague and tularemia vaccines and subcutaneous injection of brucellosis vaccine.
(3)	As in group (2) except that the plague vaccine (0.2 ml) was administered intracutaneously.

The animals of each of these groups were dived into five sub-groups, which 25 days after immunization were challenged respectively by the intra-tracheal route with (a) 1,000 DCL of P. pestis; (b) 10,000 DCL of B. tularensis; (c) 20 infective doses of Br. melitensis; (d) 1,000 DCL of B. anthracis and (e) mixtures of all these challenge doses.

Results of these challenge tests may thus be summarized:

	Percentages of Survival after Challenge with				
Group	P. pestis	B. tularensis	Br. melitensis	B. anthracis	Mixture
(1)	58.3	75.0	75.0*	16.6	8.3
(2)	83.3	75.0	90.0*	16.6	18.1
(3)	41.0	91.0	81.8*	16.6	0.0

* Absence of generalized infection.

Commenting upon these results, which they set forth in a detailed manner in tables, the authors stated that combined vaccination with the three live vaccines against plague, tularemia and brucellosis was found effective also in the case of intratracheal challenge. The resistance of the animals to plague was influenced by the mode of administration of the vaccine, cutaneous inoculation proving best (see group 2). No such differences were observed in the case of tularemia vaccination. Anthrax vaccines gave

poor results and this accounted also for the high mortality rate in the animals challenged intratracheally with mixtures of all four strains used for the challenge tests.

In their conclusions, Vereninova and her associates stressed the superior value of the cutaneous method of plague vaccination. This is in accord with the experiences of Kalacheva[53] and of Pilipenko and associates,[58] to which reference has been made earlier in this review.

Combined administration of plague vaccine and anatoxins.

In the course of a study on the compatibility of simultaneous immunization with anatoxins and with live plague or tularemia vaccines, Saltikov and Zemskov[63] subcutaneously injected guinea-pigs with ad hoc prepared mixtures of EV vaccine (in doses of 1, 10 or 1,000 million organisms) and a penta-anatoxin against tetanus, botulismus (a and B) and gas gangrene caused by Clostridium perfringens and oedematiens (novyi) and challenged the animals 27 days later with 100 DCL of a virulent plague culture. All test animals and also controls immunized with the EV vaccine alone withstood the challenge.

Practical Aspects of Plague Vaccination

Manufacture, standardization and dosage of plague vaccines*

As can be gathered from recent publications, particularly those of Klez and associates,[37,38] Mikhaleva and co-workers[64] and Skalov[33] of the Irkutsk Anti-Plague Institute, the method of submerged cultivation in reactors under aeration is now generally adopted for obtaining 2 days growth of the strains to be used for immunization with live plague vaccines. To obtain the now mainly utilized bivalent vaccines, suspensions of each of the two components (usually of the strains 1 and 17) are prepared separately so as to contain 750,000 organisms per 0.5 ml of normal saline and are then mixed in equal parts so as to produce a vaccine with a titer of 1.5 billion per ml. To obtain a stable product, the vaccine mixtures are subjected to freeze-drying in vacuo in a saccharose-gelatin substrate.[37,64]

According to the existing regulations, quoted by Mikhaleva and her colleagues,[64] the vaccines are assayed by (a) ascertaining the presence and percentage of viable plague bacilli (cultivation of 0.1 ml amounts of adequately diluted re-suspensions of the vaccines on agar plates with a pH of 7.2); and (b) assessing the immunogenicity of every tenth series of the vaccines by subcutaneously injecting batches of 10 guinea-pigs with 1 billion doses of the lots under test and challenging the animals 21 days later with the aid of a virulent plague strain, presumably with 200 lethal doses.

---

* Since the use of killed vaccines, formerly prepared in the universally adopted manner, has been given up in the Soviet Union in favor of immunization with live plague vaccines, the methods concerning the latter alone will be considered in this section.

As quoted by Mikhaleva and her associates from the instructions, the dosages in which the leve plague vaccines are used for human immunization depend upon the percentage of viable organisms found: if this equals or exceeds 20%, 1.5 billion doses are administered, while double doses (3 billion) are given if 10-20% of the organisms are found viable. Lots containing less than 10% of viable organisms are apparently discarded.

Mikhaleva and her associates maintained that the above mentioned criteria for determining the potency of the live plague vaccines were inadequate and recommended therefore additional tests, consisting of the injection of guinea-pigs with vaccine doses ranging from 1,000 to 1 billion organisms so as to determine the minimal immunizing dose of each series of vaccines. The conclusions reached by trials of this method were that

> "1. The method of determining the minimal immunizing doses of bivalent vaccines gives a proper understanding of the immunological efficacy of each series of vaccines.
>
> 2. The minimal immunizing dose of highly effective vaccines is 1 million and 100,000 microbes.
>
> 3. Bivalent vaccines with a minimal immunizing dose of 1 billion, even with a high percentage of viable organisms, are less effective than vaccines with minimal immunizing doses below 1 billion organisms."

Modes of vaccination

Recommendations to replace the usual method of vaccination by the subcutaneous route or that of intradermal injection by epidermal (cutaneous) inoculation of live plague vaccines have been made by several modern Soviet workers, particularly by Korobkova,[21] Novikova and associates,[28] Demina and co-workers[65] and Pilipenko and his colleagues.[58]

Korobkova[21] who, as recorded earlier in this review, found cutaneous plague vaccination with the EV strain innocuous as well as effective in guinea-pigs, tried this method also in human volunteers. She used for this purpose a suspension made from a 2 days' agar culture of the strain which contained 100 billion organisms per ml. Using a technique similar to that of smallpox inoculation, she rubbed drops of this material into 3 cruciform scarifications made on the flexor surface of the forearm. Local reactions began to appear 5-10 hours later and became usually maximal within 24-30 hours. They consisted of redness, slight swelling and sometimes also of the formation of small vesicles. General reactions remained absent as a rule, but in a small number of the vaccinated one could note a slight increase of the body temperature lasting for a few hours or quickly passing signs of lymphangitis and lymphadenitis.

As noted before, Korobkova came to the conclusion that cutaneous vaccinations against plague "facilitate the implementation of specific prophylaxis, do not cause marked reactions and reduce the number of contraindications to vaccination."

In an article dealing with the reactions produced by the new bivalent plague vaccine 1-17, to which further reference will be made below, Novikova and her associates[28] stated that according to an instruction promulgated in December 1953 it was recommended to vaccinate persons from 7 to 60 years intradermally, those from 2 to 7 years and above 60 years as well as women in the first half of pregnancy epidermally. After an exhaustive comparative study of these two methods the authors came to the conclusion that in place of the intracutaneous inoculation, which produced severe reactions and was moreover technically difficult to use on a large scale, preference ought to be given for all age groups to the much better tolerated and expedient method of cutaneous inoculation.

Demina and her colleagues[65] also expressed the belief that in practical work preference ought to be given to the epidermal method of plague vaccination, because it produced much milder and chiefly local reactions. However, they pointed out that often even these reactions remained absent. In their opinion

> This could be partly explained by the fact that the vaccinators, recruited from the general medical personnel, do not use the method in a proper manner. Sometimes, as is done in smallpox inoculation, they make small scratches with scarification. In other cases the vaccinated wipe off the vaccine deposited on the skin which has not yet become dry. As a result of such deviations from the rules for vaccination in the majority of the cases a reaction remains absent.

It is important to note, however, that according to the 1960 publication of Pilipenko and his associates,[58] in view of the undesirably severe local reactions caused by the subcutaneous administration of live plague vaccines

> presently for the purpose of human vaccination against plague increasing use is being made of the method of cutaneous inoculation, because this is more areactogenic, simple and not less effective than the other methods.

Postvaccinal reactions

Besides by Korobkova[21] (see above) observations on the reactions caused by the administration of live plague vaccines have been made by the following authors:

Novikova and her colleagues[28] thus summarized the results of an exhaustive study of this subject:

(a) All persons intradermally inoculated with the plague vaccine 1-17 developed within 6-8 hours after vaccination headache, general debility and fever. At the end of the first day after vaccination the temperature became normal in 52.7% of the vaccinated but there remained some weakness, headache, sometimes also pains in the joints, and the local reaction.

(b) 45% of the vaccinated had a marked general reaction lasting 2-3 days or longer, when the temperature remained elevated and their working capacity was so much impaired that they had to stay in bed.

(c) In about 1.5-2% intradermal plague vaccination led to a most severe reaction, manifested by high fever and other signs of a serious illness. The patients were confined to bed for periods of up to 10 days. Occasionally the high fever was associated with a loss of consciousness and delirium.

(d) The local reaction to intradermal vaccination was manifested by reddening of the skin and considerable subcutaneous edema at the site of inoculation.

(e) In about 5-6% of the vaccinated there appeared after 48 hours at the site of inoculation a vesicle filled with turbid serous, seropurulent or even sero-hemorrhagic contents, from which plague bacilli (invariably the glycerol-positive organisms of strain 17 only) could be cultivated.

(f) As a rule epidermal inoculation did not lead to a general reaction, while the local reaction was so slight as to cause no serious disturbance of health and no loss of working capacity.

Details of the findings made at the site of inoculation are set forth in the tabulation on page 44.

Demina and her co-workers[65] found that in persons who were inoculated intracutaneously with the bivalent plague vaccine 1-17 for the first time, a local reaction was present in 98.4%, a general reaction in 74.2%. The former consisted of hyperemia of the skin and more or less painful swellings. Enlargement or at least tenderness of the lymph nodes were quite frequent. Often it came to the formation of a papule or pustule at the site of injection.

The general reactions consisted of fever (up to 39° C), headache, general debility and loss of appetitie. Nausea (sometimes leading to vomiting) was not rare. Pains in the muscles of the legs or in the lumbar region were also noted.

Local reaction	Age Groups (years) 2 - 7	8 - 15	16 - 20	Over 20
None	13.8%	7.0%	-	5%
Congestion round the scarifications	3.6%	-	-	2.3%
Congestion and slight edema round the scarifications	17.7%	17.7%	-	7.7%
Congestion and slight infiltration	1.3%	-	-	4.8%
Congestion, moderate infiltration and vesicle formation	8.1%	-	7.3%	47.7%
Congestion, more extensive infiltration and vesicle formation	-	-	5.8%	25 %
Lymphangitis	9.3%	-	11.8%	66 %

N.B. The local reactions in the adult group, receiving presumably increased vaccine doses, were thus comparatively more marked.

As mentioned above, Demina and her colleagues found that, in contrast to these serious or at least unpleasant after-effects of intracutaneous plague vaccination, the reactions following cutaneous inoculation of the bivalent vaccine were slight, if at all manifest.

Using the plague strain 17 for the inoculation of 18 volunteers with various doses and by different routes (subcutaneously, intracutaneously or cutaneously), Mikhaleva[29] found the resulting local and general reactions to be of a mild character, though more marked than those following the administration of the EV strain.

An interesting study of the reactions following the simultaneous administration of live plague and tularemia vaccines and the separate inoculation with the two corresponding monovaccines was made by Kalacheva.[57] She selected for the former purpose 60 persons aged from 15 to 55 years giving a negative tularin reaction who were cutaneously inoculated wity
(a) live dry plague vaccine manufactured in the Saratov 'Mikrob' Institute;
(b) live dry tularemia vaccine prepared in the Gamaleia Institute; and
(c) through a third scarification with a mixture of the two vaccines.

The local reactions caused by the administration of the two vaccines were as follows:

Kind of Vaccine	Marked	Weak	Negative
Plague	34	23	3
Tularemia	50	7	3

As stated by Kalacheva, the local reactions to plague vaccination

> appeared at the end of the first day and lasted for 3-4 days. A marked reaction was characterized by an intensive reddening of the skin at the site of the injection, swelling and the presence of vesicles along the scarifications. In the case of a weak reaction it did not come to vesicle formation.

There were no marked general reactions.

Results in the two control groups cutaneously inoculated with either the plague or the tularemia vaccine were shown below:

Reaction	Monovaccine	Number of Reactions			Remarks
		Marked	Weak	Negative	
Local	Plague	7	10	3	
	Tularemia	10	8	2	
General	Plague	-	3	17	Headache in 3 persons
	Tularemia	1	3	16	Headache in 4 persons + enlargement of the regional lymph nodes in one

As described by Kozlov and his associates[66] in an article to which further reference will be made in the following section of this review, they studied the postvaccinal reactions in a group of 291 healthy individuals (155 males and 136 females 25-35 years old) who were intracutaneously inoculated with identical doses of one lot of the plague vaccine 1-17 prepared in the Irkutsk Anti-Plague Institute. There followed a local reaction in all, in 38.4% also a general reaction. As noted also by some previous observers like Demina and her colleagues,[65] there existed no constant correlation between the intensity of the local and general reactions.

The general reactions consisted of debility, headache for two days and, in 18%, of an increase of the body temperature to 37° - 39.4°C, which usually lasted for 18-20 hours. Enlargement and tenderness of the regional lymph nodes were noted in 8% of the vaccinated.

The local reactions, which usually became manifest 6-8 hours after the vaccination and completely disappeared after 5-7 days, consisted of congestion of the skin and formation of an infiltrate at the site or inoculation, and also of formation of a papule which sometimes became transformed into a vesicle. The local reaction was slight in only 7.6%, of a moderate character in 87%, marked in 5.4%.

Commenting upon these findings made in a group of adults, the authors noted that, according to their own experiences as well as to the observations of other workers, children up to 15 years reacted less intensively to plague vaccination than grown-up persons.

On the whole the character of the postvaccinal reactions varied markedly. As the authors postulated, this variance was conditioned by individual peculiarities of a non-specific nature.

Allergic reactions

The following observations demonstrating or suggesting the development of an allergic state subsequent to the administration of live plague vaccines have been recorded in the Soviet Union:

Korobkova[67] established that intradermal administration of an antigen consisting of a heat-killed culture of the EV strain (exposure to 60° C for 1 hour) produced a marked reaction in immunized guinea-pigs, while normal control animals failed to react. She postulated, therefore, that this reaction to 'pestin' could be used to determine the appearance and also the degree of immunity produced through inoculation with live plague vaccines. An interesting observation made in this connection was that intracutaneously or cutaneously inoculated animals reacted earlier and more strongly than subcutaneously vaccinated animals.

Positive skin reactions were obtained as well in human volunteers who had been vaccinated against plague 5-11 months previously.

Most severe reactions following intracutaneous vaccination with live plague vaccine (? vaccine 1-17), observed in one instance each by Kozlov and Norov[68] and by Medinski and Razumeenko[69] were ascribed by these authors to the presence of an allergic state in the persons concerned. The individual observed in Mongolia by the first mentioned two workers was stated to have been vaccinated against plague by the subcutaneous route 9 months previously, but no such statement could be found in the short communication of Medinskii and Razumeenko.

In order to study the relation between postvaccinal and allergic reactions in persons inoculated with the 1-17 plague vaccine, Kozlov and his co-workers[66] divided the individuals under their observation at random into four groups and determined the reaction to pestin (prepared according to the method of Korobkova[67] from the vaccine batch used for inoculation) in the first group 15 days after vaccination, in the second group after one month, in the third after 6 months and in the last group one year after the immunization. Readings of the tests showed the following results:

Character of the Allergic Reactions	Percentages of the Reaction in the Groups Tested at Various Intervals after Vaccination			
	After 15 days	After 1 month	After 6 months	After 1 year
Negative	-	2.5	7.0	60.6
Weakly positive	100.0	5.9	13.9	39.4
Positive	-	46.2	29.2	-
Markedly positive	-	42.2	47.9	-
Intense	-	3.4	2.0	-

Commenting upon these findings, Kozlov and his associates accepted the view of Korobkova[67] that a parallelism existed between the results of pestin reactions and the state of immunity of the persons tested. The authors maintained accordingly that 1 month after intracutaneous inoculation with 1.5 billion doses of live plague vaccine an overwhelming majority of the vaccinated had become markedly immune to the infection. It was noted in this connection that the frankly or markedly positive allergic reactions found one month after vaccination in over 91% of the inoculated corresponded almost invariably to a marked reaction to the vaccination, whereas an early disappearance of the allergic reaction always took place in individuals reacting weakly to the inoculation.

The formal conclusions reached by Kozlov and his colleagues were that:

1. The allergic reaction, indicating an immunological reconstitution of the body after plague vaccination, stood in a majority of the cases in direct relation to the postvaccinal reaction: like the immunity it was most marked between the first and sixth months after vaccination and almost disappeared one year after vaccination. Thus the pestin reaction forms a satisfactory test to assess the degree of immunity in plague-vaccinated persons.

2. The character of the reaction to vaccination with live dry plague vaccine depended to a large degree upon the individual reactivity of the vaccinated.

3. The character of the allergic reactions indicated that the doses for intracutaneous plague vaccination, as given in the instructions of the 'Mikrob" Institute, were 91.2% sufficient to produce in the vaccinated a high degree of immunological reconstitution.

Vaccination campaigns

From the publication of Osolinker[6] who, as far as is known, was the only recent writer supplying information on the scope of vaccination campaigns in any given area of the Soviet Union,* the following information may be gathered:

As stated in the introduction to Osolinker's article, the south-eastern part of the focus between the Volga and Ural rivers, which was under the control of the Gur'ev Anti-Plague Station, was from 1938 until 1952 the scene of perennial wild rodent epizootics. The varying intensity of these during the final part of the above mentioned period is illustrated by the data of the following table:

Year	Number of Rodents Examined	Plague Cultures Isolated	Year	Number of Rodents Examined	Plague Cultures Isolated
1941	49,608	78	1947	84,354	25
1942	52,177	3	1948	56,796	6
1943	83,464	1	1949	55,937	7
1944	80,846	11	1950	62,476	31
1945	74,902	410	1951	76,609	29
1946	78,444	188	1952	56,037	54

N.B. It is not stated whether these epizootics led to the appearance of plague in man.

The anti-plague program adopted to deal with this situation consisted of (a) emergency measures aiming at the prevention of human infections and (b) large-scale anti-rodent campaigns undertaken with the aim of eradicating the infection. The former program comprised, besides the vaccination campaigns described below, also anti-flea and anti-rat work in the settlements, public health education and 'zonal' eradication of the wild rodents (i.e. apparently the creation of rodent-free belts round the human settlements).

*According to a statement made by B.N. Pastukhov in the report on the "Natural focality and epidemiology of specially dangerous infectious diseases" (Prirodnaia ochagovost'i epidemiologiia osobo opasnikh infektsionikh zabolevanii, Saratov, 1959 p. 14), the total number of inoculations with live bivalent plague vaccine made in the Soviet Union during the period 1954-1956 amounted to 2,013,100.

The vaccination campaigns were conducted first with the killed AD vaccine, from 1943 with the live EV vaccine and finally from 19[illegible] wards with the bivalent vaccine 1-17 manufactured in the Saratov 'M[illegible] Institute. The scope of these campaigns, which were intensified wh[illegible] situation in the wild rodents became threatening, is shown in the f[illegible] tabulation:

Year	Number of Vaccinations	Year	Number of Vaccinations
1941	18,124	1949	30,956
1942	22,019	1950	35,780
1943	31,991	1951	34,660
1944	23,981	1952	81,377
1945	72,305	1953	43,031
1946	82,906	1954	35,409
1947	78,478	1955	8,190
1948	35,564	1956	6,659

As Osolinker stated, these campaigns, implemented in com[illegible] with the other above mentioned emergency measures, "fully permitted [illegible] remove the threat of epidemic manifestations of plague in the Volga [illegible] interfluvial region."

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(Editor's Note: The References to which the numbers in the text re[illegible] will appear in the "Bibliography" installment of th[illegible] Report to be issued next.)