SOVIET VIABLE PASTEURELLA TULARENSIS VACCINES

A REVIEW OF SELECTED ARTICLES

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I. Preface

Efforts to develop methods of vaccine prophylaxis for prevention of tularemia began some 30 years ago. Various nonliving preparations have been used in animals and man. Because of the apparent inadequacy of killed vaccines, Soviet investigators turned to a viable preparation, first used in man in 1942. Today viable vaccines form a major portion of those Soviet public health measures intended for the control of tularemia.

It is the purpose of this report to describe the development of these viable vaccines. Since such studies in man had not been conducted elsewhere, the list of literature cited obviously is limited almost entirely to articles and monographs by Soviet authors. The sources were determined, in part, by their availability in translation, and it must be appreciated that the coverage is not complete.

Gratitude is expressed to the numerous individuals and agencies through whose efforts source materials were assembled.

II. Introduction

Ampoules of "viable" tularemia vaccine were brought to the United States from Russia from the Institute of Epidemiology and Microbiology imeni N. F. Gamaleia (Gamaleia Institute) by Shope in 1956 (88). The label indicates series no. 672, control no. 3207, date of expiration January 25, 1958, and the name "cutaneous tularemia live vaccine." Accompanying was a set of provisional instructions (9) for the control and use of the dry live cutaneous vaccine made from Pasteurella tularensis strains 155 and 15 "recovered" (also translated as "renewed" or "restored"). These instructions bear an originating date of August 9, 1955. It is specified therein that the expiration date, if the vaccine is held at 4 C, is 2 years from the date of manufacture.

The two strains (155 and 15 "restored") mentioned in the title of the provisional instructions are described by Olsuf'ev et al. (69-71). Either may be used and either strain, dependent on the method of culture, will dissociate.

 Cultures from these ampoules have been shown in several American laboratories to contain two colony types. One of these, termed "white" in Soviet publications (or "blue" when colonies are viewed with oblique light), is immunogenic for guinea pigs. The other colony type, termed "gray," does not appear to be immunogenic.

In the 1955 control procedures, the "immunogenic" type must constitute 20 to 30% of the total number of organisms present in the vaccine. Discrimination is on the basis of colonial morphology after incubation for 4 to 5 days, followed by refrigeration for 24 hr.

The 1955 provisional instructions specify that guinea pigs should tolerate, without death for 1 month, the subcutaneous inoculation of 1,000,000 total cells of the vaccine, while it is permissible to lose up to half of the total of 25 test white mice receiving a subcutaneous inoculation of 1,000 cells of the vaccine. When the surviving
mice are challenged with a dose of 1,000 test strain cells, 90% must survive for a period of 15 days.

Olsuf'ev (68) states that the test strain used in the above described challenge is called 503. It was isolated in June 1949 from Dermacentor pictus Herm. in the Moscow area (37, 38) and has had 405 guinea pig passages. In the 1955 provisional instructions (9) it is indicated that 1 cell is capable of producing death in mice in 5 to 7 days. The test strain is grown for 2 days on conglutated egg medium. Olsuf'ev and Emelianova (67) characterize this strain by stating that the lethal dose of subcutaneous administration is 1 microorganism for white mice and guinea pigs, while $10^4$ or $10^5$ organisms are required to kill white rats. Apparently some rabbits are capable of surviving an intravenous administration of 1,000 organisms, and the subcutaneous LD₅₀ is $10^4$. (Olsuf'ev will be referred to extensively in this review. He is Director of the Tularemia Laboratories, Department of Natural Focal Infections, in the Gamaleia Institute of the Academy of Medical Sciences of the U. S. S. R., Moscow. Gamaleia Institute is the largest unit of its kind in the Soviet Union (12, 13). According to Sil'chenko (92, 93), this tularemia laboratory was inaugurated in 1929 under the direction of Khatenever. Olsuf'ev's early work was on the vectors of tularemia in Alma-Ata, beginning in 1934. He was appointed to his present position in 1948 upon the death of Khatenever. He is a Corresponding Member of the Academy of Medical Sciences of the U. S. S. R. (69, 70). Emelianova, associated with Olsuf'ev in various publications, will also be quoted. She, too, is a member of the staff of Gamaleia Institute and her publications on tularemia and P. tularensis began as early as 1942 (37, 38).)

III. TULAREMIA AND Pasteurella tularensis IN THE U. S. S. R.

Tularemia first was identified in the U. S. S. R. in 1926, occurring in epidemic form along the Volga in the Astrakan District (92, 93). There was initial recognition in, or perhaps extension of the disease to, areas east and west of this district during the period 1929 to 1938. During 1940, all of the foci in the south of Russia became active, and the disease appeared to spread to the west as far as the Dnieper River (45). Sil'chenko (92, 93) relates these outbreaks to an increase in voles and house mice, associated with the disruption of war (also, see Tsareva (97)). In the winter of 1941 and 1942, Jusats states that nearly 30,000 cases were reported in the lowlands near the Caucasus and some 37,000 cases occurred in the area around Rostov. In some villages, the entire population was involved. Epizootics and epidemics are stated by Borodin et al. (16) to have occurred in the Stalingrad area in 1934, 1937, 1940 to 1942, and 1945 to 1946.

In the volume on Epidemiology in Practical Experience of Soviet Medicine in World War II, there is a considerable section on the "great experience in preventing and combating tularemia" acquired by the service (62). Sil'chenko (92, 93) described investigations done under difficult front-line conditions and control of the disease in the trenches and in the civilian population behind the lines. Mikat and Kuhlmann (61) described the clinical picture in 900 German soldiers who developed tularemia as a result of participation in the "War in the East." (For a description in English of a preantibiotic and prevaccination epidemic in deportees in Siberia, see Glass (41)).

Another example of the magnitude of the problem is given by Shmuter (87), who describes tularemia as it occurred in sugar factory workers in 1948 and 1949. At various factories the overall attack rate ranged from 10 to 20%, and in certain sections up to 75% of the workers became ill. He cites figures of 1,207 workers on the sick list because of tularemia infection with a total loss of 24,353 working days, and specifies that reporting of cases was incomplete.

The public health significance of this disease is likewise attested to by the large amount of medical talent which has been devoted to its control through large-scale vaccination, treatment, and control of hosts and vectors.

The geographic distribution of tularemia in the U. S. S. R. can be appreciated readily by a glance at the map in Fig. 1. Laboratories, tularemia control centers, or reported endemic or epidemic foci are underlined thereon. Except as they may bear incidentally on the main subject of this review, i.e., viable vaccines, no effort will be made to discuss the several different types of endemic foci or the programs intended to control or eradicate hosts and vectors. It is generally agreed that case fatality rates of tularemia in Russia are lower than in the U. S. A. U. S. S. R.
FIG. 1. Map of the U. S. S. R. Localities (underlined are reported foci of tularemia or tularemia control stations.

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over-all rates are about 1.0% (72). Yet Sil’chenko (92, 93) specified that Stradowskii succumbed to the disease while investigating water-borne outbreaks in 1933, and also says that the study of the disease in the Omsk area resulted in the death of Krol’ in the early 1930’s. In a recent summary the death of two or four members of a family is recorded, the infection having resulted from ingestion of an infected hare (82). However, Elkin (32, 33) seems to summarize current opinion with this statement: “It is true that when tularemia was first studied it seemed very dangerous, but at present scarcely anyone considers it so.”

Several authors have advanced the belief that a part of the difference between Old-World and New-World tularemia was due to a difference in virulence of the respective strains. Recently Olsuf’ev et al. (72) have compared the U. S. S. R. strain 503 (described above) and the U. S. A. reference strain Schu. They state that Schu is capable of killing all rabbits in very low doses while 503 kills only with doses of 10⁶ cells. Schu also produced more deaths in rats than did 503 at comparable dose levels. For various wild rodents the lethal doses were very similar, but the time to death was shorter with Schu. Russian strains usually did not ferment glycerol, while Schu did. Immunologically no differences were detected.

IV. Development of Vaccination against Tularemia in the U. S. S. R., 1934 to 1956

Sil’chenko (91) states that the development of tularemia vaccines was begun in the U. S. S. R. by Khatenev, El’bert (26, 27) notes that he, Gaikskii, and Kudo first demonstrated in 1934 that experimental animals inoculated with a weakly virulent tularemia culture were protected when subsequently inoculated with a virulent culture and suggested the possibility of such a procedure in man. According to Sil’chenko (91), Gaikskii and El’bert began work aimed toward such a vaccine in 1935, and by 1942 at the Irkutsk Anti-Plague Institute had developed a strain, “Moscow,” which had a weakened virulence and high immunogenic characteristics.

Elsewhere (50) it is stated that Gaikskii used two methods of attenuation: “(a) by the action on Bact. tularensense of immune serum; and (b) by drying the strains at thermostat temperature.” This strain was tested in volunteers and proved to be completely harmless. Six months later six of these inoculated persons were subjected to an experimental infection with a virulent tularemia culture. The review article states that the test fully confirmed the experimental data obtained earlier on animals and proved the harmlessness of the vaccine as well as its high protective effectiveness. After use on several thousand individuals this strain apparently was lost.

By 1944 Gaikskii had succeeded in obtaining new strains of P. tularensis “according to a special method he developed,” namely, attenuation on artificial culture media (see Emel’ianova (37, 38)).

One of the attenuated strains which was nonvirulent to guinea pigs and relatively virulent to white mice was called by Gaikskii “Bovillon strain 15.” (This is probably a typographical error; “bouillon” seems more likely.) A second strain of this type was named “Ondatra (musk-rat) IV.” In a report by Faibich and Tamarina (39), it is indicated that Khatevner claimed that he provided the already attenuated strain 15 to Gaikskii. As originally prepared, this product had to be used within a week after preparation. To overcome this limitation, Gaikskii then developed a “dry” vaccine.

Clinical tests with this vaccine were reported by Kosmachevsky (55). Strains 15 and Ondatra IV were inoculated subcutaneously; the latter strain was dry. When these vaccine strains were employed separately, it was not possible to distinguish the reaction of one from the other. Kosmachevsky concluded that vaccination with attenuated strains was harmless.

El’bert et al. (25, 30) developed the method of inoculation of tularemia vaccine by the dermal route (comparable to vaccination for smallpox). In 1945 this investigator, with various workers at the Rostov Anti-Plague Institute, prepared a “liquid yolk” vaccine (ZhTV). The original publication of this “liquid yolk” media by Droshkevina (19) has not been reviewed. In later papers, reference is made to the use of an egg yolk suspension in normal saline for vaccine production, as well as the preparation of a vaccine from a 2-day-old tularemia culture on the “solid egg medium of McCoy,” suspending the preparation in equal volumes of the fluid yolk medium and normal saline (20, 21). (Instructions for preparation of vaccine issued in 1957 (18) specify that after the egg yolk and saline have been mixed the material should be coagulated in
a water-jacketed hot air incubator for 1 hr. The temperature of the water is 80 C.)

After this liquid vaccine had been used in a variety of animal studies it was applied in man. Initial work was with a 50% glycerol-virus vaccine with a titer of $2 \times 10^6$ per ml (26, 27), and the statement is made (25) that vaccination could be accomplished with as few as 3 to 4 organisms. The vaccine, which had a very short shelf life, was administered by the cutaneous route in 24 tularemia-affected localities. Local reactions occurred in most instances; e.g., in one series of 960 persons the rate was 94%. Systemic responses were mild, if noted at all, and in no instance was there time lost from work. The vaccination programs were initiated after the outbreaks of illness had reached their peak. Prior to vaccination 11.1% of the population had contracted tularemia; afterward 4.3% of those not vaccinated became ill, in contrast to a rate of 0.36% among the vaccinated. The cases in the vaccinated persons occurred only during the first 12 days after administration. It was thus concluded that vaccination during the incubation period was harmless and apparently resulted in shorter and less severe forms of the disease or even in symptomless forms. In 1946 Gaiskii and El'bert were given a Stalin prize for their joint work (91).

Faibich and Tamarina (39) of the Scientific Research Institute for Epidemiology and Hygiene (NIIEG) describe in detail the laboratory characteristics of strain 15 and Ondatra IV. They state, “thus the experiment on white mice and guinea pigs has shown that the strains from which the dry living tularemia vaccine of NIIEG has been and is being prepared are not completely avirulent for small laboratory animals; the strain Ondatra IV is virtually avirulent for guinea pigs and weakly virulent for white mice, while the strain 15 is, conversely, more virulent for guinea pigs and less virulent for white mice. Thus both strains possess in one degree or another a ‘residual’ virulence.” These authors conclude that strain 15 is generally much better than Ondatra IV, but then hasten to point out, “at the same time it must be kept in mind that inasmuch as the vaccinal strains were not identical in antigenic and immunogenic respects, the preparation of a polyvalent dry vaccine from several vaccinal strains of tularemia is expedient.” They indicated that a spontaneously attenuated strain 10 “Bulukhta” obtained in 1944 from the Saratov Institute was available for study, and that while it was less reactive for guinea pigs and white mice than strain 15 it possessed high immunogenic properties. An intent to study strain 10 in man was stated.

The subcutaneous inoculation of 12.5 to 25 million organisms of strain 15 produced agglutination and allergic responses in man at 1 month in all individuals studied, while lower percentages were obtained with strain Ondatra IV. More than 30,000 men were vaccinated. Strain 15 could be administered to recovered human patients in dosages of 25,000,000 organisms subcutaneously without producing serious side effects.

For measuring the “allergic response” to the vaccines, Faibich and Tamarina used intracutaneous inoculation of “tularin,” a suspension of killed P. tularensis. Khatenever (50) stated that he first used this for diagnostic purposes in 1931 and 1932. His preparation was heat-killed at 65 C for 30 minutes and, following intradermal inoculation, promptly produced a reaction characterized by hyperemia and edema. El’bert and Gaikskii are credited with the validation of a similar procedure for evaluating the response to vaccination. Khatenever specified a concentration of $10^8$ organisms per ml. Kalitina (47) states that the “usual” diagnostic dose of tularin is $10^8$ killed organisms in 0.1 ml. A report in 1950 (23) specifies an identical concentration and indicates that a positive response will be manifest in 6 to 8 hr. More commonly the test was read at 24 to 48 hr (64, 65).

A second report in 1946 by Faibich and Tamarina (39) is concerned with the methods initially used for the preparation of a dry vaccine. Since current methods are a direct outgrowth of this work, certain of their statements are quoted. “Our own numerous investigations enabled us to propose the following media for mass-production of tularemia bacteria preparations: (i) Semiliquid agar consisting of pancreatic hydrolysates of liver and gelatin, sucrose, white gelatin, starch, agar and water. (ii) A thick agar medium in two variants, the first of which contains pancreatic hydrolysates of liver, blood and gelatin, milk, serum, cysteine, starch, table salt, agar and water, and the second, pancreatic hydrolysates of blood, autolysates of yeasts, milk, serum, cysteine, starch, table salt, glucose, agar and water... The hydrolysates of liver and blood, contrary to
the generally accepted method, were prepared without preliminary thermal treatment, i.e., from raw material ...[and] were treated with sulfuric acid aluminum. In the growth media a slightly alkaline reaction (pH = 7.1 to 7.2) was established and in the drying media, a neutral one. The proposed media are sterilized in an autoclave at a temperature of 120 C.” The sucrose content was 4% and the gelatin 1.25 to 1.5%. These gave a yield of 1.5 to 3.0 billion organisms per ml of media, the higher figure coming from the thick agar. Prior to lyophilization, additional sucrose up to 10% was added to the semisolid medium and the material was dispensed in 1-ml amounts. If the thick agar medium was used the organisms were “washed” with the drying medium (i.e., sucrose-agar-gelatin) and then diluted with the same material to a titer of 2 to 5 billion organisms per ml. After being placed in ampoules the vaccine was prefrozen, then dried for 14 to 18 hr without added heat, and sealed under vacuum. The authors believed that the sucrose (or maltose and lactose) “fixes the amount of water” and “safeguards the microbes against excessive dehydration.” “Agar facilitates their longer preservation in the dried state,” while “gelatin merely improved drying conditions” and permits the drying of small numbers of organisms. In any event, absence of any of these components was found to produce an inferior product, as was storage without vacuum sealing. Such a vaccine was immunogenic for man after storage at 2–4 C for 1.5 years, at 18 C for 270 days, and at 26 C for 75 days. Using NIIEG vaccine prepared in 1951, which had been stored at temperatures ranging from a winter low of −2 C to a summer high of 18 C, Martinevski (59) noted that with aging the local skin lesion and the incidence of lymphadenitis declined markedly. No correlation with viable count was recorded.

Control procedures specified by Faibich and Tamaria included testing for purity, lack of harmful effects in guinea pigs, autogenicity in mice, and the titer. The measure of the last mentioned property was the limiting dilution of the vaccine at which there was perceptible growth of tularemia organisms in tubes containing yolk agar (52).

According to Vachkov and Pronina (98), mass prophylaxis vaccination was begun in 1946. An early example is given by Borodin (15), who describes a vaccination program with the liquid product in the Volga-Akhtubinsk River valley. Because of an outbreak of tularemia, 11,622 persons were inoculated during the summer of 1946. Of those inoculated, 0.17% became ill, while in the noninoculated the incidence of disease was 3.4%. Most of the cases in the inoculated occurred within 2 weeks after vaccination, and the disease was of a mild form. In subsequent years additional vaccinations were given, and the cumulative number of individuals inoculated by 1954 was 81,739. Another early program is noted by Panaiotti (75), who states that between 1947 and 1951 21,000 persons were vaccinated in Yartsevskaya. According to Vachkov and Pronina (98), during these early years mass vaccination programs were conducted in numerous areas.

The results obtained were “excellent,” and in 1951 the practice of vaccination was changed to the interepizootic period rather than waiting until an epidemic seemed likely. U. S. S. R. public health forms dated 1955 include printed entries for the reporting of cases of tularemia and for “injections against tularemia” (94).

In a monograph edited by Olsuf’ev (64), it is clearly indicated that most of the work done in Russia before 1951 had employed the liquid egg yolk vaccine (ZhTV). The Gaikskii vaccine strain 15 was used in most instances, and the Faibich strain 10 was added (sic) to the NIIEG vaccine (i.e., the dried vaccine).

(In some recent translations (69–71, 79), reference is made to liquid yolk-sac (sic) tularemia vaccine in use from 1948 to 1952. These are translation errors, and “liquid yolk” is the proper term. Although the response of embryonated hens’ eggs to the vaccine strain has been reported (7), there is no suggestion that this medium ever has been employed for vaccine production.)

In the 1953 monograph (64) there is a long review article by Olsuf’ev. He examines the variables that must enter into an evaluation of field results in a live vaccine program, such as method of storage, method of use, source of vaccine, immunological characteristics of the population, method of selection, accuracy of observation, and type and amount of skin test antigen used. He then notes that although the number of observations with the dry NIIEG product were limited it appeared that the number of clinical and immunological responses were somewhat higher when the dried product was used. He postulated that this difference is due not only to the method of
preparation of these vaccines, "but also to the differences of the strains [sic] included in them."

Olsuf'ev then considers the relative merits of the intracutaneous and the subcutaneous routes of inoculation and concludes:

"As compared with the subcutaneous injection, the skin method of vaccination is considerably more simple in its application and produces good immunological results. The skin method is convenient, because the procedure of a skin vaccination reaction makes it possible to count readily the results of the vaccination, conducted. The subcutaneous method of vaccination makes such a count impossible. In the event of a sudden contamination (at the time of vaccination) of the vaccination material, the skin method is more safe. This permits extensive enlistment of intermediate medical personnel for the implementation of skin vaccinations at any center. Thus, the entirely satisfactory results of mass vaccinations, and the considerable simplicity of the application technique justify considering the skin method of vaccination in tularemia incidence as the more promising method, especially if the dry skin vaccine of the NIIEG type is to be used."

Olsuf'ev continues with a statement that the problem of revaccination has not been adequately studied, and with a consideration of the "Epidemiological (Prophylactic) Effectiveness of Vaccination," intermingling observations on ZhTV and NIIEG vaccines. Nearly all reports show a markedly lower incidence of disease in those vaccinated, and there is one example of a rate among the vaccinated of 0.0049% (sic). Although the number vaccinated usually is not included, he makes the point that, on an oblast scale, vaccination to be effective should include upward of 90% of the "threaten contingents." (An oblast is the first order division of an individual soviet republic and may vary markedly in size and population.) The following paragraph is also of interest.

"In conclusion, it must be noted that, thanks to the vaccinations, cases of intralaboratory tularemia infection ceased; yet prior to the injection of the vaccine it has been practically impossible to prevent them, regardless of the strictest possible personal prophylaxis."

Olsuf'ev notes that the vaccine organisms are nonviable after 1 month and that "individuals vaccinated with ZhTV or NIIEG are protected from tularemia infection through the skin, respiratory organs, alimentary tract, etc." Earlier in the paper the following pertinent sentence occurs. "The question as to the quality of the vaccinations conducted can be settled only on the basis of spot checking of the vaccinated contingents by means of a tularin test; the first checkup should be conducted a year after vaccination."

Also in the Olsuf'ev monograph (64) is an article by Shmuter (86), which specifies the laboratory control procedures of that period and indicates that vaccines prepared by two agencies could vary in potency. According to Shmuter, "Tularemia vaccine strains used in production must possess the following properties. Upon inoculation of white mice with doses up to 1 million microbe cells, 30% of the mice perish. A dose of 1 billion microbe cells fails to kill guinea pigs. An immunizing dose for white mice consists of 1,000 microbe cells, for guinea pigs, of 10,000 microbe cells."

Shmuter conducted studies with production lots of vaccine obtained from two institutes, both derived from strain 15 originally. He believed that better results were obtained in man with the product having high mouse virulence.

According to Sil'chenko by 1955 Olsuf'ev had accumulated a "great number of observations" with a dry vaccine, in which he had found responses in about 97%. It is stated that until 1956, vaccines prepared at the Gamaleia Institute used vaccine strain 15 of Gaiskii, prepared by the aeration method (52).

Detailed instructions for use of the dried vaccine were issued from Gamaleia in 1953 (8), and an example of the field program is given by Ovasapyan (74), who states that 121,204 persons in the Leninakaniskiy Basin (Armenian S. S. R.) were inoculated by scarification with dry live tularemia vaccine from October 1952 to the end of 1954. The vaccine response reported was 97.5%. Karakulov, Mertsalov, and Zhokin (48) indicate that during 1955 and 1956 about 700,000 persons in Kazakhstan were inoculated. Kucherov et al. (57), apparently describing a part of the same program, specified that the dried vaccine was obtained from Gamaleia. Their figures for four regions in West Kazakhstan are 114,000, or practically the entire population. Sil'chenko (91) makes the following additional statement. "The change to the production of dry antitularemia vaccine at the Institutes [sic] must be regarded as justified." The date coincides with the issuance
of the provisional instructions for the use of dry live cutaneous tularemia vaccine noted in the initial paragraph of this summary. It must be, then, that Elf'bert (29), writing of the immunization of 60 million individuals, is referring to a variety of types of immunization employed over a period of 12 to 14 years.

V. LABORATORY STUDIES ON VACCINE STRAINS OF Pasteurella tularensis

The identification of strains ranging from fully virulent, partially virulent but immunogenic, and avirulent, particularly as measured in the mouse, resulted in a variety of laboratory studies intended to characterize these various organisms.

Olsuf'ev and Emel'ianova (67) state that Elf'bert and Gaiskii in 1941 described a difference in the agglutination of virulent and avirulent cultures, the avirulent cultures producing a fine granular agglutination, in contrast to the clumps seen when virulent cultures were similarly examined. On the basis of these 1941 studies, Elf'bert and Gaiskii proposed that the virulent cells contained two antigens, one of which was gradually lost in the process of attenuation, and was completely missing in the avirulent strains.

Aleshina and Puchkeva (5) described a correlation between virulence and fibrinolytic activity. Lytic activity was most marked when tested against fibrin from animals fully susceptible to tularemia infection.

In 1947 Karpusidi (49) described the characteristics of certain atypical and "little virulent" strains of P. tularensis. Some of these were capable of growth on ordinary laboratory media. Biochemical reactions were variable. All strains could be agglutinated by specific antisera, and, at least in some instances, passage through animals resulted in restoration of virulence.

Tinker and Bibikova (95) report on the capsular fraction of P. tularensis, indicating that this is present only in virulent tularemia organisms and is often absent in the case of avirulent growths. This is thought to be a thermostable polysaccharide which is not a complete antigen. However, the capsular fraction of the virulent and of the vaccinal strains would result in the production of a precipitin reaction against tularemia immune serum. Both the virulent and vaccinal strains had allergenic components, while corresponding tests in sensitized guinea pigs made with capsular fraction of the avirulent strain did not cause allergenic reactions. Later there is a report from Tinker and Elífeneva (96) on three strains of P. tularensis, namely, an avirulent strain (no. 11), an attenuated strain (no. 38) used for vaccine manufacture [sic], and the highly virulent strain 47. (It is not known whether the Russian strain 38 and the American strain of the same number are identical.) In vitro the leukocytes of normal guinea pigs were more active against the avirulent strain and lowest against the virulent strain.

When inoculated into guinea pigs the virulent strain produced a maximal increase in the phagocytic index, a less intense reaction was observed with the attenuated vaccinal strain, and some of the avirulent strains showed no change at all. Similar findings were observed on the phagocytic activity of normal human blood with respect to a low response, or a low index, in terms of the virulent strain and a higher index in regard to the vaccinal and the avirulent strain. In recovered cases the phagocytic index was high for the virulent and the vaccinal strains, and there was some increase in the indices in those individuals vaccinated against tularemia.

Studies on virulence, attenuation, and colonial morphology were described by Emel'ianova (34). She states that by repeated (many) passages in laboratory animals any virulent strain can be changed to an avirulent strain. This change to "moderate virulence" was associated with the appearance of two colony types: (i) a small white opaque raised colony and (ii) a large transparent flat grayish colony. Killed cultures of both were allergenic in guinea pigs. However, type 2 (large and transparent) did not kill mice in doses of 10⁸, while the small colony type sometimes was lethal for mice.

The medium used by Emel'ianova was fish-cysteine agar and blood as formulated by Kolyaditskaya and Shmurygina (52). Such a medium, with or without aeration, was used for vaccine production at Gamaleia until 1956 and was known to produce marked dissociation. It appears also that these two types could be induced by prolonged incubation. Primary growth of the virulent opaque colonies stopped by the 4th or 5th day. Then on the 9th to 11th days transparent daughter colonies were observed about isolated colonies. Puchkeva (80) also notes that on artificial culture media at 37°C, "the virulence of the vaccinal strain for mice gradually increases and again diminishes in relation to the length of
culture." Maximum lethality for white mice was noted with a 24- to 48-hr culture. Three- to 15-day-old cultures showed a material decrease in lethality for this animal. The 24- to 48-hr cultures showed maximum immunogenicity (measured by subsequent challenge) in guinea pigs, while the 10- to 15-day cultures had very poor immunogenicity. Emel'ianova and Shipitsina (35) report detailed chemical studies on virulent strain 21 (S form) and an R variant derived from 21 as a result of 250 passages on agar. In a 1957 paper (67), Olsuf'ev and Emel'ianova describe their concepts of the "Antigenic Structure of Pasteurella tularensis." They use conventional terminology for colonial types of the organism, referring to S and R, and indicating that S is virulent, while R is avirulent. They visualize the fully virulent tularemia organisms in the terms employed for the description of typhoid bacteria. Thus, there is a somatic complex symbolized O, and an envelope antigen symbolized Vi. The avirulent strain has lost the envelope or Vi antigen. Tests are described indicating that antisera against the R antigen will not agglutinate S organisms. On the other hand, S antisera agglutinate R organisms poorly, if at all. Absorption studies are described to support this concept further. "The virulence and immunogenic properties of tularemia bacteria are due to the presence of the Vi antigen. If this antigen is lost the bacteria become avirulent and nonimmunogenic. The Vi antigen may be destroyed by heating or by the action of alkaline solutions. Both the Vi antibody and the O antibody are thermolabile." There is a description of the "living culture of a 'renewed' Gaiskii 15 vaccine strain." This strain was agglutinated by S and R sera at a dilution of 1:1,280. The reaction with the S serum produced a stable agglutinate and, with R serum, an unstable agglutinate. The authors assume that while an envelope antigen is present, it is present in such a small amount that agglutination by both S and R sera is possible. The vaccine strain is therefore described by them as being SR and in terms of antigens to O (Vi). In continuation, the S strains of P. tularensis are denoted O cultures.

Biochemically, the capsular antigen contains protein, polysaccharide, and lipids. Verenikova, Kontorina, and Bakhrakh (100) also describe the separation of polysaccharides from virulent and vaccine strains of P. tularensis by heat and acetic acid treatment. They state that the "vaccine strain 15" on a weight basis provided more polysaccharide than did the virulent strains. This polysaccharide is allergic and can be used for skin testing but was not antigenic and did not protect rabbits.

In an article submitted on May 29, 1957, Emel'ianova (36) describes "Characteristics of Tularemia Vaccine Strains According to Laboratory Indices." It is stated that the work reported therein "served as the basis for the present operating specification in production, control and use of cutaneous, dry live tularemia vaccine." The complete paper should be consulted by anyone specifically concerned with this vaccine. She examines the problem of dissociation, identifies the immunogenic colony type, and specifies methods of examination to insure that an adequate number of immunogenic cells is included in any preparation.

She then turns to an examination of the requirement for "retention of residual virulence" in vaccine strains, a concept originally stated by El'bert and Gaiskii. By this is meant the retention of the capability to kill a proportion of test mice while failing to kill guinea pigs. She states that if graded doses (10^4 to 10^6 cells) of an appropriate product are given subcutaneously to equal-sized groups of white mice, the number of animals that die is proportional to the size of the dose and that 30 to 50% of all animals so inoculated should die. At least 90% of the survivors should withstand the subsequent inoculation of 1,000 cells of strain 503. Guinea pigs should tolerate 1 billion cells. At least 80% of guinea pigs vaccinated subcutaneously with 10,000 cells should withstand the subsequent inoculation of 1,000 cells of strain 503. Usable vaccines must also elicit a cutaneous lesion when inoculated by scarification.

Emel'ianova then notes that the original Gaiskii strain 15 apparently had become so attenuated that it no longer met these criteria. "After passage through animals highly sensitive to tularemia the vaccinating effectiveness of the strain increased significantly." This variant was named "no. 15 restored" ("regenerated" or "renewed") and meets the laboratory indices noted. Finally, mention is made of "refinements in production technology" resulting in products with a high percentage of immunogenic cells. Kolyaditskaia, Kuchina, and Shmurygina (53) state that as early as 1954 phenomena suggestive
of phage infection were noted in the Gaiskii 15 strain. Similar findings were also observed in 1957 in the “restored” strain. Apparently the white (immunogenic) colonies are the type usually involved; the changes vary from an “alveolar colony morphology” to complete lysis.)

Coincident with the efforts to restore the Gaiskii strain 15, a search was made for new vaccine strains (69). “By means of attenuation on artificial culture media and selection of colonies a new strain 155 was obtained which when tested on laboratory animals showed properties answering the requirements.” In the title of the paper this is called the Emel’ianova no. 155 strain.

Vaccines were prepared at the Gamaleia from strains 15 and 155. Cultures were grown by the deep method with aeration (see below for details of methodology). At the time these were prepared the vaccine production methods had not been changed, so that the total counts averaged $2 \times 10^6$ per ml with an average of 20 to 40% immunogenic cells. The vaccines were used 4 to 6 months after drying (69). Seven laboratories participated in the field program, which involved 7,324 subjects, mostly adults. Vaccines were employed in normal dosages, at 1:2 dilution, and at 1:10 dilution. No significant difference in reactivity as measured by the skin tests was found. With undiluted vaccines the average conversion rate was 97%, and at 1:10 dilution the rates were 65.8% for strain 15 and 69.1% for strain 155. Systemic reactions were rarely encountered with either product. Agglutinin titers at 30 days postvaccination were slightly lower, on the average, in those who had received strain 155. The paper concludes with a statement that “at present, strains 15 (restored) and 155 have been introduced into the production of tularemia vaccine.” This paper was received for publication in July 1957. Official instructions for the production of cutaneous, dry, live anti-tularemia vaccine published by the Ministry of Health of the U. S. S. R. in 1957 (18) include methods of calculation of dosage for these two strains as well as Gaiskii 15 (old).

VI. Production Techniques

Data concerning the “refinement in production technology” (36) and the “changes in vaccine production methods” (69–71) were described by Kolyuditskaya and Shmurygina in 1957 (but submitted for publication, July 10, 1956) (32). Prior to 1956 production of tularemia vaccine at Gamaleia was carried out by “growing a culture for 9 days, by a series of successive subcultures in a variety of media (two generations in yolk medium, then inoculating onto semisolid agar, solid agar, and semisolid or liquid medium for aeration).” Using fish-cysteine agar with blood as a check medium for demonstration of dissociation, these investigators found that original growth on coagulated yolk gave the greatest percentage of “antigenic” colonies (white and opaque). Coagulated yolk gave 69 to 89% of such colonies, while the “standard vaccine growth medium” gave approximately 3% antigenic colonies. Consequently a change was made so that seed cultures were grown on trays of fish-cysteine agar overlaid with a thin layer of liquid yolk media (3 to 5 mm) and heated to 80 C for 70 min. With cultures grown on this as seed, production batches of vaccine were prepared, and in these the percentage of antigenic organisms rather regularly exceeded 50% of the total. It is noted that this method materially decreases the number of eggs required.

The 1957 instructions previously cited indicate that seed lots so prepared may be dried in a menstruum essentially comparable to that of Faibich and Tamarina (39).

For vaccine production 48-hr cultures are washed from the trays of fish-cysteine yolk agar with normal saline containing 0.2% agar. This material is used for the inoculation of liquid or semiliquid media.

These final media contain 20 to 30% hydrolyzates of fresh fish, liver, meat or fish-mushroom, 10% gelatin hydrolyzate, 1 to 5% yeast hydrolyzate, 1.5% gelatin, 0.5% sodium chloride, 1.0% glucose, and 0.1% cysteine with a pH of 7.2 to 7.3. For the liquid medium the gelatin is omitted. These media are dispensed so as to half fill 5-liter flasks equipped to permit aeration with preheated air at the rate of 1 liter of air per liter of medium per minute.

Such flasks are inoculated with sufficient organisms to provide an initial concentration of $5 \times 10^6$ to $10^7$ cells per ml. They are then incubated for 12 to 18 hr at 36–37 C. Incubation terminates when the density reaches 2.5 to 3.0 $\times 10^9$ organisms per ml. (All concentrations are determined optically according to standards based on typhoid bacteria.) At this time the cultures and medium are added to “media for
drying,” which serves to increase the amount of sucrose and, if necessary, gelatin. This final material may be held for 10 days at a temperature of 4–6 C.

For drying, the material is dispensed into ampoules so as to provide a film less than 1 mm in thickness. After freezing at temperatures of −30 or −40 C, drying is accomplished on either manifold or chamber lyophilization equipment at a pressure of approximately 100 μ. The maximum permissible ampoule temperature at any time is −30 C. A final moisture content of less than 4% is attained. Final sealing is under a vacuum pressure not exceeding 100 μ. Shishkin (85), in a review article concerning the activities of the Rostov Anti-Plague Research Institute, states that workers there use a “rational 18-hr drying schedule” for tularemia vaccine.

At various stages in the preparation, tests for purity are employed. Tests for potency have been described in the previously noted article by Emel’ianova (36). Samples from each lot are sent for checking to the Moskovskaya Gosudarstvennaya Kontrol’naya Laboratoriya. The 1955 provisional instructions specified that each Institute preparing vaccine should examine three to four series each year in humans not immune to tularemia. Such vaccines must have produced positive responses in 23 of 25 persons. This requirement does not appear in the 1956 instructions (10), apparently having been replaced by a similar type of test on the vaccinal seed strains. Such strains, when checked in man, should not produce general reactions and should result in a local reaction in 24 of 25 individuals, as well as in a positive allergic test and an agglutinin response in 30 days.

The vaccine is stored at a temperature no higher than 4–6 C and has, under these conditions, an expiration date of 1 year from the time of drying. Freezing is permissible. It may be re-controlled at this time, and, if potency is retained, the date can be extended for an additional 6 months.

VII. Vaccination, Skin Testing, and Revaccination

For vaccine administration the dried product is rehydrated with distilled water and used within 4 hr. It is applied by the cutaneous route only (10). The shoulder of the individual to be vaccinated is cleaned with alcohol. After this dries, 2 drops of vaccine are applied 3 to 4 cm apart.

Through each drop two parallel scratches 1 cm in length are made in such a manner as to result in the appearance of only tiny drops of blood. The vaccine is then rubbed into these scratches for half a minute. A special quill is used.

In a successful vaccination small vesicles appear near the end of the first week and hyperemia and some infiltration persist to the end of the second week. The area then develops a crusted appearance and all symptoms subside within a total elapsed period of 3 to 4 weeks.

There are numerous articles evaluating the extent of these various reactions. The results seem to vary with the source of the vaccine, and there is correlation as well with the size of the inoculum. In the 1958 report by Olsuf’ev (68), general reactions were noted in 4.5% of those inoculated and enlargement of lymph nodes was reported in 3.0%. All nodes returned to normal size. In earlier reports figures up to 10-fold these are sometimes given.

Vaccination of individuals recovered from tularemia produces an increased percentage of general reactions. El’bert et al. in 1954 (28) recorded a 3-fold greater incidence in recovered cases than that seen in those without a history of tularemia. Thus, when vaccine is administered to large population groups, those individuals known to have had tularemia are not revaccinated.

Those obviously ill from any cause are excluded from vaccination, as are known asthmatics and women during the last half of pregnancy or during the period of breast feeding. (With minor variations these latter general exceptions to vaccination are applied to all viable bacterial vaccines used in the United States.) Ovasapyan (73) describes a case of tularemia in a woman at term. The agglutination titer in the infant was 1:2,000 a few days after birth. By the third month the titer was negative, as was the response to tularin. Kaltina (47) states that in vaccinated guinea pigs pregnancy causes a lowering of the response to intradermal tularin.

In the large program described by Kucherov et al. (57), children less than 4 years of age were excluded. The 1956 instructions specify that the amount of vaccine used for children of preschool age is half that used for adults. Successful vaccination of children down to 1 year of age is reported (81). Olsuf’ev (68) notes that there is “lesser stability of the immunologic reorganization of the organism in children from 7 to 14 years of age immunized against tulare-
mia.” Piotrovskiaia (79) describes the vaccination of 642 children (6 to 12 years old) and adolescents in 1948 and 1949. Six years after vaccination with a liquid product, 84% had positive reactions to tularin and 90% had agglutinins.

The 1956 regulations also specify that other vaccinations should not be carried out coincident with that for tularemia (but see below).

In a study conducted in a mental hospital Matveets et al. (60) report that in individuals with long-standing catatonic stupor “the vaccination process and the resulting immunological reaction developed in the majority of cases to a lesser extent than in healthy individuals.” In advanced parkinsonism the reaction was also less than normal, while in imbeciles and morons the response was identical with that of the healthy controls. (Bisikalova, Predtechenskii, and Zhdanovskii (14) report modification of the immune response in the rabbit under narcosis.)

An interesting observation is recorded by Klepko (51). In a military unit an outbreak of influenza was observed 4 to 13 days after the group had received tularemia inoculations. Thirty-eight of 92 men developed influenza, and in these the incidence of extensive lymphadenitis (from the tularemia vaccine) was much greater than in those not developing influenza. The agglutinin response at 2 months was not modified.

The influence of irradiation on the outcome of administration of the P. tularensis vaccine strain to white mice is described by Shevelev (84). A dose of 374 r was given at varying intervals after inoculation of 50,000 organisms (vaccine was obtained from Gamaleia). Maximum enhancement of fatality occurred when irradiation was given 2 to 24 hr after infection. Shevelev considers that this results in a coincidence of maximum organism multiplication with the climastric stage of irradiation sickness. Irradiation at a later date was without influence.

The duration of the period of immunity following vaccination has been examined by numerous investigators. According to Sil’chenko (90), the first Conference to Study the Effectiveness of Tularemia Vaccine, sponsored by the Ministry of Public Health, was held in 1949. At this meeting annual revaccination was recommended. At the 1951 Conference there was agreement that the period between vaccinations could be extended to 4 years. This represents an average figure, since Olsuf’ev (65) records “rare” cases of tularemia among vaccinated individuals in whom an initial skin reaction to vaccination had been noted. These people had received liquid vaccine a year prior to their illness.

The 1956 instructions state that the period of immunity conferred is 3 to 6 years (18). An editorial footnote to an article published in May 1958 (79) states that “at the present time the period of revaccination for children and adults living in natural foci of infection is officially set at five years.” Olsuf’ev in December of 1958 (68) makes an identical statement.

Persistence of the immune response has usually been on the basis of a continued allergic response to tularin. As early as 1953 Sil’chenko indicated (90) that a considerable portion of vaccinated people retained this reaction for as long as 8 years. (There is one report (17) of a laboratory worker, known to have had a sharply positive skin test, who developed tularemia following a laboratory accident. She had been vaccinated 5 years previously, apparently with one of the first vaccines produced.)

If previously vaccinated persons who have maintained an allergic response are reimmunized (superimmunized), the entire response proceeds at a more rapid rate than on initial vaccination, and the percentage of general reactions may be increased (28, 68). In those who have lost their allergy the revaccination proceeds in the same identical manner as in a primary inoculation.

Mention has been made of the intradermal use of tularin to measure the existence of an immune response. Sil’chenko (92, 93) and Olsuf’ev et al. (69–71) note that such a procedure frequently has undesirable side effects. Both authors cite work reported in 1953 by Maiskii and Shipiteina (58), who administered tularin intradermally to a group 9 months after vaccination. They observed tissue necrosis at the site of injection in 10%, chills in 3%, malaise and headache in 14%, and some lymph node enlargement in 29%. Kalitina (47) suggests that these reactions may be decreased by a 1,000-fold dilution of tularin without significantly decreasing the number of positive local reactions noted. In individuals who had recovered from tularemia, even more dilute tularin gave a positive test. However, in 1958 (69–71) full strength tularin (10^7 cells per injection) intracutaneously was in general use. (Dunaeva (22) notes that intradermal tularin inoculation should not be used in experimental animals prior to the conduct of studies in tulare-
mia since it may alter the immunological reactivity."

Because of these adverse reactions efforts were made in 1954 to evaluate the dermal administration of tularin. This route of inoculation for diagnostic purposes had been used as early as 1934. This method had also been used for research studies in man but had not been accepted for use in the field as a general rule. In evaluating this procedure, Olsuf'ev et al. (69–71) showed that tularin could be prepared either from a vaccine strain or from a fully virulent strain.

In 1956, Olsuf'ev et al. (66) administered tularin from a vaccine strain percutaneously. They noted that, in 3,867 previously vaccinated individuals, a positive reaction was obtained in about 90%, while side reactions occurred in only 1 to 3%. No necrosis was seen. Side reactions were more common in 202 individuals tested who had recovered from tularemia. Sil'chenko reported similar data in 1957 (92, 93) and noted good correlation between the responses to the dermal and intradermal routes. He recommended that dermal tularin should be put into widespread practical use for measuring the effectiveness of vaccination procedures. According to Muromtsev (63), during 1958 a directive for the preparation, control, and use of dermal tularin from a vaccine strain was prepared. Olsuf'ev et al. (69–71) specify that the technique of administration of the tularin by the epidermal route is identical with the procedure used for vaccination, except that a single drop of tularin with two parallel incisions is employed. Reading is done at 48 hr and evaluation is on the intensity of the local hyperemia and edema. The test material should be stored at 4 C (64, 65).

In 1952 Maiskii and Shipitsina (58) demonstrated that a polysaccharide component of P. tularensis obtained by the Boivin type of extraction could be employed for skin testing intradermally with a very prompt response. Similar studies were reported in 1956 by Verenikova et al. (100), who used both the intradermal and the dermal methods of inoculation. Although considered relatively specific, this method apparently has not been used on any large scale.

VIII. CURRENT STATUS AND FUTURE PROGRAMS

According to numerous reports, the value of viable tularemia vaccine in reducing incidence of disease and controlling epidemics is amply confirmed. Elkin (32, 33) states that it is equal in effectiveness to smallpox vaccine. Olsuf'ev credits mass inoculations, in combination with other measures, with the reduction of the animal incidence of tularemia in the U. S. S. R. by 19-fold when the period 1945 to 1950 is compared with 1951 to 1957. Furthermore, according to Olsuf'ev, the effectiveness of the vaccine has been confirmed in foci of every type and in the presence of diverse routes of transmission. Zhdanov (102), speaking at the 13th World Health Assembly, gave to the viable tularemia vaccine primary credit for the reduction of the disease in the U. S. S. R. He stated that vaccination had been so extensively practiced that the number of cases of tularemia, "which in the middle forties used to be as high as 100,000 per year, has now been reduced to a few hundred cases annually."

Yet the problem of control of tularemia still continues. In a 1958 conference (56), it was stated that the number of tularemia outbreaks in the R. S. F. S. R. increased in 1957 over 1956. In several oblasts (mainly in the Moscow area), 85.3% of all illnesses were said to be due to tularemia. These outbreaks were attributed to complacency of public health organs, curtailment of prophylaxis, inadequate training for medical personnel, and insufficient attention to vaccination. Vachkov and Pronina (98) specified that an inadequate program was being conducted in these oblasts. Panaiotti (75) indicated in 1959 that in Yartsevskaia (Smolensk Oblast) no vaccinations had been conducted since 1953.

El'bert, one of the original investigators (31), also comments on the proved effectiveness of the viable vaccine but goes on to say, "The next task is to obtain new vaccine strains capable of establishing a defense against cultures of P. tularensis with maximum virulence." Muromtsev (63), in discussing the future program of the Gamailea Institute, specifies a requirement for the development of "protective antigens suitable for prevention of certain infections against which previously immunity could be conferred only by live vaccines." Elkin (32, 33) states the need to work on "combined vaccines and simpler methods of inoculation (dermal, by aerosol, etc.) since without these there are insurmountable difficulties in the way of mass vaccination against a large number of diseases."
In the next few paragraphs some of the work now in progress to improve or simplify this vaccination procedure is noted. For example, Faibich (40) considers the possible additive value of the subcutaneous inoculation of strain 10 in "adjuvants." He concludes that agar, gelatin, gum arabic, Malva decoction, and glycerine were effective. For mice the minimum immunizing dose with agar or gelatin was 10 organisms, whereas in physiological saline the dose was 1,000 organisms. Faibich credits Zlatovskii with showing a similar effect in man.

The possibility of combining viable tularemia and smallpox vaccines and administering them simultaneously by the epidermal route was examined in guinea pigs by El'bert et al. (25). The addition of other immunizing agents (against tetanus, cholera, typhoid, parathyroid A and B, and bacillary dysentery) was recommended as being effective by Akimenko (1).

Filipenko, Polvakova, and Shchekina (76-78) described animal studies on the simultaneous inoculation of live vaccines for tularemia and brucellosis and showed that animals so vaccinated were resistant to challenge with virulent strains of either Brucella or P. tularensis. Amanzhulov and Rementsova (6) in 1955 administered vaccines for tularemia and brucellosis to humans simultaneously but at separate sites. The procedure was well tolerated. At 1 month 89% reacted to tularin; at 5 months the figure was 97%; and at 1 year it was 81%. A similar trend was observed with brucellin. Antibody titers generally paralleled the allergic reaction rate.

Studies on the simultaneous inoculation of live vaccines for tularemia and brucellosis were initiated at the Gamaleia Institute in 1953 (42, 43). It was established that the procedure produced a satisfactory response in guinea pigs to both antigens. Shulygina (89) records a continuation of this program in animals with the no. 15 strain (enhanced) of P. tularensis and the Brucella abortus BA strain, the two being mixed before drying. After drying and rehydration, 1.0 ml contained 10 P. tularensis cells and 22.5 x 10^6 B. abortus. Comparable vaccines were then tested in men by the dermal route (44). A positive inoculation reaction was obtained in 183 out of 185, and mild lymphadenopathy was observed in only 6.0%. On the basis of a report by Gubina, the Research Council of Gamaleia Institute accepted a resolution in 1957 "to recommend the use of combined brucellosis and tularemia vaccine in man (subject to approval of the Serum and Vaccine Committee)" (12). It would appear that "official instructions" have been issued for the production of a dried brucellosis-tularemia vaccine (13).

According to Vakker (99), who apparently was describing an immunization program in the Kazakh S. S. R., 42,300 people were given a simultaneous vaccination by the skin method against brucellosis and tularemia. Serologic positivity was noted in 95.1% for the former and in 86.3% for tularemia.

Studies on combining viable vaccines against plague, tularemia, brucellosis, and anthrax in guinea pigs have been reported (101). In the combination the response to anthrax was unsatisfactory, while in the other three the protection claimed was said to be the equivalent of a monovalent immunizing product. Kalscheva (46) described the immunization of mice with a combination of live plague and tularemia vaccines. Subsequently the animals were infected intraperitoneally with Pasteurella pestis or P. tularensis. It is claimed that the combination vaccination produced a more intense leukocytic reaction in the peritoneal fluid and a greater reduction in the number of organisms obtained on blood culture than did either vaccine alone.

Considerable attention is being devoted to immunization by inhalation. In 1954 El'bert et al. (28) described the comparative effectiveness of nasal and cutaneous vaccination against tularemia in the guinea pig. They could demonstrate no immunological difference between the two routes. The report also states that El'bert et al. in 1952 showed the harmlessness and immunogenicity of dye and liquid yolk nasal vaccination with strain Ondatra. Aleksandroff and Gefen (2) summarize a series of animal studies involving vaccination by inhalation of dried viable P. tularensis, strain 15 (restored). Guinea pigs, after inhaling large amounts, are reported to produce agglutination and allergic responses comparable in all ways to those noted following the subcutaneous inoculation of 20 million similar organisms. In a later paper, Aleksandroff et al. (3) indicate that the dosages ranged from "several hundred thousand to tens of millions of living microbes." Guinea pigs so immunized survived in 67 to 80% of cases after the subcutaneous or aerogenic infection with 100 to 1,000 times the minimal lethal dose.
of a virulent *P. tularensis* culture. Animals vaccinated subcutaneously survived in 88 to 100% of cases, while all unvaccinated controls died on challenge. Small laboratory animals are not considered particularly satisfactory for such studies because of the narrow cross section of their respiratory passages, the small minute volume, and their superficial type of respiration.

In 1957 studies in aerogenic vaccination of man were initiated at the Kirov Military Medical Academy. Dry tularemia vaccine (strain 15, restored), in a computed dose of 750,000 organisms, was inhaled by 128 persons. Cubicles having volumes of 3.8 and 10 m³, as well as a tent of 12 m³, were used. The exposure time was 15 min. "A general moderately severe reaction was noted in two individuals." This apparently developed 8 to 12 hr after vaccination and was manifested by fever, headache, and muscle and joint pains. One individual also had "an insignificant local reaction in the form of a rapidly progressive bronchitis. After three days these signs completely disappeared." Immunological changes appeared as early as 1 week after vaccination. At the 30-day serologic examination the agglutinin titers were reported as higher than in the subjects receiving percutaneous vaccine.

Further data on reactions to aerogenic vaccination with attenuated *P. tularensis* are given by Korostovtsev, Onikiyenko, and Khokhlov (54). They describe a brief febrile response in 1 of 16 individuals inhaling 750,000 organisms and in 3 of 8 persons inhaling 7,500,000 cells. In 1 (or 2) such individuals there was an associated decrease in the maximum pulmonary ventilation. There were no sequelae.

Aleksandrov et al. (4) describe studies on respiratory immunization of man with various combinations of dried living agents. Brucellosis-anthrax-tularemia triple vaccine was given to 17 persons with three general reactions. Brucellosis-anthrax-tularemia-plague tetravaccine was administered to 13 individuals with nine general reactions. These reactions were of short duration and did not prevent the authors from recommending aerogenic immunization of people with the combined vaccines. Subsequent skin tests with tularin showed positive reactions in most individuals participating.

Olsuf’ev (68) offers the following on respiratory immunization of man: "The nasal, aerogenic, and alimentary methods of vaccination do not display advantages over the cutaneous method and clearly yield to it on account of the non-availability of external observations for inoculability; they are not without danger, and it is impossible to recommend them for practical use.” However, it is evident from recent reports by Aleksandrov et al. (4) that aerogenic immunization of man is under continuing study in accord with a resolution of the Vaccine-Serum Committee of the Ministry of Health.

Finally, in addition to summarizing these continuing efforts to improve the usefulness of biologicals in the control of tularemia, this section relative to current work would be incomplete without noting that other methods of control of this disease are also receiving continuing study and application. These include the ecology of the animal hosts, the methods of transmission, whether by vector or other routes, diagnostic tools, and therapeutic agents.

The recognized history of tularemia in the Soviet Union began in the valley of the Volga-Akhtubinsk Rivers. Extensive vaccination programs were initiated in this area in 1946, 20 years later. During the period from 1951 to 1957 no cases of tularemia were reported (15). In addition to vaccination of all personnel and revaccination as indicated, the plan for the control of tularemia in this area includes annual extensive epizootiological, zoological, and parasitological investigations, rodent extermination, control of water rat activity, anti-ixodial tick measures, protection against blood sucking diptera, and cultivation of areas which remain as primary foci of tularemia (11).

Thus, Soviet investigators have provided a capability to deal with this disease in an acceptable manner. Major reliance is placed on the viable vaccine, supplemented, at least in critical areas, with a multidisciplinary control program. The complexity of the ecology of tularemia, and the need for simplified methods of its control, serve to generate problems that are receiving, and probably will continue to receive, the attention of the organized Soviet research effort.

IX. Literature Cited


1 To avoid confusion, references include the abbreviation “Vol.” when this is given, and “No.” precedes the issue number. For the Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii the


Preservation of the vitality, virulence, and agglutinability of the tularemia culture kept in fluid yolk media. Rostov Vol. 6:82-88.


of Soviet Medicine, Excerpta Medica, Part B, 1960, item 565.)


71. Olsuf'ev, N. G., V. P. Borodin, and Y. M. Tsevteyova. 1958. Epicutaneous skin test with tularin from vaccine strain to determine immunity in those inoculated against tularemia and for diagnosis of this infection. In N. G. Olsuf'ev, B. N. Pastukhov, and V. V. Kucheruk, [ed.]. Problems of epidemiology and prophylaxis of tularemia. MEDGIZ, Moscow.


89. Shulygina, K. N. 1958. Experimental study on dermal inoculation with live combined brucellosis and tularemia vaccine.
90. Sil'chenko, V. S. 1953. The problem of reinoculation subsequent to tularemia vaccinations. ZhMEI No. 6, 47-50.