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**EFFECTIVENESS OF VARIOUS ANTI TULAREMIA VACCINATION
METHODS**

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The comparative evaluation of subcutaneous, cutaneous (transdermal), and intradermal methods of administering live vaccine in relation to biological features of various vaccine strains is of great interest from the standpoint of improving vaccination methods and studying several problems of immunity connected with tularemia. The first two methods are widely used in the specific prophylaxis of tularemia. The intradermal method is not used in practice. This vaccination method was evaluated in the works of Nikolaeva (1940), Purgasov (1952), and Savostin and co-authors (1956). Nikolaeva indicated that tetanus antoxin given subdurally or intracutaneously has greater effectiveness than with a subcutaneous injection. The higher immunity-producing property of the preparation found in the first two methods of administration is explained by the author on the basis of I. V. Pavlov's theory; he considers that the significant feature in immunity formation is the amount of nerve endings at the site of vaccine injection; there are more of these in the cutaneous than the subcutaneous tissue. On this basis, Purgasov applied intracutaneous immunization with killed tularemia vaccine and observed in this an immunity more strongly expressed than in subcutaneous inoculation. In experiments on guinea pigs inoculated with live plague vaccine, Savostin and co-authors demonstrated that the intracutaneous vaccination method was more effective in comparison with the subcutaneous method; in this regard a better-expressed reaction was observed in the inoculated animals (cited from the book by E. I. Korobkova).

In experiments on the comparative study of the three methods of vaccination against experimentally-induced tularemia conducted on white mice and guinea pigs, we used a two-day culture of live vaccine grown in egg yolk agar and washed in saline solution. The vaccine was prepared from subcultures isolated from dry, standard cultures of vaccine strains No. 15 (Gaishii), No. 10 (Faibich and Tamarina), No. 33 (Faibich and Saltykov), and No. 53 (Saltykov). In several experiments the vaccine used was a mixture of the above-mentioned 4 strains, and also strains No. 15 and 53, with a ten-fold passage through guinea pigs; this was designated as strains No. 15/10 and 53/10. Guinea pigs were vaccinated once, subcutaneously, intradermally, or cutaneously (transdermally); white mice were inoculated subcutaneously or transdermally. Doses of 10,000 to 10 million bacteria were used for subcutaneous or intradermal vaccination; and 2 billion per ml. for cutaneous vaccination.

Thirty days after vaccination the animals were inoculated subcutaneously, by inhalation(aerially), or through the conjunctiva. Subcutaneous inoculation was given in a dose of 1,000 to 10 million Dlm. and was recorded so that the significance of the vaccination method would be clear from the degree of immunity tension. Aerial infection was accomplished with aerosol spraying of a 1 ml. suspension of 250 million or 1 billion virulent tularemia bacteria; exposure time was 10 sec. and 30 sec. In the transconjunctival inoculation, one drop of a 1 ml. suspension of 500 million bacteria was inserted into the conjunctival sac. Doses used [Begin p.44] for aerosol and conjunctival inoculation caused the death of all vaccinated animals.

White mice, vaccinated subcutaneously (10,000 and 100,000 bacteria) or by scarification with vaccines separately prepared from 4 strains or from a mixture of the 4 strains, were identically resistant to infection from a 10 million Dlm. dose of virulent culture (Table 1). In experiments on white mice inoculated subcutaneously with vaccine strain No. 15 or by scarification, a difference was also established in the effectiveness of these two methods through aerosol inoculation of the animals (Table 2).

Table 1. Effectiveness of vaccines in relation to the method used (experiments on white mice inoculated subcutaneously with 10 million Dlm. of tularemia pathogen after vaccination)

Vaccine strain No.	Survival of mice vaccinated by various methods	
	subcutaneously (10,000 bacteria)	transdermal
15	23/15 (92%)	15/15 (100%)
10	21/21 (100%)	14/15 (93.3%)
33	21/33 (91.3%)	21/25 (96%)
53	21/22 (95.4%)	20/22 (90.9%)
Mixture of the four strains. . .	17/17 (100%)	17/17 (100%)
Control--non-vaccinated animals	1/5 0/5 0/5 0/5	Dlm=1 bacterium

Legend: (in all tables) numerator = number of surviving animals;
denominator = number of infected animals.

Table 2. Effectiveness of vaccinations in relation to vaccination method in experiments on white mice inoculated aerinly (duration 30 sec.

Vaccine strain no.	Method of vaccination	Vaccine dose	Number surviving of the total number inoculated
15	subcutaneously cutaneously	100,000	12/20
15		suspension, with concentration of 2 billion per ml.	12/12

Note: Upon inoculation of non-vaccinated white mice, serially, for 10 and 30 seconds, with 100 million bacteria, all animals in the control group died.

Thus, the white mice vaccinated subcutaneously or by scarification were identically resistant to subcutaneous as well as aerial infection. No difference of any kind was established in the effectiveness of subcutaneous and cutaneous vaccination in comparative experiments on white mice.

Experiments on the comparative study of the effectiveness of subcutaneous, cutaneous, and intradermal application of 4 strain-vaccine and their mixture were set up in several variations, using guinea pigs.

In one of these experiments, guinea pigs were vaccinated by two methods: subcutaneously with 10 million bacteria, or through scarified skin with a standard vaccine dose. Control inoculation was subcutaneous, with a virulent culture of doses from 1,000 to 500 million Dlm. In these experiments we were not successful in establishing an essential difference in vaccines. It should be mentioned that the most effective vaccine in these experiments was the standard No. 15 strain and the vaccine from the mixed strains, especially when applied cutaneously.

In the following experiments we determined the comparative effectiveness of the three vaccination methods. For this guinea pigs were immunized subcutaneously with strain No. 15 vaccine and intradermally with three doses of vaccine (10,000, 1 million, and 10 million bacteria), and cutaneously with one standard dose. Thirty hours after vaccination, the guinea pigs were inoculated with a 200 Dlm. dose of virulent culture. In these experiments (Table 4), no basic difference in the results was observed with the methods tested. [Begin p. 45]

In further experiments on vaccinated guinea pigs, they were inoculated with a 1 ml. suspension containing 500 million bacteria applied to the conjunctiva. The stated dose of tularemia culture caused the death of all non-vaccinated guinea pigs. In these experiments the animals were immunized with vaccine from strains No. 15, 53, and 53/10 (ten-fold passage through guinea pigs in order to increase the immunogenic properties). The vaccine was administered subcutaneously and intradermally, at 10 million bacteria, and cutaneously applied in a standard suspension.

Table 3. Effectiveness of vaccinations in relation to vaccination method and vaccine strain in experiments on guinea pigs inoculated subcutaneously with various doses of virulent culture

Number of vaccine strain	Survival of vaccinated animals after inoculation with various doses (in Dlm) by various methods							
	subcutaneously, 10 million				cutaneously			
	1,000	1 million	10 million	500 million	1,000	1 million	10 million	500 million
15	10/10	4/5	2/3	2/3	9/10	4/5	1/2	2/3
10	7/10	3/5	2/3	1/3	7/10	4/5	0/3	2/3
33	6/10	3/5	2/3	1/3	7/10	3/5	2/3	1/3
53	6/10	4/5	1/3	1/3	8/10	4/5	2/3	2/3
Mixture of 4 strains. . .	10/10	4/5	2/3	2/3	10/10	5/5	2/3	2/3
Control--non-vaccinated animals. . . .		1 10 100 1 million		0/3 0/3 0/8 0/5				Dlm = 1 bacterium

Table 4. Effectiveness of vaccinations with subcutaneous inoculation, 200 Dlm., in relation to vaccination methods used in experiments on guinea pigs.

Number of vaccine strain	Method of vaccination	Survival after vaccination with various doses		
		10,000	1 million	10 million
15	subcutaneous	3/4	4/4	4/4
15	intradermal	4/4	4/4	4/4
15	cutaneous	---	4/4	---
Control--non-vaccinated animals..		1 5 10 100 1000	2/3 0/3 0/3 0/3 0/3	Dlm = 1 bacterium

With conjunctival inoculation of vaccinated animals no essential difference appeared in the effectiveness of vaccination by cutaneous, intradermal, or subcutaneous methods with strain No. 15 vaccine. In an experiment with vaccine from the standard strain No. 53 and from the strain passaged through guinea pigs, it became evident that the varying effectiveness was dependent on the immunogenicity of the cultures, and not on the method of vaccination (Table 5).

In the arial inoculation of vaccinated guinea pigs, we used a 1 ml. suspension of virulent tularemia culture containing 250 million bacteria; animals were exposed to the infected air for 30 seconds. Guinea pigs were vaccinated subcutaneously, intradermally, and cutaneously, with vaccine from strains No. 15, 15/10, 53 and 53/10. In these experiments (Table 6), as in the preceding, no substantial difference was observed between vaccination methods.

Our attention was drawn by the fact that in multiple passage of vaccine strains No. 15 and 53 (Tables 5 and 6) through guinea pigs, their immunogenic properties were increased. Our data on this problem coincided with the results of the work of Encl'yanova (1957), which established the possibility of increasing the immunogenicity of strain No. 15 by its passage through the body of animals highly sensitive to tularemia and by selection of the most immunogenic cells. The passage of tularemia vaccine strains through guinea pigs may be regarded as a method of restoring the immunity of strains in which it has decreased while being preserved. [Egin p.46]

Table 5. Resistance of guinea pigs vaccinated by various methods to infection through the conjunctiva.

Vaccine strain No.	Survival of animals vaccinated by various methods after inoculation through the conjunctiva		
	Subcutaneous, 10 million	Intradermal, 10 million	cutaneous, 2 billion
15	7/8	8/8	8/8
53	7/8	7/8	6/8
53/10	8/8	8/8	8/8

One hundred percent of the control animals, non-vaccinated, died upon application to the eye of one drop of suspension containing 1 ml. of 500 million bacteria.

Table 6. Resistance of guinea pigs vaccinated by various methods to arial infection.

Vaccine strain No.	Survival of animals vaccinated by various methods after inoculation			
	subcutaneous, 10 million	intradermal, 10 million	cutaneous, 2 billion	control non-vaccinated)
15	9/13	10/13	10/13	1/7
15/10	5/5	5/5	5/5	0/4
53	1/8	3/8	4/8	0/4
53/10	5/8	5/8	5/8	0/4

Of some interest are the experiments on arial infection of guinea pigs vaccinated by various methods (subcutaneously, intradermally, and cutaneously) with vaccine from a mixture of the passaged strains. In these experiments, as in the previous ones, we were not successful in finding an advantage for any one particular method of immunization (Table 7). Animal survival was dependent on the inoculum dose: vaccinated animals inoculated, regardless of the vaccination method, arially by means of exposure to an aerosol suspension of virulent culture for 10 seconds survived at the rate of 90-100%, and with aerosol spraying for 30 seconds, in 70-80% of the cases.

Table 7. Tension of immunity in vaccinated guinea pigs upon arial inoculation

Vaccine strain	Length of exposure (in sec.)	Survival of guinea pigs vaccinated by various methods						Control--inoculation of non-vaccinated animals.
		Inoculation by various doses						
		subcutaneous	intradermal	cutaneous				
		1 million	10 million	1 million	10 million		2 billion per 1 ml.	
Mixture of strains 1/10, 10/10, and 53/10. . .	10	9/10	10/10	9/10	10/10	10/10	0/15	
	30	7/10	8/10	7/10	8/10	7/10	0/15	

Finally, in order to clarify the situation with regard to tension of immunity relating to the method of applying vaccine to guinea pigs vaccinated subcutaneously with 1 ml. of 100,000, 1 million, or 10 million bacteria, and cutaneously with a standard dose, a subcutaneous injection was administered 4-6 months after vaccination. The vaccine used consisted of a mixture of the four strains. Guinea pigs were inoculated with a virulent culture in mass doses of 500-100 million Dlm. In these experiments, the animals that were vaccinated subcutaneously (10 million bacteria) and cutaneously, upon subcutaneous inoculation with 10,000 Dlm. of virulent culture 4 months after vaccination, appeared to be highly resistant and survived in 100% of the cases, regardless of the method of vaccination (Table 8). With an increase in dosage to 100,000 Dlm. and higher, or, in cases where they were vaccinated subcutaneously, with doses of less than 10 million, the number of vaccinated animals that survived was decreased. Inoculations after 6 months indicated that the degree of immunity tension was substantially reduced in guinea pigs that were vaccinated either subcutaneously or cutaneously. Thus, the guinea pigs vaccinated with 10 million bacteria or cutaneously with a standard dose, tolerated only 500 Dlm. in 100% of the cases, while after inoculation with 1,000 and 10,000 Dlm. they survived in 75-80% of the cases. It should be mentioned that [Begin p.47] guinea pigs vaccinated subcutaneously with doses of 100,000 and 1 million bacteria appeared to be of low resistance to infection after 4-6 months.

Table 8. Immunity tension in guinea pigs vaccinated subcutaneously 4-6 months after vaccination in various dosages.

Vaccine	Method of vaccination	Vaccine dose	Time of inoculation after vaccination (months)	Survival of vaccinated guinea pigs after inoculation in varying doses					
				500	1,000	10,000	100,000	1 million	10 million
Mixture of strains	subcutaneous	100,000	4					5/12	10/12
			6	10/15	9/13	9/12	7/12	3/12	1/12
		1 million	4					4/12	3/12
			6	12/15	10/14	10/12	7/12	4/12	3/12
		10 million	4					6/12	4/12
			6	15/15	10/15	12/12	10/12	5/12	3/12
Control--non-vaccinated animals		1	4					7/12	4/12
			6	15/15	12/15	12/12	10/12	4/12	3/12
		5	4					6/12	4/12
			6	15/15	12/15	12/12	10/12	4/12	3/12
		10	4					6/12	4/12
			6	15/15	12/15	12/12	10/12	4/12	3/12

Control--non-vaccinated animals

Survival of vaccinated guinea pigs after inoculation in varying doses

1 5 10 10,000 100,000 1 million 100 million

Control--non-vaccinated animals

Survival of vaccinated guinea pigs after inoculation in varying doses

1 5 10 10,000 100,000 1 million 100 million

Thus, comparative study of the subcutaneous (dose of 10 million) and cutaneous methods of vaccination in experiments on guinea pigs inoculated 4-6 months after vaccination has shown that during 4 months the animals retained resistance to infection from 10,000 Dlm. of virulent culture, after which time immunity tension was substantially reduced, regardless of the vaccination method, but was made apparent with inoculation of 500 Dlm.

Conclusions

1. In a comparative study of the effectiveness of subcutaneous, cutaneous (transdermal) methods of applying live tularemia vaccine in experiments on white mice and guinea pigs it was established that 30 days after immunization of the animals, regardless of the method of vaccination, immunity was apparent with the subcutaneous and conjunctival inoculation, and also with aerial inoculation.

2. Regardless of the method of vaccination with live vaccine from a mixture of strains, guinea pigs developed immunity which, 4 months after inoculation of the vaccinated animals, prevented infection from a dose of 1,000 Dlm.; after 6 months the degree of immunity to doses of 1,000 Dlm. or more of virulent culture was substantially reduced (75 percent survival of animals).

3. The degree of immunity tension in vaccinated guinea pigs was dependent on the quality of the vaccine strain used in preparing the vaccine and on the dosage used, and not primarily on the method of vaccination.

4. The cutaneous method of vaccination is no less effective than other methods in all conditions of inoculation and is, moreover, simpler in comparison with the subcutaneous method.

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