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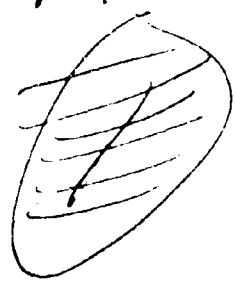

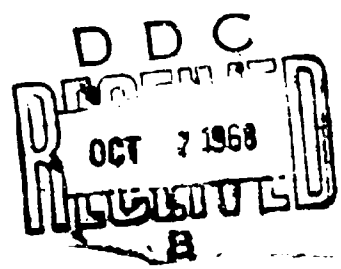

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IMMUNIZATION OF WHITE MICE TO ECTROMELIA WITH VACCINE VIRUS

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Abstract

1. Immunization to inoculated infectious ectromelia can be provided by intraperitoneal or intravenous administration of vaccine virus ("Ankara" strain) in suitable dilution.

2. The degree of immunization depends on the dose administered.

3. After peritoneal injection of vaccine virus, distinct immunization can be demonstrated within three days and immunity is established after six days.

4. Five months after a single injection of vaccine virus, more than 50% of the test animals still show immunity to a massive injection of ectromelia virus.

I. Introduction

As prophylactic measure for mouse strains with latent infectious ectromelia, universal vaccination with an effective pox vaccine has been repeatedly suggested (Ref. 1, 2, 3). Earlier protective vaccination of mice against ectromelia was made primarily as in man by intracutaneous injection of the vaccine virus; for mice generally in the area of the base of the tail (Ref. 1, 4, 5, 7). However, this method has the disadvantage in routine vaccination of a large number of mice that it is not possible to avoid external contamination with the virulent pox vaccine. Each individual vaccination also requires a relative large amount of time since at least 10 to 30 fine pricks

must be made at the base of the tail (Ref. 1, 7). The development of a vaccination pustule at the point of scarification in all vaccinated mice is successful only with the use of adequately high virus concentrations and perfect technique. Even intranasal (Ref. 8) and/or intravenous (Ref. 2) vaccination requires a relatively large expenditure of time in routine vaccination of all mice received by an institute.

In order to reduce this time, we turned for practical considerations to the intraperitoneal injection of vaccine virus already proposed by other authors (Ref. 1,3) because the method is relatively simple and requires little time. Although we could hardly expect, from the investigations of Ref. 3, any appreciable propagation of the vaccine virus by this method and accordingly also only minor formation of antibodies, we did turn to this method because protection against ectromelia infection was to be expected also with intraperitoneal injection of vaccine virus on the basis of the findings obtained with intravenous injection by Ref. 2.

The following discusses in detail the investigations for immunization of white mice to ectromelia through intraperitoneal injection of virulent vaccine virus.

II. Material and Methods

1. Test Mice

For the investigations described below, we utilized mice of the NMRI strain from the non-ectromelic strain of the Federal Research Institute for Virus Diseases of Animals in Tubingen (colony strain).

2. Incubation Eggs

Incubation eggs (white Leghorn) of the Zimmermann Farm, Wiesloch were employed as culture medium for vaccine and ectromelia virus on the chorio-allantois membrane.

3. Vaccine Virus

For immunization we utilized the vaccine-virus strain 10-54 "Ankara", adapted to the chorio-allantois membrane (CAM), after the 52nd passage which was made available by the Federal Research Institute for Virus Diseases of Animals in Tubingen. The infected membranes were stored in sterile glycerine/Ringer solution (1:3) at -30° C until further processing of the standard suspension.

Evaluation of the intensity and extent of the pox formation on the CAM produced by the vaccine virus was made with the symbols 1 +, 2 +, 3 +, 4 + and simultaneous determination whether only the point of application showed any pox formation or whether the latter had become general over the entire membrane.

Preparation of Virus Vaccine

A CAM with a large number of pox (3 + and 4 + with generalized pox formation) was macerated under sterile conditions in the Ultra-Turrax after washing 3 times with buffered physiological sodium-chloride solution, drying between sterile gauze and the addition of 1 ml Ringer solution per membrane. Subsequently it was further triturated in a motor-driven refrigerated glass homogenizer and finally in a Ten Broek mortar. The resulting suspension was centrifuged for 15 min at 3,500 rpm. The supernatant liquid is designated as "standard suspension." The latter was filled into ampoules after having been examined for toleration by mice and/or pox formation in the egg. Only such standard suspensions were utilized with which 0.2 ml of a dilution of 1:10,000 infected the CAM of 10-day old chicken embryos so that the membranes obtained had a large number of pox (3 + to 4+; generalization). The standard suspension in the ampoules retained its virulence for more than 2 years when stored between - 25 and - 30°C.

The vaccine virus was propagated by inoculating the CAM of 10 day-old chicken embryos with 0.2 ml of a dilution of 1:10,000 of the standard suspension. Under sufficient virulence of the inoculated virus, part of the embryos died 4 or 5 days after infection. 7 days after infection, the still living embryos were procured and only such membranes were stored for vaccine production which exhibited a large number of pox (3 +, 4 +; generalization).

Vaccine Tolerance of Mice

A dilution of the standard suspension of 1:10 was injected intraperitoneal at doses of 0.2, 0.4, 0.8 and 1.6 ml per mouse. The control mice received the same doses of a corresponding centrifuged product from sterile non-infected membranes (cf. Table 1). The vaccine virus utilized for immunization to ectromelia consequently was non-pathogenic for white mice even at a dose of 1.6 ml (dilution 10^{-1}) per animal.

Table 1

Test of Vaccine Virus Tolerance of White Mice Until 28 Days After Injection

a= Tierzahl	b= Vaccine-Virus: Dosis ml Stammsuspens./Maus	c= tot/gesamt
10	0,2	0/10
10	0,4	0/10
10	0,8	0/10
10	1,6	0/10

Key: a - number of animals; b - dose in ml/mouse; c - died/total

Demonstration of Vaccine Virus in the Organs of Mice

Vaccine virus (dilution 10^{-1}) was given to 4 groups of 6 mice each at a dose of 0.2 ml/animal by intraperitoneal administration. Within 1, 3, 5, and 7 days after infection, all animals of 1 group were killed and the liver, spleen and lung of all of them was homogenized together in a Ten Broek mortar. After incubating the homogenized product for 25 minutes with the addition of 100 EI penicillin and 100 gamma/ml streptomycin and after control of bacteriological sterility, the product was diluted 1:10, 1:100 and 1:1,000 with buffered physiological sodium-chloride solution and transferred in amounts of 0.2 ml to the CAM of chicken embryos incubated for 10 days (11 embryos per test). The vaccine virus suspension for infection of the mice was employed as control. 0.2 ml of the dilution 10^{-3} and 10^{-4} were placed on the CAM. The infected embryos were observed for 7 days after infection. From the embryos which died and/or were killed after 7 days, we obtained the CAM which was examined for pox formation (cf. Table 2).

Table 2

Pox Formation on Chicken Embryos after Application of Triturated Organs of Mice Inoculated with Vaccine Virus

a — Organverreibungen gewonnen x Tage nach der Infektion	b — Verdünnung der Organsusp. (0,2 ml/Embryo)	c — tot/gesamt nach 7 Tg.	d — Pocken auf CAM pos./ges.
e — 1 Tag nach Infektion: Leber/Milz/Lunge	10^{-1}	2/11	4/11
	10^{-2}	2/11	4/11
	10^{-3}	0/10	0/10
e — 3 Tage nach Infektion: Leber/Milz/Lunge	10^{-1}	1/10	2/10
	10^{-2}	1/10	1/10
	10^{-3}	0/10	0/10
e — 5 Tage nach Infektion: Leber/Milz/Lunge	10^{-1}	1/10	1/10
	10^{-2}	0/9	0/0
	10^{-3}	0/10	0/10
e — 7 Tage nach Infektion: Leber/Milz/Lunge	10^{-1}	0/10	0/10
	10^{-2}	0/10	0/10
	10^{-3}	0/10	0/10
f — Kontrollen: Zur Impfung der Mäuse verwendetes aktives Vaccinevirus	10^{-3}	10/10	10/10
	10^{-4}	10/10	10/10

Key: a - triturated organs obtained x days after infection; b - dilution of suspension (0.2 ml/embryo); c - dead/total after 7 days; d - pox on CAM positive/total; e - days after infection: liver/spleen/lung; f - controls: vaccine virus utilized for inoculation

The vaccine virus injected intraperitoneal could still be demonstrated in the triturated organs of the infected animals 1 and 3 days after infection through its formation of pox on the CAM. After 5 days, isolated pox were found only on 1 membrane after inoculation of the triturated organs and no

further formation of pox was observed after 7 days. The vaccine virus (0.2 ml at a dilution of 10^{-3} and 10^{-4}) inoculated as control directly in the CAM caused definite pox formation in all embryos and death of the embryos within 4 to 5 days.

The vaccine virus utilized for immunization against ectromelia of the NMRI mice (Tubingen) produced no perceptible symptoms of the disease. The vaccine virus injected intraperitoneal could no longer be demonstrated, 7 days after infection, in an active form in the liver, spleen and lung of the mice infected with it.

4. Ectromelia Virus

The ectromelia (E-) virus utilized for infection was cultured from the liver and spleen of a commercially purchased CFW mouse strain with endemic ectromelia. Mice with a clinical appearance of ectromelia (poor general health, swelling of the head, conjunctivitis, ulcerations of tail or extremities) were dissected. Obduction showed in almost all animals enlargement of the liver with an occasional dirty-gray discoloration and fine hemorrhages and/or whitish foci, enlarged spleen with whitish foci, and ample exsudate in the abdominal cavity. Necrotic foci in the liver and oxyphile occlusion bodies were demonstrated histologically.

Liver and spleen with such characteristic changes were suspended in a Ten Broek mortar with the addition of buffered physiological sodium-chloride solution (1 ml per liver and spleen of 1 animal). After centrifuging at 2,000 rpm for 10 minutes, 100 gamma streptomycin and 100 IE penicillin per ml were added to the supernatant. After exposure at 37° C for 15 minutes, the CAM of 10-day chicken embryos were inoculated with 0.2 ml each of this suspension and incubated at 36-37° C. After 79 hours, the surviving embryos were removed from the egg after cooling, the membranes gathered and examined for pox. All membranes had ample pox formation of pin-head size, occasionally confluent to the blood vessels. The typical central necrosis typical for vaccine pox was absent in all of the pox. Repeated alternate passages between CAM and non-ectromelic mice were made with the virus prepared from the diseased organs of the CAM. Clinical and pathological changes typical for acute electromelia were found in the mice infected intraperitoneal with the virus. The pox-containing membranes were stored in glycerine/Ringer solution (1:3) at - 25 to 30° C. Under continued passage of the virus on the CAM of 10-day embryo chicks produced pronounced pox formation (3 + to 4 +; generalization) on the membranes.

In order to test the constant infectivity of the cultured E-virus, the standard suspensions obtained after 2, 11, and 21 egg passages and prepared by the method described for vaccine virus, were diluted in buffered physiological sodium-chloride solution at powers of ten and the dilutions (10^{-3} to 10^{-8}) were injected in amounts of 0.2 ml/animal intraperitoneal in non-vaccinated mice. ID_{50} was determined as in Ref. 6. In each case, double determination was made. Post observation time was 21 days (cf. Table 3).

Table 3

Determination of ID_{50} of E-Virus Standard Suspensions for Mice After 2, 11, and 21 Egg Passages of the E-Virus

a- Stammsuspension der	b- 1. Versuch	c- 2. Versuch
d- 2. Eipassage	$10^{-6.8}$	10^{-6}
e- 11. Eipassage	$10^{-6.8}$	$10^{-6.8}$
f- 21. Eipassage	$10^{-6.4}$	10^{-6}

Key: a - standard suspension after; b - first test; c - second test; d - 2 egg passages; e - 11 egg passages; f - 21 egg passages

LD_{50} of the 3 tested standard suspensions remained practically unchanged. The virulence of the virus practically did not change in spite of storage for 13 months at - 25 to 30° C and 21 egg passages.

Fig. 1 shows the high infectivity of the E-virus after intraperitoneal injection in mice:

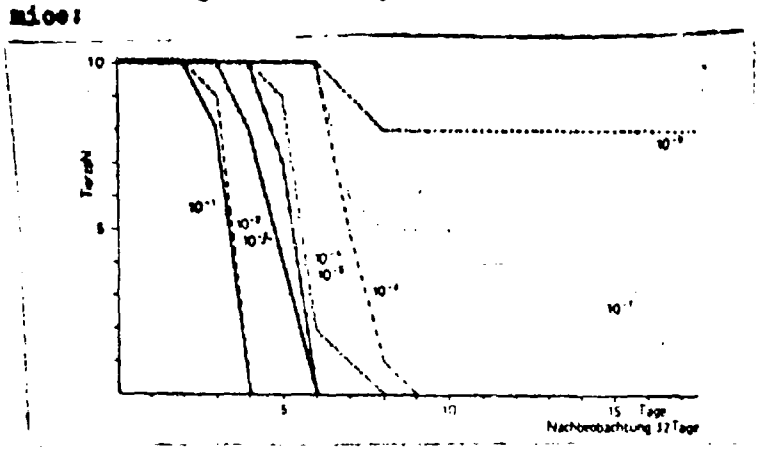

Fig. 1 Mortality Curve After Intraperitoneal Infection with Decreasing Dilutions (10^{-1} to 10^{-8}) of the Standard E-Virus Suspension; dose = 0.2 ml/animal; post observation interval = 32 days.

In a dilution of 10^{-1} to 10^{-6}, 0.2 ml caused death of all mice within 4 to 9 days. Infection with 0.2 ml of a dilution of 10^{-7} was still lethal for 9 of 10 mice within 17 days. The dilution of 10^{-8} killed only 2 animals.

The high infectivity of the E-virus for NMRI mice could be demonstrated also with intravenous, subcutaneous, intraplantar and intracerebral administration. Even after oral administration (1.0 ml/animal at a dilution of 10^{-1}

to 10^{-5}), a large number of the test animals died but it could not be decided whether the infection entered by way of the gastrointestinal tract or by contact and/or pernasal through the virus contained in feces.

In order to clarify the extent to what extent transmission of the E-virus by contact is possible, 5 ml of an E-virus dilution (10^{-1}) were sprayed on the litter (wood shavings) in pots (dia. 30 cm, 35 cm high). 10 males each were placed in 2 pots and 15 females in 1 pot. The infected wood shavings were removed after 10 days and replaced by fresh non-infected litter. Post observation interval of the animals was 9 weeks from start of test. As control, mice of the same strain were infected with the same virus used to infect the litter at a dose of 0.2 ml/animal of a dilution of 10^{-5} intraperitoneal. Normal-animal control was made with non-treated mice of the same strain which were kept and observed in the same room for the duration of the test. Finally the virus dilution utilized to infect the litter was inoculated on the CAM of 10-day chicken embryos.

Of the mice kept on the infected litter, 18 of the 20 males (90%) and 6 of 15 females (40%) died within 32-64 and/or 19-36 days. All of the control animals infected intraperitoneal with the same virus suspension died within 12 days whereas the non-treated controls survived. The CAM of 10-day chicken embryos infected at various dilutions with the virus suspension utilised for infection of the litter, displayed well developed pox.

Random specimens of liver and spleen of different animals killed by the test were triturated and suspended. 0.2 ml of the supernatant left after centrifuging was diluted (10^{-3} and 10^{-4}) and inoculated in the CAM of 10-day chicken embryos. All of the CAM showed pronounced pox formation.

Consequently, the ectromelia virus isolated from a CFW mouse strain with latent ectromelia is highly pathogenic for NMRI mice (Tubingen). The virulence of the E-virus adapted to CAM does not decrease after 21 egg passages under the test conditions described. The E-virus can be transmitted not only parenteral (intravenous, intraperitoneal, subcutaneous, intraplantar and intracerebral) but also peroral and/or by contact or pernasal.

III. Vaccination Tests

The vaccine-virus suspension stored in ampoules at - 25 to 30° C was freshly diluted with buffered physiological sodium-chloride solution as a function of dose prior to every inoculation. After addition of 100 gamma streptomycin and 100 IE penicillin per ml, the mixture was left to stand at 37° C for 20 minutes and subsequently injected into the mice intraperitoneal at a dose of 0.2 ml/animal after prior disinfection of the point of entry with iodine-alcohol solution.

1. :t Test

Test Arrangement

Dilutions of 10^{-1}, 10^{-2}, and 10^{-3} of the vaccine-virus standard suspension were injected into 10 non-ectromelic mice per group (0.2 ml/animal intravenous and/or intraperitoneal). In adaptation of the tests of Ref. 2, a vaccine virus inactivated by formaldehyde was also injected into 10 mice each (0.2 ml/animal intravenous and/or intraperitoneal).

The vaccine inactivated with formaldehyde was prepared by triturating pox-containing CAM (3 + to 4 +; generalization) in a Ten Broek glass homogeniser with the addition of 1 ml per membrane of buffered physiological sodium-chloride solution (pH 7.4) and the mixture centrifuged at 3,000 rpm for 7 minutes. The standard suspension obtained was diluted with 5-% formaldehyde solution so as to produce a terminal formaldehyde concentration of 0.2%. The mixture was left in the vibrator for 30 minutes and subsequently left to stand at 25° C for 48 hours.

16 days after vaccination (none of the test animals died during this time), all mice were infected with E-virus (0.2 ml diluted 10^{-2} intraperitoneal) with the inclusion of non-treated control animals and normal-animal controls. Post observation time was 10 weeks (cf. Table 4).

Table 4

Survival Rate after Single Vaccination with Vaccine Virus and Subsequent Ectromelia Infection After Observation for 10 Weeks

a Tierzahl	b Impfdosis 0,2 ml	c Infektionsdosis 0,2 ml	d Z. Tage post infect. sterben 3	4	5	6	7	e tot/gesamt	f Überlebensrate Zahl
10	g Lebendvaccine 10^{-1} i.v.	10^{-3} i.p.	-	-	-	-	-	0/10	10
10	" 10^{-2} i.v.		-	-	1	-	-	1/10	9
10	" 10^{-3} i.v.		-	1	-	-	-	1/10	9
10	g Lebendvaccine 10^{-1} i.p.		-	-	-	-	-	0/10	10
10	" 10^{-2} i.p.		-	3	-	-	-	3/10	7
10	" 10^{-3} i.p.		1	6	-	-	-	7/10	3
10	h Formalvaccine 10^{-1} i.v.		4	5	1	-	-	10/10	0
10	" 10^{-1} i.p.		1	6	1	2	-	10/10	0
10	i Infektionskontrollen		-	9	1	-	-	10/10	0
10	k Normaltiere		-	-	-	-	-	0/10	10

Key: a - number of animals; b - vaccination dose 0.2 ml; c - infection dose 0.2 ml; d - died x days after infection; e - dead/total; f - survival rate; g - live vaccine; h - attenuated vaccine; i - infection controls; k - normal animals

Intravenous and intraperitoneal administration of active vaccine virus in a dose of 0.2 ml diluted at 10^{-1} furnished protection of all mice against a massive infection with E-virus (0.2 ml at 10^{-2}) made 16 days later. 0.2 ml

of the vaccine-virus dilution of 10^{-2} and 10^{-3} still protected some of the animals against subsequent induced infection under the same test conditions. Prior treatment with inactivated (formaldehyde) vaccine virus furnished no protection to induced intraperitoneal ectromelia infection.

Second Test

Test 1 was repeated with the same arrangement but without the groups pretreated with formal vaccine and by reducing the intravenous dose to 0.2 ml of a vaccine-virus dilution at 10^{-1}. The dose of ectromelia infection was also reduced (0.2 ml at 10^{-3}, 10^{-4}, 10^{-5}) (cf. Table 5).

If the infective dose of E-virus was reduced from 0.2 ml at a dilution of 10^{-2} (Test 1) to 0.2 ml diluted at 10^{-3}, 10^{-4} and/or 10^{-5}, all mice pretreated with vaccine virus at a dose of 0.2 ml diluted at 10^{-1} and 10^{-2} were protected against the different degree of the infective dose of ectromelia. The death rate of the mice inoculated with lower doses of live vaccine (0.2 ml diluted at 10^{-3} intraperitoneal) decreased as a function of decreasing infective dose. The time before death was prolonged not only in the control animals but also in the vaccinated mice with decreasing infective dose.

Third Test

This examined the point in time at which vaccine protection to massive induced intraperitoneal ectromelia infection occurs in the animals vaccinated with vaccine virus. Groups of 10 mice each were immunized with active vaccine virus at 2 ml diluted at 10^{-1} intravenous and/or 10^{-1}, 10^{-2} and 10^{-3} intraperitoneal, and were then infected after 3, 6, 9 and 13 days with E-virus intraperitoneal (0.2 ml diluted at 10^{-4} and/or 10^{-5}). Post observation time was 4 weeks after infection (cf. Table 6).

In comparison to the nontreated controls, protective vaccination against intraperitoneal ectromelia infection existed already after 3 days in part of the mice treated with vaccine virus intraperitoneal and intravenous (under intraperitoneal application as a function of vaccination dose). If intraperitoneal ectromelia infection was made 6, 9 and 13 days after protective vaccination, practically all of the animals pretreated with active virus survived whereas all infection controls died within 5 to 9 days.

Fourth Test

In order to obtain data on the duration of vaccine protection which had been observed previously only up to 16 days (Test 1 and 2), 347 mice vaccinated once (0.2 ml/animal of a vaccine-virus dilution of 10^{-1} intraperitoneal) were infected with infectious ectromelia (0.2 ml/animal diluted at 10^{-2} intraperitoneal) 21 weeks after vaccination (cf. Fig. 2).

Table 5

Survival Rate After Single Inoculation with Vaccine Virus and Ectromelia Infection 16 Days Later After Observation for 4 Weeks.

a Tiernahl	b Impfdosis 0,2 ml	c Infektionsdosis 0,2 ml	d x Tage post infektionem starben 4	5	6	7	8	9	10	11	12	13	14	15	e Tot/gesamt	f Überlebensrate-Zahl
10	g Lebendvaccine 10^{-1} i.v.	10^{-3}	–	–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-1} i.p.		–	–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-2} i.p.		–	–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-3} i.p.		6	1	2	–	–	–	–	–	–	–	–	–	9/10	1
10	h Infektionskontrolle		–	6	2	2	–	–	–	–	–	–	–	–	10/10	0
10	g Lebendvaccine 10^{-1} i.v.	10^{-4}	–	–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-1} i.p.		–	–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-2} i.p.		–	–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-3} i.p.		–	2	1	–	–	–	1	–	–	–	–	–	4/10	6
10	h Infektionskontrolle		–	3	5	2	–	–	1	–	–	–	–	–	10/10	0
10	g Lebendvaccine 10^{-1} i.v.	10^{-5}	–	–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-1} i.p.		–	–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-2} i.p.		–	–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-3} i.p.		–	–	–	1	–	–	–	–	–	–	–	–	1/10	9
10	h Infektionskontrolle		–	3	3	1	1	1	–	–	–	–	–	1	10/10	0
10	i Normaltierkontrolle		–	–	–	–	–	–	–	–	–	–	–	–	0/10	10

Key: a - number of animals; b - vaccination dose 0.2 ml; c - infective dose 0.2 ml; d - died x days after infection; e - deal/total; f - survival rate; g - live vaccine; h - infection control; i - normal-animal control

Table 6

Survival Rate after Single Inoculation with Vaccine Virus and Subsequent Ectromelia Infection after 3, 6, 9 and 13 Days 4 Weeks After Infection.

a– Tierzahl	b– Impfdosis 0,2 ml	c– Infektion nach x Tagen Dosis 0,2 ml	d– x Tage post infektionem starben 5	6	7	8	9	10	11	12	13	14	15	e– tot/gesamt	f– Überlebensrate Zahl
10	g– Lebendvaccine 10^{-1} i.v.	3 Tage 10^{-3}	–	1	–	–	–	–	–	–	–	–	–	1/10	9
10	" 10^{-1} i.p.		–	–	–	–	–	–	–	1	–	–	–	1/10	9
10	" 10^{-2} i.p.		–	–	–	–	–	–	–	–	1	–	–	1/10	9
10	" 10^{-3} i.p.		–	3	–	–	–	–	–	–	–	–	1	4/10	6
10	h– Infektionskontrolle		–	2	1	3	–	–	1	–	2	1	–	10/10	0
10	g– Lebendvaccine 10^{-1} i.v.	6 Tage 10^{-4}	–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-1} i.p.		–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-2} i.p.		–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-3} i.p.		–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	h– Infektionskontrolle		–	4	3	2	1	–	–	–	–	–	–	10/10	0
10	g– Lebendvaccine 10^{-1} i.v.	9 Tage 10^{-4}	–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-1} i.p.		–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-2} i.p.		–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-3} i.p.		–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	h– Infektionskontrolle		3	6	1	–	–	–	–	–	–	–	–	10/10	0
10	g– Lebendvaccine 10^{-1} i.v.	13 Tage 10^{-4}	–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-1} i.p.		–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-2} i.p.		–	–	–	–	–	–	–	–	–	–	–	0/10	10
10	" 10^{-3} i.p.		–	–	–	1	–	–	–	–	–	–	–	1/10	9
10	h– Infektionskontrolle		2	7	1	–	–	–	–	–	–	–	–	10/10	0
10	i– Normaltierkontrolle	–	–	–	–	–	–	–	–	–	–	–	–	0/10	10

Key: a – number of animals; b – vaccination dose 0.2 ml; c – infective dose after x days 0.2 ml; d – died x days after infection; e – dead/total; f – survival rate; g – live vaccine; h – infection control; i – normal-animal control

GRAPHIC NOT REPRODUCIBLE

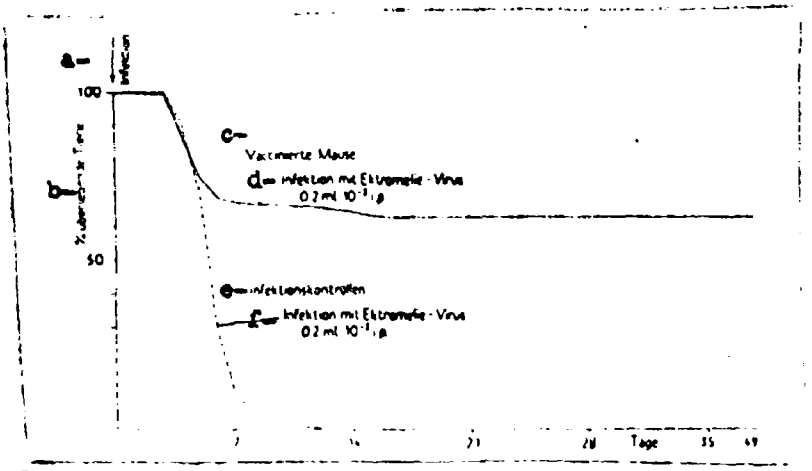

Fig. 2 Mortality Curve of Mice After Single Intraperitoneal Inoculation with Vaccine Virus (0.2 ml diluted at 10^{-1}) and Intraperitoneal Infection with E-Virus (0.2 ml diluted at 10^{-2}) after 5 Months

Key: a - infection; b - % of survivors; c - vaccinated mice; d - ectromelia infection; e - infected controls; f - ectromelia infection; g - days

Among 347 mice massively infected with E-virus intraperitoneal (0.2 ml diluted at 10^{-2}) 5 months after single intraperitoneal vaccination (0.2 ml of vaccine-virus dilution at 10^{-1}), 111 (30%) died within 7 days from acute ectromelia. After 35 days, another 10 mice died so that the total mortality was about 35%. Consequently, 65% of the infected animals vaccinated once 5 months prior to infection survived the massive intraperitoneal ectromelia infection. All non-vaccinated infected controls died within 4 to 12 days.

In grouping the individual findings of this test, it was noted that differences existed in the death rate between male and female mice within the test group so that the mortality curves of Fig. 2 were plotted separately by sex (cf. Fig. 3). The test group of 347 mice consisted of 294 females and 53 males.

Whereas 94 (32%) of 294 female mice died of ectromelia after 7 weeks, 35 (66%) of the male animals died under the same test conditions. The non-vaccinated infected controls showed no difference in the death rate between male and female animals.

Discussion

Our investigations for immunizing white mice against ectromelia were dictated entirely by practical considerations. In order to reduce as far as possible the time required for vaccination, intraperitoneal vaccination was

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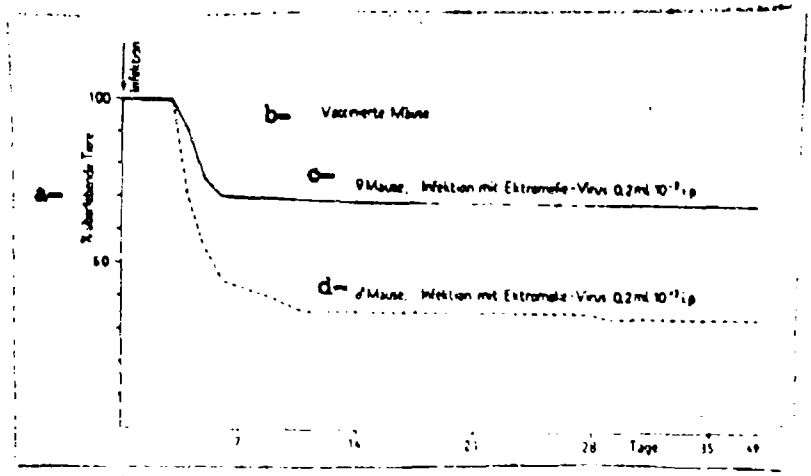

Fig. 3 Mortality Curves of Male and Female Mice Vaccinated Once Intraperitoneal with Vaccine Virus (0.2 ml diluted at 10^{-1}) and Infected Intraperitoneal with E-Virus (0.2 ml diluted at 10^{-2}) 5 Months Later

Key: a - % survivers; b - vaccinated mice; c - females infected with ectromelia; d - males infected with ectromelia

selected although data in literature available to us (Ref. 1, 3) indicate that only minor antibody formation takes place with this manner of administration. However, the present findings indicate that it is possible to reliably protect mice against even massive ectromelia infection by intraperitoneal administration of appropriate doses of the vaccine virus. The saving of time with intraperitoneal in comparison to intradermal, intranasal, or intravenous vaccination makes an eventually higher dose of the vaccine virus for achieving protective vaccination immaterial.

After intraperitoneal application, the vaccine virus is found at most after 5 days in liver, spleen and lung of the test animals which indicates that only a very minor or no propagation of the vaccine virus takes place in these organs. Ref. 3 also found only isolatedly active vaccine virus 6 days after intravenous, intraperitoneal, subcutaneous and intracutaneous application in these organs. The low capability of propagation of the vaccine virus in the mouse organism also seems to be the reason why relatively high doses of active vaccine virus are necessary for developing immunity to ectromelia. The relatively high vaccination dose utilized can very probably be further reduced, however, since the induced massive ectromelia infection does not correspond to conditions in nature.

Our findings on start and duration of immunity to ectromelia after intraperitoneal application of vaccine virus correspond to the findings of Ref. 3 from serological examinations. Not less than 6 days after vaccination,

full immunity can be demonstrated from the present findings and, even after 20 weeks, immunity to massive intraperitoneal ectromelia infection exists in more than 50% of the animals. It appears that female mice develop under the same conditions a higher immunity to ectromelia than male mice.

The attempt of breeding SPF (specific pathogen-free) mice offers the hope that scientific institutes and laboratories will have available in future non-ectromelic mice. However, as protection against intercurrent ectromelia, vaccination of such SPF mice against ectromelia will still be indicated particularly in tests which extend over the entire life of the mice. In the present state of development, we agree with Ref. 1 that only general vaccination of test mice with vaccine virus represents effective protection against infectious ectromelia.

In our opinion, such protective vaccination can be abandoned only then when SPF breeders can furnish non-ectromelic mice in sufficient numbers and then when proper maintenance of such SPF mice becomes possible in the individual laboratories.

In the meantime there are available our own satisfactory practical experiences with vaccination over an interval of 5 years which indicate moreover that even much lower doses of vaccination than utilized in the present tests are sufficient to continuously protect the stock of mice against ectromelia. Details on this will be published in a future communication.

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