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Arthus Phenomenon Like Skin Reaction and Antibody Pattern in Rabbits Immunized with Various Myxovirus Fractions

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Summary

Rabbits immunized with measles- and mumps viruses grown in monkey kidney cells and chick embryo fibroblasts and with their water- and organic solvent fractions obtained by Tween-ether treatment developed immediate type skin hyperreactivity (Arthus phenomenon-like) to challenge with the complete viruses and calf serum, but not to challenge with tissue antigens prepared in the absence of calf serum. The skin reaction in animals immunized with ether fractions was stronger than in animals immunized with water fractions or complete virus, respectively. The intensity of individual skin reactions in each animal was better correlated to the presence of precipitating antibody against calf serum or a component thereof than to the HI titer against the viruses used for immunization.

1. Introduction

The recent reports on local and systemic reactions after administration of live attenuated measles vaccine and also on atypical severe measles after exposure to wild virus in prior recipients of the formalin-killed alum-adsorbed measles vaccine of monkey kidney culture origin prompted this present investigation.

This study in rabbits was designed to determine the nature of the allergic skin reaction and the responsible sensitizing factor in experimental lots of tween ether split and live measles and mumps virus preparations.

[1] The seriological tests for measles and mumps were supported by the Bundesminister für Jugend, Familie und Gesundheit, Bonn, BRD.

2. Materials and Methods

2.1. Animals

Adult white New Zealand rabbits of either sex, obtained from a commercial breeder were used in the experiments. Their weight range was 2200 to 4200 g (mean 2800 g) at the time when immunization was started.

2.2. Antigens

2.2.1. Viruses

Four virus batches of different hemagglutinin- and tissue-specificities were prepared by growing measles virus and mumps virus in monolayer cultures of chick embryo fibroblasts and monkey kidney cells, each:

1. Measles virus, attenuated Schwarz-strain (17) was grown in primary or secondary fibroblastic monolayer cultures prepared from 9—12 day old chick embryos originating from a normal commercial flock (Antigen MEA/CEF).

2. Measles virus, strain 1677, described by Enders-Ruckle (16) was grown in primary Cercopithecus monkey kidney cell cultures (Antigen MEA/MK). Most of this material was obtained by courtesy of Dr. R. Mauler, Behringwerke AG, Marburg (Lahn).

3. Egg adapted mumps virus, strain Enders (6), kindly supplied by Dr. J. F. Enders, Boston, was grown in chick embryo fibroblasts (Antigen MU/CEF).

4. A mumps virus strain was isolated in Frankfurt (Main) from throat washings of an infected child and adapted to multiply in primary monkey kidney cells by 5 serial passages. It was identified serologically as mumps virus and used for virus production in its 6th passage in Cercopithecus monkey kidney cell cultures (Antigen MU/MK).

2.2.2. Propagation of Viruses

A technique similar to that described in the US-regulations for attenuated measles vaccines (20) was used. This includes the use of calf serum during the phases of cell-outgrowth until after infection with the virus and multiple harvests from washed cells.

2.2.3 Measurement of Virus Concentration

Each harvest was checked for virus concentration by hemagglutination tests at 37° C before further processing. Cercopithecus monkey red cells were used for measles tests and Cercopithecus and/or guinea pig red cells for mumps tests. Approximately the same titers for mumps virus were obtained in some parallel tests with either type of erythrocytes.

2.2.4. Purification and Concentration

Individual harvests were concentrated and partially purified by low speed (30 minutes/1,600—2,500 × *g*) and subsequent high speed centrifugation (1 hour/26,000—68,000 × *g*). Pellets were resuspended in a volume of Parker medium 199 (without serum, but containing penicillin, streptomycin and neomycin) to give an at least 30-fold concentration numerically. The increase of hemagglutinin titers, however, achieved by this procedure was only about eightfold or tenfold maximally.

About 1/3 of the volume of each virus concentrate was stored at —60° C without further processing to be used as antigens for immunization, skin testing, and immunodiffusion tests (antigens: MEA/CEF/V, MEA/MK/V, MU/CEF/V, and MU/MK/V). About 2/3 was subjected to fractionation by Tween 80 and ether.

2.2.5. Tween-ether Treatment

The virus concentrates were treated with Tween 80 (final concentration 0,025%) and fresh anaesthesia grade ether (half the volume of virus concentrate). The mixture was shaken mechanically for 15—45 minutes (depending on volume) at room temperature and was then centrifuged in a refrigerated centrifuge for 20 minutes at 1,500—

2,400× *g*. The water phase was separated from the gelatinous interphase and the ether supernatant fluid. Residual ether in the aqueous phase was removed by streaming nitrogen gas over the surface of the sample (during stirring) until the odor of the ether had disappeared. To prevent a reduction of HA titer bubbling of the gas through the solution was avoided carefully.

The water phase of each virus concentrate was stored at −60° C until used as antigens for immunization (antigens: MEA/CEF/WF, MEA/MK/WF, MU/CEF/WF, and MU/MK/WF).

The ether phases were concentrated by evaporating ether in a rotating vacuum evaporator at room temperature. About 10 ml Hanks-solution were added before evaporation was started to keep the lipoproteins in suspension. After ether was evaporated within less than 1 hour the volume of ether fraction was brought to about 50 ml by adding Hanks-solution. The milky suspension was stored for a few days in a refrigerator prior to use for immunization (antigens: MEA/CEF/EF, MEA/MK/EF, MU/CEF/EF, and MU/MK/EF). During storage some creamy material separated from the suspension which was easily homogenized by shaking the vials before use.

2.3. Immunization

Groups of 4 rabbits were immunized with each of the 12 antigens described above. Immunization consisted of 4 intraperitoneal injections of 2.0 ml of antigen given at 7 days intervals. Blood was taken for antibody determinations and skin tests were performed 7–10 days after the last immunizing injection. All sera were stored at −20° C until testing.

2.4. Skin Tests

The trypan blue test, originally described by MENKIN (11) was employed for skin testing. The technique was similar to the modification used by STICKL *et al.* (18) in their experiments with vaccinia virus.

Rabbits were shaved at the abdominal area and treated with a depilatory cream the day before skin tests were performed. Immediately before skin tests the animals received 0.6 ml per kg body weight of a prewarmed 1% trypan blue solution in phosphate buffered saline intravenously. They then were injected intracutaneously with 0.1 ml of the 4 untreated virus antigens (MEA/CEF/V, MEA/MK/V, MU/CEF/V, and MU/MK/V) and with a control fluid.

In addition 4 non-immunized rabbits originating from the same shipment and housed together with the test animals were subjected to the same procedure (K 1 – K 4).

The composition of control fluid varied at different testing days. Medium 199 without serum and antibiotics was used for the animals tested at days 7 and 8 after the last injection and the control animal K 1. The same medium but containing 2% calf serum and antibiotics (penicillin, streptomycin and neomycin) was used for the rabbits tested at day 9 and control K 2. The animals tested at day 10 and the controls K 3 and K 4 received as control fluid medium 199 containing antibiotics only.

Skin reaction was measured as the stained area 2, 4, and 6 hours after intracutaneous injection. Two diameters of the blue area were measured and the extent of skin reaction was expressed by its surface calculated by the ellipse formula. The individual skin reaction for each animal and each injection site was calculated as the arithmetic mean of the blue areas in square millimeters measured on the three occasions.

Individual skin reactions were expressed by a grading system according to the following scheme:

−: less than 10 mm²
(+): 11–50 mm²
+: 51–100 mm²
++: 101–200 mm²
+++: more than 200 mm²

2.5. Serologic Tests

The antibody titrations for measles and mumps virus were carried out by the hemagglutination inhibition test.

Measles HI tests were performed as described previously (3), but using a microtiter procedure in disposable U-type plastic microtrays (purchased from Fa. Greiner, Nürtingen, Germany). The HA antigen was prepared by Tween-ether treatment from lyzed cells and fluids of Fl-cultures infected with measles virus strain 1677. The rabbit sera were inactivated for 30 minutes at 56°C at a dilution of 1:2 and then absorbed with an equal volume of a 10% suspension of Grivet monkey red cells for removal of non-specific agglutinins. Serial twofold dilutions of sera in 0.025 ml phosphate buffer (pH 7.2) were mixed with equal volumes of HA antigen diluted so as to contain 4 HA units per cup. After incubation for 20 hours at 4°C and subsequently for 1 hour at room temperature 0.025 m of a 0.75% suspension of Cercopithecus monkey red cells was added. Readings were done after incubation for 90 minutes at 37°C. HI titers were recorded as the reciprocal of the highest serum dilution inhibiting hemagglutination completely or almost completely. The titers given refer to 0.025 ml of the initial serum dilution. A hyperimmune rabbit anti-measles serum and the international human measles standard serum were included for reference purposes. All sera were analysed in one test.

The *mumps HI tests* were also performed by the microtiter method. The virus antigen used was amniotic fluid from eggs infected with the Enders strain of mumps virus. The sera were pretreated with receptor destroying enzyme, according to the technique recommended for influenza serology (15). The sera then were absorbed with equal volumes of a 20% guinea pig red cell suspension.

Serial 2-fold dilutions of serum in 0.025 ml phosphate buffer (pH 7.2) were mixed with equal amounts of antigen containing 4 HA-units per volume. After incubation for 20 hours at 4°C and 1 hour at room temperature 0.025 ml of a 1% suspension of guinea pig red cells was added. Readings were done after incubation for 1 hour at 4°C. Titers were expressed as the reciprocal of the highest serum dilution giving complete inhibition of hemagglutination. The lowest serum dilution tested as 1:10 and titers refer to 0.025 ml of the initial dilution. A human mumps convalescent serum pool, a negative human serum and an anti-mumps hyperimmune serum from guinea pigs served as references.

2.6. Immunodiffusion Tests

The serum from each animal was tested by immunodiffusion methods against the following antigens:

1. antigen MEA/CEF/V as used for immunization,
2. antigen MEA/MK/V as used for immunization,
3. antigen MU/CEF/V as used for immunization,
4. antigen MU/MK/V as used for immunization,
5. a chick embryo cell antigen, prepared from secondary chick embryo cell cultures grown without calf serum after the 1st trypsinization and lyzed by 3 cycles of freezing and thawing (1 ml corresponding to approx. $2-5\times10^5$ cells),
6. a monkey kidney cell antigen, prepared from primary Cercopithecus monkey kidney cell cultures grown in absence of calf serum for 14 days and lyzed by 3 cycles of freezing and thawing (1 ml corresponding to about 2×10^6 cells),
7. a control fluid consisting of medium 199 containing 5% calf serum, and
8. medium 199 without calf serum.

Immunodiffusion tests were performed in 0.8% agarose (purchased from Fa. Serva, Heidelberg, Germany) jelly in barbiturate buffer, pH 8.5 containing merthiolate 1:10,000 by two different techniques:

a) a microprecipitation test on microscopic slides covered with an agarose layer of about 1 mm thickness using the LKB well cutter with 8 wells surrounding the central hole. Two drops of undiluted serum were placed into the central well and 1 drop

of each antigen (diluted 1:4 for all antigens except No. 5 which was used in 1:2 dilution) into the outer wells.

b) a microprecipitation test similar to that described by BEALE and MASON (2). One drop of serum and antigen (diluted 1:2 for all antigens except No. 5 which was used undiluted) was placed into holes cut into a piece of plexiglass fixed on an agarose layer of 1 mm thickness on a microscopic slide.

Both precipitation tests were incubated for 18 hours at room temperature and stained with Amidoblack 10 B by the routine methods after excess antibody was washed out by 0.85% sodium chloride solution (3 hours) and distilled water (2—3 hours).

Technique b has proven to be more sensitive since more precipitation lines were seen than with method a. This allowed to grade the intensity of the precipitation lines seen in each system in a semiquantitative manner according to the following scheme:

$-$ = no lines in either technique.
$(+)$ = a discrete line in the more sensitive technique.
$+$ = discrete lines in both techniques.
$++$ = clear lines in both techniques.
$+++$ = thick or double lines in both techniques.

All immunodiffusion tests were run twice on one microscopic slide. The first test was performed with the original rabbit serum and the second one with the same serum absorbed with calf serum. One drop of calf serum was added to 0.2 ml of rabbit immune sera in order to remove all antibodies with specificities directed against calf serum.

3. Results

48 rabbits were immunized with the various antigen preparations. 4 animals died during the period of immunization, thus only 44 could be tested for skin reaction and antibodies. 4 animals served as non-immunized controls.

Table 1 presents in detail the results of the studies on the immediate type skin-reaction, the HI antibody response and of the immunodiffusion tests.

3.1. Skin Reactions

Skin reactions of the type of Arthus phenomenon, leading to extravasation of the dye injected previously, were observed in 40 of 44 immunized rabbits. One of the non-reacting animals (No. 830) apparently was in poor condition and suffered from diarrhoea, the remaining 3 showed no abnormal clinical signs.

In most of the rabbits the skin reaction was recognizable 2 hours after intradermal injection of the test antigens and usually reached its maximum between the 2nd and the 4th hour. It was diminishing already after 6 hours.

No skin reactions of the delayed type were observed in this study. All animals were reexamined at days 2, 4, and 7 following intradermal injection of test antigens. Neither indurations nor other local reactions could be detected during this period except the slowly disappearing blue maculae due to the Arthus phenomenon-like skin reaction.

No skin reaction at all was seen in the non-immunized control rabbits, neither of the immediate nor of the delayed type.

Although there is considerable variation from animal to animal, it is immediately evident that for measles and mumps virus the Arthus phenomenon-like skin reaction was much stronger in animals immunized with ether fraction as compared to the animals immunized with the complete virus or the water fraction. No clear difference could be detected between the groups of animals immunized with the

Table 1. *Detailed Results of Skin Tests and Studies for HI and Precipitating Antibodies*

Material used for immunization	Rabbit		Skin reaction against					HI antibody against		Precipitin reaction against				
	No.	Day of bleed.	MEA CEF V	MEA MK V	MU CEF V	MU MK V	CS	Measles	Mumps	MEA CEF V	MEA MK V	MU CEF V	MU MK V	CS
Rabbits immunized with complete virus														
Measles, grown	837	7	++	++	+	++		8		—	—	—	(+)	—
on chick embryo	838	8	++	+++	+++	+++		64		+	++	(+)	++	++
fibroblasts	839	9	(+)	(+)	(+)	(+)	(+)	no serum		(+)	(+)	—	(+)	+
(MEA/CEF/V)	840	10	(+)	+	(+)	+		64		—	(+)	—	—	+
Measles, grown	841	—	died, not tested											
on monkey	842	8	+	+	(+)	++		1024		+	+++	++	++	++
kidney cells	843	9	—	—	(+)	(+)		256		(+)	+	(+)	+	+
(MEA/MK/V)	844	10	(+)	(+)	(+)	+		4096		—	(+)	—	+	+
Mumps, grown	845	7	++	++	++	+++		<4		—	++	—	+++	++
on chick embryo	846	8	(+)	+	+	++			40	—	(+)	—	(+)	(+)
fibroblasts	847	9	(+)	++	(+)	+	++[2]		320	—	(+)	—	+	+
(MU/CEF/V)	848	10	—	—	—	—			160	—	(+)	—	+	(+)
Mumps, grown	849	7	(+)	(+)	—	(+)			40	—	+	—	+	+
on monkey	850	8	(+)	++	—	++			40	+	++	+	+++	++
kidney cells	851	9	(+)	(+)	+	+	(+)		80	—	++	—	++	++
(MU/MK/V)	852	10	(+)	—	(+)	+			40	—	(+)	—	(+)	+
Rabbits immunized with water fraction of virus														
Measles, grown	805	7	(+)	(+)	(+)	(+)		64		—	(+)	—	(+)	[2]
on chick embryo	806	8	++	++	+	++		512		(+)	+++	(+)	+++	+++
fibroblasts	807	9	(+)	+	(+)	+	+++	512		(+)	+	—	(+)	++
(MEA/CEF/WF)	808	10	—	—	—	—		8		—	—	—	—	—
Measles, grown	809	7	(+)	(+)	—	(+)		32		—	(+)	—	(+)	(+)
on monkey	810	8	(+)	(+)	(+)	(+)		64		—	(+)	—	+	+
kidney cells	811	9	+	+	+	++	+	32		(+)	(+)	—	(+)	+
(MEA/MK/WF)	812	10	+	++	+	++	+	64		—	+	—	+	++

Mumps, grown	813	7	++	++	+	+++			80	–	+	—	+	+
on chick embryo	814	8	(+)	(+)	(+)	(+)			40	—	+	—	(+)	+
fibroblasts	815	9	—	—	—	—	—		20	—	—	—	—	—
(MU/CEF/WF)	816	10	+++	++	++	+++			20	—	+	—	+	+
Mumps, grown	817	—	died, not tested											
on monkey	818	8	+	+	+	++			20	—	(+)	—	(+)	—
kidney cells	819	9	—	+	—	—	—		20	—	+	—	+	+
(MU/MK/WF)	820		died, not tested											
Rabbits immunized with ether fraction of virus														
Measles, grown	821	7	+++	+++	+++	+++		32		++	+++	++	+++	+++
on chick embryo	822	8	++	++	++	+++		512		++	++	+	+++	+++
fibroblasts	823	9	++	++	++	+++	+	128		++	++	++	++	++
(MEA/CEF/EF)	824	10	+	++	+	++		1024		+	+	+	+	+
Measles, grown	825	7	+++	+++	+++	+++		32		—	+	—	+	+
on monkey	826	8	+++	+++	++	+++		2048		(+)	+++	—	+++	+++
kidney cells	827	9	++	+++	+++	+++	++	64		—	++	—	++	++
(MEA/MK/EF)	828	10	++	+++	++	+++		512		+	+++	(+)	+++	+++
Mumps, grown	829	7	+++	+++	+++	+++			40	+++	+++	++	+++	+++
on chick embryo	830[1]	8	—	—	—	—			20	—	—	—	—	—
fibroblasts	831	9	++	++	++	++	++		20	+	+++	++	+++	+++
(MU/CEF/EF)	832	—	died, not tested											
Mumps, grown	833	7	++	++	++	+++			80	—	+	—	+	++
on monkey	834	8	+	+	+	++			160	++	+++	++	+++	+++
kidney cells	835	9	+++	+++	+++	+++	+++		40	+	++	—	++	++
(MU/MK/EF)	836	10	++	++	+	+++			160	++	+++	++	+++	+++
Control rabbits — non immunized														
	K 1	—	—	—	—	—		<4	<10	—	—	—	—	—
	K 2	—	—	—	—	—	—	<4	<10	—	—	—	—	—
	K 3	—	—	—	—	—		<4	<10	—	—	—	—	—
	K 4	—	—	—	—	—		<4	<10	—	—	—	—	—

Explanation of symbols for the degree of skin reaction and the intensity of precipitation see section materials and methods.

[1] Animal in poor condition, diarrhoea.

[2] Test could not be read exactly.

two latter types of antigens. There also was no clear difference between skin reactions evoked by immunization with measles or mumps viruses grown on monkey kidney epithelial or chick embryo fibroblast cells.

Both viral antigens grown on monkey kidney cells following intradermal injection caused a little more severe skin reactions than the antigens from chick embryo cells. There is, however, no evidence that homologous testing for hemagglutinin- or tissue specificities would have revealed more pronounced skin reactions than heterologous testing.

All animals injected intradermally with a control fluid containing 2% calf serum in medium 199 (indicated as CS in the head of the Table) presented skin reactions of almost the same average intensity as those injected with the various viral antigens. No such reactions could be observed with serum free medium 199, even if it contained penicillin, streptomycin and neomycin in the same concentration as the viral antigens.

3.2. HI Antibody Response

All animals developed HI antibodies to the viral antigens used for immunization. No antibody reaction to either virus could be demonstrated in the 4 non-immunized control rabbits at the end of the experiment. Furthermore no heteros logous antibody was found in the 3 animals tested. The HI titers for measles varied between 8 and 4096, the geometric mean being 149.1. HI titers for mump- ranged between 10 and 320 with a geometric mean of 49.2.

Due to the small numbers of rabbits within the individual test groups (maximum 4 animals) mean titers between groups cannot be compared. Thus estimates of the immunogenic potencies of the various viruses and fractions thereof used in the study cannot be made.

3.3. Immunodiffusion Tests

Precipitation lines against at least one of the test antigens were seen in immunodiffusion tests with the sera of all but 3 immunized animals. Sera of the rabbits presented precipitin reactions of varying intensity to 3—5 of the eight test antigens used in the study. No precipitin reactions were seen with the uninfected monkey kidney- and chick-embryo fibroblast antigens grown in the absence of calf serum and with medium 199 only. However, all but 5 rabbit sera produced precipitation lines with a control antigen consisting of medium 199 containing 5% calf serum. The intensity of such lines varied from animal to animal but generally corresponded to that of the lines produced by the viral antigens. In some instances identity reactions between the lines produced by the control fluid and the viral antigens were observed. No precipitation lines appeared against test antigens containing no calf serum.

Correspondingly no precipitin lines were detected if one drop of calf serum was added to 0.2 ml of rabbit immune serum prior to testing. The latter findings and the results with serum free tissue antigens (see above) are not included into the Table, since they were consistently negative in all tests performed.

3.4. Skin Reaction and HI-titer

Attempts to correlate the intensities of maximum skin reaction and the HI antibody titers are presented in Fig. 1. The left part of the diagram gives the

results in animals immunized with measles virus preparations and the right part such of rabbits immunized with mumps virus preparations. Although an exact statistical analysis is prevented by the small numbers of animals per group it can be seen at least for the right diagram that no correlation seems to exist. For the animals immunized with measles virus there is a tendency that some rabbits

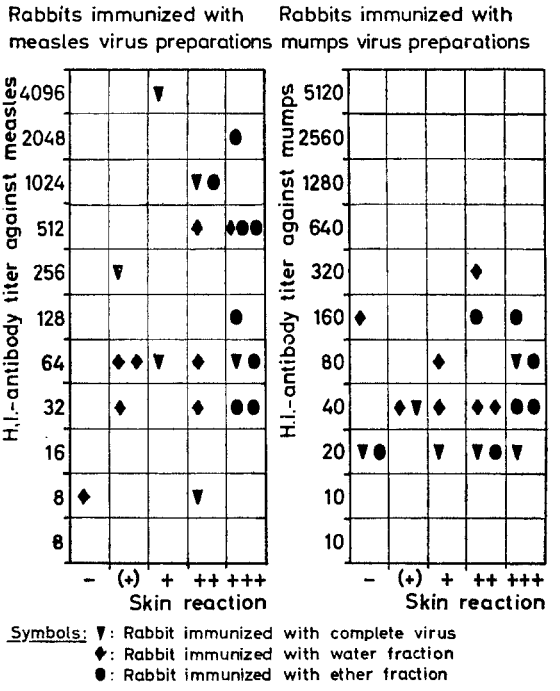

Fig. 1. Maximum individual skin reaction and HI antibody titer in rabbits immunized with measles and mumps viruses and their fractions

with high HI titers also present severe skin reactions. However, a clear correlation between skin reaction and concentration of HI antibody does not exist. Both diagrams confirm the observation that immunization with the ether fraction (circles) evoked more severe skin reactions than immunization with complete virus or water fraction (triangles and squares).

3.5. Skin Reaction and Precipitating Antibody

A similar attempt to correlate the intensities of skin reaction and precipitin reaction for each animal is presented in Fig. 2 (assuming that these reactions are due to a common non-viral antigen the maximum individual skin reaction of each animal was selected for this comparison). Some degree of correlation seems to exist between these two parameters. It is of interest that 3 of the 4 non-reactors in the skin test had also no precipitating antibodies against this common antigen and that the fourth non-reacting animal possessed only very limited amounts of such antibody.

In addition Fig. 2 shows that the intensity of skin reaction and the concentration of precipitating antibody were highest in rabbits immunized with ether

fraction and less in animals immunized with complete virus and water fraction — irrespective, whether measles or mumps preparations were used for immunizations. With the only exception of one rabbit in poor condition (No. 830) all animals immunized with ether fractions presented skin reactions ranging from ++ to +++ intensity and precipitin reactions graded between + and +++. The reactions in animals immunized with complete virus or water fraction varied much more in intensity. Rabbits showing weak skin reactions, however, generally also had less amounts of precipitating antibodies in their blood serum.

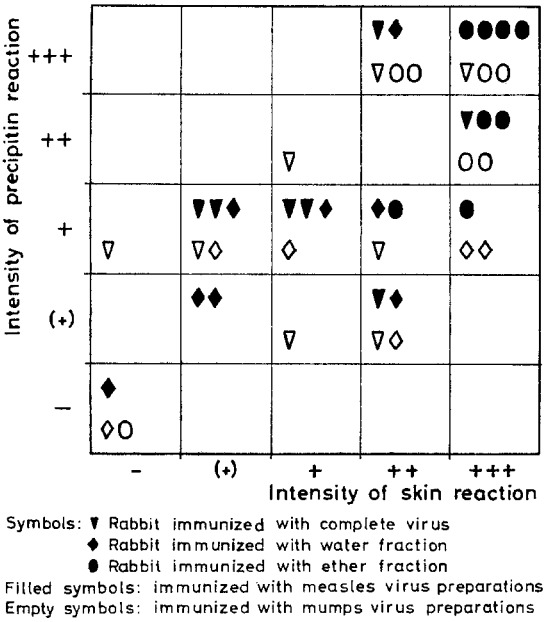

Fig. 2. Maximum individual skin reaction and precipitin reaction in rabbits immunized with measles and mumps viruses and their fractions

4. Discussion

The allergic skin reactions elicited with various measles- and mumps virus antigens and non-viral control preparations in rabbits immunized with the respective materials differ from those described in children pre-immunized with the alum precipitated inactivated measles vaccine. Lennon *et al.* (9), Fulginiti *et al.* (5) and Fulginiti and Arthur (4) have reported in 1968 on skin reaction of the delayed type in children immunized with 3 doses of killed and 1 dose of live measles virus vaccine on re-immunization or intradermal skin tests with live measles vaccine. Development of delayed type skin hyperreactivity in guinea pigs was reported by Sheldon *et al.* (18), Matte *et al.* (10) and other authors mentioned below.

In our experiments in rabbits immunized with various fractions of measles- and mumps-virus no similar reaction could be noticed although the animals were observed for at least 7 days. The rabbits presented a cutaneous hypersensitivity of the immediate type (Arthus phenomenon-like) which resembles more that described in the studies of Hennessen and Mauler (7) and Aprile *et al.* (1). The former authors reported that guinea pigs immunized with complete measles

virus of monkey kidney culture origin presented a strongly positive Schultz-Dale-reaction when tested with Tween-ether extract of the virus, but only minimal reactions in tests with the water fraction (from which the lipoproteins were removed by such treatment). The latter authors reported about the sensitizing ability of different inactivated and live adsorbed commercial and experimental measles virus vaccines to non-viral protein components of the various tissue culture systems as shown by the passive cutaneous anaphylactic (PCA) test and the Schultz-Dale-reaction. In contrast the non-adsorbed inactivated vaccines of monkey kidney culture origin and live attenuated measles vaccine from chick embryo tissue culture showed minimal sensitizing activity against tissue culture components of monkey kidney and chick embryo fibroblast cultures. However, if the attenuated measles virus of CETC origin used for immunization contained human albumin as stabilizer the guinea pigs showed a positive PCA reaction on challenge with 0.1% human albumin, 10% monkey sera and measles virus fluid of CETC origin containing human albumin. The observation that the water-phase of the Tween-ether treated measles virus of monkey kidney culture origin provoked a stronger reaction than the ether-phase on challenge in guinea pigs sensitized with inactivated adsorbed and also live attenuated measles vaccine is in contrast to the results of HENNESSEN and MAULER (7) and our present findings. This discrepancy may be due to technical factors of Tween-ether treatment and purification methods or may depend on the presence or absence and the concentration of adjuvants.

Specificity of skin reaction: The available clinical and experimental data indicate that the allergic hyperreactivity causing skin reactions in children is neither due to measles virus specific antigens nor to host tissue specific components of the challenge antigens. All authors working in this field therefore suspect that a common tissue culture component must be the cause of such allergic hyperreactivity. The hypothesis that trace amounts of calf serum or other proteins present in the tissue culture fluids could be the sensitizing antigen has already been discussed by LENNON and ISAACSON (8), NORRBY *et al.* (13) and APRILE *et al.* (1); convincing evidence for this suggestion, however, was lacking so far.

The experiments reported in this paper confirm this hypothesis. They substantiate the present knowledge by reasonable evidence that an antigenic component related to calf serum proteins is the sensitizing antigen for allergic skin reactions, at least of the immediate, Arthus phenomenon-like type.

In our study such reactions were produced in sensitized animals not only with measles- and mumps virus preparations grown in chick embryo fibroblast or monkey kidney cell cultures but also with a control fluid containing only low molecular weight substances and 2% calf serum. No such reactions occurred at the sites of injection of medium 199 alone or containing 100 IU penicillin, 100 γ streptomycin, and 100 γ neomycin per ml; sensitization against antibiotics, therefore, seems not to play a major part in development of skin hyperreactivity.

Further evidence on the antigenic effect of calf serum may be derived from the results of the immunodiffusion studies. All precipitation lines seen in such tests may be explained by an antigen-antibody reaction the specificity of which is indistinguishable from that of calf serum (or a component thereof).

Evidence for this opinion is based on four observations:

1. Whereas all skin-test antigens (concentrates of TC fluids from infected cell cultures) gave precipitations of varying intensities, no precipitation at all was seen with pure tissue-culture antigens prepared in absence of calf serum (details see "Materials and Methods").

2. All sera reacting positive in the immunodiffusion test with one or more of the skin test antigens also gave precipitation lines with a control antigen consisting of medium 199 containing small amounts of calf serum. In some instances identity reactions were observed between the precipitation lines produced by such antigens and the calf serum control.

3. The immune sera lost their ability to form precipitates with the skin test antigens and the calf serum control antigen after 1 drop of calf serum was added to each 0.2 ml of immune serum.

4. Sera of animals immunized with one specific virus preparation (measles or mumps from chick embryo- or monkey kidney origin) did not reveal stronger precipitation in homologous test systems (for hemagglutinin- and tissue specificity) than in heterologous systems or in the control system containing calf serum as the only antigenic component.

It seems easy to understand that the antigen preparations used for immunization and skin tests may have contained trace amounts of calf serum. The virus suspensions used for preparation of these antigens were grown by the multiple harvest method from cells which have been in contact with such serum until after infection with the virus. Furthermore, infected cell cultures cannot be washed as vigorously as uninfected cells to avoid loss of the infective centers in the cell sheet.

Production of antibodies directed against calf serum in rabbits by immunization with measles virus grown in monkey kidney tissue culture has been demonstrated in unpublished experiments of v. Mettenheim and Göing (personal communication). Van Ramshorst and Polak (14) have reported that guinea pigs hyperimmunized with poliovirus D-antigen preparations also developed precipitating antibodies against calf serum. Aprile *et al.* (1) have shown that human albumin contained in the immunizing antigens may lead to delayed-type skin hyperreactivity to such protein.

Further evidence for the hypothesis that the calf serum rather than the viral antigen was the sensitizing component in the virus preparations used for immunization may be derived from the two graphs presented in section "Results". There was no, or almost no clear correlation between the HI antibody titers of individual rabbits against measles- or mumps virus and their skin reaction. The intensities of skin reaction and of precipitin formation seem to correlate rather well. The small numbers of animals per group prevent an exact statistical analysis of these correlations.

Intensity of skin reaction and precipitin reaction: From the Table presented under "Results" it is evident that rabbits immunized with ether fractions developed stronger dermal hypersensitivity than animals immunized with complete virus or water fractions. This may be due to the following factors:

1. The lipoproteins of the viral particles and particularly of the tissue culture components accumulate after Tween-ether treatment mainly in the ether frac-

tion. HENNESSEN and MAULER (7) showed that the former evoked a stronger Schultz-Dale-reaction and our studies demonstrate that the ether fraction seems to be the stronger skin-sensitizing antigen. That the ether fractions used in these experiments were no pure lipoprotein preparations is shown by the fact that they induced HI antibodies in rabbits of a similar level as did the water fractions or the complete virus preparations. Experiments with more purified ether- and water fractions are necessary to prove this hypothesis and to clear up the controversial results of APRILE *et al.* (1).

2. The ether fraction could represent a greater antigenic mass. Actually one immunizing dose (2 ml) of ether fraction was derived from a greater volume of original virus suspension than the same dose of water fraction or complete virus antigen. This hypothesis, however, seems not very likely, since the ether fraction only induced stronger skin hypersensitivity and higher concentrations of calf serum precipitating antibodies and gave no rise to significantly higher titers of antibodies directed against the viral hemagglutinins.

3. The lipoproteins contained in the ether fraction could have some adjuvant effect. The importance of aluminium adjuvants for the development of skin hyperreactivity of the delayed type has been stressed already by WILSON and APRILE (20) and APRILE *et al.* (1). The ether fractions used in our experiments were milky suspensions with a relatively high content of lipids which could have acted as oil adjuvants.

Further work is required to define the antigenic components of measles virus vaccines necessary for adequate immunization and those responsible for adverse skin sensitization and systemic reaction. In addition, the mechanism for the occurrence of the atypical severe illness in prior recipients of inactivated measles vaccine — so far only observed after use of the formalin-killed alum-adsorbed type — on later exposure to wild virus has to be clarified.

It appears from our rabbit experiments and also from a survey (3) of children immunized with the Tween-ether split measles vaccine (Quintovirelon) and revaccinated with attenuated live (Schwarz) virus vaccine that at least for the avoidance of local reactions the use of a vaccine consisting mainly of the water phase of the Tween-ether split product is a better approach to the ultimate goal of highly purified vaccines than the formalin-inactivated vaccines containing whole virus. This is particularly the case for inactivated vaccines which require for adequate immunogenicity concentration of antigen and addition of adjuvant.

If the Tween-ether inactivated and $Al(OH)_3$ adsorbed German and the purified HA-fluid measles vaccine of NORRBY and GARD (12) are also advantageous in regard to prevention of the atypical measles, is less sure and warrants further surveillance.

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