

Detection of sensitizing tissue culture components in viral vaccines

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Adsorbed measles and poliomyelitis vaccines of monkey kidney tissue culture origin sensitize guinea pigs to protein components of the tissue culture, as shown by the passive cutaneous anaphylactic test. Unadsorbed vaccines are not sensitizing, and there is a correlation between the production of anaphylactic antibodies in guinea pigs by different vaccines and their ability to sensitize infants to a subsequent injection of attenuated measles virus vaccine.

Several reports have indicated that children immunized with an alum-precipitated, inactivated measles vaccine of monkey kidney tissue culture origin may subsequently display local reactions when given live attenuated measles virus vaccine parenterally¹ or contract an atypical measles illness when exposed to the natural infection.^{2, 4} Delayed dermal reactions have also been obtained with inactivated measles and poliomyelitis vaccines in subjects immunized with the alum-precipitated vaccine.⁵ The latter authors (Lennon and associates) have suggested that the dermal reactions could be due to the presence of tissue culture antigens in the viral vaccines, and we felt that the same explanation might also serve as a working hypothesis for the reactions provoked by attenuated measles virus in humans vaccinated with the inactivated measles vaccine.

This paper describes the development of a test involving passive cutaneous anaphylaxis in guinea pigs as an indicator for the semiquantitative measurement of tissue culture antigens in inactivated measles, poliomyelitis, and rubella vaccines. The test has been used for monitoring the production of a purified measles vaccine which does not sensitize children.

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MATERIALS AND METHODS

Preparation of sera

Groups of 10 to 12 guinea pigs (250 to 300 grams) were injected intramuscularly with 0.5 ml. of vaccine in 3 doses, one week apart, and bled by cardiac puncture one week after the third dose. The pooled sera were heated at 56° C. for 30 minutes, and samples were adsorbed with *Cercopithecus* monkey erythrocytes prior to measurement of hemagglutination-inhibition antibody titers.⁶ For determination of measles-neutralizing antibodies in the sera, 0.25 ml. of twofold serial dilutions were mixed with 14 TCID₅₀ (tissue culture infectious doses, at 50 per cent end point) of measles virus; after 1½ hours at 4° C., the mixture was added to human amnion⁷ roller-tube tissue cultures and incubated for 8 days at 37° C. The antigens used for preparation of the guinea pig antisera included Pfizer-Vax measles K (Chas. Pfizer & Co. Inc.), measles vaccine inactivated (Eli Lilly & Co.), and 3 experimental inactivated vaccines prepared in these laboratories (measles vaccines Lots 101, 1117-4 and DPT polio measles vaccine* Lot 5001). Antisera to 2 experimental DPT polio vaccines 353A and 353B were obtained similarly but with 2 doses one week apart. All of the vaccines used were of MKTC origin except measles vaccine inactivated (Eli Lilly & Co.), which was derived from chick embryo TC. The aluminium content of vaccines containing mineral adjuvant was estimated by the Solochrome Cyanine R method.⁸

Challenge samples derived from monkey kidney tissue culture (MKTC)

Measles vaccines 101, 1117-4, and DPT polio measles vaccine 5001 were experimental preparations obtained from the Edmonston strain of measles virus grown in monolayers of *Cynomolgus* monkey kidney TC, clarified by filtration and inactivated with formaldehyde. Measles virus fluid M1201 (titer 10^{-4.9}) and uninfected control tissue culture 1201 were also derived from *Cynomolgus* MKTC. Part of M1201 was clarified by filtration; another sample was treated with protease CA7 from *Aspergillus oryzae*, at an enzyme concentration of 0.025 per cent, for 19 hours at room temperature to yield M1201-protease digest. A portion of the virus fluid was also treated with Tween 80 and ether,⁹ yielding aqueous and organic solvent fractions. The aqueous phase was dialyzed for 3 days against daily changes with Hanks' balanced salt solution. The organic phase was mixed with Hanks' B.S.S., concentrated against polyethylene glycol 20M (PEG, Union Carbide), and dialyzed against Hanks' B.S.S. to obtain an aqueous preparation. For preparation of the experimental Lot 1163, measles vaccine was adsorbed to a calcium phosphate gel, the supernatant fluid removed by centrifugation, and the vaccine solubilized with versene.¹⁰

In the preparation of poliomyelitis (Salk) vaccine, the monovalent vaccines were concentrated 36 times for storage. DPT polio vaccines Nos. 353A and B

*Diphtheria and tetanus toxoids combined with pertussis, poliomyelitis, and measles vaccines.

were experimental vaccines prepared for animal tests in which the vaccine No. 353B contained a minimal amount of aluminium hydroxide (0.4 mg. per milliliter) for adsorption of the poliomyelitis, diphtheria, and tetanus components.

Unfiltered poliovirus fluid, Type 1 (Mahoney, titer $10^{-7.8}$) and attenuated poliovirus vaccine, Type 3 (Sabin, titer $10^{-8.5}$) were prepared from Cynomolgus and Cercopithecus monkey kidney tissue cultures, respectively. Attenuated rubella virus fluid M33 HPV80 (titer $10^{-4.4}$) was grown in Cercopithecus MKTC in medium containing 0.5 per cent human albumin and inactivated by heating at 37°C . for 19 hours.

An attempt was made to solubilize Pfizer-Vax measles K by dialysis against 0.05M disodium ethylenediaminetetracetate (pH 4.5) for 2 hours. Since a small amount of precipitate remained, the dialysis was continued against 0.28M citric acid (pH 1.98) for one hour and then against several changes of phosphate-buffered saline ($\mu = 0.15$) at pH 7.4 when a clear solution was obtained.

Fractionation of MKTC by molecular sieve filtration

A 120 ml. sample of Cynomolgus MKTC No. 1201 was frozen and thawed (FT) twice, concentrated against PEG, dialyzed against phosphate-buffered saline for 3 days, and made up to 20 ml. with buffer. The sixfold concentrated MKTC/FT was clarified by centrifugation (one hour at $25,000 \times g$), and 10 ml. of the clear supernatant fluid (containing 11.8 mg. protein) was applied to the top of a 2.5 by 35 cm. column (volume 130 ml.) of Sephadex G-100 equilibrated with phosphate-buffered saline. The material was eluted from the column with the same buffer in 10 ml. aliquots and the ultraviolet absorption at 280μ measured in one centimeter silica cells.

Preparation of alum-adsorbed, alum-precipitated, and aluminium hydroxide-adsorbed MKTC

A 10 per cent aqueous alum solution (4 ml.) was brought to pH 7.2 by the addition of 2N sodium hydroxide and the resulting suspension added to 100 ml. MKTC/FT No. 1201. The pH was adjusted to 7.6, the mixture left overnight at 1°C ., and centrifuged for one hour at $25,000 \times g$ to yield alum-adsorbed MKTC. The supernatant fluid from the alum adsorption was concentrated sixfold against PEG and dialyzed against phosphate-buffered saline.

A portion (10 ml.) of the concentrated supernatant fluid was stirred magnetically while 2.4 ml. of 10 per cent aqueous alum solution was added and the pH quickly adjusted to 7.4. The heavy suspension was then stirred for 15 minutes and centrifuged for 15 minutes at $25,000 \times g$ in order to obtain the supernatant fluid from alum-precipitated MKTC.

For the preparation of aluminium hydroxide-adsorbed material, 5 ml. of 1.2 per cent $\text{Al}(\text{OH})_3$ gel at pH 8.2 was added to 100 ml. MKTC/FT No. 1201, the pH adjusted to 6.5, and the mixture left overnight at 1°C . and centrifuged for one hour at $25,000 \times g$. The supernatant fluid from the $\text{Al}(\text{OH})_3$ -adsorbed MKTC was concentrated 6 times as for the alum-adsorption experiment.

Other challenge samples

Monolayers from 10-day-old chick embryos were grown in medium 597 containing 0.5 per cent bovine serum to yield the uninfected control tissue culture. Measles virus fluid (titer $10^{-5.3}$) was obtained from chick embryo tissue culture infected with Edmonston strain 749D but with the use of unenriched medium. Uninfected and measles-infected monolayers of human amnion serial tissue culture⁷ were grown to yield FLTC (Fogh-Lund tissue culture) and measles virus fluid FLTC (titer $10^{-5.8}$).

Passive cutaneous anaphylaxis test¹¹

The backs of normal albino guinea pigs (250 to 300 grams body weight) were shaved and depilated. The following afternoon, the animals were injected intracutaneously with 0.1 ml. aliquots of various sera in 4 to 6 sites, 5 cm. apart. Between 20 and 25 hours later, the passively immunized guinea pigs were injected intravenously with 0.5 ml. of a 1:1 mixture of the test sample and a 10 mg. per milliliter solution of pontamine sky blue 6BX dye in phosphate-buffered saline. The diameters of blue spots appearing at the site of serum injections were measured 10 minutes later and the relative intensity of each spot noted.

RESULTS

The virus-neutralizing and hemagglutination-inhibition (H.I.) titers of the guinea pig antisera to the 4 measles vaccines of MKTC origin (Pfizer-Vax, No. 101, No. 1117-4, and No. 5001) were similar (Table I) but were significantly higher than the titers of the antiserum against vaccine of chick embryo TC origin.

Table I. *Properties of vaccines and resulting guinea pig antisera*

<i>Original antigen</i>	<i>Mineral adjuvant</i>	<i>Amount of Al+++ (mg./ml. vaccine)</i>	<i>Reciprocal antibody titer of pooled serum</i>	
			<i>Neutral- izing*</i>	<i>Hemagglutina- tion-inhibiting†</i>
Pfizer-Vax measles K	Alum-precipitated	0.93	237	323
Measles vaccine of chick embryo TC origin	AlPO ₄ -adsorbed	1.07	32	102
Measles vaccine No. 101	None		178	323
Measles vaccine No. 1117-4	Al(OH) ₃ - adsorbed	0.32	178	323
DPT polio measles vaccine No. 5001	None		422	256
DPT polio vaccine No. 353A	None		724 (polio)	
DPT polio vaccine No. 353B	Al(OH) ₃ - adsorbed	0.14 (total for all components)	362 (polio)	

*Challenge doses: 14 TCID₅₀ of measles virus, 316 TCID₅₀ of Poliovirus, type 1.

†Average titer from 3 tests at a dose level of 4 hemagglutinating units.

When guinea pigs, passively immunized with antisera to the measles vaccines No. 101, 1117-4, and Pfizer-Vax, were challenged with unfiltered measles virus fluid of MKTC origin, only the antisera to the 2 adsorbed vaccines gave passive cutaneous anaphylactic (PCA) reactions (Table II). The anti-Pfizer-Vax serum diluted one-ninth gave a stronger reaction than the undiluted antiserum to the adsorbed vaccine No. 1117-4. Since there was no significant difference between the antibody titers of all 3 antisera, it appeared that the PCA test did not necessarily involve viral antigens. This was confirmed by the positive reactions of diluted antiserum to Pfizer-Vax with MKTC and poliovirus fluid. The size and intensities of the reactions to the 4 challenges suggested that growth of measles virus in MKTC was associated with the appearance of more antigenic material than either freezing and thawing the MKTC or growth of poliovirus and that processing of measles virus fluid reduced the amount of sensitizing antigens considerably.

Since the *Bordetella pertussis* vaccine component of DPT polio measles vaccine is an effective adjuvant for the viral antigens and the toxoids, it appeared reasonable to expect that the bacterial vaccine would also act as an adjuvant for MKTC antigens. In order to examine this possibility, guinea pigs were passively immunized with undiluted antiserum to DPT polio measles vaccine and with diluted antiserum to Pfizer-Vax measles K. No PCA reaction was obtained when the guinea pigs were challenged with measles virus fluid from chick fibroblast TC (Table III), whereas uninfected tissue culture of human amnion origin (FLTC), measles virus fluid from FLTC, uninfected MKTC, and measles virus fluid from MKTC all provoked PCA reactions only with the diluted antiserum to Pfizer-Vax measles K. These findings indicated that the antigens in Pfizer-Vax measles K cross-reacted with antigens from tissue culture of human origin.

The greatly reduced PCA activity of measles virus fluid of MKTC origin, treated with the proteolytic enzyme CA7, indicated that the sensitizing antigens were proteinaceous. Three passages of fluid M1201 through an epoxy-coated filter, a procedure used in the production of measles vaccines in these laboratories, also appeared effective in removing most of the MKTC antigens.

Throughout these tests, the antiserum to the unadsorbed multiple vaccine was uniformly nonreactive, indicating that the *B. pertussis* component was ineffective as an adjuvant for stimulating the sensitizing antigens. It therefore seemed reasonable to examine the striking contrast between adsorbed and unadsorbed monkey kidney-derived vaccines further with the use of guinea pigs whose skins had been passively immunized with antisera to unadsorbed and $\text{Al}(\text{OH})_3$ -containing DPT polio vaccine. Whereas a control challenge of chick embryo TC concentrated 20 times gave no PCA reactions with antisera to Pfizer-Vax measles K, unadsorbed DPT polio measles, DPT polio, and $\text{Al}(\text{OH})_3$ -containing DPT polio vaccines (Table IV) challenges with poliomyelitis and rubella vaccines provoked PCA reactions with diluted antiserum to Pfizer-Vax and undiluted antiserum to DPT polio vaccine containing a low level of mineral carrier. Unconcentrated measles vaccine No. 101 reacted only with the former and to a lesser extent than Pfizer-Vax measles K solubilized with 0.28M citric

Table II. Comparison of antisera to adsorbed and unadsorbed vaccines in the PCA

Intravenous challenge (0.25 ml. sample + 0.25 ml. dye)	No. of guinea pigs	
		Diluted 1/3
Measles virus fluid M-1201†	3	28 s
Monkey kidney tissue culture No. 1201†	4	20 m
Poliovirus fluid (Mahoney, Type 1)	2	12 f
Measles vaccine No. 101	2	11 f

*Where intensity of each spot is given by s = strong, m = moderate, and f = faint.

†Samples frozen and thawed before addition of dye solution.

Table III. PCA reactivities of measles virus fluids from monkey kidney, human amnion, and chick embryo tissue cultures

Intravenous challenge (0.25 ml. frozen and thawed sample + 0.25 ml. dye)	No. of guinea pigs	Average diameter (mm.) and intensity* of blue spot with antiserum to:			
		Pfizer-Vax measles K alum-precipitated		Measles vaccine (chick) AlPO ₄ - adsorbed, undiluted	DPT polio measles vaccine, unadsorbed, undiluted
		Diluted 1/3	Diluted 1/9		
Measles virus fluid from chick embryo TC	2	0	0	0	0
FLTC	3	20 m	13 f	0	0
Measles virus fluid from FLTC	3	25 m	19 m	0	0
MKTC No. 1201	3	20 m	10 f	0	0
Measles virus fluid M1201	4	22 m	12 f	0	0
M1201—protease digest	2	8 f	3 f	0	0
M1201—filtered	3	9 f	3 f	0	0
Bovine serum diluted 1/10	2	0	0	0	0

*Where intensity of each spot is given by m = moderate and f = faint.

acid or unfiltered measles virus fluid. In all cases, except the rubella challenge, the reactions with anti-Pfizer-Vax serum diluted one-ninth were stronger than those with undiluted antiserum to DPT polio vaccine. Treatment of measles virus fluid with Tween-ether had no significant effect on the PCA activity of the aqueous phase, and the fivefold concentrated ether phase gave only a faint reaction with antiserum to Pfizer-Vax.

Two major peaks with a minor peak between them were obtained by molecular sieve filtration of sixfold concentrated and centrifuged MKTC on Sephadex G-100, and the eluate was divided into 4 fractions as shown in Fig. 1. PCA tests on the highest and lowest molecular weight fractions (F1 and F4) indicated that they contained only small amounts of the MKTC antigens (Table V). The material in the minor peak (F3), representing 19 per cent of the total protein applied to the column, had the highest PCA activity, and a challenge dose containing 9 μ g protein provoked a positive reaction. It was of note that F3

test

Average diameter (mm.) and intensity* of blue spot with antiserum to:				
Pfizer-Vax measles K alum-precipitated		Measles vaccine No. 101 unadsorbed		Measles vaccine No. 1117-4 Al(OH) ₃ - adsorbed, undiluted
Diluted 1/9	Diluted 1/27	Undiluted	Diluted 1/3	
19 s	13 f	0	0	19 f
11 m	0	0	0	2 f
7 f	0	0	0	0
0	0	0	0	0

concentrated 25 times gave very strong reactions with antisera to Pfizer-Vax and Al(OH)₃-containing DPT polio vaccine but did not react with the antisera to unadsorbed DPT polio and DPT polio measles vaccines.

Concentrated supernatant fluid from MKTC either precipitated in situ with alum or adsorbed with Al(OH)₃ gel was devoid of PCA activity, indicating that the sensitizing antigens were effectively adsorbed on the mineral adjuvant with either method. In contrast, the supernatant liquid from MKTC, treated with a preformed alum suspension at pH 7.6, gave strong PCA reactions. This suggested the possibility of separating the immunogenic portion of measles vaccine from the MKTC antigens by an adsorption-elution process. The high PCA activity in the supernatant fluid from the calcium phosphate-treated measles vaccine Lot 1163 and the nonreactivity of the solubilized fraction indicated that the bulk of the sensitizing antigens had been removed from the vaccine.

DISCUSSION

The PCA results indicate that injection into guinea pigs of alum-precipitated or aluminium hydroxide-adsorbed measles vaccines of primary monkey kidney tissue culture origin causes production of anaphylactic antibodies directed against proteinaceous components of MKTC, whereas unadsorbed measles and DPT polio measles vaccines have no apparent sensitizing activity. The ability to sensitize guinea pigs to monkey kidney antigens is not a unique property of adsorbed measles vaccines since DPT polio vaccine, containing only a small amount of aluminium hydroxide, also elicits production of anaphylactic antibodies, although in lower titer. The antisera to alum-precipitated measles and Al(OH)₃-containing DPT polio vaccines react in the PCA test with challenges consisting of poliomyelitis or rubella vaccines derived from Cynomolgus and Cercopithecus MKTC, respectively, indicating that similar antigens are present in poliomyelitis, rubella, and measles vaccines from both types of MKTC. The antisera to unadsorbed measles, DPT polio, and DPT polio measles vaccines have high titers of virus-neutralizing antibodies and are uniformly unreactive in the PCA test. It is unlikely, therefore, that viral antigens are involved in the latter test at the levels of challenges used in these studies. This is confirmed by the non-reactivity of the antiserum to adsorbed measles vaccine of chick embryo origin.

Table IV. Detection of monkey kidney tissue culture antigens in various viral vaccines

Intravenous challenge (0.25 ml. frozen and thawed sample + 0.25 ml. dye)		No. of guinea pigs	Average diameter* (mm.) and intensity† of blue spot with antiserum:		
			Pfizer-Vax measles K alum-precipitated		DPT polio vaccine Al(OH) ₃ -adsorbed, undiluted
			Diluted 1/3	Diluted 1/9	
Chick embryo TC	Conc. ×20	3	0	0	0
Poliomyelitis vaccine	Conc. ×36	3	26 s	22 s	16 f
Poliovirus vaccine (Sabin, Type 3)	Conc. ×2	2	27 s	23 s	0
Heated rubella virus fluid HPV 80	Conc. ×5	2	26 s	17 s	29 s
	Uneconc.	2	3 f	0	14 f
Measles vaccine No. 101	Conc. ×5	4	20 s	12 s	0
	Uneconc.	2	7 f	3 f	0
Solubilized Pfizer-Vax measles K	Uneconc.	3	19 m	12 f	0
Measles virus fluid M-1201	Uneconc.	3	19 m	11 f	0
Tween 80: ether-treated M-1201—					
Aqueous phase	Uneconc.	2	23 m	17 m	0
Organic phase	Conc. ×5	3	13 f	9 f	0

*In no case was there a reaction with undiluted antisera to either unadsorbed DPT polio or DPT polio measles vaccines.

†Where intensity of each spot is given by s = strong, m = moderate, and f = faint.

Uninfected MKTC appears to be almost as effective a challenge as measles virus fluid in the PCA test, suggesting that the sensitizing antigens are normal components of MKTC. Although the antigens show considerable molecular heterogeneity on Sephadex G-100 gel filtration, they appear to have molecular weights mainly in the range 10,000 to 100,000. In view of the well-known adjuvant effect of *B. pertussis* vaccine on low molecular weight antigens, it is of interest that unadsorbed DPT polio and DPT polio measles vaccines are non-sensitizing, whereas Al(OH)₃-adsorbed measles vaccine provokes production of anaphylactic antibodies in low titer against the MKTC antigens, reflecting differences in the action of the bacterial and mineral adjuvants in the respective vaccines.

The present studies indicate that the PCA test is a sensitive monitor of non-viral antigens in measles, poliomyelitis, and rubella vaccines grown in MKTC. The contamination of poliomyelitis with tissue culture antigens could explain the local reactions in children immunized with Pfizer-Vax measles K and challenged intradermally with poliomyelitis vaccine,⁵ but there would appear to be no direct relationship between the findings with the PCA test and the dermal reactions in children challenged with live attenuated measles vaccine of chick embryo origin (Rubeovax). However, a rough correlation has been found in these laboratories between the local reactions of children to a Rubeovax booster after immunization with inactivated measles vaccines and the PCA reactivities of guinea pig antisera to the inactivated vaccines. In a trial with infants,¹² 7 out of 8 subjects immunized with Pfizer-Vax measles K gave erythematous reactions with diameters greater than 12 mm. (average 20 by 30 mm.) within 3

Table V. Size and adsorption properties of monkey kidney tissue culture antigens

Intravenous challenge (0.25 ml. frozen and thawed sample + 0.25 ml. dye)		No. of guinea pigs	Average diameter* (mm.) and intensity† of blue spot with antiserum to:		
			Pfizer-Vax measles K alum-precipitated		DPT polio vaccine Al(OH) ₃ -adsorbed, undiluted
			Diluted 1/3	Diluted 1/9	
Monkey kidney TC	Conc. ×6	3	28 s	17 s	16 f
MKTC/G100-Sephadex filtration—					
F1	Conc. ×25	2	24 m	15 m	14 f
	Conc. ×6	2	14 f	7 f	0
	Uneconc.	1	0	0	0
F2	Conc. ×25	2	33 s	26 s	25 m
	Conc. ×6	2	23 s	17 s	0
	Uneconc.	1	0	0	0
F3	Conc. ×25	2	41 s	31 s	34 s
	Conc. ×6	2	45 s	40 s	30 f
	Uneconc.	2	22 f	11 f	0
F4	Conc. ×25	3	5 f	4 f	0
Supt. from alum-precipitated MKTC	Conc. ×5	2	0	0	0
Supt. from Al(OH) ₃ -adsorbed MKTC	Conc. ×6	2	0	0	0
Supt. from alum-adsorbed MKTC	Conc. ×6	2	29 s	21 s	14 m
Supt. from No. 1163	Conc. ×5	3	29 s	19 s	5 f
Measles vaccine No. 1163	Conc. ×5	5	0	0	0

*In no case was there a reaction with undiluted antisera to either unadsorbed DPT polio or DPT polio measles vaccines.

†Where intensity of each spot is given by s = strong, m = moderate, and f = faint.

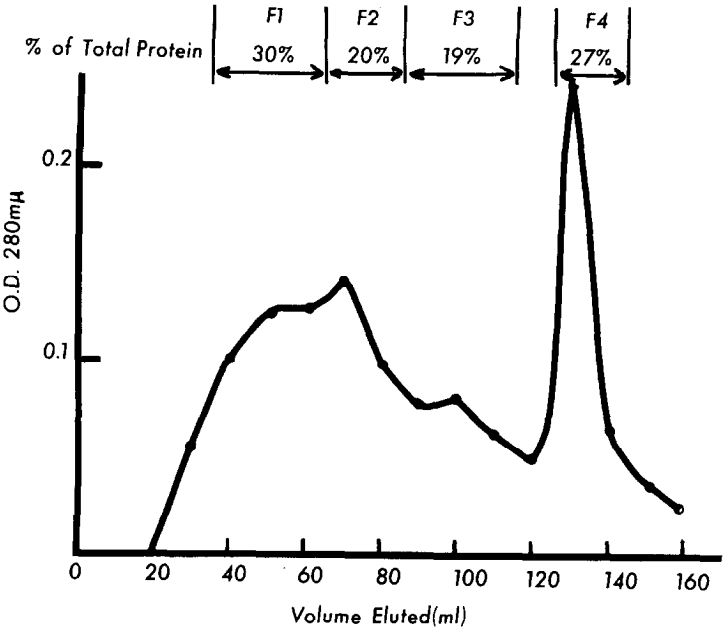


Fig. 1
Molecular sieve filtration of frozen and thawed monkey kidney tissue culture on Sephadex G-100.

days of receiving a Rubeovax injection, whereas only 3 out of 14 infants given $\text{Al}(\text{OH})_3$ -adsorbed measles vaccine No. 1117-4 reacted (average size 11 by 22 mm.), and there were no detectable reactions in the children given unadsorbed DPT polio measles vaccine No. 5001 (cf PCA results in Tables III and IV). It is evident that procedures which reduce the amount of MKTC antigens in measles vaccines also reduce the local reactions of infants to live attenuated measles vaccine, and these findings suggest the desirability of the use of unadsorbed measles vaccine for human immunization. It appears likely that the vaccination of children with Pfizer-Vax measles K provokes production of either cell-bound or reaginic antibodies directed against MKTC components of the vaccine, but occasionally these antibodies may also react with autologous tissue components released by the cytopathogenic effect of measles infection or an injection of attenuated measles virus vaccine.

The provocation of cross-reactive immunity by foreign related tissue antigens is well documented,¹³ and examples include the sensitization of rabbits to autologous tissue components by immunization with rat tissues¹⁴ and induction by Group A streptococcal infection in human subjects of antibodies which can react with human heart tissue as well as the bacterial cell-wall antigens.¹⁵

A recent note¹⁶ has suggested that the sensitizing factor in measles vaccines grown in MKTC may be removed in the organic phase by Tween 80-ether treatment, as shown by the Schultz-Dale technique in guinea pigs. Results in these laboratories¹⁷ with Pfizer-Vax-sensitized guinea pigs in the Schultz-Dale test have largely paralleled those obtained with the PCA test, and, in both cases, the bulk of the antigenic activity was in the aqueous phase. Furthermore, an unadsorbed DPT polio measles vaccine grown on MKTC which does not sensitize children to a Rubeovax booster injection will not sensitize guinea pigs to MKTC antigens either in the Schultz-Dale or PCA tests. Both tests can be used as monitors for the removal of tissue culture components from measles vaccines by a procedure¹⁰ which does not greatly reduce the amount of viral antigens. The MKTC material is therefore not intimately associated with the measles virus, in contrast to the suggestion¹⁸ that myxoviruses incorporate host macromolecules into the viral envelope.

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