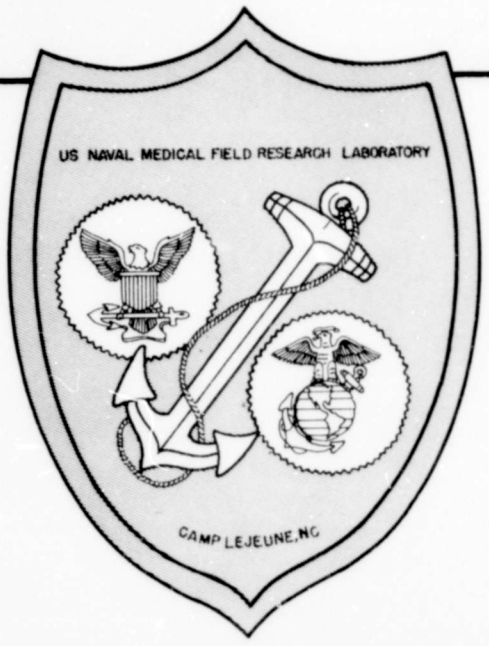

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RESEARCH LABORATORY
CAMP LEJEUNE, NORTH CAROLINA

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Bureau of Medicine and Surgery, Navy Department
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SUMMARY PAGE

THE PROBLEM

An epidemic of acute respiratory disease (ARD) associated with adenovirus type 4 occurred in recruits who had been vaccinated with living adenovirus type 4 vaccine (LAV) by the gastrointestinal route. The vaccination utilizing a potent LAV during the ARD epidemic unexpectedly did not reduce the incidence of disease.

FINDINGS

The original vaccination with LAV type 4, lot number 16-CI-00103, was only partially effective due to low potency of that lot. Administration of a potent vaccine induced antibody as expected in men vaccinated for the first time but the response rate was much less than expected in men who were revaccinated. The epidemic treated in which adenovirus type 4 was anticipated was subsequently found to be associated with adenovirus type 7.

RECOMMENDATIONS

The duration of the protection provided by LAV type 4 should be evaluated. The response to revaccination should be studied, and the use of alternate routes of vaccine administration (intranasally) should be considered for both primary vaccination and revaccination.

Potency of adenovirus vaccines should be monitored to prevent inadvertent use of low potency vaccines.

ADMINISTRATIVE INFORMATION

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This restriction will be removed and the report may be released on 1 March 1970.

ABSTRACT

Acute respiratory disease (ARD) associated with adenovirus type 4 occurred in recruits vaccinated with low titered living adenovirus type 4 vaccine (LAV). Only 29% of the vaccinees had neutralizing antibodies two months postvaccination. Upon revaccination with higher titered LAV, there was an 86% response in men vaccinated for the first time but only a 47% response in men who were revaccinated.

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INTRODUCTION

Beginning in October 1967 all recruits entering the Marine Corps Recruit Depot at Parris Island, South Carolina received living adenovirus type 4 vaccine (LAV). This program was continued through the end of December 1968, at which time the existing supply of vaccine had become exhausted. The first unvaccinated recruits were to arrive at Camp Lejeune in early March 1969.

There was an epidemic of acute respiratory disease (ARD) associated with adenovirus type 4 in late January 1969. The hospitalization rate for ARD began to peak again in early March and this was thought to be a continuation of the adenovirus type 4 epidemic in unvaccinated recruits reporting to Camp Lejeune. Attempting to control the epidemic, 7000 doses of adenovirus type 4 vaccine were administered. Recruits training at Camp Lejeune who had arrived during the preceding three weeks and all recruits scheduled to arrive at Camp Lejeune during the subsequent four weeks were vaccinated (Tables 1 and 2). This approach to epidemic control had been demonstrated by this laboratory to be effective during the 1967 ARD epidemic (Figure 1). However, the 1969 ARD epidemic was uninfluenced by the vaccine.

A retrospective serologic study was undertaken to determine why men who had been vaccinated only months before were sick with adenovirus type 4 ARD and to evaluate the individual response to the adenovirus type 4 vaccine administered in March 1969.

Table 1

Living Adenovirus Type 4 Vaccine Administered at ITR,* 13-14 March 1969, Lot No. 16-CI-00101 (2300 doses available)

Company	Date Formed	No Pills Admin.	ITR* Strength (weekly)
Z	12 Feb 69	224	4662
I	18 Feb 69	222	5064
W	19 Feb 69	228	
F	25 Feb 69	253	4962
A	4 Mar 69	222	4685
N	5 Mar 69	216	
Y	5 Mar 69	223	
P (1st)	11 Mar 69	214	4932
(2nd)		168	
X	11 Mar 69	215	
		2185	

*1st Infantry Training Regiment, Marine Corps Base, Camp Lejeune, N. C. (4932 recruits in training week of 13 March 1969 at ITR; 2185 doses administered, or 44.3% received the vaccine).

MATERIALS AND METHODS

Titrations

Virus titrations and serum neutralizing antibody assays were performed by a micromethod utilizing HEp-2 tissue culture. Disposable plastic plates containing 96 "U"-shaped wells were washed in ethanol and sterilized by ultraviolet irradiation. Adenovirus titrations were carried out with five wells per dilution. Virus dilutions and HEp-2 cells suspended in growth media were inoculated simultaneously. The wells were then overlayed with mineral oil to provide a bacteriological and CO_2 seal. Serum neutralization tests were carried out with three or four wells per dilution. Challenge virus was diluted in tissue culture growth medium and inoculated at approximately 32 $TCID_{50}$ per well. The virus-serum mixture was allowed to incubate at room temperature for one hour, HEp-2 cells were added and the plate was sealed with mineral oil. Plates were incubated at 35°C and read with an inverted microscope at 24 and 48 hours. Adenovirus cytopathic effect end point was usually completed at 48 hours.

Table 2

Parris Island Platoons Administered Living Adenovirus Type 4 Vaccine
13 March 1969,* Lot No. 16-CI-00101 (4800 Doses)

Platoon	Date	R.O.S.†	Platoon	Date	R.O.S.†	Platoon	Date	R.O.S.†
1052	11/10/68	7999	1068	12/16/68	7124	115	1/27/69	7449
1053	to		2066	to		116	to	
1054	11/16/68		2067	12/22/68		117	2/2/69	
1055						118		
3052			1069	12/23/68	7749	119		
			1070	to		216		
2052	11/17/68	7211	1071	12/29/68		217		
2053	to		3068			218		
2054	11/23/68		3069			219		
2055			3070			312		
3055			3071			313		
						314		
1056	11/24/68	7043	100	12/30/68	7838	315		
1058	to		101	to		316		
1059	11/30/68		102	1/5/69		317		
2056			103			318		
2057			300					
2058			301			120	2/3/69	8089
3056			302			121	to	
3057			303			122	2/9/69	
3058						123		
3059			208	1/6/69	7717	220		
			209	to		221		
1060	12/1/68	7418	304	1/12/69		222		
1061	to		305			223		
1062	12/7/68		306			319		
1063			307			320		
1064								
2059			108	1/13/69	7392	124	2/10/69	7993
2060			109	to		125	to	
2061			110	1/19/69		224	2/16/69	
2062			111			225		
2063			210			226		
3060			211			227		
3061			308			321		
3062			309			322		
3063						323		
			112	1/20/69	7334			
1065	12/8/68	7462	113	to		126	2/17/69	7628
1066	to		114	1/26/69		127	to	
1067	12/15/68		212			228	2/23/69	
2064			213			229		
2065			214			230		
3064			215			231		
3065			310					
3066			311					
3067								

*Week of 13 March 1969 there were 8691 recruits on Station: 1st Bn., 2626; 2nd Bn., 3087; 3rd Bn., 2978; 4796 or 55.2% received the vaccine.
†Recruits on Station.

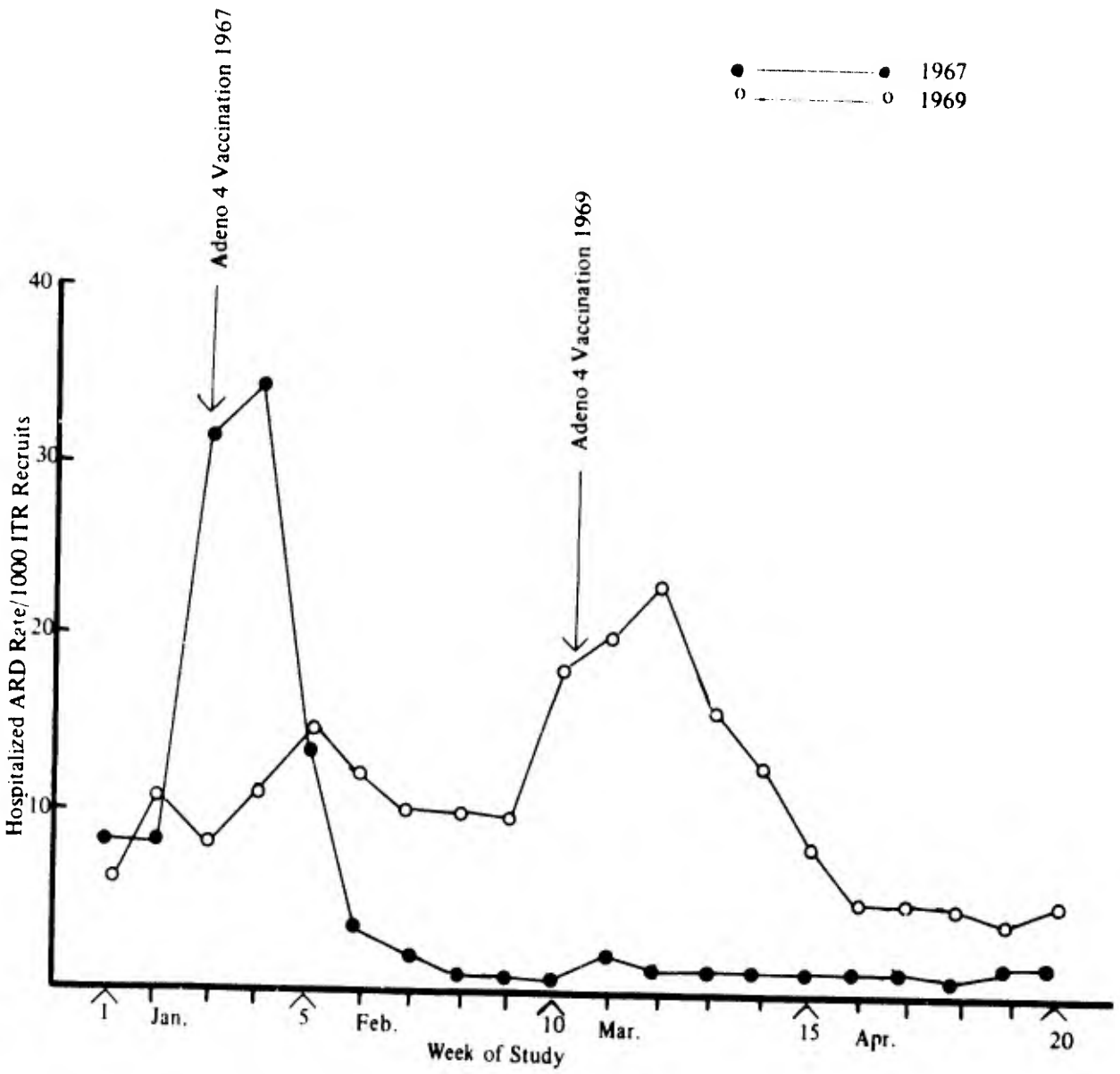

Figure 1. Adeno 4 ARD Epidemic Control by Mass Vaccination.

Titrations of vaccine pills were carried out in human embryonic kidney tissue culture tubes.

Subjects

All subjects in these studies were recruits who either appeared at sick call or were hospitalized for respiratory disease. Acute and two-week convalescent sera as well as throat and rectal cultures were collected. Men were selected for vaccine evaluation if they had both received the vaccine and were subsequently collected by our surveillance within the two weeks postvaccination.

RESULTS AND DISCUSSION

The pattern of ARD for 1969 was particularly interesting. There are three peaks in the hospitalization rates for ARD (Figure 2). The first, in early January, was associated with a high isolation rate of Hong Kong influenza. Adenovirus type 4 isolation corresponded with the second peak. All recruits training at Camp Lejeune at that time had been vaccinated against adenovirus type 4. The third peak appeared in late February and early March at the time the first unvaccinated recruits arrived at Camp Lejeune. Isolation rates shown in Figure 3 reveal that the third peak was not associated with adenovirus type 4 as expected but was instead associated with adenovirus type 7. Vaccination for adenovirus type 4 in mid-March had no effect on the hospitalization rates and little effect on the isolation rate for adenovirus type 7. Adenovirus type 4 isolation was probably prolonged by recovery of vaccine virus shed in the stool.

The first problem to be answered was why were men who had been vaccinated in October becoming sick with adenovirus type 4 in January. There were only a few possibilities: (1) they had not been vaccinated, (2) the vaccine had failed to produce immunity, and (3) the level of immunity induced by the vaccine had decreased and some of the men were now susceptible.

Neutralizing antibody levels were determined in 62 men from four Parris Island platoons vaccinated in October 1968. The number of susceptibles in all four platoons was high and nearly the same (Table 3). It was assumed that the vaccine had not been omitted. If only some of the men in these platoons had escaped vaccination, the prevalence of immunity should have been high. That the number of men not having adenovirus type 4 neutralizing antibody was high in all four platoons implies that the vaccine had failed to produce antibody in most of the men or that the level of neutralizing antibody had decreased in many of the men. Upon titration of a few of the pills left over from the lot used at Parris Island (Lot 16-CI-00103), we found that it contained only $10^{2.7}$ $TCID_{50}$ of viable virus. This was much lower than the original titer. Based on data published from this laboratory by Gutekunst *et al.*,[1] vaccination with $10^{2.7}$ $TCID_{50}$ adenovirus type 4 vaccine should have produced neutralizing antibody in approximately 50% of the vaccinees within 30 days of vaccination. At three months, however, we demonstrated only

Table 3

Adenovirus Type 4 Neutralizing Antibody Status Three Months After Vaccination

Platoon No.	No. Men	Percent Without Antibody
3036	12	67%
3039	23	70%
1040	12	59%
1041	15	87%
Total:	62	Mean: 71%

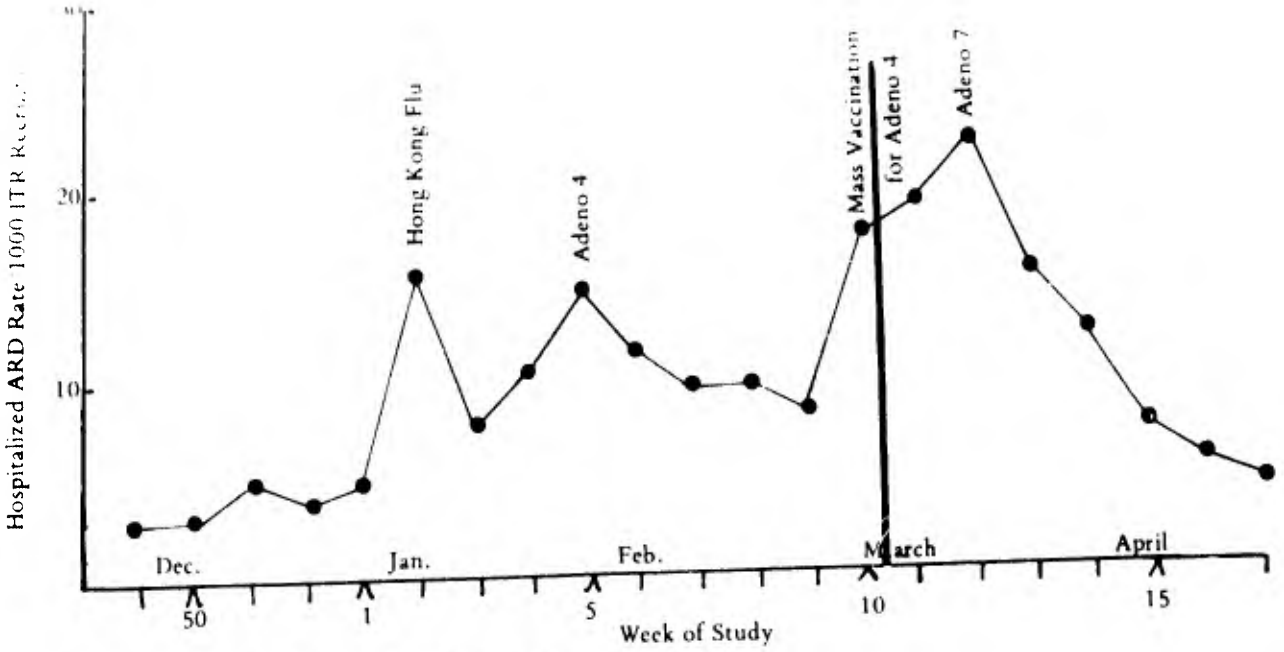

Figure 2. Hospitalization for ARD 1969

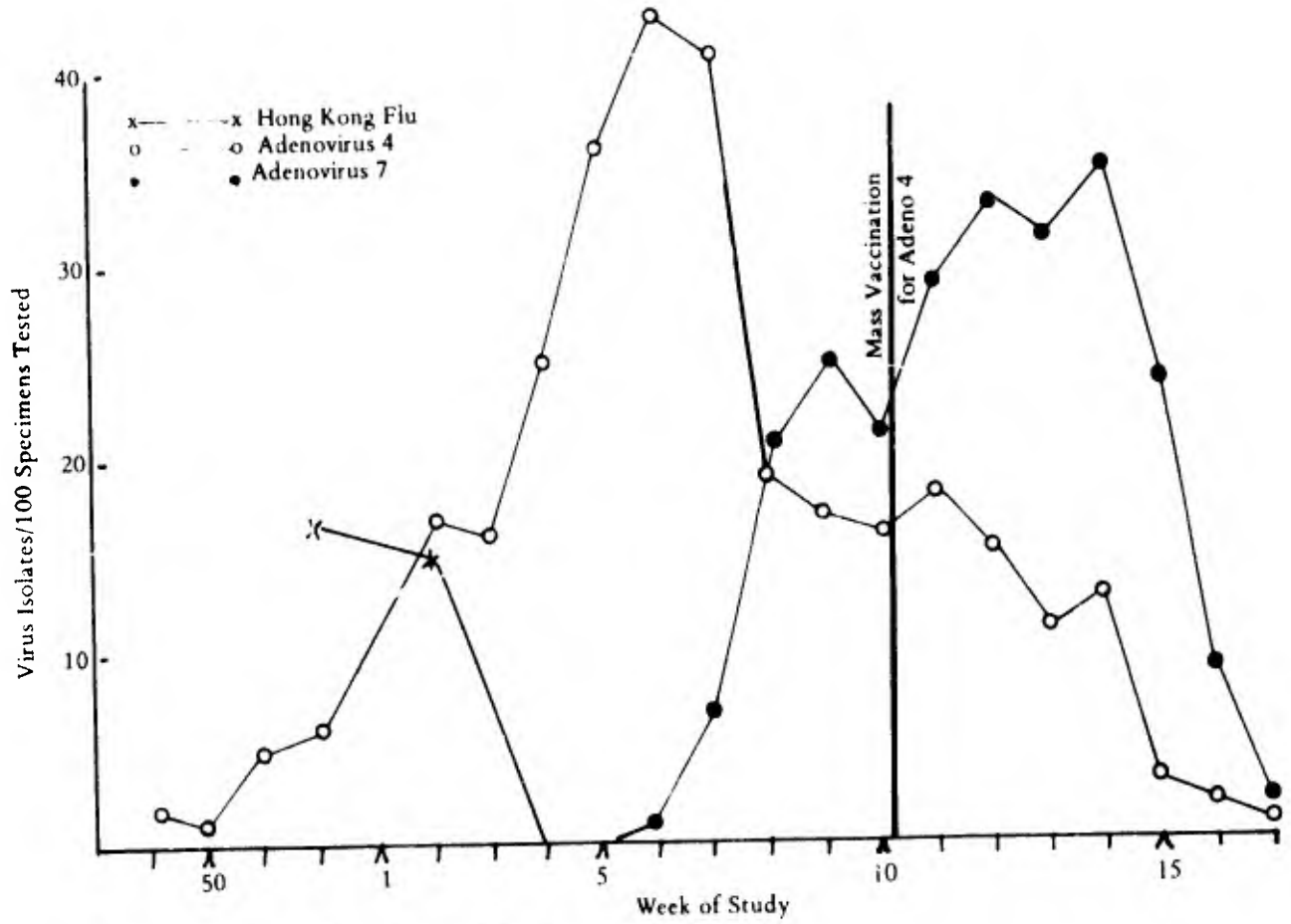

Figure 3. Virus Isolation Rate ARD Season 1969

29% were immune in our four platoons. Therefore, it appears that two factors were operating to allow an epidemic in vaccinated men. There was deterioration of the vaccine, thus inducing a low level of immunity and there was some loss of neutralizing antibody.

The vaccine administered in March 1969 was from lot number 16-CI-00101 which titered $10^{3.7}$ $TCID_{50}$ per pill. Great care was taken to ensure that each recruit swallowed the vaccine. A group of recruits were studied to assess the response to the new vaccine. For one-third of these men this was a primary vaccination and for two-thirds this was a revaccination. Acute phase sera were evaluated for neutralizing antibody levels (Table 4). In the primary vaccination group, 94% had titers less than 1:10. As expected from the four Parris Island platoons studied previously, 85% of the men already vaccinated once at Parris Island had adenovirus type 4 neutralizing antibody levels 1:10 or less. We have found that titers of 1:10 or less are not uniformly protective and that the antibody response to adenovirus type 4 disease does not appear to be anamnestic with a vaccine induced neutralizing antibody level of 1:10. The response to the vaccine is demonstrated in Table 5. Of the primary vaccination group, 86% developed adenovirus type 4 neutralizing antibody. Only 47% of the revaccinated men developed a neutralizing antibody response. One man in the revaccination group had an apparent anamnestic response. As expected, both groups developed antibody to adenovirus type 7 as a result of the ARD epidemic.

Table 4

Primary vs Revaccination Adenovirus Type 4 Neutralizing Antibody Status

	No. Men	Without Acute Phase Antibody
Primary Vaccination Group	15	14 (94%)
Revaccination Group*	26	22 (85%)

*Primary vaccination 2 months prior to revaccination.

Table 5

Primary vs Revaccination Neutralizing Antibody Response to Adenovirus Type 4 Vaccination

	No. Paired Sera	Developing Adeno 7 Antibody	Developing Adeno 4 Antibody
Primary Vaccination Group	7	4 (57%)	6 (86%)
Revaccination Group*	17	13 (76%)	8 (47%)†

*Primary vaccination 2 months prior to revaccination.
†Expect 70-82% response rate.

Based on dose response data published by Gutekunst *et al.*,[1] Figure 4 was constructed. From this we would expect 70-80% of the men to develop neutralizing antibody in response to the March vaccine which titered $10^{3.7}$ $TCID_{50}$. This was the case with the primary vaccination group. The 47% response in the revaccination group was well below that expected and was approximately the number of men, extrapolated from the dose response curve, that should have remained susceptible after their first vaccination with the low titered lot.

Why only 47% of the men in the revaccination group responded to the adenovirus type 4 vaccine, while 86% of the primary vaccination group responded, is of great interest. It is unlikely that the adenovirus type 7 epidemic altered the revaccination response.[2] The

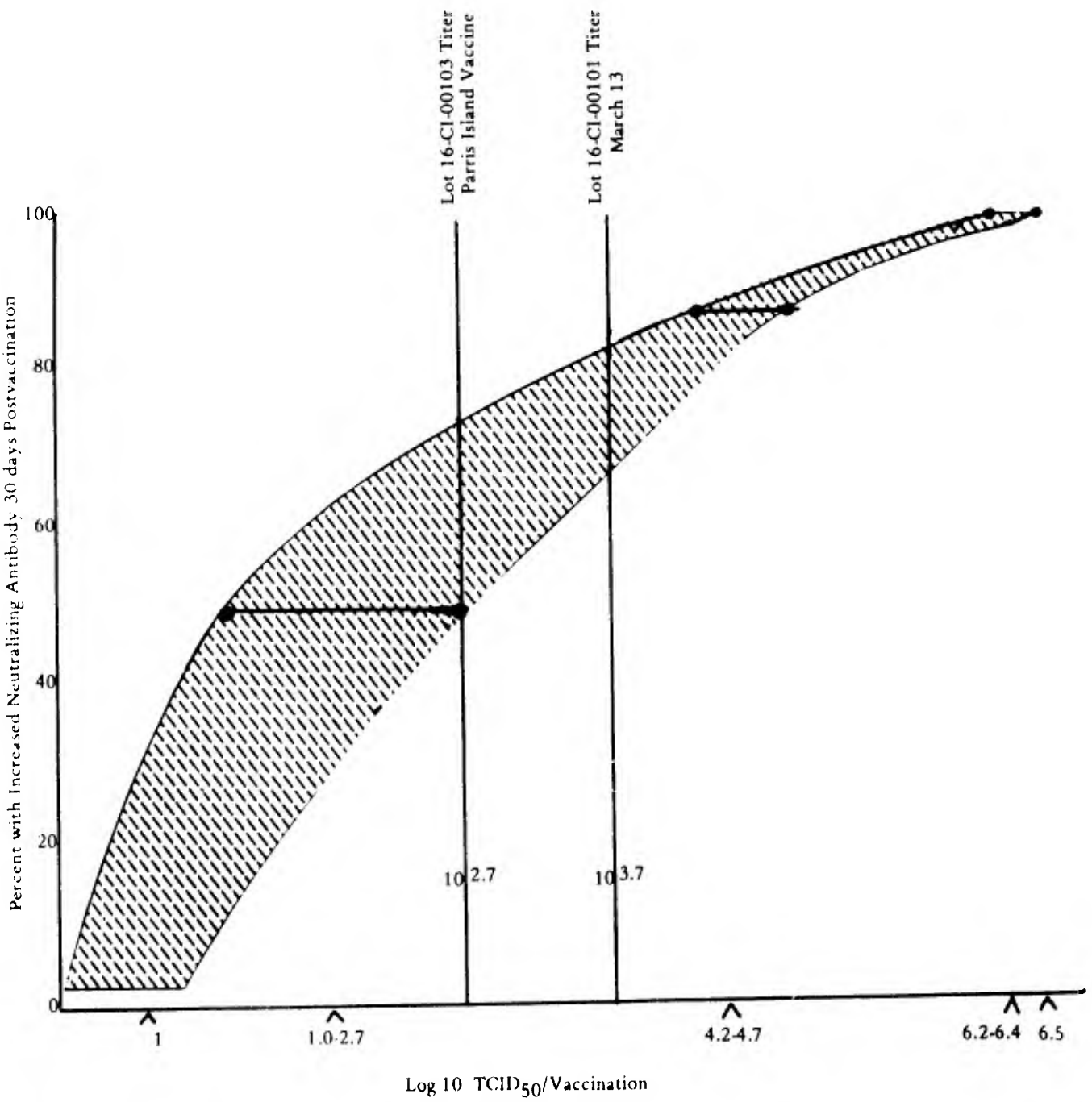

Figure 4. Dose Response Curve for Adenovirus Type 4 Vaccine

hypothesis we put forth is that the response to initial vaccination in December was as expected with approximately 50% of the men developing antibody. With vaccination via the gastrointestinal tract, these men developed serum neutralizing antibody and local secretory antibody.[3] The levels of serum antibody in some men fell to 1:10 or less but we suspect that their gastrointestinal secretory antibody remained. Therefore, upon revaccination approximately 50% of the men showed no response because they were protected by gastrointestinal antibody. These men with no protective serum antibody and no respiratory tract secretory antibody were susceptible to invasion of the respiratory tract and developed adenovirus type 4 ARD. We are presently evaluating this hypothesis by directly measuring secretory antibody response to the oral vaccine and its effect upon revaccination.

RECOMMENDATIONS

The duration of the protection provided by LAV type 4 should be evaluated. The response to revaccination should be studied, and the use of alternate routes of vaccine administration (intranasally) should be considered for both primary vaccination and revaccination.

Potency of adenovirus vaccines should be monitored to prevent inadvertent use of low potency vaccines.

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13. ABSTRACT

Acute respiratory disease (ARD) associated with adenovirus type 4 occurred in recruits vaccinated with low titered living adenovirus type 4 vaccine (LAV). Only 29% of the vaccinees had neutralizing antibodies two months postvaccination. Upon revaccination with higher titered LAV, there was an 86% response in men vaccinated for the first time but only a 47% response in men who were revaccinated. (U)

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