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Received Nov. 6, 1967. P.S.E.B.M., 1968, Vol. 127.

Immune Response Following Simultaneous Administration of Attenuated Trivalent Poliovirus and Type 4 Adenovirus Vaccines* (32826)

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The efficacy of a live oral adenovirus type 4 vaccine in controlling acute respiratory disease due to this agent in military recruits is well established (1,2). Since adenovirus infections occur early in training and cause a costly interruption of the training cycle, this vaccine should be administered shortly after arrival at a recruit camp (3). Marine recruits at Parris Island, South Carolina currently receive seven separate immunizations, during their first training week, including oral trivalent poliovirus, and smallpox vaccines.

The question of diminished immune response following simultaneous administration of live virus vaccines has not been completely resolved. The Public Health Service Advisory Committee on Immunizations suggests that whenever feasible attenuated poliovirus and measles virus vaccines should be administered separately with at least 1 month between immunizations (4). Other reports, however, indicate that attenuated measles vaccine and smallpox vaccine may be simultaneously administered (5). Serologic and epidemiologic evidence of interference by enteric viruses with attenuated poliovirus vaccination is well documented (6–10). Attenuated adenovirus type 4 immunizes by establishing a silent infection in the lower gastrointestinal tract but interference of this infection by enteric viruses has not been reported. In 1966 successful simultaneous immunization with monovalent type 1 attenuated poliovirus and the oral live adenovirus type 4 vaccine was achieved (11). This paper describes the results of simultaneous administration of live attenuated poliovirus and adenovirus vaccines; no serologic evidence of interference was noted.

Methods. Three platoons of recruit volunteers at Marine Corps Recruit Training Depot, Parris Island, South Carolina were vaccinated simultaneously according to the following schedule: (a) Platoon 240 received live attenuated adenovirus type 4 as a tablet containing $10^{5.3}$ $TCID_{50}$.[1] (b) Platoon 246 received a single dose of the attenuated trivalent poliovirus vaccine.[2] (c) Platoon 247 was vaccinated simultaneously with both the attenuated poliovirus and adenovirus vaccines.

All recruits were bled immediately prior to vaccination and 21 days later. Sera were stored at —20°C before use. Serum neutralizing antibody titers to adenovirus type 4 were determined in human embryonic kidney tissue culture employing 10–32 $TCID_{50}$ adenovirus type 4. Prevaccination sera from platoons 240 and 247 were screened at a 1:4 dilution for adenovirus type 4 neutralizing antibody. Quantitative antibody response to the attenuated adenovirus vaccine was determined on sera from 25 recruits lacking initial neutralizing antibody at the 1:4 dilution.

Poliovirus neutralizing antibody titers were determined on pre- and postvaccination sera

* The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

[1] The experimental vaccine (Lot No. 16CI-00101) was prepared by Wyeth Laboratories. This was supported by Public Health Service Contracts 43-62-842 and 43-62-40 from the Vaccine Development Branch, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland.

[2] Orimune (Lederle) containing $10^{5.9}$, $10^{5.0}$, and $10^{5.7}$ $TCID_{50}$ Types I, II, and III poliovirus, respectively.

TABLE I. Neutralizing Antibody Response Following Administration of Live Type 4 Adenovirus Vaccine.

Platoon	Vaccine group	No. without preexisting antibody (<1:4)	No. with a fourfold rise	Geometric mean titer
240	Adenovirus vaccine (68 total men)	46	41 (89%)	119.5
247	Trivalent poliovirus vaccine (1 dose) + adenovirus vaccine (65 total men)	35	28 (80%)	92

from 25 men in platoons 246 and 247. One hundred $TCID_{50}$ of each poliovirus serotype was employed in a standard tube neutralization test employing HEp-2 tissue. Destruction of 50% or greater of the cell sheet was interpreted as lack of virus neutralization. The polioviruses used in the test were Mahoney, MEF, and Saukett for types 1, 2, and 3 respectively.

Results. Forty-six recruits in platoon 240, receiving only attenuated adenovirus, had prevaccination antibody titers less than 1:4. Of these 46, 41 or 89% developed fourfold or greater rises in neutralizing antibody titers. Thirty-five men in platoon 247, immunized with both vaccines, lacked initial neutralizing antibody to adenovirus type 4, and of this group, 28 or 80% developed fourfold or greater rises in titer. Geometric mean antibody titers of 119 and 92 were obtained on sera from 25 men each in platoons 240 and 247, respectively. The difference in the number of men immunized and in the geometric mean titers is not significant (Table I).

To determine the antibody response to the trivalent poliovirus vaccination, sera from 25 men from platoons 246 (poliovirus only group) and 247 (combined adenovirus and poliovirus vaccines) were tested quantitatively for neutralizing antibody response to each of the poliovirus serotypes. There was no significant difference in the number of men in each platoon developing a fourfold antibody rise to any of the poliovirus serotypes (Table II). The geometric mean fold increases in antibody titer were of the same order of magnitude in both platoons for all serotypes. The post vaccine geometric mean titers were lower in men receiving both vaccines, but were still well above minimal protective levels (456 and 675 for platoons 247 and 246, respectively). The difference between these two titers is not significant.

The effect of preexisting poliovirus neutralizing antibody on vaccine response is presented in Table III. As noted previously, the number developing fourfold or greater rises in specific neutralizing antibody titers is similar for both platoons. Prior to vaccination only six individuals had low levels of antibody (1:4 or less) to any of the three poliovirus serotypes, and of these 6, 5 responded to the

TABLE II. Poliovirus Neutralizing Antibody Responses Following Simultaneous Administration of Trivalent Poliovirus and Type 4 Adenovirus Vaccines.

			No. of men with 4-fold or greater neutralizing antibody responses to:					
Platoon	Vaccine	No. of men tested	Poliovirus I	Geometric mean fold antibody rise	Poliovirus II	Geometric mean fold antibody rise	Poliovirus III	Geometric mean fold antibody rise
246	Trivalent poliovirus	25	10	17	14	8.8	12	7.1
247	Trivalent poliovirus plus type 4 adenovirus	25[a]	10	8	13	11	14	7.2

[a] All men showed 4-fold or greater rises to adenovirus type 4.

TABLE III. Number of Men Developing a Fourfold or Greater Antibody Rise Following Administration of Trivalent Poliovirus Vaccine.

	Poliovirus serotype:							
	I		II		III		Total of I, II, III	
Prevaccine titer	246[a]	247[b]	246	247	246	247	246	247
4	1/1[c]	1/1	1/1	0/0	0/0	2/3	2/2	3/4
8	0/0	0/0	0/0	0/0	1/1	0/0	1/1	0/0
16	3/3	1/2	2/2	6/6	3/3	2/3	8/8	9/11
32	0/0	1/2	0/0	0/0	1/1	1/1	1/1	2/3
64	3/8	3/3	5/8	5/7	1/1	3/3	9/17	11/13
128	0/0	2/5	2/4	1/1	2/2	0/9	4/6	3/7
256	3/8	2/8	4/6	1/9	4/14	6/7	11/28	9/24
512	0/1	0/2	0/0	0/0	0/1	0/2	0/2	0/4
1024	0/4	0/2	0/4	0/2	0/2	0/5	0/10	0/9
Total	10/25	10/25	14/25	13/25	12/25	14/25	36/75 (48%)	37/75 (49%)

[a] Platoon 246 immunized with attenuated type 4 adenovirus vaccine.

[b] Platoon 247 immunized simultaneously with attenuated type 4 adenovirus and trivalent poliovirus vaccines.

[c] Denominator is number of men in group; numerator is number developing 4-fold or greater rise in specific neutralizing antibody.

vaccine. The antibody response in men with preexisting titers of 1:16 or greater was similar for both vaccine groups. Two exceptions were noted in the group with preexisting antibody levels at 1:256: (i) Only 1 of 9 recruits in platoon 247 (combined vaccine group) developed a rise to poliovirus type II, whereas 4 of 6 recruits in platoon 246 (poliovirus vaccine group) responded to vaccination; (ii) In contrast, 6 of 7 men in platoon 247 developed a rise to poliovirus type III but only 4 of 14 men in platoon 246 showed a response to this serotype. The cumulative number responding to all serotypes was almost identical in the two platoons.

Discussion. Interference in the immune response to attenuated poliovirus by enteric viruses, and other poliovirus serotypes has been documented by epidemiologic and serologic studies (6,8,9). Epidemiologic evidence also indicates interference by attenuated type II poliovirus with a wild epidemic type I strain (7). Interference of vaccine response by naturally occurring enteric viruses may be overcome on a community level by sequential mass vaccination with attenuated trivalent poliovirus vaccine (12).

In vitro interference of poliovirus infection by a DNA virus, vaccinia, has been reported (13), but there are no reports of *in vivo* interference of attenuated poliovirus by a DNA virus; Report of RNA (rabies) interference by DNA vaccinia (See Excerpta Med.). Since the immune response to attenuated adenovirus type 4 vaccine follows intestinal infection, the possibility of interference by other enteric virus infections was very real (2).

In 1966 Lennette reported successful simultaneous vaccination with attenuated adenovirus type 4 and monovalent type I poliovirus (11). In this present study interference in the development of protective levels of antibody to adenovirus type 4 by attenuated trivalent poliovirus could not be detected. Furthermore, adenovirus intestinal infection did not interfere with the acquisition of protective levels of neutralizing antibody to the three poliovirus serotypes.

With but one exception, all men in both platoons who failed to respond to poliovirus vaccination had initial titers of 1:16 or greater (Table III). This one man, with a prevaccine titer of 1:4 or less to poliovirus type III failed to develop a fourfold rise in antibody to this serotype. The number of men developing a fourfold antibody rise to adeno-

virus is comparable to that seen in other studies (1,2,3).

The high level of prevaccination antibody to poliovirus reflects the high proportion of personnel entering military service with positive vaccination histories (14).

Summary. No interference was noted in the acquisition of neutralizing antibody to either attenuated poliovirus or adenovirus in a group of Marine recruits simultaneously vaccinated with attenuated adenovirus type 4 and trivalent poliovirus vaccines. This study would indicate that combined vaccinations with these agents is feasible in the military.

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Received Nov. 10, 1967. P.S.E.B.M., 1968, Vol. 127.

Biosynthesis of Immunoreactive Insulin by the Duct-Tied Dog Pancreas* (32827)

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Several different systems have been described to study the biosynthesis of insulin through the incorporation of labeled precursors into the active hormonal fraction. These have included the use of the isolated islet tissue (Brockmann bodies) of the marine teleosts (1–3), rat or rabbit pancreas slices (4–6), human islet cell tumors (7,8), and the separated islet of the rat pancreas (8). Each of the methods has inherent limitations as mentioned previously (9). Some of the problems encountered have been circumvented by the use of the isolated, recirculated, perfused dog pancreas as a model system to study the incorporation of ^{14}C labeled amino acids into insulin (9).

Another approach to this problem is described in this paper which presents the results of incubating the duct-ligated "acinar free" dog pancreas with ^{14}C labeled amino acids. This preparation actively incorporates ^{14}C labeled amino acids into fractions that have insulin activity by both biological and immunological assays.

Methods. Pancreas preparation. Adult mongrel dogs of either sex were used. Each dog was anesthetized with pentobarbital sodium (30 mg/kg) and the pancreatic ducts were exposed, doubly ligated and cut under

* This work was supported by USPHS Grant No. AM09135.

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