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Studies on Vaccination against Bacillary Dysentery

4. Oral Immunization with Live Monotypic and Combined Vaccines

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Results of tests made in 1964 confirm the previous findings that live oral vaccine, prepared from streptomycin-dependent strains of shigellae, confers a strong, type-specific protection against acute bacillary dysentery. This vaccine did not reduce the carrier rate of shigellae. Observations on soldiers treated with a vaccine of Shigella flexneri *serotypes 2a and 3 combined revealed no antagonizing effects from the type 3 component upon the protective effect of the 2a component contained in the same vaccine.*

In a controlled field trial carried out in 1963, results of investigations of a live oral dysentery vaccine, prepared from streptomycin-dependent strains of *Shigella flexneri* 2a have shown that a significant degree of protection can be achieved with such a vaccine (Mel, Terzin & Vukšić, 1965). It was concluded that these results justified further experiments and that tests should be made to determine whether strains of other *Shigella* serotypes, administered in the doses that proved effective with *Sh. flexneri* 2a would also confer protection. Results of such investigations, carried out in 1964, are presented in this paper.

Materials and methods

Unless it is stated otherwise, the preparation and administration of the vaccine, the determination of the number of live organisms, the bacteriological examination of stools and the criteria for determining post-vaccinal reactions and recording of morbidity rates were all as described in previous reports (Mel et al., 1965; Mel, Terzin & Vukšić, 1965).

Experimental groups

Of the 2 experimental military garrisons observed in 1964, one was stationed in a hyperendemic area (previously described under Group V) and the other was in an endemic area (previously described under Group IV). Both localities have already been described in detail (Mel, Terzin & Vukšić, 1965).

The trial included only soldiers recruited after September 1963, i.e., soldiers who during the preceding dysentery season were not in the army and who had never before been vaccinated against bacillary dysentery.

In the "hyperendemic garrison" 2 monotypic vaccines were administered, each in 5 doses. A vaccine of *Sh. flexneri* 2a was given to 319 soldiers, and a vaccine of *Sh. flexneri* type 4 was given to 403 soldiers. As shown in Table 1, both vaccines were administered in comparable doses and contained a known number of live organisms. The total number of live organisms administered in 5 doses to each of the 722 soldiers in this group amounted to 157×10^9 organisms of one type or the other. It was known from previous experiments that the immunogenicity of our vaccine was strictly type-specific. The 2 groups of soldiers vaccinated with type 2a or with type 4 vaccine were intended to serve as control groups, one to the other.

In the "endemic garrison", a combined vaccine containing a comparable number of *Sh. flexneri* types 2a and 3 organisms, was administered to 278 soldiers in doses shown in Table 1. A control group of 282 soldiers was designated, each man received 5 doses of placebo, as described by Mel et al. (1965).

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TABLE 1
INCIDENCE OF REACTIONS FOLLOWING ADMINISTRATION OF VACCINES

Doses	*Sh. flexneri* 2a or 4 [a]				*Sh. flexneri* 2a and 3 [b]			
	No. of organisms	Date	Cases	Percentage	No. of organisms	Date	Cases	Percentage
1st	27×10^9	1.4.64	13	1.8	40×10^9	15.4.64	8	2.9
2nd	28×10^9	4.4.64	10	1.4	49×10^9	18.4.64	5	1.8
3rd	29×10^9	7.4.64	5	0.7	54×10^9	21.4.64	5	1.8
4th	32×10^9	10.4.64	2	0.3	59×10^9	24.4.64	3	1.1
5th	41×10^9	13.4.64	0	0.0	60×10^9	27.4.64	0	0.0
Total	157×10^9	1–13.4.64	30		262×10^9	15–27.4.64	21	

[a] Administered to men in the hyperendemic garrison.
[b] Administered to men in the endemic garrison.

Vaccination

In view of our earlier observations, the vaccine was administered in gradually increasing doses in order to minimize post-vaccinal reactions.

Allocation of experimental units to vaccinated and control units was arranged, according to a random system, by dormitories. In each dormitory about half of the randomly selected soldiers were vaccinated, while the other half served as controls.

RESULTS

Post-vaccinal reactions during the 1964 tests were weaker than those in 1963. No cases of exacerbations were observed in 1964. Post-vaccinal reactions

TABLE 2
SHIGELLA CARRIERS IN 2 VACCINATED GARRISONS

	Hyperendemic garrison; 722 men vaccinated with:		Endemic garrison; 560 men vaccinated with:	
	Sh. flexneri 2a	*Sh. flexneri* 4	*Sh. flexneri* 2a+3	Placebo
Positive for *Sh. flexneri* 2a:				
No.	9	10	4	5
%	(1.3)	(1.3)	(0.5)	(0.6)
Positive for *Sh. flexneri* 3:				
No.	0	0	2	2
%			(0.2)	(0.2)
Positive for *Sh. flexneri* 4:				
No.	0	1	0	0
%		(0.1)		
Positive for other *Shigella* serotypes:				
No.	6	14	18	19
%	(0.9)	(1.9)	(2.2)	(2.2)
Total *Shigella* positive:				
No.	15	25	24	26
%	(2.2)	(3.4)	(2.9)	(3.0)
Numbers of swabs tested	691	743	822	877

manifested themselves as 1–6 soft or liquid stools; the reaction disappeared spontaneously within 24 hours.

Incidence rates of reactions following vaccination in the 2 experimental groups observed are shown in Table 1. As expected, the incidence of post-vaccinal reactions proved to be dose-dependent; the reactions occurred most frequently after the administration of the first dose of the vaccine, gradually becoming less frequent and finally absent in all the 1000 vaccinated soldiers after the administration of the fifth dose of vaccine. None of the 282 soldiers treated with placebo in the endemic garrison had detectable reactions.

The carrier rate was followed by examination of a total of 3133 rectal swabs, taken from the randomly selected soldiers at monthly intervals. In the hyperendemic garrison (722 soldiers), a total of 1434 swabs was examined, and in the endemic garrison (560 soldiers) a total of 1699 swabs. Data presented in Table 2 show that the carrier rates for individual types of shigellae were comparable both in the vaccinated and the control groups of each garrison.

TABLE 3

SHIGELLA MORBIDITY ACCORDING TO SEROTYPE IN THE HYPERENDEMIC GARRISON FROM 1 MAY TO 15 SEPTEMBER 1964

Diagnosis	No. of men vaccinated against *Sh. flexneri* serotypes:[a]		Total
	2a	4	
Sh. flexneri 1	1	1	2
Sh. flexneri 2a	7 (2.2)	57 (14.1)	64
Sh. flexneri 3	0	2	2
Sh. flexneri 4	0	0	0
Sh. flexneri 6	1	1	2
Sh. sonnei	1	1	2
Sh. schmitzi	2	3	5
Sh. boydi 1–15	25 (7.8)	26 (6.5)	51
Clinical diagnosis only	20 (6.3)	23 (5.7)	43
All dysentery cases	57 (17.9)	114 (28.3)	171
No dysentery	262 (82.1)	289 (71.7)	551
Total	319 (100.0)	403 (100.0)	722

[a] Percentages given in parentheses.

Morbidity rates

In 1964, no case of *Sh. flexneri* type 4 infection was observed in either of the 2 experimental groups. Consequently, the 403 soldiers in the hyperendemic garrison (see Table 3), vaccinated with type 4 vaccine, could serve only as controls to those vaccinated with type 2a vaccine, but the protective effect of the type 4 vaccine could not be evaluated in 1964.

Tables 3 and 4 show the morbidity observed in the hyperendemic garrison, where 319 soldiers were vaccinated with type 2a vaccine, and the 403 soldiers vaccinated with type 4 vaccine served as controls. The morbidity rate due to *Sh. flexneri* 2a observed in the control group (57 cases, representing an incidence rate of 14%), was significantly higher ($\chi^2=31.4$; $P<0.0001$) than that observed among soldiers vaccinated with type 2a vaccine (7 cases, representing an incidence of 2%). All these findings seem to confirm our previous observations.

As shown in Tables 5 and 6, a total of 560 soldiers was observed for incidence of dysentery cases over a period of 4 months in the endemic garrison. The control group treated with placebo consisted of 282 soldiers, while 278 soldiers were vaccinated with a combined types 2a and 3 vaccine. The total morbidity due to *Sh. flexneri* types 2a or 3 among the unvaccinated amounted to 33 cases (11.7% incidence rate). On the other hand, among the vaccinated soldiers only 5 cases (1.8%) of acute dysentery due to *Sh. flexneri* types 2a or 3 were detected. The difference between the 2 morbidity rates in the 2 groups of soldiers is highly significant ($\chi^2=21.7$; $P<0.0001$). These results seem to suggest that 2 serotypes combined into 1 vaccine display no antagonistic effects; moreover, our live dysentery vaccines, if administered as a mixture containing organisms of 2 serotypes, still confer an effective type-specific protection.

In conclusion, it may be stated that our type 2a vaccine conferred to men in the hyperendemic garrison an 84.3% protection against type-specific infection, while vaccine of types 2a and 3 combined administered to men in the endemic garrison gave an 84.7% protection against infection with *Sh. flexneri* types 2a and 3.

DISCUSSION

The type-specific protection against bacillary dysentery conferred by trial vaccinations in 1964 seems to have been somewhat weaker than that observed in 1963 (Mel et al., 1965). A possible reason for the difference observed in comparing the 2 series of trials

TABLE 4

SHIGELLA MORBIDITY AT FORTNIGHTLY INTERVALS IN THE HYPERENDEMIC GARRISON

Diagnosis		May	June		July		August		September
		15–31	1–14	15–30	1–14	15–31	1–14	15–31	1–14
319 men vaccinated against *Sh. flexneri* 2a									
Sh. flexneri 2a dysentery:	No.	0	2	5	0	0	0	0	0
	%		(0.6)	(1.6)					
Other dysentery:	No.	1	9	26	8	5	0	1	0
	%	(0.3)	(2.8)	(8.2)	(2.5)	(1.6)		(0.3)	
Total dysentery:	No.	1	11	31	8	5	0	1	0
	%	(0.3)	(3.4)	(9.7)	(2.5)	(1.6)		(0.3)	0
403 men vaccinated against *Sh. flexneri* 4									
Sh. flexneri 2a dysentery:	No.	0	4	28	20	3	1	0	1
	%		(1.0)	(6.9)	(5.0)	(0.7)	(0.3)		(0.3)
Other dysentery:	No.	5	5	27	9	6	4	1	0
	%	(1.2)	(1.2)	(6.7)	(2.2)	(1.5)	(1.0)	(0.3)	
Total dysentery:	No.	5	9	55	29	9	5	1	1
	%	(1.2)	(2.2)	(13.6)	(7.2)	(2.2)	(1.2)	(0.3)	(0.3)

TABLE 5

SHIGELLA MORBIDITY ACCORDING TO SEROTYPES IN THE ENDEMIC GARRISON FROM 14 MAY TO 15 SEPTEMBER 1964

Diagnosis	No. of men vaccinated against *Sh. flexneri* 2a and 3 [a]	Un-vaccinated	Total
Sh. flexneri 1	6	4	10
Sh. flexneri 2a	4 (1.4)	23 (8.2)	27
Sh. flexneri 3	1 (0.4)	10 (3.5)	11
Sh. flexneri 4	0	0	0
Sh. flexneri 6	2	2	4
Sh. sonnei	0	0	0
Sh. schmitzi	19 (6.8)	12 (4.3)	31
Sh. boydii 1–15	2	1	3
Clinical diagnosis only	7 (2.5)	10 (3.5)	17
All dysentery cases	41 (14.7)	62 (21.9)	103
No. dysentery	237 (85.3)	220 (78.0)	457
Total	278 (100.0)	282 (100.0)	560

[a] Percentages given in parentheses.

could be that a smaller number of organisms was used in our vaccines during 1964.

As shown in Tables 2, 3 and 5, in both experimental garrisons both the vaccinated and the control groups of soldiers showed comparable carrier rates as well as comparable morbidity rates due to various shigellae, except for morbidity rates due to serotypes contained in the vaccine. These findings seem to justify the random system of stratification that we applied in allocating epidemiologically comparable groups. Moreover, these findings imply that, in both experimental garrisons, both the vaccinated and control group of soldiers were exposed to a comparable extent to all *Shigella* types, including the types contained in the vaccine.

As already described, one of the greatest difficulties experienced in conducting field trials was the production of the vaccine in large quantities in field laboratories. Experiments performed in 1965, 1966 and 1967 have shown that lyophilized samples of our vaccine were as effective as the freshly prepared samples in the field and that they induced a significantly lower incidence of post-vaccinal reactions (less than 1 % after the first dose) compared with the freshly prepared vaccine. In subsequent reports, the preparation of lyophilized vaccines and their protective effect in field trials will be described.

TABLE 6

SHIGELLA MORBIDITY AT FORTNIGHTLY INTERVALS IN THE ENDEMIC GARRISON

Diagnosis		May	June		July		August		September
		15–31	1–14	15–30	1–14	15–31	1–14	15–31	1–14
278 men vaccinated against *Sh. flexneri* 2a and 3									
Sh. flexneri 2a dysentery:	No.	0	0	1	1	2			
	%			(0.4)	(0.4)	(0.7)			
Sh. flexneri 3 dysentery:	No.	0	0	0	1	0			
	%				(0.4)				
Other dysentery:	No.	1	1	9	11	13	1	0	0
	%	(0.4)	(0.4)	(3.2)	(4.0)	(4.7)	(0.4)		
Total dysentery:	No.	1	1	10	13	15	1	0	0
	%	(0.4)	(0.4)	(3.6)	(4.7)	(5.4)	(0.4)		
282 unvaccinated men									
Sh. flexneri 2a dysentery:	No.			3	9	10	1		
	%			(1.1)	(3.2)	(3.5)	(0.4)		
Sh. flexneri 3 dysentery:	No.			1	5	3	1		
	%			(0.4)	(1.8)	(1.1)	(0.4)		
Other dysentery:	No.	2	1	7	10	8	1		
	%	(0.7)	(0.4)	(2.5)	(3.5)	(2.8)	(0.4)		x
Total dysentery:	No.	2	1	11	24	21	3		
	%	(0.7)	(0.4)	(3.9)	(8.5)	(7.4)	(1.1)		

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RÉSUMÉ

De précédentes recherches ayant montré qu'un vaccin antidysentérique vivant préparé à partir de souches de *Shigella flexneri* 2a qui ne croissent qu'en présence de streptomycine conférait un degré de protection satisfaisant, des essais similaires ont été effectués en utilisant d'autres sérotypes.

Deux vaccins monotypiques ont été administrés par voie orale en 5 doses successives à des soldats casernés dans une région de Yougoslavie où la dysenterie bacillaire est hyperendémique: 319 d'entre eux ont reçu une préparation à base de *Sh. flexneri* 2a, et 403 un vaccin à base du sérotype 4. Les doses totales étaient dans les deux cas de 157×10^9 organismes. Chaque groupe a servi de témoin pour l'autre. Dans une autre garnison moins exposée, 278 soldats ont pris 5 doses d'un vaccin mixte préparé à partir des sérotypes 2a et 3, cependant qu'un groupe témoin de 282 hommes recevait 5 doses de placebo.

Des réactions postvaccinales, avec émission de 1 à 6 selles molles ou liquides, ont été notées dans un petit nombre de cas, surtout après administration de la 1re dose de vaccin; elles se sont atténuées progressivement pour disparaître lors de la prise de la 5e dose. Des examens

de selles destinés à dépister les porteurs de *Shigella* chez les vaccinés ont fourni des pourcentages de porteurs de 2,2 et 3,4% dans la 1re garnison et de 2,9% dans la seconde. Chez les soldats non vaccinés, 3% de porteurs ont été découverts.

Aucun cas de dysenterie à *Sh. flexneri* type 4 n'a été signalé dans les deux garnisons. Chez les soldats vaccinés par le vaccin 2a, le taux de la morbidité par dysenterie (*Sh. flexneri* 2a) a été de 2%; dans le groupe témoin, il a été de 14%. Dans le groupe traité par placebo, on a observé 11,7% de cas de dysenterie (*Sh. flexneri* types 2a et 3), alors que dans le groupe vacciné par la préparation mixte, 1,8% seulement de cas d'infection par ces souches ont été décelés.

On peut conclure de ces résultats que dans une collectivité très exposée, le vaccin du type 2a a conféré un taux de protection de 84,3% contre l'infection homologue. La vaccination par une préparation mixte 2a et 3 a prémuni les vaccinés contre les infections correspondantes dans la proportion de 84,7%.

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