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Study of Live Typhoid Vaccine in Chimpanzees*

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Streptomycin-dependent Salmonella typhi *containing O and H antigens was administered as a live oral antityphoid vaccine to chimpanzees. Five animals served as controls; 5 others received the vaccine 4 times at 3-day intervals; 4 further animals were given 4 doses of vaccine at 3-day intervals together with streptomycin; and 1 animal received the 4 doses of vaccine and a daily dose of streptomycin. The individual vaccine doses varied between* 36×10^9 *and* 82×10^9 *organisms, totalling about* 258×10^9 Salm. typhi *per animal. The chimpanzees were challenged with* 26×10^9 *cells of the virulent* Salm. typhi *Ty2 strain 10 days after immunization and followed up bacteriologically, serologically and clinically. It was observed that after this very heavy challenge the immunized animals that had received streptomycin with the vaccine were protected to some degree against the infection and showed fewer symptoms. The animal that received vaccine and streptomycin daily did not develop the disease.*

The authors point out that, while the strain used may have potential usefulness for the protection of man, further studies are needed to confirm the innocuity of the vaccine, to reduce the strain's reversion to streptomycin-independence, and to determine the relative effectiveness of different immunization dosages and schedules.

Although certain parenterally administered vaccines have been observed to produce a high degree of immunity against typhoid fever (Cvjetanović & Uemura, 1965), there are no reliable data on the effectiveness of oral typhoid vaccines.

Oral immunization with killed typhoid organisms has been attempted by many workers (Thompson & Thompson, 1948), although the first controlled field trial on man was performed only recently in India (Dr C. S. Chuttani, personal communication). Laboratory studies on small experimental animals and serological studies in man have indicated that killed oral vaccines stimulate antibody production (Vlădoianu et al., 1965) and may possibly give some protection, but a field trial and a clinical trial on human volunteers, undertaken in India and the USA respectively, have cast serious doubts on their efficacy (Dr C. S. Chuttani and Dr R. B. Hornick, personal communications).

Oral immunization with live antityphoid vaccine was considered promising by some workers but, to our knowledge, has never been tried in man or in susceptible non-human primates.

Reitman (1967) developed a streptomycin-dependent strain of *Salmonella typhi* and suggested that it could serve for oral immunization against typhoid fever because of its antigenicity. The fact that a streptomycin-dependent strain of *Shigella flexneri* has been shown to protect man against dysentery caused by the same serotype of *Shigella* (Mel et al., 1965) indicates that this suggestion is worth considering.

It was deemed appropriate, therefore, to attempt experimental immunization with streptomycin-dependent *Salm. typhi.* Although numerous animal species have been used in attempts to simulate human typhoid fever since Eberth's discovery of the typhoid bacillus, only chimpanzees appear to respond similarly to man after infection with *Salm. typhi* (Edsall et al., 1960).

The purpose of this study was to determine whether chimpanzees could be immunized with orally

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administered streptomycin-dependent *Salm. typhi* vaccine.

The experiment was so designed that it put the effectiveness of vaccine to a severe test, since only a vaccine of high efficacy would be of practical interest because an effective parenteral typhoid vaccine is already available. Moreover, an attempt was made to explore the course of the immunological response by determining the relationship of antibodies to immunoglobulins, in a search for a possible serological indicator of immunity.

MATERIALS AND METHODS

Vaccine

The original *Salm. typhi* 19 VSD strain of Reitman (1967), obtained from Dr L. S. Baron, possesses O, H and Vi antigens. It was grown on media containing 200 μg streptomycin per ml. Our vaccine strain, designated *Salm. typhi* 20SD, was derived from this strain. It was grown on Brain-Heart Infusion Agar (BHA) (Difco) medium with 400 μg streptomycin sulfate per ml because it was thought that a higher streptomycin content would eliminate reversion to streptomycin-independence and thus render the vaccine safer for use. The strain had a tendency to lose its Vi antigen when cultured on this medium. It also appeared that should this vaccine reach the stage of practical use, a micro-organism that does not require special precautions might be more practical for vaccine production. Besides, earlier studies had shown that Vi antigen has little relation to human protection (Edsall et al., 1959; Cvjetanović & Uemura, 1965). Therefore, a Vi-negative variant of strain 20SD was employed. It did not show reversion to streptomycin-independence *in vitro* during 2 months of daily laboratory transfers, nor after a passage in chimpanzees.

While we observed no reversions of strain *Salm. typhi* 20SD on solid media during the preparation of the vaccine and when recovering this vaccine strain from immunized chimpanzees, Dr E. S. Anderson (personal communication), who studied its reversion in nutrient broth containing 300 μg per ml streptomycin (which is the minimum required for the growth of *Salm. typhi* 20SD), noted a frequency of reversion of at least 3×10^{-6}. One-third of the streptomycin-independent colonies thus recovered were smooth. Further studies of reversion and additional efforts to derive a stable streptomycin-dependent line are therefore necessary before *Salm. typhi* 20SD can be considered for studies in man.

Fresh vaccine was prepared for each immunization. All media contained 400 μg/ml streptomycin. The strain grown on BHA was transferred to Brain-Heart Infusion Broth (BHI) (Difco). After 18 hours' culture at 37°C, 2.0 ml of the growth were inoculated on to Roux bottles containing BHA. These were incubated for 24 hours at 37°C. The growth from each bottle was collected with 20 ml of sterile saline. The washings were immediately used for immunization as well as for bacterial counts by serial dilution in BHI and by plating to BHA containing streptomycin. Duplicate counts were always performed. The concentration of the organisms varied between 18×10^9 and 41×10^9 per ml in different batches. Admittedly, however, some animals might have ingested slightly different amounts of organisms. It is also possible that the survival time of the bacteria varied in some of them.

Experimental animals

The animals were healthy chimpanzees of either sex weighing 14 kg to 21 kg, of an age estimated at between 3 and 5 years. They were examined for the presence of *Salmonella* before immunization and challenge, and proved free of such infection.

Fifteen chimpanzees were divided into three similar groups of 5 individuals each. Each group was composed of 3 males and 2 females. The first group served as the control. This group, as a whole, weighed approximately 10% more than the other two groups. The second group received vaccine alone. The third group, which comprised an animal which had had a resection of about 5 cm of the ileum for a surgical experiment 10 months before this project was carried out, received vaccine plus streptomycin. One animal belonging to the third group received, in addition, streptomycin every day during the immunization period. The control group was caged separately during the immunization period, but on the day of challenge the chimpanzees were taken to the premises occupied by the two vaccinated groups. All three groups were challenged at the same time, and were caged, fed and treated identically afterwards.

Immunization schedule

While a group of 5 animals were kept as controls, 10 animals were immunized with 4 doses of the vaccine administered 3 days apart in the morning in apple juice, or in bananas if the juice was refused. Each dose consisted of a 2-ml suspension of *Salm. typhi* 20SD in saline. The number of organisms

TABLE 1
IMMUNIZATION SCHEME AND DOSAGE

Day of immunization	Dose	No. of organisms
0	1st	60×10^9
3	2nd	36×10^9
6	3rd	80×10^9
9	4th	83×10^9
Total	4	258×10^9

given on each occasion is shown in Table 1. (The total number of vaccine organisms given is comparable to the number of organisms contained in oral killed vaccine studied recently in field and clinical trials and found ineffective (C. S. Chuttani and R. B. Hornick, personal communications).) Of these 10 animals, one group of 5 received only vaccine, while the other 5 were given vaccine and 40 mg streptomycin orally with each dose of the vaccine. One chimpanzee in the last group was also given 40 mg of streptomycin orally once daily during the entire immunization period of 18 days. The small dose of streptomycin was given to facilitate the survival of the vaccine organisms in the intestine in order to determine whether this affected subsequent immunity. The distribution of animals into the various vaccinated and control groups is given in Table 2.

Stool examinations

The survival of the vaccine strain in the gut was determined by culturing the stools daily on BHA with and without 400 μg streptomycin per ml respectively, and on SS Agar (Difco). Two plates were streaked directly from each specimen. The stools were also added to Selenite F Medium (B.B.L.) from which BHA and SS plates were streaked after 20–24 hours' incubation. While practically all animals had positive stools 24 hours after immunization, some shed typhoid bacilli on the second day also. A few had bacteriologically positive faeces on the third day, but none on the fourth day after the last vaccination.

The results of daily stool cultures after immunization are presented in Table 2, which shows that the duration of the excretion and the survival of the vaccine strain in the intestine varied. Those chimpanzees that received streptomycin with each dose of the vaccine did not excrete the organism longer than those that did not receive it. Although the chimpanzee that was given streptomycin daily did not excrete organisms significantly longer than other animals that did not receive this antibiotic, there is no doubt that a greater number of organisms survived and probably multiplied in the gut, because stool cultures from it revealed abundant growth of the vaccine organism on the bacteriological plates. Sometimes practically pure cultures were obtained from this chimpanzee.

All strains isolated from the experimental animals were plated to BHA without streptomycin for checking reversion to streptomycin-independence. None was observed by this method.

Side-reactions, such as diarrhoea, loss of appetite and vomiting, were not observed.

Challenge

The animals were challenged with virulent *Salm. typhi* strain Ty2 10 days after the fourth dose of the vaccine was administered. The strain was obtained from Dr W. H. Ewing, National Communicable Disease Center, Atlanta, Ga. It had Vi antigen, was not agglutinable with 9,12 O sera, and belonged to phage group E1.

BHA plates without streptomycin were heavily inoculated from this culture. After 12 hours' incubation at 37°C, the plates were washed with 3 ml BHI, and 1.5-ml amounts transferred to each of 2 Roux bottles with BHA. These were incubated at 37°C in an inverted position for 5½ hours. The growth from the Roux flasks was harvested with sterile saline. The number of organisms was determined by the same method as used for the estimation of the bacterial count in the vaccine. Two parallel determinations were made.

Irrespective of their weight (14 kg to 21 kg), after 24 hours of having food withheld, each chimpanzee was fed with 0.75 ml of a suspension containing 26×10^9 viable organisms of the challenge strain Ty2 injected into unpeeled bananas. This dose was apparently delivered in an effective manner because 24 hours later all animals excreted the organisms in their stools, and some also 48 hours later as shown in Table 2.

Bacteriological and clinical follow-up of the challenged animals

All chimpanzees were inspected daily, and signs of any illness recorded.

Stools were collected every day and blood samples every second day. Both were examined for *Salm. typhi* by the method used during the vaccination. The temperature was recorded every second day. Animals were weighed before and 10 days after the challenge.

Immunological procedures

The animals were bled once before and after immunization as well as every second day during the first 12 days after the infection, and at longer intervals thereafter. The sera were kept at −20°C, without a preservative, until tested.

Agglutinin titres were determined by the routinely employed 2-fold tube-dilution technique against boiled (O) and formolized (H) antigens prepared from the *Salm. typhi* strain Ty2 used for challenge.

The bactericidal test was performed with the aid of the method of Muschel & Treffers (1956), based on the photometric growth assay in the presence of complement in the modification of Karolček et al. (1963), in which the bacterial multiplication between determinations is slowed down by keeping the tubes at 0°C.

The opsonic index was estimated by comparing the effect of the tested sera with that of a mixture of normal chimpanzee sera.

Immune globulins IgG, IgA and IgM in the sera were estimated with the aid of the Manzini et al. (1965) single radial-diffusion method, using Hyland plates.

The participation of 7S and 19S-type immune globulins was determined by separating these two groups by diluting the sera 1 : 10 with 0.01 M phosphate buffer, pH 7.4, containing 0.1 M 2-mercaptoethanol (ME) and subsequent dialysis against iodacetamide, then against 0.01 M phosphate buffer. The sera were restored to their initial volume by pre-evaporation in the cold room under a fan. Parallel agglutination experiments were set up with the ME-treated and the untreated fractions.

Antibody-antigen precipitates were analysed after precipitation at previously determined equivalence for 2 hours at room temperature, then for 4 days at +2°C in tared test-tubes, using a radioactive antigen prepared from *Salm. typhi* Ty2 cultured in Eagle's minimum essential medium (MEM) containing 28 mg per litre L-lysine-1-^{14}C to give 2 mCi of radioactivity per litre. The organisms were centrifuged at 15 000 *g* and +2°C, disrupted in a Branson sonicator, and centrifuged again. The supernatant was employed as the antigen at equivalence. The antigen–antibody complex was collected on a Millipore filter-paper with 0.45 μ mean pore diameter, and the bound radioisotope determined in the Beckman gas-flow low-beta-scintillating counter.

The antibody avidity (firmness of the antigen–antibody complex) was tested in precipitates prepared in several tubes from each of 3 selected sera and *Salm. typhi* strain Ty2 sonicated supernate as the antigen by 2 hours' incubation at room temperature (22°C) and 4 days at 2°C–4°C, centrifuging at 15 000 *g* and 2°C for 30 minutes, and dissolving the sediment in a citrate buffer with a different pH (3.5 to 5.5) in each tube. Ascending chromatography on CM Sephadex columns with carbonate buffer, according to the technique of Tozer et al. (1962), permitted the determination of the point at which 50% of antigen and antibody were separated.

All immunological tests were made in duplicate, whereas the antibody avidity estimations were carried out in triplicate.

RESULTS IN TERMS OF PROTECTION

Typhoid fever in man is a severe and relatively long-lasting disease, while in chimpanzees it is milder and shorter (Edsall et al., 1965). As in man, the infection in chimpanzees results in various clinical pictures. Typhoid infection may produce mild symptoms in man as it did in most of the chimpanzees, although some became quite ill. One of the animals in the control group had a temperature exceeding 39°C for a week, and lost more than 3 kg in weight.

It was difficult to classify accurately the severity of the illness in each chimpanzee and to define the degree of protection the vaccine might have given, except from the results of the antibody–antigen complex analysis.

Table 3 presents the principal symptoms and bacteriological findings in control and in immunized animals.

All control animals became ill, as well as the chimpanzees that received vaccine only. Among the animals that received vaccine and streptomycin, two remained healthy; one of them was the animal that received streptomycin daily throughout the immunization period.

Table 3 shows that illness seemed to have been more severe in the control group, judging by the duration of the febrile period, loss of appetite, dehydration, and lethargy. Two animals became

TABLE 2

BACTERIOLOGICAL FINDINGS OF VACCINE STRAIN DURING IMMUNIZATION PERIOD AND OF VIRULENT STRAIN AFTER CHALLENGE IN FAECES AND BLOOD

Group	Animal No.	Sex	Immunization period [a] (stools [b])																				Challenge period (stools [b] and blood [c])										Treatment period (stools [b])				
			Day 0 [a]	Day 1	Day 2	Day 3 [a]	Day 4	Day 5	Day 6 [a]	Day 7	Day 8	Day 9 [a]	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	No. of positive days per animal	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 1	Day 2	Day 3	Day 4	Day 5
Control	1895	M																					S+			B+		S+ B+	S+	S+ B+	S+	S+ B+	S+		S+		
	1833	F																					S+			S+ B+		B+	S+	B+		S+ B+					
	1860	F																					S+			B+		B+		B+			S+				
	1765	M																					S+			S+		B+		B+							
	1861	M																					S+			B+		B+		B+		S+ B+			S+		
Vaccine only	1830	M		S+	S−	S−	S+	S+	S+	S+	S−	S−	S+	S+	S−	S−	S−	S−	S−	S−	S−	7	S+									B+					
	1826	F		S+	S−	S−	S+	S+	S+	S+	S−	S−	S+	S+	S−	S−	S−	S−	S−	S−	S−	7	S+			B+				B+							
	1832	M		S+	S+	S−	S+	S+	S+	S+	S+	S−	S+	S+	S+	S−	S−	S−	S−	S−	S−	10	S+	S+	S+	S+ B+				B+							
	1854	M		S+	S−	S−	S+	S−	S−	S+	S+	S−	S+	S−	S−	S−	S−	S−	S−	S−	S−	5	S+	S+		S+ B+			S+	S+ B+	S+					S+	
	1894	F		S−	S−	S−	S+	S+	S+	S+	S+	S−	S+	S+	S+	S−	S−	S−	S−	S−	S−	8	S+			B+	S+		S+	S+ B+							
Vaccine with streptomycin	1829	F		S+	S+	S−	S−	S−	S−	S−	S−	S−	S−	S−	S−	S−	S−	S−	S−	S−	S−	2	S+	S+													
	1825	F		S+	S+	S−	S+	S−	S−	S+	S−	S−	S+	S−	S−	S−	S−	S−	S−	S−	S−	5	S+			B+		B+		B+	S+						
	1856	M		S+	S+	S−	S+	S−	S−	S+	S−	S−	S+	S−	S−	S−	S−	S−	S−	S−	S−	5	S+	S+		S+ B+	S+	B+		S+ B+	S+	B+					
	1826	M		S+	S−	S−	S+	S−	S−	S−	S−	S−	S+	S−	S−	S−	S−	S−	S−	S−	S−	3	S+	S+	S+	S+ B+	S+	S+ B+	S+	S+ B+	S+	S+					
Vaccine-with-streptomycin and streptomycin daily	1831	M		S+	S+	S+	S+	S+	S+	S+	S−	S−	S+	S+	S+	S−	S−	S−	S−	S−	S−	10	S+	S+													

[a] Immunization was carried out immediately after the stool sampling in respect of the four days shown in bold type.

[b] S+ = stool positive; S− = stool negative (stool was examined every day).

[c] B+ = blood positive (blood was examined every other day).

TABLE 3

SYMPTOMS AND BACTERIOLOGICAL FINDINGS IN CHIMPANZEES AFTER CHALLENGE

Group	Total no. of animals	No. of animals remaining healthy	No. of sick animals with bacteraemia, positive stools and blood, rise of temperature, and loss of weight	No. with positive stools	No. with positive blood	No. febrile [a]	Mean duration (days) of bacteraemia per animal	Mean weight loss or gain (kg) per animal	No. with stools positive 3 or more days after treatment
Control	5	0	5	5	5	5	6.5	−1.20	2
Vaccine only	5	0	3	3	5 [b]	5 [b]	5.6	−0.65	1
Vaccine with streptomycin	4	1	3	3	3	3	4.5	+0.55	1
Vaccine with streptomycin and streptomycin daily	1	1	0	0	0	0	0	0	0

[a] Criterion was a rise of 2 degC above the normal average body temperature for the individual animal.
[b] One of these animals had a mild illness and gained weight.

seriously ill, dehydrated and lethargic. One lost over 1½ kg in weight; and in 2 the illness had a milder course.

Among the animals that received vaccine, 2 became dehydrated and ill while 3 had no particular clinical symptoms except elevated temperature.

Among those given vaccine and streptomycin, only 1 became dehydrated. This animal was earlier subjected to a resection of part of the ileum. The other did not show clinical symptoms.

The only 2 animals that did not develop the disease were: 1 among the 4 that received vaccine and streptomycin on the days of vaccination, and the single animal that received vaccine and, throughout 17 days, daily streptomycin.

Considering the weight loss according to the groups of the experimental subjects, 5 chimpanzees in the control group lost a total of 6 kg (3 of them more than 1 kg). The group that received only vaccine lost 3.20 kg (1 animal more than 1 kg) while the group that received vaccine and streptomycin gained 2.25 kg even though 3 developed bacteraemia and had elevated temperatures.

After treatment and after becoming negative and apparently recovering, 2 animals died; one from the vaccine group and the other from the vaccine and streptomycin group.

The results of the determinations of agglutinin titres determined against boiled O and formolized H antigens prepared from the challenging organism, and the bactericidal titres, are presented in Table 4 together with the opsonic index expressed in reciprocal serum dilutions which showed opsonic activity equal to that of undiluted normal chimpanzee sera. On the 60th and 20th–25th days before challenge animals had no detectable antibody titres and they did not show increasing levels during the immunization. One control animal (No. 1833) had developed relatively high agglutinin titres 4 days after challenge. Lower agglutinin levels were observed also in 2 of the immunized animals as early as day 6 after challenge. On day 15, 3 each of the control and vaccinated animals had O titres of 1 : 40 or 1 : 80. Elevated H titres were encountered in the same number of chimpanzees serving as controls and in those receiving streptomycin and vaccine. The situation had not changed by day 17, except for the appearance of higher H titres also in the animals receiving vaccine but not streptomycin. The vaccinated chimpanzee receiving streptomycin daily during the immunization period (No. 1831) had the highest H values. All animals showed declining agglutinin titres after that day.

Three of the immunized animals (among them No. 1831) showed serum bactericidal and opsonic activity against the challenge organism 2 days before infection.

The examinations of the 7S and 19S-type immunoglobulins and their relationship to agglutinins, as well as the radioactivity counts of the antibody–antigen complexes, indicate that reduction of 19S-type antibodies, which are said to be ME-sensitive,

TABLE 4

ANTIBODY LEVELS IN CHIMPANZEES CHALLENGED WITH *SALM. TYPHI* TY2

Group	Animal No.	Antibody levels before challenge [a]				Antibody levels after challenge [a]																			
		3 days before				2 days after				4 days after				15 days after				17 days after				25 days after				44 days after			
		O[b]	H[c]	Bact.[d]	Ops.[e]	O[b]	H[c]	Bact.[d]	Ops.[e]	O[b]	H[c]	Bact.[d]	Ops.[e]	O[b]	H[c]	Bact.[d]	Ops.[e]	O[b]	H[c]	Bact.[d]	Ops.[e]	O[b]	H[c]	Bact.[d]	Ops.[e]	O[b]	H[c]	Bact.[d]	Ops.[e]
Control	1895									0	0	5	10	40	160	[12	20	40	640	50	160	0	80	47	60	0	40	13	20
	1833									120	120	16	0	80	640	0[c]	0	80	160	35	80	80	320	38	40	0	80	24	120
	1860			0				0		0	0	0	0	40	160	10	120	40	160	33	120	0	160	22	60	0	80	15	20
	1765									0	0	0	0	0	80	19	20	40	640	27	40	0	80	12	80	0	40	10	40
	1861									0	0	0	0	0	80	15	40	40	320	39	80	0	160	41	40	80	80	25	60
Vaccine only	1830	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	20	0	40	20	30	40	40	29	60	0	0	10	60
	1826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0[f]	0	0	0	40	10	Died [g]							
	1832	0	0	0	0	0	0	0	0	0	0	10	10	0	80	15	30	40	80	23	40	0	80	30	40	0	0	20	120
	1854	0	0	5	10	0	0	13	20	0	40	15	40	0	80	10	80	40	640	22	80	0	320	35	60	0	240	30	120
	1894	0	0	0	0	0	0	8	10	0	0	7	20	0	0	18	20	40	40	13	20	0	0	15	80	0	0	16	0
Vaccine with streptomycin	1829[h]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1825	0	0	11	10	0	0	28	20	0	0	24	40	40	160	27	40	0	160	33	60	0	80	38	60	0	40	30	40
	1856	0	0	0	0	0	0	0	0	0	0	0	0	40	80	10[f]	0	40	160	25	20	80	160	49	120	40	40	38	160
	1526	0	0	0	0	0	0	0	0	0	0	0	0	0	160	0	0	0[i]	160	0	0	40	80	10	0	0	40	10	10
Vaccine with streptomycin and streptomycin daily	1831[h]	0	0	5	10	0	0	18	30	30	0	32	60	40	640	21	80	40	960	42	160	40	160	47	160	0	40	21	60

[a] All values are averages of 2 determinations.

[b] O = O agglutinin reciprocal titres; 0 indicates titre <30.

[c] H = H agglutinin reciprocal titres; 0 indicates titre <30.

[d] Bact. = bactericidal activity expressed in percentage reduction of light transmission of culture; 0 indicates <5.

[e] Ops. = opsonic index expressed in serum dilution matching the opsonic activity of undiluted normal chimpanzee serum; 0 indicates <10.

[f] These animals required lactated Ringer and glucose solution infusions.

[g] This animal was free of *Salm. typhi* from the 10th day after challenge.

[h] This animal did not develop the disease.

[i] Blood culture positive on day 17 after challenge.

was greatest in the vaccinated. The largest amount of antibody fixed under these conditions occurred in the chimpanzee receiving streptomycin daily in addition to vaccine, even when compared with the results in a very rapidly reacting unvaccinated animal (Table 5).

Table 5 demonstrates also the firmness (avidity) of antibody–antigen bonds. It was evident that avidity increased with the length of time after infection, and that IgG formed a firmer complex than IgM if the bond was broken by a low pH buffer.

It appears from Table 5 that the antigen–antibody complex was firmest in animal No. 1831, which showed elevated titres also in other tests.

Table 2 shows the bacteriological findings in faeces and blood in all animals. The duration of bacteraemia was longest in the control group; shorter in the vaccine group; and lasted even fewer days in the group receiving vaccine with streptomycin.

After 10 days of observation, i.e., on day 11 after challenge, chloramphenicol treatment of all sick animals began.

After 3 days of treatment, 3 animals were still bacteriologically positive; 2 were in the control group. One immunized animal that developed the disease became bacteriologically positive 20 days later. This could be considered a relapse.

TABLE 5

ANALYSIS OF ANTIBODY–ANTIGEN (AB–AG) PRECIPITATES IN SELECTED CHIMPANZEES

Animal No. (and group)	Day after infection	Reciprocal agglutinin titre [a]				Precipitate with whole cell lysate		Avidity of antibody (pH at which 50%± 3%AB–AG complex dissociated)
		Before 2-mercaptoethanol		After 2-mercaptoethanol		AB–AG weight [b] (mg)	Radioisotope emission count (counts/min)	
		O	H	O	H			
1833 (control)	6	120	120	0	0	1.7	12.8	4.5
	15	80	640	0	40	7.3	100.3	4.5
	25	80	320	80	80	5.4	64.6	4.0
	30	80	160	0	30	3.3	32.9	3.4
	44	0	80	0	30	2.5	12.8	3.5
1854 (vaccine only)	6	0	40	0	0	2.7	15.7	5.4
	15	40	80	0	30	6.9	61.7	5.2
	25	0	320	0	120	4.1	32.5	4.0
	30	0	320	0	120	4.4	11.5	4.0
	44	0	240	0	80	1.9	8.7	3.7
1831 [c] (vaccine and streptomycin daily)	6	30	0	0	0	0	1.3	4.1
	15	40	640	0	120	8.9	115.2	4.0
	25	40	160	0	120	6.2	100.3	3.6
	30	0	80	0	30	3.2	64.7	3.5
	44	0	40	0	30	2.9	21.8	3.2
Control serum	0	0	0	0	0	0	1.5	
Antigen extract subjected to total procedure						0	2.1	
Antigen extract alone							5 059.5	

[a] 0 indicates titre <30.

[b] 0 indicates <0.5 mg.

[c] This animal did not develop disease.

The immune globulin values fluctuated somewhat during the experiment. IgG varied initially between 880 and 1100; IgA between 240 and 315; IgM between 75 and 165, according to the individual animals. There was an increase of IgG, usually not exceeding 20% for 2–4 weeks after challenge; IgA remained essentially unchanged, whereas IgM was higher in some sera during the first 2–4 weeks after challenge. When the groups of chimpanzees were considered as entities, there were no differences permitting conclusions to be drawn because the standard deviation was ±8% to 10%. Moreover increasing blood concentrations in the sick animals due to fluid loss and the use of infusions in two of them may have further influenced the proportion of immune globulins and serum. Immunoglobulin determinations, therefore, were not helpful.

DISCUSSION

It is likely that with a smaller challenge dose, more solid protection could have been demonstrated. Moreover, the administration of only 4 doses of vaccine may not have been a satisfactory stimulus for the production of sufficient effective antibody. If streptomycin-dependent live dysentery vaccine is given without streptomycin, for example, 5 doses are needed for protection (Mel et al., 1965). Better results might be expected, therefore, after the administration of more doses of the vaccine.

Streptomycin may play an important adjuvant role by allowing the vaccine organism to survive and possibly to multiply in the intestine, and perhaps also by suppressing part of the competing intestinal flora. Animals given streptomycin, especially one chimpanzee that received it daily during immunization, seemed to show a higher degree of immunity as judged by morbidity symptoms and several immunological tests.

It is rather difficult to correlate the serological tests, immune globulin results and their relationship to the degree of protection. The non-reacting animal and that with immediately developing high titres may have had a *Salmonella* infection in the past. Such disease is not rare in non-human primates. Chimpanzee No. 1831, which received both vaccine and streptomycin daily and was protected against infection, showed a more vigorous serological response than any of the others. If H antibodies are considered valuable indicators of the immunity, this finding may be significant in pointing to the effectiveness of oral live vaccine when given with streptomycin.

Since streptomycin is not absorbed from the gut into the bloodstream, streptomycin-dependent *Salm. typhi* may multiply in the gut and survive longer there but cannot penetrate into the circulation and cause bacteraemia (unless streptomycin is administered parenterally). Perhaps if more and higher doses of streptomycin had been administered at closer regular intervals, better protection could have been achieved, as the results show that the greatest immunity was obtained by the greatest amount of actually living vaccine organisms.

The real effectiveness of the live vaccine could have been clouded in particular by two factors:

(*a*) the control group weighed more than the other groups, and one animal in the vaccine-with-streptomycin group had had a partial ileal resection; thus the control group may not have represented a true parallel of the immunized animals;

(*b*) the challenge dose was very heavy and tended to overcome any protection, as has been shown by Hornick & Woodward (1966).

The shortcomings of this study are evident. Not all the results can be interpreted with ease, but from the first experiments with a new type of vaccine one cannot expect much more than information on the potential value of that vaccine. As further studies are carried out, more light may be shed on this problem.

CONCLUSIONS

This study indicated that live, orally administered vaccine, prepared from a streptomycin-dependent strain of *Salm. typhi*, especially when administered with small doses of streptomycin, may protect chimpanzees to a certain degree against challenge with live wild-type *Salm. typhi*. This live streptomycin-dependent oral typhoid vaccine may have potential usefulness for the protection of man. Further studies are needed, however, to confirm the innocuity of the vaccine, to reduce the reversion of the strain to streptomycin-independence, and to determine its protective effect with various immunization schedules and dosages.

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RÉSUMÉ

EXPÉRIMENTATION CHEZ LE CHIMPANZÉ D'UN VACCIN ANTITYPHOÏDIQUE VIVANT

On a administré par voie buccale à des chimpanzés un vaccin antityphoïdique vivant préparé à partir de la souche vaccinale *Salmonella typhi* 20SD qui renferme les antigènes O et H et qui ne se développe qu'en présence de streptomycine. Quinze chimpanzés de 3 à 5 ans ont été utilisés au cours de cette étude: 5 ont servi de témoins; 5 ont reçu 4 doses de vaccin vivant à 3 jours d'intervalle, et 4 des doses identiques de vaccin avec, lors de chaque prise, 40 mg de streptomycine par voie orale; le dernier animal, également traité à quatre reprises par l'association vaccin-streptomycine, a reçu en outre une dose quotidienne de 40 mg de streptomycine. Chaque dose de vaccin renfermait de 36×10^9 à 82×10^9 *Salm. typhi*, la dose totale par animal atteignant 258×10^9 organismes viables. Dix jours après la dernière prise de vaccin, tous les chimpanzés ont subi une infection d'épreuve comportant l'administration par voie buccale de 26×10^9 organismes de la souche virulente *Salm. typhi* Ty2. Ils ont ensuite été suivis sur les plans clinique, bactériologique et sérologique.

Tous les animaux témoins, de même que leurs congénères traités par le vaccin seul, ont présenté des symptômes de maladie, plus accentués chez les premiers. Les singes qui avaient reçu le vaccin et, concomitamment, de la streptomycine ont été dans une certaine mesure protégés contre l'infection et n'ont souffert que de symptômes bénins. L'animal qui avait bénéficié d'un traitement quotidien par la streptomycine est resté indemne de toute affection.

Il est possible que la streptomycine joue un rôle adjuvant non négligeable en permettant à la souche vaccinale de survivre et de se multiplier dans l'intestin et, peut-être, en inhibant la croissance d'une partie de la flore intestinale. De nouvelles recherches sont nécessaires pour vérifier l'innocuité de ce vaccin antityphoïdique vivant, réduire les possibilités de retour de la souche vaccinale à la non-dépendance vis-à-vis de la streptomycine et évaluer l'efficacité relative de différents schémas d'immunisation, avant d'entreprendre des essais pratiques contrôlés chez l'homme. Bien qu'on ait relevé une certaine corrélation entre les résultats des tests sérologiques, comme le titrage des agglutinines anti-H et la mesure de l'avidité des anticorps, et le degré de protection obtenu, on ne peut en tirer actuellement aucune conclusion.

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