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Intradermal cholera vaccination

By J. E. NOBLE*
David Bruce Laboratories, East Everleigh, near Marlborough, Wilts

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INTRODUCTION

Immunization by the intradermal route against the enteric group of diseases and against tetanus has been shown to be particularly free from post-inoculation reactions (Barr, Sayers & Stamm, 1958; Noble, 1963). The employment of this method of inoculation during the past 4 years has led to a considerable saving of manpower in Army personnel.

Cholera vaccine is still administered by the subcutaneous route with a significant number of both local and general reactions.

Bearing in mind the successful reduction in number and severity of reactions experienced in enteric and tetanus vaccination, it was decided to investigate the possibility of producing an intradermal cholera vaccine. This article is an account of the experiments carried out to compare and contrast the biological effects produced by cholera vaccine administered by both the subcutaneous and intradermal routes.

Investigating the inoculation of cholera vaccine by the intradermal route, Panja & Das (1947) expressed the opinion that this method appeared to be superior to inoculation by the subcutaneous route. These authors also stressed the economy of materials which resulted, and the negligible reactions which followed injection of cholera vaccine by the intradermal route.

Singer, Wei & Hoa (1948) reported better antibody formation after intradermal vaccination than after inoculation by the subcutaneous route in human volunteers. These authors, however, reported abscess formation at the site of inoculation in a few cases following the administration of a third dose of *Vibrio cholerae* vaccine by the intradermal route. The intradermal inoculations consisted of 1500×10^6 heat-killed *V. cholerae* organisms administered in a dose of 0·2 ml. As 0·1 ml. is now used as the standard intradermal dose it is likely that ulceration was caused by the volume rather than the contents of the inoculations.

All virulent strains of *V. cholerae* possess a common O antigen. By agglutinin–absorption techniques subsidiary O antigenic components can be demonstrated. Gardner & Venkatraman (1935) classify *V. cholerae* strains into three subtypes, designated according to the standard strains, Inaba, Ogawa and Hikojima. Inaba and Ogawa each possess distinct subsidiary O antigens. Hikojima possesses both these subsidiary O antigens. All strains of *V. cholerae* and some other non-pathogenic vibrios possess a common H antigen (Pollitzer, 1959).

Cholera vaccine prepared at the David Bruce Laboratories, for administration

* Lt.-Col., Royal Army Medical Corps.

to Service personnel, is made in accordance with the recommendations laid down by World Health Organisation (1959) and contains *V. cholerae* strains Inaba and Ogawa. The vaccine consists of a heat-killed and phenol-preserved suspension of 4000 million Inaba and 4000 million Ogawa organisms per ml. in 0·5% phenol-saline.

Vella (1963), in his experiments with the El Tor vibrio, showed that the mouse strain C/57/Black was suitable for experimental studies with *V. cholerae*. As he had established a readily available supply of this strain of mice to these laboratories, it was decided to use C/57/Black mice in these studies.

MATERIALS AND METHODS

Preliminary animal virulence tests

These tests were performed by challenging mice and guinea-pigs intraperitoneally with various strains of *V. cholerae* in order to assess their suitability as challenge organisms, and to establish the lethal dosage for 100% of animals inoculated (LD 100).

(*a*) *Mice.* Groups of mice (C/57/Black—ten mice, of average weight 22 g., in each group) were inoculated by the intraperitoneal route with graded doses of challenge organism contained in 0·5 ml. of isotonic saline. The mice were observed for 72 hr. and the numbers dead, at the end of that time, were recorded. Mucin was not used. The challenge organisms used were National Collection of Type Cultures' strains Inaba (7258, 7260 and 8039) and Ogawa (5596 and 8040).

(*b*) *Guinea-pigs.* Animals of the Dunkin–Hartley strain, weighing 250–300 g., were injected intraperitoneally with graded doses of *V. cholerae* Inaba (NCTC 7260) suspended in 1·0 ml. physiological saline. Mucin was not used. The number dying in 72 hr. was recorded.

Active mouse protection tests

This group of experiments was devised to compare the protection afforded to mice by the administration of *V. cholerae* vaccine by both the subcutaneous and intradermal routes. Dosage and time interval between inoculations and between immunization and challenge were varied, as were the strains used for preparation of the vaccines and subsequent challenge.

In each experiment two immunizing inoculations only were given; subcutaneous injections were given in a standard dose volume of 0·5 ml. introduced into the loose areolar tissue of the 'scruff' of the neck; intradermal injections were administered in constant dose volume of 0·05 ml. introduced into the shaved flank of the animals.

Challenge doses were suspended in sterile isotonic saline and administered in a constant dose volume of 0·5 ml. injected intraperitoneally. Mucin was not used.

(*a*) Two groups of mice (C/57/Black strain—forty mice in each group) were inoculated with current issue David Bruce Laboratories (D.B.L.) cholera vaccine batch no. 398, one group subcutaneously and the other by the intradermal route. Seven days later a second immunizing dose was given. As the vaccine contained 8000 million organisms per ml., the group injected with 0·5 ml. subcutaneously received 4000 million organisms per mouse at each inoculation, whereas those

inoculated with 0·05 ml. intradermally received 400 million organisms per mouse at each inoculation. Ten days after the second immunizing dose all mice were challenged with 1 LD 100 (1000×10^6 organisms) *V. cholerae* strain Inaba (NCTC 7260), together with twenty unimmunized control mice. The mice were observed for 72 hr. and survivors recorded.

(*b*) The previous experiment was repeated with twenty mice in each group and a challenge dose of 2 LD 100 (2000×10^6 organisms).

(*c*) A further experiment was performed, with ten mice in each group, in which each vaccine was so diluted as to give an immunizing dose of 100 million organisms by both inoculation routes. The interval between the first and second inoculation and between the second inoculation and the 1 LD 100 challenge dose was 14 days.

(*d*) Two vaccines were prepared, both containing 4000×10^6 organisms per ml. of *V. cholerae* strain Ogawa (NCTC 8040) in 0·5 % phenol-saline. One contained in addition Inaba (NCTC 7260) of 4000×10^6 organisms per ml. whilst the other contained Inaba (NCTC 8039) of the same strength. Both vaccines therefore contained 8000×10^6 organisms per ml. Two groups of forty-five mice (C/57/Black Porton) were immunized with these vaccines by two intradermal injections of 0·05 ml. at an interval of 7 days. Seven days later, the mice were challenged in groups of 15 by the intraperitoneal route, in doses of 0·5 ml., with three strains of Inaba (NCTC 8039, 7258 and 7260), 900×10^6 organisms in each dose. Unimmunized control challenges were set up in parallel.

Active guinea-pig protection test

Animals of the Dunkin–Hartley strain of guinea-pigs, weighing between 250 and 350 g., received two immunizing doses of D.B.L. issue *V. cholerae* vaccine batch no. 398 containing 8000 million organisms per ml., at an interval of 5 days. Five animals received 0·1 ml. and 0·2 ml. injected by the intradermal technique, and five animals received 0·5 ml. and 1·0 ml. by the subcutaneous route.

Seven days after the second immunizing dose these ten animals, together with five unprotected ones as control, received a challenge of live *V. cholerae* (NCTC 7260) intraperitoneally, 5000 million organisms suspended in 1·0 ml. isotonic saline. The animals were observed and the survivors at 72 hr. noted.

Agglutination response in rabbits

This experiment was devised to compare the antibody response in rabbits after immunization with cholera vaccine given subcutaneously and intradermally.

Six rabbits (Porton coloured) were divided into three groups (A, B and C) of two animals in each group.

Two vaccines were prepared containing equal amounts of *V. cholerae* strains Inaba and Ogawa (NCTC 7260 and 8040, respectively) to strengths of 20,000 million and 8000 million organisms per ml.

All rabbits were bled to establish a base-line titre of agglutination. No agglutinins to several *V. cholerae* suspensions were detected. Group A rabbits received two inoculations of 0·1 ml. of the 20,000 organisms per ml. vaccine intradermally, with an interval of 16 days. They were bled 7 days after the second injection. Eight

days after the second injection a further dose of 0·1 ml. of the same vaccine was given intradermally. After a further 7 days they were bled again.

Rabbits of groups B and C were given two inoculations of 0·5 ml. of the 8000 million organisms per ml. vaccine subcutaneously, with an interval of 16 days between each injection. Seven days after the second injection they were bled. On the following day group B received a third dose of 0·5 ml. of the 8000 million organisms per ml. vaccine by the subcutaneous route and group C received an intradermal dose of 0·1 ml. of the vaccine of 20,000 million organisms per ml. Seven days after these inoculations both groups B and C were bled again.

Agglutinations were performed on all sera by the Felix & Bensted (1954) technique using an antigenic suspension of equal parts of *V. cholerae* strains Inaba and Ogawa. The titres were expressed as the reciprocal of the lowest dilution of serum in the last tube showing granularity of deposit visible to the naked eye.

Agglutination response in human volunteers

Eighteen human volunteers, from the staff of the David Bruce Laboratories, were found to have no agglutinin titres to several *V. cholerae* suspensions.

These volunteers fell into two groups: group A who had received no previous experience of cholera vaccination, and group B who had previously been given various cholera injections, but none within the previous 12 months.

Group A was subdivided into two further subgroups of five volunteers each and group B was divided into two groups of four volunteers in each.

Half of group A received two subcutaneous injections of D.B.L. current issue vaccine batch no. 400, in doses of 0·5 and 1·0 ml. at an interval of 10 days. The other half received two intradermal inoculations, of 0·1 ml. on both occasions, of the same vaccine, at the same interval.

The two subgroups of group B were inoculated in a similar manner except that they received one dose only of 0·1 ml. intradermally or 0·5 ml. subcutaneously.

All volunteers were bled at intervals of 4, 8 and 14 weeks after their last inoculation, and the agglutination titres of their sera determined.

Passive mouse protection tests

Rabbit immune serum was obtained by inoculating Porton coloured rabbits, either intradermally or subcutaneously, with three injections of current issue D.B.L. cholera vaccine batch no. 400, at intervals of 7 days. Intradermal inoculations were of 0·1 ml. volume and subcutaneous inoculations of 0·5 ml.

Seven days after the third inoculation the rabbits were bled and the agglutination titres determined.

The titres of agglutination produced by intradermal and subcutaneous inoculation were 1:320 and 1:160 respectively.

Two groups of twenty mice (Ajax albino), and one group of ten mice, were inoculated subcutaneously with immune serum from rabbits inoculated either intradermally or subcutaneously, and with normal rabbit serum, respectively. Twenty-four hours later all mice were challenged intraperitoneally with *V. cholerae* strain Inaba (NCTC 7260), in a saline suspension of 1500 million organisms

contained in a dose of 0·5 ml. Unprotected mice were challenged in parallel. All mice were observed and survivors at 72 hr. noted.

A further passive mouse protection test using sera obtained from human volunteers was carried out.

Two pairs of human volunteers were given issue D.B.L. cholera vaccine batch no. 400 in two inoculations of either 0·5 and 1·0 ml. subcutaneously or 0·1 ml. intradermally, at an interval of 10 days. Fourteen days later the volunteers were bled and the agglutination titres of their sera determined.

The titres of the individuals inoculated by the subcutaneous route were 1:640 and 1:320 whilst those injected intradermally were 1:320 and 1:160.

The sera were pooled and filtered and gave final titres of 1:320 and 1:160 respectively. Serum obtained from non-immunes tested at the same time showed no agglutination in the lowest dilution (1:10).

Two groups of twenty C/57/Black mice were inoculated with the immune sera and one group of ten mice received normal human serum.

The protective inoculation consisted of 0·5 ml. of serum administered subcutaneously, 24 hr. before challenge.

All mice were challenged with *V. cholerae* Inaba (NCTC 7260) live suspensions in a dose of 0·5 ml. given intraperitoneally, and containing 1500 million organisms suspended in isotonic saline.

Intradermal skin tests

This group of experiments was designed to study the effect of cholera vaccine and its components when inoculated intradermally into the skin of guinea-pigs.

Cholera vaccines consisting of Inaba and Ogawa strains (NCTC 7260 and 8040 respectively) mixed in equal proportions were used. The vaccines were heat-killed, preserved with 0·5% phenol-saline, and made up to strengths of 64,000, 32,000, 16,000 and 8,000 million organisms per ml. Controls included isotonic saline, 0·5% phenol-saline, and stock TABT intradermal vaccine (D.B.L. batch no. 31).

Guinea-pigs were inoculated intradermally on the shaved abdomen with 0·1 ml. of each of these preparations. The inoculation site and any skin lesions produced were observed and measurements taken daily.

The effect of repeated intradermal inoculations was studied in both guinea-pigs and rabbits by injecting those animals with doses of 0·1 ml. intradermally at weekly intervals for 9 successive weeks. The vaccine used was D.B.L. issue cholera vaccine batch no. 395. The injections were all placed into a 1 in. diameter shaved area of abdominal skin but not into the exact site of previous inoculations. The inoculation sites were examined daily for evidence of ulceration, and the presence or absence of indurated nodules assessed by palpation. Daily records of these observations were kept.

RESULTS

Animal virulence tests

These tests (see Tables 1 and 2) show that the strains used were lethal for mice and guinea-pigs, conform to the standards of virulence laid down by W.H.O. (1959), and were suitable as challenge organisms in active and passive protection tests.

The *V. cholerae* Inaba strains were more virulent for mice (C/57/Black) than the Ogawa strains. The virulence of *V. cholerae* strains was found to vary slightly from one experiment to the next and a preliminary virulence test was performed with each experiment in order to adjust challenge doses to an accurate LD 100.

Table 1. *Number of mice killed by graded challenge doses of various strains of* Vibrio cholerae

(Expt. 1. Virulence test. *V. cholerae*. C/57/Black mice. Av. wt. 22 g.)

Organisms used	Challenge dose live organisms in millions						
	250	500	1000	1500	2000	2500	3000
Inaba (7258)	1	4/7	9	10	9	—	—
Inaba (7260)	0	5	6	9	10	10	—
Inaba (8039)	0	5	10	10	—	—	—
Ogawa (5596)	0	1	4	8	7	10	10
Ogawa (8040)	1	0	6	—	10	—	—

Challenge dose given in a standard volume of 0·5 ml. saline intraperitoneally. Mice: C/57 Black—10 mice in each group unless otherwise stated. Controls: no deaths occurred with dead organisms inoculated at the same time, in the highest dose used for live challenge.

Table 2. *Number of guinea-pigs killed by graded doses of* Vibrio cholerae, *Inaba type* (NCTC 7260)

(Expt. 2. Virulence test. *V. cholerae*. Av. wt. 250–300 g.)

Challenge Inaba 7260—millions of organisms	2000	3000	4000
Deaths at 72 hr.	1/3	2/3	3/3

Numerators represent animals killed. Denominators represent number of animals challenged Challenge given in standard dose of 1·0 ml. intraperitoneally.

Active mouse protection tests

V. cholerae vaccine, inoculated by both the subcutaneous and intradermal routes, gave almost complete and equal protection against an LD 100 challenge of live *V. cholerae* organisms. Table 3, Expt. 3 (*a*), shows the results obtained. As the same vaccine, containing 8000 million organisms per ml., was used for inoculation by both routes, the organism content of the intradermal injections (400 million organisms in 0·05 ml.) was only 1/10 of the strength of the subcutaneous inoculation (4000 million in 0·5 ml.) yet, in this experiment, showed equal protection.

Expt. 3 (*b*), Table 3, showed that about half the mice were still protected when the lethal dose was doubled to 2 LD 100, irrespective of the route of inoculation.

Expt. 3 (*c*), Table 3, demonstrated that the organism content of the inoculum could be lowered to only 100 million organisms, injected by either route, and still give absolute and equal protection to an LD 100 challenge of live organisms.

Expt. 3 (*d*), Table 4, shows that protection with *V. cholerae* vaccine by the intradermal route in mice (C/57/Black) was almost complete, and independent of the Inaba strains used both in the preparation of the vaccine and as challenge organism. Inaba strains were selected as being the most virulent strains of *V. cholerae* available in these laboratories at the time.

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Table 3. *A comparison of the number of mice surviving a challenge of* 1 *LD*100 *and* 2 *LD*100 *Inaba organisms* (*NCTC* 7260) *after two inoculations of cholera vaccine administered by the subcutaneous and intradermal routes*

(Active mouse protection test. C/57/Black mice. Av. wt. 20–30 g.)

	Protection ...	Subcutaneous 0·5 ml. × 2 at 7 days	Intradermal 0·05 ml. × 2 at 7 days	Nil (control group)
	No. orgs × 10^6 ...	4000	400	Nil
Expt. 3 (*a*)	Challenge at 10 days—no. orgs. × 10^6	1000	1000	1000
	Survivors at 72 hr.	39/40	38/40	2/20
	Increased challenge dose			
Expt. 3 (*b*)	Challenge at 10 days—no. orgs. × 10^6	2000	2000	2000
	Survivors at 72 hr.	10/20	6/20	0/20
	Decreased protection dose—longer interval			
	Protection ...	0·5 ml × 2 at 14 days	0·05 ml × 2 at 14 days	Nil (control group)
	No. orgs. × 10^6 ...	100	100	
Expt. 3 (*c*)	Challenge at 14 days—no. orgs. × 10^6	1000	1000	1000
	Survivors at 72 hr.	10/10	8/10	0/10

Numerators represent survivors at 72 hr. Denominators represent number of animals challenged. Challenge organism Inaba (NCTC 7260) was given in a standard dose of 0·5 ml. intraperitoneally.

Table 4. *Active mouse protection test comparing two strains of* Vibrio cholerae *Inaba incorporated in the vaccine. Vaccine administered intradermally. Challenge—three strains of* V. cholerae *Inaba.*

(Expt. 3 (*d*). Active mouse protection test. C/57/Black mice. Av. wt. 18–25 g.)

Protected 0·05 ml. intradermally × 2 at 7 days.	Ogawa plus 7260 vaccine			Ogawa plus 8039 vaccine		
At 7 days challenged Inaba 900 × 10^6 in 0·5 ml. intraperitoneally	7258	7260	8039	7258	7260	8039
Survivors at 72 hr.	15/15	15/15	12/15	14/15	15/15	12/14

Control virulence test

Inaba strain	7258		7260		8039	
Dose in 0·5 ml. intraperitoneally × 10^6	450	900	450	900	450	900
Survivors at 72 hr.	5/10	1/10	7/10	0/10	0/10	0/10
Plate count viability	67 %		60 %		47 %	

Numerators represent the number of animals to survive. Denominators represent the number of animals inoculated.

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Active guinea-pig protection tests

Table 5 demonstrates that equal and good protection was afforded to guinea-pigs when vaccinated by either the intradermal route or the subcutaneous route with *V. cholerae* vaccine, and challenged with live virulent cholera organisms.

Table 5. *The numbers of guinea-pigs protected by* Vibrio cholerae *vaccine when challenged by a lethal dose of live* Vibrio cholerae

(Expt 4. Guinea pig active protection test. Av. wt. 250–300 g.)

Protected D.B.L. cholera vaccine. Batch no. 398, 2 doses at 5 days	Intradermal, 0·1 and 0·2 ml.	Subcutaneous, 0·5 and 1·0 ml.	Nil (control group)
Challenge Inaba 7260 orgs. $\times 10^6$ intraperitoneally in 1·0 ml.	5000	5000	5000
Survivors at 72 hr.	5/5	5/5	1/5
Plate count viability	49%		

Numerators represent number of animals to survive. Denominators represent the number of animals inoculated.

Table 6. *Agglutinin titres produced in rabbits after two and three doses of cholera vaccine given either intradermally or subcutaneously.*

(The antigen suspension contained both Inaba and Ogawa strains mixed in equal quantities.) (Expt. 5).

Rabbits		Titre after two inoculations	Titre after three inoculations
Group	No.		
A	R 6	80	320
	R 27	160	320
B	R 40	320	320
	R 48	160	320
C	R 5	640	320
	R 22	160	640

Group A received 3 doses intradermally ($20{,}000 \times 10^6$ orgs. per ml.). B received 3 doses subcutaneously (8000×10^6 orgs. per ml.). C received 2 doses subcutaneously and 1 dose intradermally. Titres expressed as reciprocal of the lowest dilution of serum in the test tube showing granularity of deposit visible to the naked eye.

Agglutinin response in rabbits

Agglutinin titres in previously unprotected rabbits, inoculated with two or three doses of cholera vaccine, were comparable, independent of whether the animals were inoculated by the subcutaneous or intradermal routes. Table 6 shows the responses obtained.

Agglutinin responses in human volunteers

No material difference could be detected in the agglutinin response in human volunteers when they were inoculated with issue D.B.L. cholera vaccine by either the subcutaneous or intradermal routes (Table 7). Previously unprotected

volunteers showed good agglutinin responses, and others requiring booster dosage, after at least 1 year's lapse since previous inoculation, showed an equally good response by either vaccination route. The actual agglutinin titres obtained were very much the same as those obtained with a similar vaccine, by Singer *et al.* (1948).

Table 7. *Agglutinin response in human volunteers to D.B.L.* Vibrio cholerae *vaccine, batch no.* 400, *administered by either the intradermal or subcutaneous route*

Expt. 6 Volunteers	Route	No.	Weeks after second inoculation or booster dose: 4	8	14
Group A. No previous cholera inoculations	Subcutaneous 0·5 and 1·0 ml., interval 10 days	5	20	80	80
		6	160	80	40
		7	160	160	160
		17	40	80	160
		18	80	160	320
	Intradermal 0·1 ml. × 2, interval 10 days	2	320	160	80
		3	160	80	40
		4	40	80	40
		15	20	20	20
		16	20	40	20
Group B. Various previous cholera inoculations. None during the past 12 months	Subcutaneous 0·5 ml. once only	11	80	160	80
		12	20	80	40
		13	160	320	80
		14	320	160	160
	Intradermal 0·1 ml. once only	1	320	40	40
		8	40	160	160
		9	20	20	20
		10	160	160	160

Titres expressed as the reciprocal of the lowest dilution of serum in the test tube showing granularity of deposit to the naked eye.

Passive mouse protection tests

This experiment demonstrates that about half the mice passively protected with immune rabbit serum survive a challenge of an LD 100 dose of live *V. cholerae*, as compared with no survivors in the control group receiving serum from unimmunized rabbits. No difference could be demonstrated in the protection afforded by sera obtained from rabbits immunized by either the subcutaneous or intradermal routes.

A temporary shortage in the supply of C/57/Black mice necessitated the use of Ajax (Porton) albino mice in the experiment described. The result was confirmed later in C/57/Black mice. The inclusion of the white mice experiment also illustrates that strains other than C/57/Black are equally effective in this type of cholera experiment. Table 8 shows the results obtained.

Table 9 shows the results with sera from human volunteers, and it is seen that the passive protection of C/57/Black mice is identical with that afforded by immune rabbit serum described above. Again the fact emerges that no material difference could be demonstrated between immunization by either the intradermal or subcutaneous routes.

Table 8. *Passive mouse protection test using sera from rabbits inoculated with* Vibrio cholerae *vaccine by the intradermal and subcutaneous route*

(Expt. 7. Passive protection test. Ajax white mice. Av. wt. 25 g.)

	Serum			
Protection 0·5 ml. subcutaneous	Rabbit 1 (intradermal titre 1:320)	Rabbit 2 (subcutaneous titre 1:160)	Neutral serum titre 0	Unprotected (control group)
Challenge Inaba 7260 0·5 ml. intraperitoneally orgs. $\times 10^6$	1500	1500	1500	1500
Survivors at 72 hr.	9/20	11/20	0/10	1/10

Numerators represent survivors. Denominators represent number of animals inoculated.

Table 9. *Passive mouse protection test using serum from human volunteers inoculated with* Vibrio cholerae *vaccine by the subcutaneous and intradermal routes*

(Expt. 8. Passive protection test. C/57/Black mice. Av. wt. 22 g.)

	Serum		
Protection 0·5 ml. subcutaneous	Pool I, subcutaneous, titre 1:320	Pool II, intradermal, titre 1:160	Neutral serum, titre 0
Challenge Inaba 7260 0·5 ml. intraperitoneally orgs. $\times 10^6$	1500	1500	1500
Survivors at 72 hr.	11/20	12/20	0/20

Numerators represent survivors. Denominators represent the number of animals inoculated.

Intradermal skin tests

(*a*) Cholera vaccine, inoculated intradermally, in doses of 0·1 ml. of vaccines containing 64,000 and 32,000 million organisms per ml. produced severe persistent reactions in the shaved abdominal skin of guinea-pigs. Firm, indurated nodules of average diameter 12 and 8 mm. respectively were produced. For the first few days the nodules were accompanied by a narrow band of erythema. In 10 days the nodules produced by the larger dose were about half their original size whereas those of the lower dose resolved in 5 days.

Guinea-pigs inoculated with vaccine containing 16,000 and 8000 million organisms per ml. showed no reactions at all. The reaction at the inoculation site measured less than 5 mm. 8 hr. after injection, and was not detectable 18 hr. later.

The TABT vaccine given intradermally produced no reactions, and was indistinguishable from the two lower cholera vaccine doses.

The phenol-saline (0·5 %) and isotonic saline produced no reaction, and were absorbed in 4 hr.

(*b*) Repeated intradermal inoculations in doses of 0·1 ml. of an 8000 million organisms per ml. cholera vaccine, at weekly intervals for 9 weeks into a limited

area of both rabbit and guinea-pig skins, failed to produce ulceration due to local tissue hypersensitivity. No reactions of any type occurred, except that early in the series it was noted that the inoculum was completely absorbed in approximately 8 hr., whereas after the sixth injection the inoculum tended to require a little longer time to absorb, which never exceeded 24 hr.

(*c*) During the course of the experiments in this paper some hundreds of intradermal inoculations, usually in pairs at an interval of 7–10 days, have been administered to mice, guinea-pigs, rabbits and human volunteers, and no reactions, except where provoked in (*a*) above, have been detected, and at no time has ulceration occurred at the site of injection.

DISCUSSION

The important methods available in the laboratory for assessing the immunizing properties of a vaccine may be classified as follows: (1) active immunization experiments in laboratory animals; (2) serological tests to determine the presence of agglutinating antibodies in the serum of actively immunized animals and man; (3) passive protection tests with the serum of vaccinated animals and man. Each of the three methods has its advocate. Burrows, Mather, Elliott & Havens (1947) stressed the value of active immunization tests, but formed an unfavourable opinion of passive mouse protection tests in assessing cholera vaccines. Griffitts (1944), on the other hand, strongly recommended passive mouse protection tests for assaying the results of cholera vaccination. Panja & Das (1947) and Singer *et al.* (1948) not only confirmed the value of active protection tests, but found excellent agglutination responses with cholera vaccine administered by both the subcutaneous and intradermal routes.

In the foregoing experiments, all three methods have been used to compare the immunizing properties of cholera vaccine injected both intradermally and subcutaneously. It should be noted that, except where otherwise specifically stated, the cholera vaccines contained a fixed organism content, and consequently mice injected intradermally with 0·05 ml. received one-tenth of the immunizing dose compared to those injected subcutaneously with 0·5 ml. Similarly, human volunteers injected with 0·1 ml. intradermally received one-fifth of the subcutaneous immunizing dose. No material difference in the protection afforded by cholera vaccine could be demonstrated between the two routes of administration. Active and passive mouse protection tests, active guinea-pig protection tests, and the agglutination responses in humans and rabbits, all gave excellent and parallel results independent of the inoculation route.

Miles (1958) studying the toxicity of intracutaneous TAB vaccine concluded that provided the dose of TAB bacilli was about half the minimum dose inducing necrosis at the centre of the injection site, it could be as safely injected in a 0·05 ml. volume as in a volume of 0·5 ml. or greater. He further stated that, in spite of some pronounced differences in structure, there is *prima facie* evidence that, so far as intradermal inoculations are concerned, human skin reacts in a similar way to the skin of guinea-pigs and rabbits.

In the experiments described above doses of cholera vaccine as large as

6400 million organisms (0·1 ml. of $64{,}000 \times 10^6$ organisms per ml.) intradermally inoculated in rabbits failed to cause ulceration, although unacceptable indurated nodules did occur. Smaller doses produced no such unacceptable reactions. Doses of 800 million organisms (0·1 ml. of 8000×10^6 organisms per ml.) in human volunteers produced no reactions of any kind.

Singer *et al.* (1948), whilst finding better antibody production with cholera vaccine administered by the intradermal route than by subcutaneous injection, rejected the former method as they experienced some cases of ulceration at the injection site when giving a third injection. This was not our experience. Doses of 0·1 ml. of a vaccine containing 8000 million cholera organisms per ml., injected intradermally at weekly intervals for 9 weeks into the skin of both rabbits and guinea-pigs, failed to produce ulceration.

Panja & Das (1947), whilst noting the economy of materials and negligible reactions resulting from the intradermal inoculation of cholera vaccine, considered the method impracticable in mass inoculation programmes. Combined enteric and tetanus prophylactic by intradermal inoculation has been in use in the Army for 4 years (Noble, 1963). During this time large numbers of intradermal inoculations have been given by Service medical officers without any technical difficulties being experienced.

It is the experience of the author, and a generally accepted fact among Service medical officers, that cholera vaccine administered by the subcutaneous route produces both local and general reactions in about 10% of patients inoculated. No reactions have occurred when cholera vaccine was administered by the intradermal route either to animals or to a small number of human volunteers.

Barr *et al.* (1958) demonstrated a dramatic fall in the incidence of reactions to combined enteric–tetanus prophylactic. The use of the intradermal route of inoculation for this prophylactic in the Army since 1959 had led to a considerable saving of manpower, due to the decrease in numbers and severity of reactions (Noble, 1963). As cholera vaccine, administered by the intradermal route, gives excellent protection, equal to that afforded by subcutaneous vaccination, it is suggested that reactions to cholera vaccine would be greatly reduced if the intradermal route of inoculation were adopted. Furthermore, in order to reduce the number of inoculations required by individuals proceeding overseas, it should be possible to combine intradermal TABT prophylactic with intradermal cholera vaccine, and still retain the freedom from undesirable inoculation reactions, shown separately by the two preparations.

SUMMARY

The protection afforded by cholera vaccine administered by the intradermal route, and demonstrated by active and passive mouse protection tests, active guinea-pig protection tests, and agglutination titres, is excellent and equal to that given by subcutaneous inoculation.

It is suggested that cholera vaccine administered by the intradermal route would greatly reduce the incidence of reactions which occur when the vaccine is given subcutaneously.

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