THE IMMUNIZING POTENCY OF ALCOHOL-KILLED AND ALCOHOL-PRESERVED TYPHOID VACCINE AFTER STORAGE FOR TEN YEARS

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CONTENTS

INTRODUCTION

The contention that alcohol-treated typhoid vaccine is superior to ordinary heat-killed phenolized vaccine is based primarily on the demonstration of its capacity to stimulate development of circulating Vi antibody in the rabbit and in man. At the time the alcohol-treated vaccine was introduced, it was stated that this type of vaccine 'still contains typhoid Vi antigen in its effective form after storage in the cold for at least 9 months, and probably much longer' (Felix, 1941). It was, therefore, important to determine for how long this distinctive property of alcoholized vaccine was maintained.

During the war of 1939–45 several batches of vaccine that had been stored at 1–2° C. for periods up to 4 years were examined, and no significant loss of their power to stimulate circulating Vi antibody could be detected. The expiry date of this type of vaccine could, therefore, be extended well beyond the 12–18 months' period generally accepted for the old type of typhoid-paratyphoid vaccine. In the present communication an account is given of the tests carried out with alcohol-killed and alcohol-preserved typhoid vaccine after storage for 10 years.

COMPARATIVE TESTS OF TYPHOID VACCINE TEN YEARS' OLD AND FRESHLY MADE

The four vaccines employed in the following experiment were monovalent typhoid vaccines, all made from the same *Salmonella typhi* strain Ty 2. Three of the vaccines had been prepared in February 1940 and served in one of the experiments described in a previous paper (Felix, 1941, Table III, p. 394). The remaining portions of these vaccines had been stored in the refrigerator at 1–2° C. until November 1949, i.e. approximately 10 years. Great care was taken throughout these years not to allow the vaccines to remain outside the refrigerator for any considerable period of time. On several occasions during the war years, when the laboratory was

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transferred to another place or when a break-down occurred in the refrigerating plant, it was impossible to avoid keeping the vaccine bottles at a temperature higher than 1–2° C. for periods of a few hours or, at most, for a day or two.

The fourth vaccine was freshly prepared before the start of the experiment in November 1949, using the same *Salm. typhi* strain Ty 2 and exactly the same method of alcohol treatment as that employed in the preparation of the corresponding vaccine in 1940. The four vaccines are listed in Table 1.

Table 1. *Giving details of the four typhoid vaccines used in the experiment*

(Monovalent typhoid vaccine containing 1000×10^6 organisms per ml. of *Salm. typhi* strain Ty 2.)

Vaccine	Sterilized by	Suspended in	Prepared	Stored at 1–2° C. for
A	75 % alcohol	Saline	February 1940	10 years
B	75 % alcohol	Saline + 0·5 % phenol	February 1940	10 years
C	75 % alcohol	Saline + 25 % alcohol	February 1940	10 years
D	75 % alcohol	Saline + 25 % alcohol	November 1949	2 weeks

Note. Vaccines A, B and C represent the remaining portions of the first three vaccines listed in Table III in the paper by Felix (1941).

The three vaccines made in 1940 were from small experimental batches, and the quantities available in 1949 were not sufficient to carry out active-immunity tests on groups of mice of adequate numbers. Moreover, these tests were not considered to be essential, for the reasons discussed in the preceding paper of this series (Felix, 1951). The three old vaccines and the freshly prepared control vaccine were compared for their antibody-stimulating properties in rabbits, and the rabbit sera were examined in passive-immunity tests in mice.

(*a*) *Vi- and O-antibody response in the rabbit*

Four groups of five rabbits, all from the same stock and weighing 2000–2300 g., were selected on the basis of preliminary tests for the presence in their serum of 'normal' H, O and Vi agglutinins for *Salm. typhi*. It has been shown previously (Felix, 1951) that the use of rabbits harbouring coliform organisms which share with *Salm. typhi* its characteristic Vi antigen (Kauffmann, 1941) may vitiate the test for the estimation of the potency of typhoid vaccines. Such rabbits contain in their serum pre-formed Vi agglutinins, and do not elaborate further Vi antibody in response to immunization with even the best preparation of the Vi antigen.

Three out of thirty rabbits that were examined in the preliminary tests had to be rejected, two because of a raised TVi-agglutinin titre and one because of an unusually high TO titre; none of the rabbits showed a significant TH agglutination. The sera from the twenty rabbits selected for immunization with the four different vaccines all gave readings in the agglutination tests below what is considered to be the limit of 'normal' TH, TO and TVi agglutinins in this animal, namely:

TH 'standard' agglutination in a dilution 1 in 20;
TO 'standard' agglutination in a dilution 1 in 500;
TVi 'standard' agglutination in a dilution 1 in 10.

It is not proposed to discuss here the details of the technique followed in the

agglutination tests summarized in Table 2. When the first experiments with alcohol-treated T.A.B.C. vaccine were published, it was stated that the technique of these agglutination tests had not yet been standardized satisfactorily and that the titres recorded by various workers might differ, and in fact did differ, very considerably. The opinion was then expressed that 'this difficulty will probably not be overcome until standard agglutinating sera are employed along with the standard agglutinable suspensions that are at present in use' (Felix, Rainsford & Stokes, 1941). The necessity for standardizing these and other agglutination tests by international agreement has been stressed recently (Felix, 1950), and the suggestion that 'International Serum Standards' be adopted has been accepted by the Expert Committee on Biological Standardization of the World Health Organization (World Health Organization, 1950).

The four groups of rabbits were immunized according to the same scheme as that which had been followed in the experiment published 10 years earlier. The rabbits were injected intravenously at weekly intervals, and the samples of blood were taken on the seventh day after each immunizing injection. The vaccines were diluted 1 in 4 with saline; the first dose contained 500×10^6 organisms in 2 ml. total volume; the second dose contained 1000×10^6 organisms in 4 ml. total volume. Large samples of blood were drawn from the jugular vein with all care for sterility—each time 30–35 ml. from each rabbit—to serve subsequently for passive-immunity tests in mice.

A glance at Table 2 shows that the O-agglutinin responses were equally good in the four vaccine groups. This is not surprising because the 'stable' O antigen is resistant to many kinds of chemical and physical treatment. On the other hand, there were significant differences in the Vi-agglutinin responses. Vaccine A, which had been stored suspended in saline alone, without any preservative, still contained a trace of agglutinogenically active Vi antigen, since two out of five rabbits in this group showed an appreciable, though rather feeble, Vi-agglutinin response. Vaccine B, which had been preserved with 0·5% phenol, did not stimulate any Vi-antibody response, whereas the alcohol-preserved vaccine C showed no deterioration at all. The five rabbits that received the 10-year-old vaccine C gave Vi-agglutinin responses as good as those in the control group that had been immunized with the freshly prepared vaccine D. No significance, of course, attaches to the fact that two of the rabbits in vaccine-group D (nos. 18 and 20) showed slightly higher Vi titres than the corresponding animals in vaccine-group C (nos. 12 and 15), so that the average titre was slightly higher in group D than in group C. The degree to which the antibody-producing capacity of the individual varies in all animals is too well known to call for further emphasis.

As a rule, the highest TVi titres were found in the sera from bleeding III, taken 7 days after the second dose of vaccine. There was only one exception to this rule, rabbit no. 19, which showed the maximum titre after the first dose. The TO-agglutinin curve does not follow the same course; in half the rabbits listed in Table 2 the TO agglutinins had already reached their maximum after the first dose of vaccine. The Vi-antibody titre also tends to fall more rapidly than the TO titre. Eleven of the twelve rabbits that developed Vi antibody showed an appreciable

fall in the titre in the serum from bleeding IV, which was drawn only 3 days after bleeding III; the one exception was rabbit no. 18. The corresponding figures for the TO antibody were: eleven rabbits showed a decrease in titre between bleedings III and IV, whereas nine rabbits maintained their TO titre at the same level. These observations are in good accord with earlier experience gained while immunizing horses (Felix & Petrie, 1938). The fact that there is a strong tendency for the Vi-agglutinin titre to decrease rapidly must be borne in mind in order to procure rabbit sera of a Vi-antibody titre sufficiently high to be suitable for the passive-immunity test in the mouse.

Table 2. *Vi- and O-agglutinin responses in rabbits immunized with variously preserved typhoid vaccines*

	Specimen of serum	Rabbits immunized intravenously with: Vaccine A (1940) 1	2	3	4	5	Vaccine B (1940) 6	7	8	9	10
Standard TVi-agglutinin titre	I	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	15	0	0	0	0	0	0
	III	0	0	0	25	15	0	0	0	0	0
	IV	0	0	0	20	0	0	0	0	0	0
Standard TO-agglutinin titre	I	0	0	0	0	0	0	0	0	0	0
	II	8,000	4,000	5,000	10,000	20,000	4,000	2,000	20,000	5,000	10,000
	III	6,000	5,000	5,000	25,000	20,000	12,000	4,000	20,000	10,000	20,000
	IV	6,000	4,000	4,000	20,000	20,000	10,000	3,000	10,000	10,000	20,000

		Rabbits immunized intravenously with: Vaccine C (1940) 11	12	13	14	15	Vaccine D (1949) 16	17	18	19	20
Standard TVi-agglutinin titre	I	0	0	0	0	0	0	0	0	0	0
	II	0	50	50	15	40	40	12	40	80	10
	III	60	70	180	30	100	180	30	120	60	120
	IV	35	35	100	25	70	120	20	120	30	60
Standard TO-agglutinin titre	I	0	0	0	0	0	0	0	0	0	0
	II	5,000	10,000	25,000	25,000	12,000	10,000	12,000	10,000	5,000	5,000
	III	5,000	10,000	20,000	20,000	10,000	20,000	12,000	10,000	10,000	10,000
	IV	5,000	10,000	20,000	15,000	5,000	20,000	5,000	10,000	5,000	5,000

'Standard' titres are based on the 'Provisional Standard Anti-typhoid Serum' (Felix, 1938). TVi-agglutinin titre O = a negative result in a dilution 1 in 10. TO-agglutinin titre O = a negative result in a dilution 1 in 500. I = serum taken before immunization. II = serum taken 7 days after first intravenous dose of vaccine (equivalent to 500×10^6 *Salm. typhi*). III = serum taken 7 days after second intravenous dose of vaccine (equivalent to 1000×10^6 *Salm. typhi*). IV = serum taken 10 days after second intravenous dose of vaccine.

Table 3 has been compiled in order to make a direct comparison of the results obtained with vaccines A, B and C after storage for 9 months and for 10 years, respectively. The relevant findings of the 1940 experiment are reprinted from the earlier paper (Felix, 1941, Table III, p. 394). It is clear that the deterioration of the Vi antigen that occurs rapidly on storage in saline alone or with the addition of 0·5% phenol does not take place in the presence of 25% alcohol in the vaccine even after 10 years.

This finding made it necessary to examine the rabbit sera in passive-immunity tests in mice. It is known from earlier work that the Vi antibody elaborated in

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response to alcohol-killed typhoid bacilli is in all respects identical with that resulting from immunization with the 'natural' Vi antigen from living Vi strains. Thus the potency in protective action of both varieties of serum runs parallel to the titre of Vi agglutination, a correlation which serves as a criterion of the functional efficacy of the Vi antibody (Felix & Bhatnagar, 1935; Felix & Petrie, 1938). Nevertheless, the importance of the finding that vaccine C after 10 years' storage apparently contained the Vi antigen in undamaged form called for a careful comparison of the protective action of the rabbit sera from vaccine-groups C and D.

Table 3. *Effect of storage on Vi-antibody response to variously preserved typhoid vaccines*

	Examined 1940 after storage at 1–2° C. for 9 months			Examined 1949 after storage at 1–2° C. for 10 years		
		No. of rabbits showing a significant increase in Vi-agglutinin titre after intravenous injection of			No. of rabbits showing a significant increase in Vi-agglutinin titre after intravenous injection of	
Monovalent typhoid vaccine sterilized by 75 % alcohol. Suspended in	No. of rabbits in group	500×10^6 organisms	1000×10^6 organisms	No. of rabbits in group	500×10^6 organisms	1000×10^6 organisms
A. Saline	4	2	2	5	1	2
B. Saline + 0·5 % phenol	4	1	1	5	0	0
C. Saline + 25 % alcohol	4	4	4	5	5	5
Control vaccine freshly prepared						
D. Saline + 25 % alcohol	.	.	.	5	5	5

Note. The figures for 1940 are reprinted from the paper by Felix (1941, Table III, p. 394).

Table 4. *Vi- and O-agglutinin titres of pooled rabbit sera from vaccine groups A, B, C, and D*

	Pooled sera from bleedings III of rabbits from vaccine groups			
	A	B	C	D
Standard TVi-agglutinin titre	1:8	<1:5	1:90	1:120
Standard TO-agglutinin titre	1:14,000	1:10,000	1:10,000	1:14,000

(*b*) *Passive-protection tests in mice*

The sera from bleedings III were used in the mouse-protection tests, since these specimens of serum had the highest Vi-agglutinin titres. The sera had been kept in the refrigerator without addition of any preservative and remained sterile. Equal quantities of serum from each of the five rabbits belonging to one vaccine group were pooled, and the Vi- and O-agglutinin titres of the four pooled sera were determined. These tests were set up in duplicate in dilutions chosen closely enough to enable accurate estimation of the two antibodies to be made. If the difference between the two parallel estimations of a given serum was more than 20 % the titration was repeated, again in duplicate. It has been stated previously (Felix, 1938) that the deviation in the readings of agglutination tests is not less than

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$\pm 20\%$, even in the hands of experienced investigators. It may be added here that the accurate reading of O-agglutination may be even more difficult than that of Vi-agglutination tests.

Table 4 shows the Vi- and O-agglutinin titres of the four pooled sera. Within the limits of accuracy of these tests the observed titres were in good agreement with the mean calculated for each group from the titres of the individual sera recorded in Table 2.

Since the main object of the comparative mouse-protection tests was to establish whether or not the Vi antibody contained in the serum from vaccine-group C was as effective as that from vaccine-group D, it was essential to employ the two sera in doses containing equal amounts of the Vi antibody. Earlier work with the 'Provisional Standard Anti-typhoid Serum' had shown that the degree of accuracy of the passive-immunity test in the mouse was such that a difference in the dose of the serum of the order of 25% was reflected by a difference in the survival rates when groups of twenty mice were employed for each dose of serum (Felix, 1938). The pooled serum III from vaccine-group D was, therefore, diluted with pooled serum from bleedings I of the corresponding rabbits, in the proportion of three parts of bleeding III to one part of bleeding I. Repeated titrations of the pooled serum C and the adjusted serum D (i.e. diluted 3 to 1) showed that their Vi- and O-agglutinin titres were, for practical purposes, identical.

The technique of the passive-immunity test followed in all essential points the procedure recommended in earlier work and need not be discussed in detail. The test culture of the typhoid strain Ty 2 was first examined for its degree of inagglutinability by pure O serum and its degree of agglutinability by pure Vi serum. Having passed these tests, the size of the challenge dose was determined in a preliminary virulence test the day before the mice were given the serum intramuscularly in the thigh. The challenge dose, always contained in 0·5 ml. of Ringer solution, was injected intraperitoneally 48 hr. later. The culture was grown on agar containing 20 g. dehydrated 'Bacto' nutrient broth per litre, incubated for 12 hr. at 37·5° C. and suspended in Ringer solution of a pH of from 7·6 to 7·4, the temperature of which had been brought to 37·5° C. Male mice only were used, all from one stock and weighing 16–20 g.

The intention was to give the mice a dose of serum that would protect less than 50% of the animals. Little published information, however, could be found to guide us in the choice of the right dose of serum. Most of the workers who have published results of passive-immunity tests with the typhoid bacillus have employed the mucin technique, and the fallacy of this method has been discussed in the preceding paper. On the other hand, the earlier work of one of us (A. F.) was concerned almost exclusively with high-titre antisera with Vi-agglutinin titres ranging from 1 in 600 to 1 in 3000, whereas the rabbit sera from groups C and D had a Vi titre of only 1 in 90.

The 'Provisional Standard Anti-typhoid Serum' has a Vi-agglutinin titre of 1 in 600, and a dose of 0·2 ml. of this serum protected approximately 50–75% of mice against 3 M.L.D. of the virulent strain Ty 2 (Felix, 1938). The pooled rabbit sera C and D, of a Vi titre of 1 in 90, could, therefore, be expected to afford an

equivalent amount of protection in a dose of 1·32 ml. Since it was intended to employ a challenge dose of 2·5 M.L.D. and to protect rather less than 50% of the animals, it was decided to use the sera C and D in a dose of 0·4 ml. per mouse. This guess proved to be a gross underestimate and the first experiment was a complete failure. In the next experiment the mice received a dose of 1 ml. of serum and this was found to give adequate protection.

Two preliminary virulence tests of the challenge strain, the first carried out 2 weeks and the second 1 day before the passive-immunity test, showed that the approximate M.L.D. of the cultures was not greater than 100×10^6 organisms (see Table 5). This is the level of virulence below which, according to earlier experience, the culture should not be allowed to fall if it is to be fully O resistant, and to serve as a reliable measure of the protective action of the Vi antibody (Felix, 1938, 1951). To maintain the cultures at this level of virulence it is essential to employ an agar medium of high nutritive value. Neither ordinary meat-infusion agar nor agar containing 8 g. 'Bacto' dehydrated nutrient broth per litre, as used in routine laboratory work, are suitable for this particular purpose. Only trypsin-digest agar of best quality or agar containing not less than 20 g. 'Bacto' dehydrated nutrient

Table 5. *Preliminary virulence tests for the determination of the challenge dose*

No. of organisms of *Salm. typhi* strain Ty 2 in dose	Tested 10 January 1950 No. of mice		Tested 24 January 1950 No. of mice	
	Tested	Died	Tested	Died
100×10^6	10	10	10	10
80×10^6	10	9	10	9
60×10^6	10	8	10	9
40×10^6	10	7	10	8

broth per litre will ensure the full development of Vi antigen and the maintenance of maximum mouse virulence. The same two media are also indispensable for growing cultures of *Salm. typhi* that are to be typed by the Vi-bacteriophage technique (Craigie & Felix, 1947; Felix & Anderson, 1951).

It should be mentioned that the results of virulence tests with strain Ty 2 are not always as regular as those obtained in the two tests recorded in Table 5. When the decrease in survival rates with increasing challenge doses is irregular, this is usually found to be due to one of the following two reasons: either the mice are from a stock of relatively high resistance to infection with *Salm. typhi*, or the technique of the intraperitoneal injection is at fault, resulting in the challenge dose being occasionally injected into the lumen of the intestines or into the bladder. Holding the mouse suspended in a vertical position during injection reduces the risk of such accidents. From experience with the strain Ty 2 during the past 17 years it can be stated that the correct determination of the dose representing the approximate M.L.D. is, as a rule, possible from a single preliminary virulence test using groups of ten mice for each dose.

On the basis of the two preliminary virulence tests (Table 5) a dose of 250×10^6 organisms was chosen as the challenge dose in the comparative protection tests recorded in Table 6. The titration of the challenge suspension in normal control

mice showed that the challenge dose did, in fact, represent approximately 2·5 M.L.D.

It is seen from Table 6 that only the pooled sera C and D afforded a certain amount of protection, whereas the other three sera were entirely devoid of protective action. Serum B from the pooled bleedings I which contained no Vi or O antibody was included as a control serum. The percentage difference between the survival rate in group AIII or BIII and that in group CIII is more than twice its standard error and is therefore statistically significant; an analysis of the data by the χ^2 method confirms this. On the other hand, the difference between the

Table 6. *Comparative protection tests* 27 *January* 1950

(Challenge dose: approximately 2·5 M.L.D. from strain Ty 2.)

Pooled rabbit sera from		Agglutinin titre of antibodies present in the serum		Dose of serum	No. of mice tested by intraperitoneal injection of 250×10^6 organisms of *Salm. typhi* strain Ty 2		Percentage of
Vaccine group	Bleeding	TVi	TO	(ml.)	Tested	Survived	survivors
A	III	1:8	1:14,000	1	20	1	5
B	I	<1:5	<1:500	1	20	1	5
	III	<1:5	1:10,000	1	20	1	5
C	III	1:90	1:10,000	1	20	8	40
D	III diluted 3 to 1	1:90	1:10,000	1	20	5	25
Controls with normal mice:							
Group receiving 120×10^6 organisms					10	1	.
Group receiving 100×10^6 organisms					10	1	.
Group receiving 90×10^6 organisms					10	0	.
Group receiving 80×10^6 organisms					10	3	.

figures for groups C and D is not statistically significant. It is, however, clear that serum C, from the group of rabbits immunized with 10-year-old alcoholized vaccine, was not less effective than serum D, from the rabbits that received freshly prepared vaccine.

The table also illustrates once again that antisera of relatively high O-antibody titre are as ineffective as normal rabbit serum, when the challenge strain possesses a sufficiently high degree of mouse virulence.

It was not considered necessary to compare the protective value of the Vi antibody contained in serum C with that of the 'Provisional Standard Anti-typhoid Serum', which has been prepared by immunization with the 'natural' Vi antigen as contained in the living bacterial cell. Freshly prepared suspensions of alcohol-treated bacilli were examined very extensively in this respect in earlier work, and were invariably found to induce production of Vi antibody with a protective value of the same order as that resulting from immunization with living Vi bacilli (Felix & Petrie, 1938). Since serum C was not less effective than serum D, it is evident that storage for 10 years had not caused any detectable change in the properties of the Vi antigen contained in the alcohol-preserved vaccine.

DISCUSSION

The finding that alcohol-preserved typhoid vaccine retained its immunogenic properties unimpaired during storage for 10 years came as something of a surprise. It was known from the work of Rainsford (1942) that this type of vaccine, after being stored for only 20 days at 37° C., failed to stimulate Vi-antibody formation in the rabbit, and similar observations were also published by Loureiro (1946). It was, therefore, expected that storage at 1–2° C. for a period as long as 10 years would have a destructive effect on the Vi antigen strong enough to be detected in the rabbit or mouse tests. The finding that this was not so was as unexpected as it was welcome.

Peluffo (1941), in a remarkable series of experiments, showed that the Vi antigen was heat-labile only in aqueous suspensions, but was highly resistant to heat when the bacilli were suspended in absolute alcohol or acetone. After dehydration *in vacuo* the Vi antigen was not inactivated even by heating for 2 hr. at 100° C. or for 1 hr. at 150° C. (Peluffo, 1941). Dried bacilli, killed by treatment with acetone and desiccated *in vacuo*, were successfully employed by Henderson, Amies & Steabben (1940) for the preparation in the horse of therapeutic anti-typhoid serum of high Vi and O titre, and Rainsford (1942) suggested the use of acetone-dried T.A.B.C. vaccine in tropical and subtropical climates under circumstances where cool storage is unobtainable. Experiments with hypertonic solutions as a means of dehydrating the bacterial cell and thereby preserving the Vi antigen were also successful. Rainsford (1942) suggested for this purpose the use of a 32% saline solution as suspending fluid. Loureiro (1946) found a molar (34%) sucrose solution to be an effective stabilizer of the Vi antigen and a suitable vehicle for typhoid vaccine. The result of the experiment described in the present paper clearly indicates that the dehydrating effect of alcohol in a 25% solution is strong enough to prevent hydrolysis of the Vi antigen taking place at 1–2° C., though not at room temperature.

The necessity of storing alcohol-killed and alcohol-preserved typhoid vaccine in the refrigerator was recognized from the start. This was emphasized at the time the new type of typhoid vaccine was first described, and it was repeatedly stated that all the vaccines used in the early experiments had been stored at 1–2° C. (Felix, 1941; Felix *et al.* 1941). With the old type of heat-killed and phenol-preserved vaccine it was customary to keep the vaccine bottles at ordinary temperatures during storage and transit. Although the words 'Store in the cold room' were prominently printed on all labels and leaflets issued with the alcoholized vaccine that was prepared at the Lister Institute, due attention was not paid to this warning by the principal users of the vaccine in this country.

During the 1939–45 war, and until quite recently, the alcoholized vaccine was stored, transported and dispensed in the same way as was used for the old type of vaccine. It is only during the last 2 or 3 years that steps have been taken by the Army and the Air Force authorities to ensure that alcoholized vaccine is handled with due precaution, i.e. stored and transported in cold storage. For this reason it is not justifiable to draw any conclusion regarding the efficacy of the vaccine from

reports of outbreaks of typhoid fever in Service personnel who were inoculated with alcoholized T.A.B. vaccine before the necessary precautions in the handling of the vaccine had been introduced. This applies also to the observations on the value of the alcoholized vaccine recorded by one of us in a report on an outbreak of typhoid fever in the Middle East in 1945 (Anderson & Richards, 1948).

Two points of practical importance emerge from the fact that alcohol-preserved typhoid vaccine does not deteriorate on storage for at least 10 years. The expiry date of this type of vaccine can be safely extended far beyond that of phenolized vaccine, which is generally fixed at 12–18 months (Perry, Findlay & Bensted, 1934). A longer period, 2½–3 years, was suggested by Mishulow, Mowry & Stocker (1937), but the mouse-protection tests on which these authors based their suggestion were carried out by the mucin technique and their results, therefore, cannot be accepted as affording a reliable guide to the state of preservation of the Vi antigen in the vaccine. The expiry date of alcoholized vaccine can be extended to a period of 10 years, provided the vaccine is kept continuously in cold storage.

Another conclusion of practical importance is that the alcohol-preserved vaccine can now be adopted as 'standard vaccine'. It has been stated in the preceding paper (Felix, 1951) that earlier attempts at standardizing the potency of typhoid vaccine by adopting an internationally agreed method of test did not materialize, because no method existed that would permit a 'standard vaccine' to be prepared which would remain stable over a reasonably long period of time. Alcohol-preserved vaccine has now been shown to remain stable for at least 10 years and can, therefore, be suggested as a suitable 'standard vaccine'.

SUMMARY

1. Alcohol-killed and alcohol-preserved typhoid vaccine retains its power of stimulating Vi- and O-antibody formation unimpaired during storage at 1–2° C. for at least 10 years.

2. The Vi antibody induced by immunization with the 10-year-old vaccine possesses full 'functional efficacy' in passive-immunity tests in mice.

3. The expiry date of this type of vaccine can be extended to a period of 10 years, provided the vaccine is kept in cold storage.

4. Alcoholized vaccine can serve as 'standard vaccine' in any future attempt at standardization of the potency of typhoid vaccine.

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