BRITISH MEDICAL JOURNAL

LONDON SATURDAY MARCH 15 1941

A NEW TYPE OF TYPHOID AND PARATYPHOID VACCINE

BY

A. FELIX, D.Sc.

(From the Lister Institute and the Emergency Public Health Laboratory Service)

Anti-typhoid inoculation is one of the old-established immunizing procedures introduced in the relatively early days of the bacteriological era. The original method of making typhoid vaccine (Wright and Semple, 1897; Pfeiffer and Kolle, 1896) has survived essentially unchanged up to the present time, and the type of vaccine still employed most extensively consists of a suspension of bacterial bodies which have been killed by heat and preserved by the addition of phenol. A great number of different methods have been described, both before and after the war of 1914-18, with a view to increasing the immunizing power of the vaccine and reducing the unpleasant systemic reactions which follow the administration of typhoid vaccines of the ordinary type. Some of these modified vaccines were suspensions of bacterial bodies killed by various chemicals instead of by heat, others were bacteria sensitized by treatment with homologous immune serum, while still other modifications consisted of bacterial extracts prepared in a great variety of ways (for references see Gay, 1918; Fleming and Petrie, 1934). These numerous types of typhoid vaccine were, however, all abandoned, since none of them was found to be superior to the ordinary vaccine made by the simple and time-honoured method of heat-killing and phenol-preservation.

Practical advances in anti-typhoid immunization, long delayed, have recently been made in quick succession as a result of our newer knowledge of the antigenic structure of the typhoid bacillus and of the different significance of different antibodies. Of the two typhoid antigens that were first differentiated the somatic O antigen is an important immunizing agent, while the flagellar H antigen is not (Felix, 1924; Arkwright, 1927; Topley, 1929; Schütze, 1930). Arkwright (1927), in an admirable discussion of the Army Council Report of the Anti-typhoid Committee (1912), showed that its main conclusions with regard to the preparation of typhoid vaccine were accounted for by the fact that the committee concentrated its attention almost entirely on one test as an index of immunity—namely, the measurement of the agglutinins produced by the inoculation of rabbits and man. The only typhoid agglutinin that could be estimated at that time was, however, the H agglutinin, and it is now known that the corresponding antigen is of little, if any, importance in the production of immunity.

Grinnell (1932) showed that the mouse is a suitable experimental animal for virulence and protection tests with the typhoid bacillus, and that the Rawlings strain—the classical laboratory strain used for more than thirty years in the preparation of typhoid vaccine in this and many other countries—had lost its immunizing capacity, although the cultures still possessed most of the commonly accepted criteria of smoothness. Perry, Findlay, and Bensted (1933a, 1933b, 1934a, 1934b) demonstrated in numerous experiments on mice that the degree of protection afforded by a typhoid vaccine was closely related to the virulence of the culture employed in its preparation. These workers also succeeded in "rejuvenating" the old Rawlings strain. It is now generally recognized that smooth mouse-virulent cultures of the typhoid bacillus possess the two antigenic constituents known as the O and the Vi antigens and that these are the essential elements which determine the immunizing capacity of typhoid vaccines (Felix and Pitt, 1934).

The balance of evidence obtained from active and passive protection experiments in mice indicates that the Vi antigen is the more effective prophylactic agent. At the same time reports have accumulated from various parts of the world showing that strains of the typhoid bacillus when freshly recovered from the blood culture invariably contain the Vi antigen, which, however, is gradually lost on subculture on ordinary media (Craigie and Brandon, 1936; Bensted, 1937, 1940). Earlier reports on the isolation from the blood of typhoid patients of strains devoid of the Vi antigen—for instance, that published by Felix, Krikorian, and Reitler (1935)—were probably incorrect and resulted from inadequate methods of growing and testing the cultures. It seems justifiable, therefore, to assume that the prophylactic value of typhoid vaccines has been greatly increased in recent years by the employment of selected strains which are particularly rich in the Vi antigen.

The Case for Changing the Old Method of Making Typhoid Vaccine

Why, then, it will be asked, should a new type of typhoid vaccine be suggested? It is perhaps not out of place to quote here two remarks from Gay's (1918) excellent monograph on typhoid fever: "It may well be questioned why further modification of typhoid vaccine

should seem necessary in view of the extremely good protective results that have already been obtained in certain large groups of individuals. . . . The only answer to such questions is and must ever remain that theoretical considerations based on the understanding of underlying principles will ever precede all practical advances" (p. 163). There can be little doubt that present-day methods of anti-typhoid immunization do not yet afford protection of a degree comparable to the immunity acquired by recovery from the disease. Gay's remarks seem to be as much to the point now as they were at the time they were written, nearly a generation ago.

The available knowledge of the properties of the Vi antigen clearly indicates that it is a remarkably labile substance which suffers very considerable damage by the treatment to which it is subjected in the customary processes of making typhoid vaccine. Sera from rabbits or horses immunized with suspensions of Vi strains sterilized by heating to 58° C. or by 0.5% phenol contain either no Vi antibody or only negligible amounts of it. Such sera are much less effective in protecting mice against virulent O + Vi bacilli than are sera which have been prepared by immunization with living bacilli and contain demonstrable Vi antibody. Treatment with formalin, such as that employed in the preparation of Grasset's (1939) "endotoxoid" vaccine or the mixture of T.A.B. vaccine and tetanus toxoid suggested by Ramon (1939) and Maclean and Holt (1940), so alters the Vi antigen that the resulting antibody exhibits a peculiar "functional deficiency," which is characterized by a reduced power of promoting phagocytosis and of protecting mice passively against infection with virulent strains of the typhoid bacillus (Felix and Bhatnagar, 1935). On the other hand, alcohol-treated suspensions stimulate in the rabbit or the horse the production of a Vi antibody which possesses the full functional efficacy of the antibody induced by immunization with the "natural" Vi antigen in living Vi strains (Felix and Petrie, 1938).

When mice are actively immunized, instead of passively protected by the injection of immune serum, the results are entirely different. In mice, vaccines sterilized in different ways induce an active immunity against infection with a virulent strain so long as cultures containing the Vi antigen are employed in the preparation of the vaccine. Schütze (1936) compared ten different methods of sterilization, including the standard method of heating to 53° C. for seventy minutes with the subsequent addition of 0.5% phenol, and found all of them of equal value in the active immunization of mice. Other workers have recorded similar results, and active-protection tests in mice are now employed in most laboratories as the sole method of assessing the protective value of typhoid vaccines. The demonstration of circulating antibodies in a suitable animal or in man, by using the serum to passively immunize mice, is not generally held to indicate the real protective value of a vaccine, and most workers discard it as unnecessary, though this technique appears to me to be of fundamental importance. The position is reminiscent of that already referred to when, in the early days of typhoid vaccination, agglutination tests were used as the sole criterion of immunity response, to the exclusion of the other suggested tests which were more difficult to perform, such as the estimation of the lytic or opsonizing power of the serum, or protection tests in the guinea-pig.

It is recognized that active antibacterial immunity depends mainly on the increased power of the tissues to elaborate specific antibodies rapidly when infection occurs, and the titre of the circulating antibodies that is found in the individual animal or man before the stimulus of infection occurs is not a reliable measure of this power.

To that extent it is probably correct to state that active antibacterial immunity is independent of circulating antibodies. It is, however, equally well established that both the increased cellular reactivity and the circulating antibody owe their existence to the specific stimulus of the same substance—namely, the specific bacterial antigen. Thus it is also correct to state that in order to induce good active immunity the vaccine used must contain the specific antigen in that form which most effectively stimulates the increase of both cellular reactivity and the circulating antibody. Recovery from the disease, it is known, produces both these signs of immunity response.

It has been mentioned already that the active immunity experiments in mice do not disclose those great differences in the antigenic value of various preparations of the Vi antigen which are so clearly demonstrated by using the serum of vaccinated rabbits or horses in passive-protection experiments with mice or even by *in vitro* laboratory tests. This may perhaps be due to the fact that the typhoid bacillus is not a natural pathogen of the mouse. The customary anti-immunity test in the mouse therefore does not represent a sufficiently sensitive method of testing different preparations of typhoid antigens, and certainly should not be accepted as the sole test. It is obvious that in comparative tests of this kind all available methods should be employed in order to detect how far the immunizing properties of the various vaccines have been preserved or impaired. At present the position seems to be unsatisfactory, since the greatest care is taken to include in the vaccine only cultures that are particularly rich in the Vi antigen but very little is done to ascertain that this substance is being preserved in its most efficient form.

Methods of Assaying the Protective Value of Different Types of Typhoid Vaccine

Three distinct methods are available for carrying out comparative tests of different types of typhoid vaccine, as follows: (1) Active immunization of mice. (2) Immunization of rabbits with a view to establishing: (a) whether circulating Vi and O antibodies are readily elaborated; (b) whether the Vi antibody produced possesses full functional efficacy or belongs to the variety which is deficient in opsonizing and protecting power. (3) Tests of the Vi and O antibody responses in man.

Technical details cannot be discussed here. The tests are essentially identical with those employed in the estimation of the potency of therapeutic anti-typhoid sera, and have been described in detail (Felix, 1938). A few remarks only need be made concerning the practical application of the various tests.

1. Active immunization of mice is the least sensitive test. A vaccine that has been found to be of low protective value in this test should be condemned, and need not be further examined.

2a. The antibody response in the rabbit is estimated by Vi and O agglutination tests. It has been shown, in work already referred to, that in sera prepared against living Vi bacilli the Vi agglutinin titre runs approximately parallel with the protective power against living virulent bacilli, while the O agglutinin titre in all sera that contain the O antibody runs, on the whole, parallel with the protective power against the toxic action of killed typhoid bacilli. Thus the two agglutinin titres provide a trustworthy measure of the protective activities of the two antibodies, and the complicated protection tests can be safely replaced by the relatively simple agglutination reaction.

2b. The functional efficacy of the Vi antibody is examined in passive-protection tests in mice or in phagocytosis experiments (Felix and Bhatnagar, 1935).

3. A vaccine that has successfully passed the tests specified under paragraphs 1 and 2 should be submitted to the final

test of its capacity to induce the formation of Vi and O agglutinins in man.

So far this more severe method of assaying the efficacy of typhoid vaccines by tests in the mouse, the rabbit, and man has not been adopted by the majority of the workers engaged in the preparation of typhoid and paratyphoid vaccines. Rainsford (1937, 1939) experimented with typhoid vaccines sterilized by silver nitrate or sodium merthiolate and tested them in the manner outlined here. His experiments, however, were interrupted by the outbreak of the war before reaching the final stage of testing the efficacy of the vaccines in the inoculated human subject.

Experiments with Alcohol-killed Typhoid Vaccine

As soon as it was found that alcohol-killed typhoid bacilli stimulated in the rabbit abundant Vi antibody of full protecting and opsonizing power (Felix and Pitt, 1936; Felix and Petrie, 1938) an experiment was arranged to test for antibody response to such vaccine in the inoculated human subject. With the kind co-operation of Dr. W. D. Nicol, medical superintendent of the Horton Mental Hospital, 50 female mental patients were given three doses of the vaccine by the subcutaneous route, and another group received large doses of the same vaccine by the mouth. Samples of blood were taken in each case before the first dose and again eighteen days after the third dose, and the sera were examined in parallel by agglutination tests, using as reagents the same batch of agglutinable suspensions. Any rise in agglutinin titre corresponding to an increase of 100% or more was taken as "significant." The results of the O and Vi agglutination tests are summarized in Table I.

TABLE I.—Percentage of Cases showing a Significant Increase in O and Vi Agglutinins after Immunization with Alcohol-killed Typhoid Vaccine containing no Preservative

	No. of Organisms in Dose	No. of Immunized Persons in Group	Percentage Frequencies of Significant Increase in Titres	
			O	Vi
Subcutaneous inoculation	1st dose, $2,000 \times 10^6$	50	98.0	48.0
	2nd " $3,000 \times 10^6$			
	3rd " $3,000 \times 10^6$			
Oral administration	1st dose, $60,000 \times 10^6$	44	31.9	4.5
	2nd " $80,000 \times 10^6$			
	3rd " $80,000 \times 10^6$			

It is seen from the table that the results obtained were very encouraging indeed, since the subcutaneous inoculation of the vaccine had induced a detectable rise in O antibodies in nearly all and of Vi antibodies in nearly half the inoculated. Though the doses were larger than those employed in the customary T.A.B. inoculation the reactions following the alcohol-killed vaccine seemed to be much less severe. The table also shows that the oral administration of very large amounts of the vaccine, which was given on the empty stomach in the form of a saline suspension without the addition of bile, only led to very feeble stimulation of either the O or the Vi antibody.

The vaccine used in this experiment did not contain any preservative. The bacilli were killed by treatment with 75% alcohol; the latter was then removed by centrifuging and washing with saline, and the bacillary bodies were made up to the required opacity with saline alone. However, re-examination of samples of this vaccine after storage for half a year in the cold room, in saline alone or with the addition of 0.5% phenol or 0.35% tricresol, gave the disappointing result that each of the samples had almost entirely lost the capacity of stimulating formation of Vi antibody in the rabbit, though O antibody invariably was produced to high titre. This alcohol-killed

vaccine could therefore not be recommended for use in large-scale inoculations, since a vaccine must contain a preservative capable of protecting it from casual contamination. Freshly prepared alcohol-killed vaccine containing no preservative was, however, employed during the past few years for the immunization of those amongst the personnel of the Lister Institute who were often handling virulent typhoid bacilli, and the impression gained in the course of time was that the reactions following the inoculations were less severe than those produced by the customary heat-killed and phenol-preserved vaccine. The experiments referred to were not published, but were recorded in the Annual Report of the Lister Institute for 1937.

Alcohol as a Preservative of Typhoid Vaccine

During the present war experiments with the alcohol-killed vaccine were continued, and the suggestion was made by Prof. G. S. Wilson of the Emergency Public Health Laboratory, Oxford, that alcohol might serve not only for the sterilization of the vaccine but possibly also for its preservation. This suggestion was soon found to be most valuable.

Long experience of alcohol-killed suspensions of the typhoid bacillus had proved beyond any doubt that a solution containing 75% absolute alcohol and 25% normal saline was a most reliable bactericidal agent. Large quantities of typhoid vaccine sterilized in this way and suspended in saline had been employed during the past few years for the immunization of horses in the course of the preparation of therapeutic anti-typhoid serum (Felix and Petrie, 1938), and in no instance did sterility tests on the numerous batches of vaccine prepared disclose the survival of either typhoid bacilli or other organisms.

The disinfectant action of alcohol was now compared with that of 0.5% phenol, using *Staphylococcus aureus* as the test organism. The comparative tests, which need not be described here in detail, were conducted both in saline alone and in saline containing 2,500 millions of alcohol-killed T.A.B. bacteria per c.cm. The clear-cut result was that the disinfectant action on *Staph. aureus* of 25% alcohol was slightly stronger than that of 0.5% phenol, while that of 20% alcohol was slightly weaker.

Next the effect of the subcutaneous injection of saline containing 25 or 30% alcohol was tried out on me and on a few volunteers. The injections of 0.5 or 1 c.cm. of these solutions were felt as a sting lasting for a fraction of a minute, but did not cause any local or general reaction. It was therefore decided that 25 or 22.5% alcohol could be employed as a suitable preservative of typhoid vaccine.

Batches of T.A.B. and T.A.B.C.* vaccine were prepared for experiments in rabbits and for comparative trials in human subjects. Two different vaccines were made in each case, both equivalent in composition and total bacterial count to the customary T.A.B. or T.A.B.C. vaccine—i.e., 2,500 million organisms per c.cm.—but prepared by one of the following two methods: (a) sterilized by treatment with 75% alcohol and preserved in saline containing 25% alcohol; (b) sterilized by treatment with 75% alcohol, washed on the centrifuge with saline, and preserved in saline containing 0.5% phenol. At the same time a quantity of the alcohol-killed typhoid suspension that served as one of the components of the mixed vaccines was retained for further experiments in

* Under the Therapeutic Substances Regulations the denomination T.A.B.C. now signifies in this country a vaccine containing typhoid bacilli along with paratyphoid bacilli of Types A, B, and C, and not, as previously, a vaccine containing typhoid bacilli and paratyphoid bacilli of Types A and B plus the vibrio of cholera; this latter vaccine is now known as T.A.B. + cholera.

rabbits, divided into five equal portions, and suspended in: (1) saline, (2) saline + 0.5% phenol, (3) saline + 25% alcohol, (4) saline + 30% alcohol, and (5) saline + 50% alcohol. All the vaccines were stored in the cold room at 1° to 2° C. and were tested for antibody response in rabbits and in human subjects after various intervals of time. The comparative trials in man are described in a paper by Felix, Rainsford, and Stokes that will appear in the next issue of the *Journal*. The experiments in rabbits are summarized in Tables II and III.

TABLE II.—Typhoid Vi Antibody Response in Rabbits Immunized with T.A.B. and T.A.B.C. Vaccines

Vaccines Sterilized with Alcohol			No. of Rabbits in Group	No. of Rabbits Showing Significant Increase in Vi Agglutinin Titre				Average Vi Agglutinin Titre after First and Second Intravenous Injections
Batch	Stored at 1° to 2° C. for	Preserved with		After Subcutaneous Injection of		After Intravenous Injection of		
				0.5 c.cm.	1 c.cm.	1 c.cm.	2 c.cm.	
T.A.B.	4 months	{ Phenol Alcohol Phenol Alcohol	3	0	0	0	0	1 : 20
			3	0	0	3	3	1 : 3
T.A.B.C.	1 month		3	0	0	1	1	1 : 7
			3	0	0	2	2	

Table II shows that one of the alcohol-killed vaccines that had been preserved with phenol failed to stimulate Vi agglutinin, while the other induced formation of Vi agglutinin in only one out of three rabbits, whereas the two alcohol-preserved vaccines did so in five out of six. It is seen from the table that Vi antibody was in no instance demonstrable after the two subcutaneous injections, but made its appearance only after intravenous injection of a dose identical with the maximum dose that is employed in the customary subcutaneous inoculation of adult men, making no allowance for the difference in body weight. This fact is noteworthy in view of the results—discussed in the subsequent paper—which were obtained in human subjects inoculated with these four batches of vaccine. On the other hand none of the twelve rabbits failed to show a significant rise in the typhoid O agglutinin even after the first subcutaneous dose. At each stage during the immunization of the rabbits the average typhoid O titres were much the same in the four groups—namely, about 1 in 500 after the first subcutaneous injection, about 1 in 1,000 after the second subcutaneous injection, and about 1 in 5,000 after the first intravenous injection. In this and the following experiment the rabbits were inoculated at weekly intervals and the samples of blood were taken on the seventh day following each immunizing injection. The degrees of agglutination that have been employed to indicate the O and Vi titres respectively were identical with those used in the examination of the sera from inoculated human subjects, and are defined in the subsequent paper.

TABLE III.—Vi Antibody Response in Rabbits Immunized with Various Preserved Typhoid Vaccines

Alcohol-killed Typhoid Vaccine Stored for 9 Months at 1° to 2° C., Suspended in	No. of Rabbits in Group	No. of Rabbits Showing a Significant Increase in Vi Agglutinin Titre after Intravenous Injection of		Average Vi Agglutinin Titre after Intravenous Injection of	
		500 × 10 ⁶ Organisms	1,000 × 10 ⁶ Organisms	500 × 10 ⁶ Organisms	1,000 × 10 ⁶ Organisms
Saline	4	2	2	1:4	1:10
Saline + 0.5% phenol	4	1	1	1:2.5	1:2.5
Saline + 25% alcohol	4	4	4	1:20	1:80
Saline + 30% alcohol	4	4	4	1:10	1:50
Saline + 50% alcohol	4	4	4	1:35	1:90

Table III corroborates the observation, already referred to, that the capacity of the alcohol-killed vaccine to

stimulate Vi antibody formation deteriorates on storage in saline with or without the addition of 0.5% phenol. At the same time the table clearly demonstrates that the presence of alcohol in the vaccine prevents this deterioration taking place for at least nine months, and probably for much longer. It is also seen that 25% alcohol seems to be as effective in this respect as the two higher concentrations tested, though the latter conclusion may have to be modified after observation over a longer period of time. Again, there was no significant difference in the O antibody response to the five different vaccines. Each of the twenty rabbits produced abundant O antibody, and the average titres in the five groups were of the same order—about 1 in 5,000 after the second dose of vaccine.

The Vi Antigens of *Bact. paratyphosum* A, B, and C

A few remarks must be made here on the other three constituents of the mixed typhoid and paratyphoid vaccine. Some time ago *Bact. paratyphosum* A and B were found to possess specific Vi antigens of their own (Felix and Pitt, 1936), while *Bact. paratyphosum* C contains a certain amount of the specific Vi antigen of the typhoid bacillus (Kauffmann, 1935). So far most workers have paid very little attention to these findings, because the presence of these antigens in cultures of paratyphoid A, B, and C bacilli has much less striking effects on their virulence, agglutinability, and immunizing properties than the typhoid Vi antigen has on the typhoid bacillus.

Experiments carried out in collaboration with Miss R. M. Pitt before the war, but not yet published, strongly suggest that the heat-labile somatic antigens of paratyphoid A and B bacilli have chemical properties essentially similar to those of the typhoid Vi antigen, though they differ from the latter in certain respects. These antigenic components must therefore be recognized as separate and distinct entities, quite different from the heat-stable somatic O antigens of paratyphoid A and B bacteria. In experiments on passive immunization of mice pure Vi serum has a powerful protective action against virulent paratyphoid B bacilli, and the same is probably the case with paratyphoid A bacilli, though experimental evidence on this point has not yet been published.

From this brief summary of the experimental data three conclusions of practical importance in the preparation of T.A.B. or T.A.B.C. vaccine seem to be quite obvious: (a) The cultures of paratyphoid A and B bacilli to be included in the vaccine should contain maximum amounts of both the O and the Vi antigen. (b) Treatment with alcohol, instead of with heat and phenol, is likely to enhance the protective power of the vaccine against infection with paratyphoid A or B bacilli, though the advantage may not be of the same magnitude as it is in the case of the typhoid bacillus, since heat-killed or phenolized suspensions of paratyphoid A or B bacilli do stimulate circulating Vi antibodies in the rabbit. (c) If, for special reasons, paratyphoid C bacteria also are included in the vaccine, further advantage is gained by selecting a strain which possesses the specific Vi antigen of the typhoid bacillus and by treating it with alcohol.

Technique employed for the Preparation of Alcohol-killed and Alcohol-preserved Vaccine

This technique is very simple indeed. The growth on plain agar from Roux bottles of the usual size is washed off with 20 c.cm. of sterile saline per Roux bottle, the suspensions are collected in flasks containing glass beads, and a count or estimation by opacity is made. The growth from one Roux bottle, 8½ in. by 4½ in., usually yields about 1,500,000 × 10⁶ organisms of the typhoid

and paratyphoid strains employed, varying according to the quality of the agar medium. Absolute alcohol is added to the suspensions in the proportion of 60 c.cm. of absolute alcohol to 20 c.cm. of saline. The flasks, which now contain the bacterial suspensions in 75% alcohol, are kept for two days at room temperature in the dark and are repeatedly shaken during that time.

The time required for the sterilization of the suspensions is very short—probably to be measured in minutes rather than in hours—nevertheless it is advisable to prolong the treatment for forty-eight hours and to ensure very thorough shaking. It is well known that absolute alcohol is a very poor disinfectant, and it might be feared that some contaminants, especially spore-bearing organisms, might gain access to the suspensions with the alcohol. It has been mentioned already that treatment with 75% alcohol for forty-eight hours at room temperature has invariably accomplished sterilization of each batch of vaccine that has been prepared up to the present time, without previous sterilization of the commercial absolute alcohol.

On the morning of the second day of the treatment with 75% alcohol the supernatants are decanted or sucked off, and the sediments are resuspended in the same quantities of fresh 75% alcohol and allowed to stand for some hours, until the bacteria have settled down completely. The solution of 75% alcohol + 25% saline to be used for this purpose should be prepared at least two days in advance and kept at room temperature, to ensure sterilization of the commercial absolute alcohol. The second supernatants are then sucked off as completely as possible. This procedure removes from the suspensions most of the constituents, mainly broth, which may have been collected from the agar medium together with the bacterial growth.

The deposits now consist of about a tenth to a fifth of their volume of solid bacteria and the remaining part of 75% alcohol. The amount of alcohol present in the deposits is estimated approximately and an appropriate adjustment is made while the dilute alcohol is being prepared, so that the deposits are finally suspended in saline containing 22.5% alcohol. This dilute alcohol should be prepared from a stock dilution of 75% alcohol which has been stored at room temperature for not less than two days. The suspensions of the three or four strains that are to be incorporated in the vaccine are then tested for sterility and again counted by opacity or some other method, and the final product is made by mixing the calculated quantities of the various suspensions and further diluting with 22.5% alcohol.

Attention must be paid to the fact that suspensions of alcohol-killed typhoid or paratyphoid bacteria have an opacity considerably less than that of suspensions equivalent in number of bacteria but killed by heat and preserved with phenol. The difference in opacity is about 10 to 20%, and is accounted for partly by the destruction of the flagella and partly by some alteration in the physical condition of the surface of the bacteria.

The methods used for the selection of the most suitable strains and the preservation and maintenance of the cultures have been described in detail in previous papers. Freshly isolated strains of typhoid and paratyphoid bacilli are not necessarily the best source of the antigens that are essential in the make-up of a potent vaccine. Though many strains at the time of their isolation are highly virulent and possess the Vi antigen in maximum amount, only few have the ability to maintain it at a high level when subcultured on artificial media. The strains employed in the present experiments—*Bact. typhosum* strain Ty 2, *Bact. paratyphosum* A strain HA 6, *Bact. para-*

typhosum B strain HB 3, *Bact. paratyphosum* C strain K 32 (Kauffmann)—are all known from earlier work, and the first three strains have been chosen for their outstanding ability to produce Vi antigen in maximum amount when grown on plain agar for periods of many years.

The routine examination of the cultures consists of agglutination tests alone, using pure O and pure Vi sera. Occasionally the precaution is taken to check the results by virulence tests in the mouse. Bensted (1937), who has had much experience of such tests, found that the estimation of the Vi content of typhoid cultures by agglutination tests was often a more accurate guide in observing slight differences in virulence than the actual mouse test itself, unless large numbers of animals were employed.

The important discovery made by Craigie and Yen (1938) of type-specific typhoid Vi phages also must be mentioned in connexion with the problem of selecting the typhoid strain or strains for inclusion in the vaccine. It is known that by means of these phages Craigie succeeded in dividing typhoid strains from different sources into a number of different types. In view of the striking stability of these phage types it was considered necessary to investigate whether serological differences could be detected in the Vi antigen of typhoid strains belonging to different phage types. The experiments included cross-agglutination and cross-absorption tests, and phagocytosis and passive-protection tests in mice, and were completed in collaboration with Miss R. M. Pitt before the war but are not yet published. No difference between the actions of homologous or heterologous phage-type sera could be disclosed by any of these methods, and it appears to be safe to continue employing a single strain of the typhoid bacillus for making typhoid vaccine.

Summary

Ordinary typhoid-paratyphoid vaccine killed by heat and preserved with phenol stimulates in the rabbit and in man only negligible amounts of demonstrable typhoid Vi antibody.

Alcohol-killed vaccine readily stimulates circulating typhoid Vi antibody when injected intravenously into the rabbit, and induces its formation in nearly 50% of human beings after subcutaneous inoculation. This Vi antibody possesses its full opsonizing and protecting power.

Alcohol-preserved vaccine still contains typhoid Vi antigen in its effective form after storage in the cold for at least nine months, and probably much longer.

The technique of the preparation of alcohol-killed and alcohol-preserved T.A.B.C. vaccine is described.

REFERENCES

- Arkwright, J. A. (1927). *J. Path. Bact.*, **30**, 345.
 Army Council (1912). Report of Anti-typhoid Committee, G.B. War Office, London.
 Bensted, H. J. (1937). *J. R.A.M.C.*, **68**, 1.
 — (1940). *Ibid.*, **74**, 19.
 Craigie, J., and Brandon, K. F. (1936). *J. Path. Bact.*, **43**, 249.
 — and Yen, C. H. (1938). *Canad. publ. Hlth. J.*, **29**, 448, 484.
 Felix, A. (1924). *J. Immunol.*, **9**, 115.
 — (1938). *J. Hyg., Camb.*, **38**, 750.
 — and Bhatnagar, S. S. (1935). *Brit. J. exp. Path.*, **16**, 422.
 — Krikorian, K. S., and Reitler, R. (1935). *J. Hyg., Camb.*, **35**, 421.
 — and Petrie, G. F. (1938). *Ibid.*, **38**, 673.
 — and Pitt, R. M. (1934). *Lancet*, **2**, 186.
 — (1936). *Brit. J. exp. Path.*, **17**, 81.
 Fleming, A., and Petrie, G. F. (1934). *Recent Advances in Vaccine and Serum Therapy*, London.
 Gay, F. P. (1918). *Typhoid Fever*, New York.
 Grasset, E. (1939). *British Medical Journal*, **2**, 58.
 Grinnell, F. B. (1932). *J. exp. Med.*, **56**, 907.
 Kauffmann, F. (1935). *Z. Hyg. Infektkr.*, **116**, 617.
 Maclean, I. H., and Holt, L. B. (1940). *Lancet*, **2**, 581.
 Perry, H. M., Findlay, H. T., and Bensted, H. J. (1933a). *J. R.A.M.C.*, **60**, 241.
 — (1933b). *Ibid.*, **61**, 81.
 — (1934a). *Ibid.*, **62**, 161.
 — (1934b). *Ibid.*, **63**, 1.
 Pfeiffer, R., and Kolle, W. (1896). *Disch. med. Wschr.*, **22**, 735.
 Rainsford, S. G. (1937). *J. Hyg., Camb.*, **37**, 539.
 — (1939). *Ibid.*, **39**, 131.
 Ramon, G. (1939). *Presse méd.*, **47**, 877.
 Schütze, H. (1930). *Brit. J. exp. Path.*, **11**, 34.
 — (1936). *J. Hyg., Camb.*, **36**, 559.
 Topley, W. W. C. (1929). *Lancet*, **1**, 1337.
 Wright, A. E., and Semple, D. (1897). *British Medical Journal*, **1**, 256.