proteins (from 6.4 to 4.8—see table), and since no diuresis had ensued, this was abandoned. Mersalyl 1 c.cm. was injected intramuscularly on October 31, and a similar dose intravenously on November 2.

Table Correlating the Treatment, Blood Pressure, Urine Excretion and Blood Estimations

Date	Blood Pressure	Blood Urea mg. per 100 c.cm.	Hb %	Haemato-crit (% R.B.C.)	Plasma Protein gm. per 100 c.cm.	Treatment	Urine Out-put
Oct. 28	—	—	—	—	—	—	4 oz.
" 29	132/100	154	124	54.0	6.4	{ 3,000 c.cm. 5% glucose—	Trace
" 30	125/90	210	94	42.0	4.8	{ saline 100 c.cm.	"
" 31	140/100	295	—	—	—	{ 4×N serum	None
Nov. 1 (a.m.)	140/100	295	98	42.5	4.3	400 c.cm. 4×N serum	"
(p.m.)	140/110	309	90	40.0	4.9	—	—
" 2	150/110	327	88	39.5	4.3	—	—
" 3	145/110	369	76	35.5	4.6	200 c.cm. 4×N serum	"

To raise the plasma proteins and because of its diuretic action (Anderson and Boyle, 1940) concentrated human serum was given intravenously. The serum used was the dried serum supplied by the Medical Research Council's serum-drying unit. It should perhaps be emphasized that the serum from Group A (II), B (III), and AB (I) donors is pooled before filtration and drying, and that the effect of this pooling is to render the agglutinin content negligible (Levinson and Cronheim, 1940). A solution of four-times-normal concentration was made. Intravenous injection of 100 c.cm. on October 31, and 400 c.cm. (by intravenous drip) on November 1, produced a rise of plasma protein from 4.3 to 4.9. Coincidentally the patient seemed more comfortable. A further infusion was started at 11 a.m. on November 3. 200 c.cm. had been administered by the time she died. (On this last occasion 0.5 gramme of evipan intravenously was necessary to restrain the patient while the cannula was inserted. Anaesthesia lasted twenty minutes. On November 1 sodium amyltal grains 1½ by mouth was sufficient.) No reaction was caused by the serum. The patient's temperature remained subnormal throughout her stay in hospital.

Pathology

The post-mortem examination was performed by Dr. J. V. Dacie. The chief findings were as follows:

General: Symmetrical oedema of body from nipples to feet. Arms oedematous below mid-point of humerus.

Thorax: No excess of fluid in pleural cavities. Slight excess of clear fluid in pericardium. Heart normal. Fluid blood in great vessels.

Lungs: Pale and bulky. Slight basal congestion (detached portions floated in water).

Abdomen: Slight excess of peritoneal fluid. Stomach, liver, spleen, and mesentery normal. Considerable injection of the mucous membrane of the bowel from the duodenum to the sigmoid colon. Kidneys large and pale, foetal lobulations well marked. On section the kidney cortex seemed paler than normal. The differentiation between cortex and medulla was very clear. The capsule stripped readily. Ureters normal, bladder empty. No other abnormalities were found.

High muscles: Oedematous but otherwise normal. No extravasation of blood, no microscopical evidence of necrosis.

- It is the microscopical appearance of the kidney that is the chief point of interest in this case. Most of the tubules contained a granular debris that stained a brick-red colour with eosin. This material could be found in all parts of the tubule from the capsular space to the duct of Bellini. Proximally it was small in amount and was often embedded in an albuminoid substance, but in the collecting tubules the granules were tightly packed, forming a solid cast of the lumen. At first sight some of these

granules seemed to be red blood corpuscles; on closer inspection and comparison with intact corpuscles in the vessels it was evident that the tubular contents were frankly granular, though "ghosts" of broken cells could be seen in some places. In an unstained section the tubular contents were the golden-red colour of unstained red cells. The tubular epithelium in some places showed evidence of degeneration. Some of the cells had lost their outline. There were some pyknotic nuclei. This degeneration seemed to bear no constant relation to the amount of material in the tubule. The glomeruli were normal; the capsular space was not dilated. Special staining showed no fat and no free iron in the kidney substance or in the tubules.

Summary

The case is reported of a patient who died from uraemia following a type of injury that often produces shock. The micropathology resembles that of incompatible transfusion. Unlike similar cases reported in this issue there can be no possibility here of incompatible transfusion, since no blood was given to our patient. Herein lies the importance of this case and the reason for reporting it.

We wish to thank Dr. A. T. Moon for allowing us access to his patient and Dr. J. V. Dacie for performing the necropsy and preparing sections of the kidney and other organs.

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ANTIBODY RESPONSE AND SYSTEMIC REACTIONS AFTER INOCULATION OF A NEW TYPE OF T.A.B.C. VACCINE

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An account was given in last week's issue (p. 391) of the experimental work that led to the preparation of a new type of typhoid vaccine killed and preserved with alcohol. The object of the present paper is to record the results of comparative trials in man, carried out with the new and the old types of vaccine. Observations were made on the local and general reactions produced by the inoculations, and the immunity response was measured by the estimation of the Vi and O antibody content of the sera.

From the experiments described in the preceding paper it seemed justifiable to expect that alcohol-killed and alcohol-preserved vaccines would stimulate Vi antibody response in an outstanding manner and could, by this property, be distinguished from ordinary heat-killed and phenolized vaccines. To test this point, and also to ensure the unbiased reading of the agglutination tests, the first three trials were so arranged that the samples of serum reached one of us (A. F.) labelled only with serial letters and numbers, without indicating which of the vaccines tested had been used for the inoculation of any particular group of volunteers. It will be seen from the following tables that in each of the three trials the group

that had been inoculated with the new type of vaccine could be told from the results of the Vi agglutination tests.

It is generally believed that typhoid-paratyphoid vaccines retain their immunizing potency undiminished for at least a year (Perry, Findlay, and Bensted, 1934) or, according to American workers, even for several years when stored at 8° to 10° C. (Mishulow, Mowry, and Stocker, 1937). In this country the expiry date of batches of T.A.B. vaccine is usually fixed at eighteen months from the date of preparation. There are reasons also for believing that the toxicity of ordinary typhoid-paratyphoid vaccine decreases gradually on storage, so that less severe local and general reactions follow its administration. For the sake of accuracy we considered it necessary to record in the tables the date of preparation of every batch of vaccine that had been employed in the comparative trials. The alcohol-killed T.A.B. and T.A.B.C. vaccines were from the four batches that had been used in the experiments on rabbits described in the preceding paper. Some of the other vaccines were from various sources, but the exact mode of their preparation was known to us, with the exception of one commercial product which is mentioned in Table I.

Antibody Response to Customary Doses of 0.5 and 1 c.cm. of Vaccine

Throughout this investigation each volunteer before receiving the first dose of vaccine had a sample of blood withdrawn from a vein in the arm, and a second sample was taken after the administration of the second dose of vaccine. The sera from the two samples were stored in the cold room without the addition of any preservative and were in each instance examined in parallel for the presence of the various antibodies, using as reagents the same batch of agglutinable suspensions. Any rise in agglutinin titre of 100% or more was taken as "significant" increase in antibody content. The intervals between the two subcutaneous injections and also those between the second injection and the second bleeding varied slightly in the different trials, and are in each case stated separately.

Preliminary Trial

A preliminary trial on a very small scale was first carried out with the kind co-operation of Air Vice-Marshal H. E. Whittingham, Director of Hygiene, Air Ministry. The volunteers were young R.A.F. recruits who had no history of previous enteric infection or inoculation. The two doses of vaccine were given at an interval of ten days, and samples of blood were taken three weeks after the second dose of vaccine. The results of the agglutination tests are summarized in Table I. The H agglutination tests are discussed in a later section.

TABLE I.—Preliminary Trial of Four Differently Prepared T.A.B. Vaccines in Volunteers among R.A.F. Recruits, May, 1940

T.A.B. Vaccines				No. Inoculated	No. of Cases showing Significant Rise in O and Vi Agglutinin Titres 21 Days after Second Dose			
Batch	Prepared	Sterilized by	Preserved with		TO	BO	AO	Typhoid Vi
No. 1	February, 1940	Alcohol	Phenol	5	5	5	3	0
No. 2	October, 1939	Heating at 53° C. for 1 hour	Alcohol	5	5	5	4	2
No. 3			Phenol	5	5	5	2	0
Dissolved T.A.B. vaccine	September, 1939	Mode of preparation unknown		5	4	5	2	0

It will be seen from Table I that there was not much difference in the O antibody response stimulated by the four different vaccines. All of them appeared to be quite satis-

factory in this respect. On the other hand, the result of the typhoid Vi agglutination test made it possible to pick out the group that had been given the alcohol-killed and alcohol-preserved vaccine, in spite of the fact that very small numbers had been employed in this trial.

With regard to the local and general reactions produced by the various vaccines, Air Vice-Marshal Whittingham had the impression that the heat-killed and phenol-preserved vaccine caused the more severe reactions. There was not much to choose between the three other vaccines. The "dissolved" vaccine was included in the trial because it was known to produce only slight reactions.

Comparative Trial of Three Different Vaccines in Male Mental Patients

The second trial was on a somewhat larger scale. Three groups of male mental patients were inoculated with three different vaccines. The customary doses of 0.5 and 1 c.cm. were given at ten days' interval and samples of blood were withdrawn from the patients three times—namely, before the immunization, on the fourth day after the second dose, and again six days later. The results of the agglutination tests shown in Table II are those obtained with the sera taken on the tenth day after the second injection.

TABLE II.—Comparative Trial of Three Different Vaccines in Male Mental Patients. August, 1940

Vaccines				No. Inoculated	No. of Cases showing Significant Rise in O and Vi Agglutinin Titres 10 Days after Second Dose			
Batch	Prepared	Sterilized by	Preserved with		TO	BO	AO	Typhoid Vi
T.A.B. No. 4	May, 1940	Heating at 53° C. for 1 hour	Phenol	21	18	20	7	1
T.A.B.C. No. 1	June, 1940	Heating at 53° C. for 70 min.	..	22	20	19	12	1
T.A.B. No. 2	February, 1940	Alcohol	Alcohol	22	20	20	13	5

Table II shows that the O antibody response was of much the same order in the three groups. The slightly lower figure for paratyphoid A in the first group may be accounted for by the fact that the corresponding vaccine was made from strains other than those employed in the preparation of the two other vaccines. The only striking difference in antibody response in the three groups was in the typhoid Vi antibody. The number of Vi positive sera in the group immunized with the alcohol-killed vaccine was five times that in each of the two other groups. The two heat-killed and phenol-preserved vaccines had been prepared only a few weeks before they were used in the trial, and the typhoid component that each of them contained was made from a properly selected "full Vi" strain. The T.A.B.C. vaccine No. 1 and the alcohol-killed vaccine T.A.B. No. 2 actually contained the same strain of the typhoid bacillus. There can be little doubt, therefore, that the much better Vi antibody response induced by the alcoholized vaccine was due to its mode of preparation.

In a recent paper Bensted (1940a) described the appearance of significant though transitory Vi agglutinins in the blood of human subjects and of animals that had been inoculated with ordinary heat-killed and phenolized T.A.B. vaccine. The Vi antibody was observed to reach its maximum titre as early as four days after the inoculation and to disappear rapidly in the course of a few days. In view of this observation samples of blood were drawn from the inoculated mental patients on the fourth day after the second dose of vaccine and the sera were examined in parallel with the samples taken, respectively, before the immunization and on the tenth day after the second injection. It is seen from Table II that 7 out of 65 inoculated subjects gave a significant Vi agglutination reaction on the tenth day after the second inoculation. Six of these cases showed the same Vi titre on the fourth and the tenth day after the injection of the second dose, while in one instance the reaction was slightly stronger with the earlier sample. The remaining 58 cases, however, gave negative results with both samples of serum.

Comparative Trial of Four Different T.A.B.C. Vaccines in Volunteers among Naval Recruits

Four groups each of about 60 volunteers were accepted in the third trial (Table III). The course of immunization consisted of the customary doses of 0.5 and 1 c.cm. given at one week's interval, and the second sample of blood was withdrawn fourteen days after the second dose of vaccine. The alcohol-treated vaccines P and A were from the batches that had been used in the experiments in rabbits described in Table II in the preceding paper. The vaccines S and F were prepared at the Royal Naval Medical School, the method of preparation being that at present employed for the T.A.B.C. vaccine now being used for the immunization of personnel in the Royal Navy. Batch F was made with the same strains of typhoid and paratyphoid bacteria that served for the preparation of the alcohol-killed batches P and A. Batch S was made from the cultures employed at the Royal Naval Medical School, except for the paratyphoid B strain, which was identical with that contained in the other three vaccines.

In the preceding paper it was mentioned that the opacity of suspensions of alcohol-killed bacteria is considerably less than that of suspensions killed by heat and preserved with phenol. The opacities of the four vaccines were compared with each other in a Spekker photo-electric absorptiometer which has been calibrated by the R.N. Medical School staff. The following figures were obtained:

Vaccine	Sterilized by	Bacterial Content estimated by Opacity Tests in the Spekker Photo-electric Absorptiometer
S	Heat	2,500 million organisms per c.cm.
F	"	2,500 " " " "
P	Alcohol	1,600 " " " "
A	"	1,400 " " " "

For reasons stated in that paper estimation by opacity does not give the correct number of bacteria in suspensions that have been treated in different ways; alcohol-treated suspensions give higher counts as reckoned by opacity alone. It is certain at any rate, from the mode of preparation, that the bacterial content of the two alcohol-killed vaccines was not greater than that of the heat-killed batches.

TABLE III.—Comparative Trial of Four Different T.A.B.C. Vaccines in Volunteers among Naval Recruits, August, 1940

T.A.B.C. Vaccines				No. Inoculated	Percentage of Cases showing Significant Rise in O and Vi Agglutinin Titres 14 Days after Second Dose				
Batch	Prepared	Sterilized by	Preserved with		TO	BO	AO	CO	Typhoid Vi
S	June, 1940	Heating at 58° C. for 1½ hours	Phenol	55	78.2	90.9	61.8	66.6	5.4
F	"	"	"	56	83.9	78.5	71.4	50.0	7.1
P	May, 1940	Alcohol	"	59	84.7	96.6	96.6	59.4	6.7
A	"	"	Alcohol	57	84.2	87.7	84.2	44.0	40.3

The results of the agglutination tests are summarized in Table III and need little comment. The O antibody response was equally good in all four groups of volunteers, but the Vi antibody response was about six to seven times greater in the group inoculated with the alcohol-killed and alcohol-preserved vaccine than in any of the other three groups. It is seen from the table that the vaccine P, which was killed with alcohol and preserved with phenol, did not stimulate more of the Vi antibody than the two batches of heat-killed and phenol-preserved vaccine. The range of rise of titres is stated later in this paper.

H Agglutination Tests

The sera from the inoculated persons were also tested for their H agglutinin content, though this antibody need not be considered in assessing the protective value of the vaccines. It was thought possible that the prolonged treatment with alcohol might possibly lead to complete inactivation of the H antigen, so that H agglutinins would not be produced as

a result of the inoculation with alcohol-killed vaccines. It is known that the high-titre H agglutinins which are formed after inoculation with ordinary T.A.B. vaccine usually persist in the blood for a very long time and render H agglutination tests useless as a means of diagnosing enteric infection in such inoculated persons. A potent typhoid-paratyphoid vaccine that would not stimulate the unwanted H agglutinins would therefore be a very welcome improvement. It was soon found, however, that each of the batches of alcohol-treated vaccines stimulated H agglutinins in a considerable proportion of the inoculated subjects. Some of the results obtained in the comparative tests in naval recruits are recorded in Table IV.

TABLE IV.—Showing H Agglutination Results in Volunteers Inoculated with Four Different T.A.B.C. Vaccines

T.A.B.C. Vaccines			No. Inoculated	No. of Cases giving a Positive H Agglutination Reaction in Dilution 1 in 400 with Suspension of		
Batch	Sterilized by	Preserved with		TH	BH (sp.)	AH
S	Heating at 58° C. for 1½ hours	Phenol	55	42	55	54
F	"	"	56	56	55	49
P	Alcohol	"	59	31	22	16
A	"	Alcohol	57	7	12	8

The table shows that the men inoculated with the two alcohol-killed vaccines produced much less H agglutinins than those who were given the two batches of heat-killed vaccine. It is also according to expectation that batch A, which was preserved with alcohol, stimulated less H agglutinins than batch P, which was preserved with phenol. It is, however, quite obvious from the results shown in Table IV that H agglutination tests cannot be relied upon for diagnostic purposes even after inoculation with a vaccine that has been killed and preserved with alcohol. Twelve of the 57 men who had received vaccine A gave standard agglutination with one or more of the H suspensions in dilution 1 in 400. In the dilution 1 in 40 the figure was 43 out of 57, and four sera in this group showed H titres of 1 in 5,000. Although hope of success in future attempts need not be abandoned, the position remains in this respect unchanged for the present.

Remarks on the Technique of O Agglutination Tests

It is not generally recognized that the technique of O agglutination tests has not yet been standardized satisfactorily and that the titres recorded by various workers may differ, and in fact do differ, very considerably. 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.

The technique of the O agglutination test has changed profoundly since the time when the difference in O and H agglutinin response to T.A.B. inoculation was first described (Felix, 1924). The permanent O variant of the sensitive typhoid strain "901," which is now employed almost universally, had not yet been isolated, and the estimation of typhoid O and H agglutinins had to be done by the use of two typhoid cultures of different O-agglutinability and of a culture of *Bact. enteritidis* Gaertner. The low-titre O agglutinins which appear in the serum after T.A.B. inoculation could not be demonstrated by that insensitive method. The permanent O variants "O 901" (Felix and Olitzki, 1926) and "BO" (strain Glasgow of *S. aertrycke*, Schütze, 1930; Felix, 1930; Gardner and Stubington, 1932) did not come into use until some years later. Gardner (1929) first recommended for the "O titre" the reading of the "last trace of clumping visible with a watch-maker's lens," and this procedure was soon adopted by many workers. It has been pointed out (Felix, 1938) that the dilution which gives the "last trace" reaction is usually ten times higher than that giving the +++ reaction, which had been employed in earlier work on O agglutination in the diagnosis of enteric fever (Felix, 1924).

For the purposes of the present investigation quantities of O suspensions were prepared sufficient for testing all the sera from inoculated human subjects referred to in this paper and also the rabbit sera mentioned in the preceding one. The suspensions were from agar-grown cultures, alcohol-treated and

suspended in buffered formalin-saline solution (Felix and Gardner, 1937). The sensitiveness of the suspensions was in each test checked against standard O agglutinating sera. The serum samples from the naval recruits were tested independently by two of us (S. G. R. and A. F.), using the same batches of suspensions and standard sera and the same technique. Thanks to this precaution the results obtained by the two observers were in good agreement.

The following serum dilutions were tested for O antibodies against the various suspensions:

Bacterial Suspensions	Sample of Serum taken before Inoculation	Sample of Serum taken after Inoculation
<i>Bact. typhosum</i>	1 : 50 1 : 100 1 : 200	1 : 50 1 : 100 1 : 200 1 : 500 1 : 1,000 1 : 2,000
<i>Bact. paratyphosum B</i>		
<i>Bact. paratyphosum A</i>	1 : 25 1 : 50 1 : 100	1 : 25 1 : 50 1 : 100 1 : 200 1 : 500 1 : 1,000
<i>Bact. paratyphosum C</i>		

In order to detect even a very slight increase in O antibody content the titre was assigned to the highest dilution of the serum which showed a "last trace" reaction, visible by means of a magnifying lens. Any difference in titre between the two corresponding samples of serum that amounted to one dilution in the series tested—i.e., an increase of 100%—was taken as "significant." With this very sensitive method of measurement the high figures of "significant" reactions that have been recorded in Tables I, II, and III were obtained.

No attempt was made to express the degree of antibody response in terms of average titres observed in the various groups. It is well known that the ability of different individuals to produce high-titre agglutinins varies over a very wide range. Groups comprising much greater numbers than those tested in these trials would be needed to counteract the effect of individual differences and to produce statistically significant figures. Most of the titres observed with the two sensitive suspensions TO and BO varied from 1 in 100 to 1 in 500, only very few reaching or exceeding a titre of 1 in 1,000. With the less sensitive paratyphoid A and C suspensions the O titres usually varied from 1 in 25 to 1 in 100, and very rarely reached or exceeded 1 in 200.

The readings of the O agglutination tests were taken after two hours' incubation at 37° C., and a further twenty-two hours at room temperature. It was found that O agglutinins of titres as low as those occurring in the serum from inoculated subjects do not withstand prolonged heating at 50° to 52° C., the temperature usually employed for incubating diagnostic agglutination tests. It is known from earlier work (Eisenberg, 1906) that diluting the serum increases the heat resistance of agglutinins, and this is of particular importance in the case of the O agglutinins, which are less resistant to heat than H agglutinins (Felix and Olitzki, 1929). Many of the O reactions recorded in Tables I, II, and III as positive would have been missed had incubation at 50° to 52° C. been employed.

Technique of Typhoid Vi Agglutination Tests

The Vi agglutination tests were carried out with a batch of suspension prepared by Lieut.-Colonel R. F. Bridges at the Standards Laboratory, Oxford, using the sensitive strain Vi I of Bhatnagar (1938). The agglutinability of the suspension was checked against the provisional standard serum which was described in connexion with the standardization of therapeutic anti-typhoid serum (Felix, 1938). It was soon found, however, that the standard of agglutination chosen in that work, and employed also in diagnostic Vi tests to indicate the Vi titre of a serum, was too high for the measurement of Vi antibodies of titres as low as those induced by T.A.B.C. inoculation. A lower standard had therefore to be adopted for the purposes of this investigation. This was chosen at half the original value, so that the standard agglutination which determined the Vi titre was that occurring in the dilution 1 in 1,200 of the provisional standard serum. The degree of this reaction with the suspension that was employed throughout this investigation was a full + reading estimated with the naked eye.

The two corresponding samples of serum taken from each volunteer before and after inoculation were tested against the Vi suspension in the dilutions 1 in 10, 1 in 20, 1 in 40, and 1 in 80, and any increase in titre of 100% or more was considered as "significant." In the series of 227 naval recruits recorded in Table III 34 significant Vi reactions were found. The following titres were observed: 15 had titres of 1 in 10, 11 of 1 in 20, 7 of 1 in 40, and 1 of 1 in 80.

Local and General Reactions

The reactions following the administration of the alcohol-killed and alcohol-preserved vaccine were compared with those produced by the other batches of vaccine employed in the trials. Notes were made separately of the reactions following the first and second doses of vaccine. Records were kept of temperature readings taken during the first two days after the injections and of the occurrence of any of the symptoms that are associated with T.A.B. inoculations. Under local reactions the diameter of erythema and the degree of tenderness were recorded, and special attention was paid to involvement of axillary glands and to any serious complaints of inability to use the arm.

In the course of the three trials described in the preceding sections, and in a few others not hitherto mentioned, about 140 adults were given alcohol-killed and alcohol-preserved vaccine in doses of 0.5 and 1 c.cm. About 100 of the inoculated were men and 40 were women, including 20 nurses. It was soon found that the reactions produced by the new type of vaccine differed in two respects from those caused by ordinary vaccine.

(i) *General Reactions.*—There was a remarkable absence of severe general reactions. Temperatures over 99° F. were recorded in a number of cases within twelve to twenty-four hours, especially among the female volunteers, but the slight malaise was transient and did not cause the nurses to go off duty.

(ii) *Local Reactions.*—The injection is felt as a sting lasting for a fraction of a minute. This is due to the alcohol contained in the vaccine. The early local reactions, consisting of erythema and swelling, were similar to those produced by ordinary T.A.B. vaccine, though they were usually milder and rarely involved the axillary glands. As a rule there was no pronounced muscular stiffness and the arm could be used freely. The inoculated naval ratings, for instance, did not require to be excused duty, which included physical training. The redness and swelling persisted for two or three days and then subsided rapidly, but a small palpable nodule was left in most cases, and this persisted for about ten days before passing off gradually. In some cases the nodule, about the size of a split-pea, could still be felt after three weeks.

In 3 cases out of 140 that were given the customary large doses of vaccine the local reaction has been different—in 1 male and 1 female after the first dose (0.5 c.cm.) and in 1 male after the second dose (1 c.cm.). In these cases there was some local induration, which appeared about the fourth day after the injection and by the sixth day had developed into a small fluctuating area at the site of the injection. This delayed local reaction was not accompanied by any general reaction, did not interfere with the free use of the arm, and disappeared spontaneously in the course of two or three weeks.

The alcohol-killed and alcohol-preserved batches of T.A.B. and T.A.B.C. were the only vaccines that produced the subcutaneous nodule. The vaccine which was killed by alcohol but suspended in saline containing 0.5% phenol (see batch P in Table III) produced both general and local reactions of the type seen after inoculations of ordinary heat-killed vaccine. There can be little doubt, therefore, that the alcohol that is injected with the vaccine is in

some way responsible for the modified reaction. Rabbits inoculated subcutaneously with alcoholized vaccine develop similar nodules. It is possible that histological examination will suggest an explanation of how alcohol and bacterial toxins, when contained in the vaccine, combine to produce the lesion. The absence of more severe general reactions is probably due to the fact that the absorption of toxic substances from the bacterial bodies is delayed as a result of the action of alcohol.

Antibody Response and Severity of General or Local Reactions

Some workers hold the view that the severe reactions that occur after inoculations with ordinary T.A.B. vaccine are of value in enhancing antibody production and thus are not wholly a disadvantage. Ramon's (1926) method of producing artificial sterile abscesses in horses undergoing immunization in order to increase the amount of antitoxin in their blood is sometimes quoted in support of this view. It has been mentioned already that according to Bensted (1940a) ordinary T.A.B. vaccine occasionally stimulates a certain amount of Vi antibody, though it is demonstrable only during a very short period after the inoculation. Bensted found such results solely in men who had suffered from a very severe reaction after receiving a dose of ordinary T.A.B. vaccine.

We have searched very carefully for a possible correlation between Vi antibody response and severity of general or local reactions but have failed to detect any evidence of such correlation. Tables II and III show a few instances of a significant Vi antibody response in subjects inoculated with different batches of heat-killed vaccine. The persons who had these significant Vi titres were, however, not among those who had developed the most severe general or local reactions. The numerous significant Vi reactions that were found amongst the volunteers inoculated with alcoholized vaccine also did not suggest any relation between typhoid Vi antibody and severity of reactions. The same was true with regard to the titres of O antibodies against the four bacterial species tested. Nor was there any constant relation between significant Vi titres and high O titres, or between high O titres for one particular bacterium and those for one or more of the other types.

It seems therefore to be safe to conclude that the severe reactions caused by the ordinary T.A.B. vaccine are not only undesirable, for a variety of quite obvious reasons, but are of no value as a factor contributing to good immunity response. It has been shown in Tables II and III that the alcoholized vaccines, which produced the subcutaneous nodule, were in respect of O antibody formation not superior to any of the other vaccines. It has not yet been established whether the presence of the nodule has any effect on the persistence of demonstrable O or Vi antibodies. This point is now being investigated.

Experiences with Smaller Doses of the New Vaccine

Those who have had personal experience of T.A.B. inoculation of large groups of civilians have long formed the opinion that the doses of 0.5 and 1 c.cm. of vaccine of the usual strength that are employed as standard doses for members of the armed Forces are too large for the less robust average adult civilian. Even half these doses may cause severe reactions, as has been recently stated by Bensted (1940b). Reduced doses of vaccine, carefully graded according to age, sex, body weight, and physical condition, must be employed in the mass inoculation of the civilian population if unduly severe reactions are to be avoided.

Very few data have been published to show the effect on antibody response of reducing the dose

of T.A.B. vaccine. Henderson Smith and Brooks (1912) showed that, up to a point, antibody response increased with increase of antigen. More recently Topley (1930) demonstrated in experiments with paratyphoid B bacilli in rabbits that a relatively large increase in the dose of antigen administered resulted in a relatively much smaller increase in the amount of antibody produced. Since, however, these experiments were concerned only with H agglutinins, the results are not necessarily applicable to O or Vi antibody response following administration of T.A.B. vaccine. Our own observations so far consist only of two preliminary trials on small groups, while comparative trials on a larger scale are at present in progress. The results of the preliminary tests, for what they are worth, are illustrated in Tables V and VI.

TABLE V.—Showing Effect on O and Vi Antibody Response of Reducing the Dose of Vaccine

T.A.B. Vaccine Batch No. 2 Killed and Preserved with Alcohol			Interval between 1st and 2nd In- jections (Days)	No. Inocu- lated	Percentage Frequencies of Significant Increase in Agglutinin Titres 10 Days after Second Dose			
Stored at 1° to 2° C. for	1st Dose (c.cm.)	2nd Dose (c.cm.)			TO	BO	AO	Typhoid Vi
6 months	0.5	1	10	22 adult males	90.9	90.9	59.0	22.7
8 "	0.25	0.5	14	20 adult females	70.0	75.0	50.0	20.0

The two groups recorded in Table V consisted of adult mental patients. It will be seen from the table that the reduction of the doses to half the amount was followed by a noticeable drop in O antibody response. The low figure for the Vi antibody showed no significant change.

TABLE VI.—Showing Effect on O and Vi Antibody Response of Reducing the Dose and Prolonging the Interval between the Injections

T.A.B.C. Vaccine Batch A Killed and Preserved with Alcohol			Interval between 1st and 2nd In- jections (Weeks)	No. Inocu- lated	Percentage Frequencies of Significant Increase in Agglutinin Titres 14 Days after Second Dose			
Stored at 1° to 2° C. for	1st Dose (c.cm.)	2nd Dose (c.cm.)			TO	BO	AO	Typhoid Vi
3 months	0.5	1	1	57 adult males (naval ratings)	84.2	87.7	84.2	40.3
6 "	0.2	0.4	2	19 adult females (nurses)	57.9	57.9	36.8	36.8
6 "	0.2	0.4	3	20 adult females (nurses)	90.0	75.0	75.0	35.0

The first group recorded in Table VI is identical with the fourth group shown in Table III, and has been included here for purposes of comparison. The table shows that the reduced doses given at two weeks' interval produced a much lower O antibody response than the full doses at one week's interval. The response was greatly improved by increasing the interval between the injections from two to three weeks. Again there was no significant difference in the figures for the typhoid Vi antibody.

These results seemed to indicate that the doses cannot be reduced drastically without a corresponding decrease in the immunizing effect produced, but that, up to a point, the effect of decreasing dosage can be counterbalanced by increasing the interval between the injections.

So far, reports have been received on the reactions observed in about 250 adult females and 150 adult males who were given reduced doses of the alcoholized T.A.B.C. vaccine. Adult males received 0.25 c.cm. of vaccine

followed by 0.5 c.cm., and adult females had 0.2 c.cm. followed by 0.4 c.cm. Among 210 inoculated nurses only 3 had to go off duty after the first dose of vaccine, none after the second dose. No case of delayed local reaction was observed amongst the 400 subjects who had been inoculated with the reduced dose of vaccine.

Suggested Doses and Recommendations for the Administration of the Alcoholized Vaccine

The alcoholized T.A.B.C. vaccine is equivalent in composition and total bacterial count to the customary vaccine—i.e., it contains 1,000 million *Bact. typhosum* and 500 million each of *Bact. paratyphosum* A, B, and C per c.cm. The following doses of this vaccine are recommended:

	First dose	Second dose
For adult males	0.25 c.cm.	0.5 c.cm.
For adult females	0.2	0.4
For children (male or female):		
From 16 to 18 years	0.2	0.4
" 13 to 15 "	0.1	0.2
" 9 to 12 "	0.05	0.1
Under 8	0.05	0.05

These are maximum doses and should not be exceeded: the next smaller dose in the series is advised for subjects in each category who are below average in physical vigour or development.

The interval between the two injections should be three weeks. If this cannot be adhered to and the choice is, say, two weeks or four weeks, it is preferable to choose the longer interval. Revaccination is advisable one year after the primary immunization: a single dose of the size of the first dose mentioned above is sufficient.

General reactions—i.e., languor with slight fever—are as a rule either absent or quite trivial after the small doses of vaccine. If the precaution is taken to make the injections as late as possible in the day—preferably not earlier than six hours before bedtime—any general reaction will go unperceived and only a very small proportion of inoculated subjects will find themselves unfit for work on the following morning.

The local reaction is hardly ever of such a degree as to prevent free use of the arm. This is equally true of the delayed reaction mentioned previously, which was observed in 3 out of 140 subjects who had been given the large dose of vaccine. So far this type of local reaction has not yet been seen in any subject who received the reduced dose of vaccine. It is to be expected, however, that certain persons who are especially susceptible may develop a small fluctuating area even after the injection of a small dose. No treatment of any kind is necessary or advisable for such a local reaction. It is nevertheless emphasized that the quantity of vaccine injected as the second dose must not be increased in these cases. Such susceptible subjects should receive as their second dose only half the quantity of vaccine injected as the first dose.

Summary

A new type of typhoid-paratyphoid vaccine, killed and preserved with alcohol, was compared with ordinary heat-killed T.A.B. or T.A.B.C. vaccine from various sources. Two marked differences were found between groups of subjects inoculated with the two types of vaccine: (1) The alcohol-killed and alcohol-preserved vaccines stimulated demonstrable typhoid Vi antibodies in a relatively high proportion of cases, whereas the Vi antibody response to ordinary vaccines was almost negligible. No significant difference in O antibody response was observed. (2) The reactions produced by the alcoholized vaccine were much milder than those caused by ordinary vaccine. General reactions were as a rule either absent or quite trivial. The local reaction was of a modified type and hardly ever prevented free use of the arm.

No correlation was found between Vi or O antibody response and severity of general or local reactions.

Recommendations are made with regard to the dose of vaccine and the interval between the two injections.

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Clinical Memoranda

An Unusual Injury to the Larynx

The injury in the following case seems to be unusual enough to be worth recording.

CASE REPORT

One night during the black-out a constable, after leaving a brightly lit police station, collided with a pillar-box in the street. The upper edge of this obstruction struck him on the thyroid cartilage. At once he experienced pain in the front of the neck and his voice became husky. The degree of huskiness gradually increased, reaching a maximum four hours after the accident. The next day his voice was still very rough, and there was tenderness to the left of the laryngeal cartilages. An examination of the larynx revealed the presence of a sausage-shaped haemorrhage under the mucosa of the left ventricular band. This haematoma almost completely obscured the view of the left vocal cord, though the extreme edge of this structure became visible when the cords were in full adduction. The possibility of a recurrent laryngeal nerve lesion was eliminated by the fact that both cords moved normally. The condition remained stationary for four days and then gradually improved. On the following day—that is, five days from the infliction of the injury—the voice still sounded deeper than usual. The haematoma was much decreased in size and the ventricular band looked purplish and swollen, but the vocal cord was clearly visible. It took twelve days for the voice to become quite normal, and in eighteen days all signs of the injury had disappeared.

Commentary.—Fatal and non-fatal cases involving injury to the neck are relatively common in police work. This particular case seems to emphasize the necessity for examining the interior of the air passages in all non-fatal medico-legal cases involving injury to the neck. Very often the assaulted person complains of pain and tenderness near or over the larynx, but apart from the tenderness that can be discovered externally there may be no concrete clinical findings that will serve as indisputable proof of injury received. Marks of finger-nails and bruising may be found externally; but this is not invariable, and the bruising may take several days to appear on the surface. In all such cases, therefore, a laryngeal examination might prove valuable.

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