STAPHYLOCOCCUS POLYVALENT SOMATIC ANTIGEN VACCINE PART II.* AN IMPROVED METHOD OF PREPARATION†

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IN AN EARLIER article[1] we reported the development of a Polyvalent Somatic Antigen Vaccine for use against staphylococcal infections. The vaccine was capable of protecting experimental animals when challenged with lethal or skin test doses against all of a wide variety of pathogenic *Staphylococcus aureus* strains. The results were so encouraging that a series of preliminary studies in humans was begun. It soon became clear that the reactions in humans after inoculations, while not dangerous, were rather severe and that they would discourage widespread use of the vaccine. A two-fold approach was used to overcome this undesirable feature. Attempts to purify and isolate the immunizing "substance" or "substances" were undertaken and are still under way. Secondly, we investigated the possibility of eliminating the reactions by preparing the vaccine in an entirely synthetic medium. Such a medium, based on Medium No. 858 of Healy *et al.*,[2] proved suitable. Its composition and method of use are described in this report.

MATERIALS AND METHOD

The composition and details of preparation of the medium used are outlined in Table I. The inoculum for each 500 ml. of medium was 0.5 ml. of a reconstituted freeze-dried culture[1] containing approximately 5 x 10^8 organisms per ml. The flasks were incubated for 24 hours at 37° C. Samples for testing for total bacterial count and for the presence of toxins were taken, and phenol to give a concentration of 0.5% was added to the remainder. It was then placed in a water bath at 56° C. for 1½ hours. Following this, samples for sterility tests were taken (0.5 ml. on each of three blood agar plates, and 0.5 ml. into each of six tubes of thioglycollate medium and trypticase soy broth medium containing meat particles and incubated at 30-32° C. for seven days) and the vaccine was refrigerated at 4° C. until required. The total count of staphylococci varied from 12 x 10^6 to 30 x 10^6 organisms, the average being 20 x 10^6 organisms per ml. To test for toxins, approximately 5 ml. quantities of the vaccine were Seitz-filtered and tested for hemolysins on rabbit and sheep erythrocytes, and for erythrogenic and dermonecrotic toxins by injecting rabbits with 0.1 ml. amounts intracutaneously. None of the vaccine preparations contained any of these toxins.

Once the results of the sterility tests were found to be satisfactory, the phenolized heat-killed vaccine was brought to 37° C., 100,000 units of dornase† added for each 500 ml. of vaccine, and incubated at 37° C. for 24 hours. The preparations were then removed from the incubator (or water bath) and held at room temperature until complete (or almost complete) lysis of staphylococci was achieved. This varied from one to six weeks, but the lysis could be substantially hastened by constant shaking. Periodic adjustment of pH to between 7.0 and 7.4, the optimum range for dornase activity, was necessary throughout this period. Prior to use each vaccine was tested for safety and sterility by the methods described previously,[1] and for toxins by the method described above.

RESULTS AND DISCUSSION

The immunizing capacity of the polyvalent somatic antigen vaccine prepared from the chemically defined medium and designated as P.S.A. No. 6 was determined by the rabbit skin challenge technique[1] prior to its use in humans. One hundred and twenty-five challenge strains were used and these included all of the known phage types, and some untypable strains. Thirty-four were from phage group I, and 16, 29, 6 and 30 were from phage groups II, III, IV and the Miscellaneous phage group, respectively. In addition, there were 10 strains which we were not able to type. The vaccine gave complete protection against all.

The course of injections for humans has been 0.1 ml. intracutaneously (either on the forearm or on the back in the medial area of the scapula), followed in 10 minutes with 0.5 ml. intramuscularly. This dosage is repeated after an interval of four to six weeks. It is our opinion, based on animal experiments, that the intracutaneous and intramuscular injections are both essential if the maximum effects of immunization are to be achieved. The intracutaneous injection, in addition, serves as a sensitivity test.

To date we have had no severe reactions (over 150 patients) after the administration of P.S.A. No. 6. The reactions have ranged from "none" to "moderate", and have been limited to pain at the site of injection which has lasted for two or three days. There were no systemic reactions. The skin reactions at the site of the intracutaneous injection have been rather unusual. A slight swelling may occur, accompanied by a shallow diffused erythema which may extend in size from a few centimetres to most of the forearm. This will generally have cleared by the third day. There have been no cases exhibiting necrosis or sloughing of skin at the sites of injection.

The reactions following the administration of P.S.A. No. 4, our earlier preparation,[1] were much more severe. Twenty-six patients received this preparation. The reactions varied but none was as moderate as the reactions following the administration of P.S.A. No. 6. A few systemic reactions accompanied by malaise and fever, which lasted for two or three days, were encountered.

P.S.A. No. 6 differs from P.S.A. No. 4 in a few minor details of manufacture and in the fact that

*Part I of this study was reported in the *Canad. M. A. J.*, 83: 143, 1960.
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†Dornavac—produced by Merck Sharp & Dohme, West Point, Pa.

TABLE I.—SYNTHETIC MEDIUM FOR STAPHYLOCOCCUS SOMATIC ANTIGEN PRODUCTION

Solution 1 (*Amino acids*)	*Mg.*
L-Arginine	140.0
L-Histidine	40.0
L-Lysine	140.0
L-Tyrosine	80.0
L-Tryptophane	20.0
L-Phenylalanine	50.0
L-Cystine	40.0
L-Methionine	30.0
L-Serine	50.0
L-Threonine	60.0
L-Leucine	120.0
L-Isoleucine	40.0
L-Valine	50.0
L-Glutamic acid	150.0
L-Aspartic acid	60.0
L-Alanine	50.0
L-Proline	80.0
L-Hydroxyproline	20.0
L-Cysteine	520.0
Glycine	100.0
$CaCl_2$*	400.0
Distilled water	250 ml.

Solution 3 (*Coenzymes*)	
Diphosphopyridine nucleotide	14.0
Triphosphopyridine nucleotide	2.0
Coenzyme A	5.0
Cocarboxylase	2.0
Flavin adenine dinucleotide	2.0
Uridine triphosphate	2.0
Glutathione	2.0
(*Nucleosides*)	
Adenine desoxyriboside	20.0
Guanine desoxyriboside	20.0
Cytosine desoxyriboside	20.0
5-Methyl desoxycytidine	0.2
Thymidine	20.0
Distilled water	250 ml.

Solution 2 (*Vitamins*)	*Mg.*
Vitamin A	0.2
Ascorbic acid (Vitamin C)	100.2
Calciferol (Vitamin D)	0.2
α-Tocopherol phosphate (Vitamin E)	0.02
Menadione	0.02
Pyridoxine	0.05
Pyridoxal	0.05
Biotin	0.02
Folic acid	0.02
Choline	1.0
Inositol	0.1
p-Aminobenzoic acid	0.1
(*Miscellaneous*)	
Sodium acetate	100.0
Sodium glucuronate	8.4
L-Glutamine	200.0
D-Glucose	3000.0
Phenol red (pH indicator)	40.0
Ethanol (as an initial solvent for fat-soluble constituents)	32.0
Tween 80	10.0
Cholesterol	0.4
Distilled water	250 ml.

Solution 4 (*Inorganic Salts*)	
NaCl	13600.0
KCl	800.0
$MgSO_4 . 7H_2O$	400.0
$NaH_2PO_4 . H_2O$	280.0
$NaHCO_3$	4400.0
Fe, as Fe $(NO_3)_3$	0.2
Distilled water	250 ml.

*Inorganic salt, but must be added to Solution 1. If added with the other inorganic salts, precipitation will occur.

Prepare four stock solutions as noted in the table. Heat solution 1 to boiling and add 0.1 ml. concentrated reagent grade HCl. Allow to cool and add to Solution 2 while agitating. Continue agitating and add to Solution 3, and finally, still stirring, add the entire amount to Solution 4. The final volume will be 1 litre. This should be adjusted to pH 7.2 - 7.4 with 0.1 $NaHCO_3$ or 0.1 N HCl.

an entirely different medium was used for growing the staphylococci. The same strains were used for its production, however, and the same proportions of the somatic antigens were incorporated in the final preparation. This is shown in Table II. The average bacterial count for P.S.A. No. 6 was 20 x 10^6 organisms per ml. whereas it was 5 x 10^8 organisms per ml. for P.S.A. No. 4, an approximate 25-fold difference. A difference in total nitrogen was also evident, but this was not as marked, being approximately four-fold. P.S.A. No. 4 averaged 250 μg./ml. whereas P.S.A. No. 6 averaged only 70 μg./ml. Some of the reduction in reactions between the two preparations could have been due to the differences in the bacterial count and total nitrogen concentration. This was not tested, but it is very unlikely that this difference in reactions, which was quite marked, could be attributed entirely to this quantitative difference in the two vaccines, for so far as can be judged in protection tests in rabbits, and in the production of agglutinins in rabbits and humans, there is very little or no difference between the immunizing capacity of the two vaccines.

TABLE II.—COMPOSITION OF POLYVALENT SOMATIC ANTIGEN VACCINE NO. 6

Strain	*Phage type*	*Proportion in vaccine* %
584763	(Group I) 80/81	30
596536	(Group II) 3A/3B/3C	30
596510	(Group III) 7/47/53/54/75+	30
591714	(Group IV) 42D	5
6032	(Group I) KS6	5

The significance of staphylococcal agglutinins has not yet been determined. We have found that strains from different phage groups possess a common agglutinating antigen. We have also found that animals (rabbits and hamsters) immunized with monovalent somatic antigen will be protected against lethal challenge with the homologous strain and a limited number of heterologous strains, provided that the challenged animal has an agglutinin level of 1:600 or more. It will not, however, be protected against challenge with all strains. On the other hand, the agglutinin levels after im-

munization with polyvalent somatic antigen are no higher than those obtained with the monovalent form, but the polyvalent vaccine will protect against a much wider range of staphylococcal strains. It is therefore clear that the presence of agglutinins, even in high titre, does not always indicate immunity against infection with a given strain but it may have some significance, or be an indication of immunity against others.

TABLE III.—THE IMMUNIZING VALUE OF COMMERCIAL STAPHYLOCOCCAL TOXOIDS AS SHOWN BY THE RABBIT SKIN CHALLENGE TEST

	Challenge strains		*Average agglutinin titres*†	
Manufacturer	*Number used*	*Number protected against*	*Pre-immunization*	*Post-immunization*
A	36	4	1:64	1:640
B*	36	8	1:128	1:2560
C	36	3	1:64	1:1280
D	36	3	1:256	1:1280
E	15	3	1:256	1:1280

*Toxoid combined with other staphylococcal antigens.
†There was no detectable antitoxin in either the pre-immunization sera or in the post-immunization sera taken two weeks later.

Attempts to isolate the substance (or substances) involved in immunity to staphylococcal infections are now in progress. The pathogenic staphylococcus produces a number of diffusible substances *in vitro*, which have been shown to have adverse reactions on tissues or on tissue constituents. These include toxins (hemolytic, dermonecrotic and lethal), leukocidins and various enzymes such as hyaluronidase, coagulase, various proteases and lipases. There seems little reason to doubt that the toxins can and do play a part in staphylococcus infections, but the part, if any, that these other substances play is uncertain. It is questionable whether the antibodies to any of them, including the toxins, play any major part in immunity or resistance to staphylococcal infections. Staphylococcal toxoids have gained in popularity in recent years and their proponents attribute whatever success that has been achieved to the production of specific antitoxins. There is some evidence, however, to suggest that this may not be the case, and that the immunity developed to staphylococcal infections might be due to other factors. In the course of our studies, we have had occasion to assay toxoids from five different manufacturers by the rabbit skin challenge test. The protection afforded by these products varied but ranged between 9 and 22% of the challenge strains. In all cases, the agglutinin titres in the test animals (rabbits) rose significantly, indicating that antibodies other than antitoxins are involved. This is shown in Table III where the number of strains used for challenge and the number protected against, and, as well, the average rise in agglutinin titres, are recorded. There is therefore good reason to question the purity of the preparations labelled "toxoid", for it is clear that other antigens can be involved.

Our work with somatic antigen has shown clearly that antitoxin is not a factor in any immunity developed following its use. The method of manufacture and our control tests preclude the possibility of the presence of toxins or toxoids. Furthermore, in our studies with humans and experimental animals we have not been able to demonstrate a rise in antitoxin levels in any of the experimental subjects. It is our contention that if immunity is ever achieved it will be due to the development of antibodies that have a marked antibacterial activity. Antibodies to the various diffusable substances may alter the course of an infection, but it is extremely doubtful whether they could stop an infection.

SUMMARY

A chemically defined synthetic medium for the production of staphylococcus somatic antigen has been described.

Vaccine produced in the above medium is relatively reaction-free. Reactions, when present, have been moderate and of short duration and have been confined to the sites of injection.

The course of immunizations for human subjects is outlined.

The relative value of the different antibodies, i.e. agglutinins, antitoxins, antibacterial, etc., is discussed.

REFERENCES

1. GREENBERG, L. AND COOPER, M. Y.: *Canad. M. A. J.*, **83**: 143, 1960.
2. HEALY, G. M., FISHER, D. C. AND PARKER, R. C.: *Proc. Soc. Exper. Biol. & Med.*, **89**: 71, 1955.

PAGES OUT OF THE PAST: FROM THE JOURNAL OF FIFTY YEARS AGO

THE RODDICK BILL

The "Roddick Bill", which provides for one registration and establishes a Dominion Council, upon whose licence physicians may practise in any province, passed a special committee of the House of Commons on March 3. Provision is made for representation on the council from universities, and by reason of this provision objections were urged by Saskatchewan, Alberta, and British Columbia, on the ground that they have no universities. By way of compromise, it will be provided that of the three members of the council appointed by the government, two shall be from these provinces. With this exception the Bill will be reported as it stood in the abstract published in the Journal in February. The congratulations and thanks of the profession will go out to Dr. Roddick for his unceasing labour in this cause during the past nine years.—*Canadian Medical Association Journal*, 1: 363, April 1911.