though in another patient (not included in this series because he had chorea only) haemolytic streptococci resistant to sulphonamide were found in routine weekly throat swabs after eight weeks of treatment. The patient was treated with bactericidal doses of intramuscular penicillin, a tonsillectomy was performed, and prophylaxis was then continued with oral penicillin.

Since that time, all patients with rheumatic fever or chorea have been given sulphonamide or penicillin prophylaxis in hospital, and this has been continued after discharge (sulphatriad, 1 g. daily, or penicillin G, 200,000 units twice daily). Our original impression of the efficacy of this prophylaxis has been confirmed, and toxic reactions have been few in number and harmless. Most have occurred within a few weeks of starting treatment—that is, whilst the patients were still in hospital. Prophylaxis has then been changed from one drug to the other and there has been no further trouble.

We have no data available on differences in the recurrence rate between cases treated with sulphonamide and penicillin. Assuming both drugs to be equally effective, penicillin would be the one of choice, for there is the possible though small danger of agranulocytosis with sulphonamide. The slight extra cost of penicillin (3.6d. a day as compared with 1.6d. a day for sulphonamide) does not seem important, and storage of penicillin presents no problems, for the tablets retain their potency for three months if kept in an airtight tin in the cool. Injections of benzathine penicillin have been advocated by some as preferable to penicillin by mouth. Though this method ensures that prophylaxis is carried out, it is difficult to impose a regime of weekly injections on children in the knowledge that this must be continued for years. The Rheumatic Fever Committee of the Royal College of Physicians (1957) recommends 200,000 units of penicillin G twice daily, or 120 mg. of penicillin V twice daily, and that benzathine penicillin should be used if for any reason oral prophylaxis cannot be given. With this we concur.

Finally, for how long should prophylaxis be continued? This is a matter for individual judgment, taking into account parental attitudes and circumstances. It is our belief, based on these figures for recurrence rates, that prophylaxis should continue for at least five years after the last attack or until the child has left school, whichever period is the longer (Rheumatic Fever Committee of the Royal College of Physicians, 1957). If, after leaving school, patients are exposed to an increased risk of streptococcal infection—that is, should they enter a university or one of the Services—then prophylaxis should be continued for a further period. If liability to reinfection is based on M antibody level, it may be that even more prolonged prophylaxis will be necessary.

Summary

Two comparable groups of patients with rheumatic fever were followed for a period of five years. One group of 96, admitted in 1949–50, were given no prophylaxis either in hospital or after discharge. The other, comprising 88 patients admitted in 1951–2, were given 1 g. of sulphonamide daily in hospital and after discharge. The period of follow-up in the two groups overlapped by two to four years.

The mean incidence of recurrences per patient-year was 5.6% in the unprotected and 1.2% in the protected. There was only small variation from year to year in each group, and no tendency for the recurrence rate to decrease with time. The reasons for the relatively low incidence of recurrences in the unprotected group and other differences between this and American series are discussed. Toxic reactions were few: none were serious. The duration, dosage, and merits of various forms of prophylaxis (sulphonamide, oral penicillin, and long-acting penicillin by intramuscular injection) are discussed.

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DESENSITIZATION TO BEE STINGS BY INTRACUTANEOUS INJECTIONS OF WHOLE-BEE EXTRACT

BY

DAVID LORDMAN, M.B., Ch.B., D.P.H.
The South African Institute for Medical Research,
Johannesburg

Reports of more or less severe reactions to bee stings are not infrequent in South Africa, and deaths therefrom are occasionally reported in the lay press. Our own information about bee-sting sensitivity and the effects of bee stings derives largely from many physicians in various parts of the country who, describing more or less severe reactions in their patients, inquire about the possibility of prophylactic desensitization.

Persons sensitive to bee stings commonly manifest local reactions in redness and swelling at the site of the sting and general reactions in rash, urticaria, oedema of face and glottis, and often anaphylactic shock, with collapse, unconsciousness, and even death. There may also be malaise, vomiting, dyspnoea, or bronchospasm. These effects appear within minutes after the sting or reach their climax some hours later.

The majority of patients who show a high degree of sensitivity to bee stings have suffered symptoms of progressively increasing severity after each sting, following a longer or shorter period of freedom from significant reactions after the initial sensitizing sting, which may have been many years ago. The following examples are given in illustration:

A farmer 44 years old had frequently been stung, as a boy and young man, with more or less severe local reactions after each sting. When he was stung again in 1952 there was little local reaction, but within three minutes he became unconscious and remained so for two hours. On recovery he had a "choking" sensation, with headache and malaise continuing for a day or two.

A medical man with no family history of allergy was stung at the age of 3½ years by about a hundred bees. The stings were removed and he suffered no ill effects. When he was 12 years old he was stung on the hand and developed a swollen face. Two years later he was stung between the toes. This resulted in swollen eyes, oedema of the glottis, and extreme dyspnoea requiring urgent medical attention. At the age of 29 he was stung once on the forearm, and, although he was injected with adrenaline within 20 minutes and with three further injections at hourly intervals, he had to have treatment at hospital, where he was detained for some time. He was stung again 10 years later, and, although adrenaline was administered within 10 minutes, he developed alarming swelling of the face.

A physician wrote from the Transvaal: "A patient of mine was stung by a bee in the soft tissue around his eye and had a severe systemic reaction. He became semiconscious and vomited profusely; rapid heart, with fall of blood pressure. He had never been affected by a bee sting previously although stung many times."

As a result of our advice and recommendations concerning the possibility of desensitization, bee-venom extracts are

Details of 24 Persons Sensitive to Bee Stings Submitted to Desensitization.

Case No.	Sex and Age	Otherwise Allergic	Reactions from Bee Stings	Whole-bee Extract Skin Sensitivity and Treatment Series	After Desensitization	
					Reactions from Bee Stings	Outcome
1	M 6	Urticaria from insect bites	Progressively more severe reactions from stings. Was stung recently and had to be treated with corticotrophin	1 unit 0.5-250 units	Stung twice during course of desensitization with hardly any effect from second sting	Successful
2	M 48	Migraine	For the past 2 years had developed palpitations, pallor, faintness, and prostration with every sting	50 units 25-500 units	Stung on finger: local oedema lasting only a few hours	"
3	M 36	Vasomotor rhinitis from inhalation of mohair dust	Local swelling and severe itching, worse with each successive sting. General reaction not marked except slight swelling of eyelids and listlessness	500 units 250-2,000 units	Local spot only. No general reaction	"
4	M 9	Not	Had become increasingly sensitive to stings. Last sting produced a marked local reaction and a very severe general reaction, including urticaria with oedema of glottis	1,000 units 500-3,000 units	Recently stung repeatedly with, at most, a slight local reaction. "This has been a most gratifying result"	"
5	F 56	"	Severe urticaria and palpitations	250 units 100-1,500 units	"Only mild reactions"	"
6	M 5	"	At age of 2 years was stung on face, gross oedema developing there. At age 4 was stung on left thumb and within a minute there was oedema of face with gross tachycardia, oedema of the larynx occurring 5 minutes later	250 units 100-1,000 units	After three separate stings on the one leg the only reaction was a local swelling	"
7	M 32	"	Stung at age 6 years and again at 13: no serious local reaction and no general effect. Recently stung by 5-6 bees on hands, with slight local swelling and itching of whole body 15 minutes later. Stung again 2 weeks later by 4-6 bees with slight local swelling but marked general itching and marked swelling of loose tissues of throat, eyelids, and armpits, with generalized urticaria and vomiting. "I firmly believe that I would have died from oedema of the throat and glottis if I did not inject myself with 1 ml. adrenaline immediately I realized that my tongue was getting thick." The patient is a physician	1 unit 0.5-25 units	Stung 6 months later on finger with only slight local swelling and itching	"
8	M ?	"	When stung on neck or face became pulseless, icy cold, and collapsed. Often found on ground by his family deathly pale after a single sting	500 units 250-1,500 units	Sting on arm: no untoward reactions	"
9	F 41	"	Always very sensitive to stings. Marked shock, breathlessness, fainting, and palpitations. The patient, a trained nurse living on a farm, gives herself adrenaline after a sting	250 units 100-1,500 units	Discontinued injections after 100 units owing to distance from doctor. Stung 9 months later, with only mild local erythema and no general reaction	"
10	F 60	" }	Both grandmother and grandson reacted similarly to stings, with rash, tachycardia, and oedema of glottis. A sting in either patient meant an emergency call to the doctor	1,000 units 500-2,000 units	Only 6 injections of 500-unit extract. Stung 2 months later: no bad reaction	"
11	M 3			1 unit 0.5-25 units	No reaction after sting 2 months later or after sting one year later	"
12	M 7	"	Several episodes of anaphylactic shock with patient in very critical condition	50 units 25-3,000 units	No shock at all after recent sting	"
13	M 50	"	Severe anaphylactic type of reaction, with vomiting, urticaria, and collapse	500 units 250-2,000 units	After two recent stings only slight swellings. One sting on neck resulted in only mild flushing. "No doubt this man has benefited very considerably from desensitization. He is extremely happy at result"	"
14	M ?	"	Increasing sensitivity to stings. On last occasion, acute oedema of glottis and unconsciousness. The oedema developed so rapidly that he was unable to give himself an injection of adrenaline	5 units 2.5-3,000 units	Small urticarial weal at site of sting. No general reaction. "Naturally the patient is delighted"	"
15	F ?	" }	The physician provided a general account of five cases under his care. "Same history in each case: Patient feels unusually bad after first sting; after second sting, urticaria with slight oedema of glottis. One patient became unconscious by the time he reached hospital. Another was laying out a golf course and was seriously thinking of giving up his job owing to the increasingly severe reactions from bee stings"	50 units 25-500 units	"The injections were given according to the suggested dosages in the pamphlet accompanying the treatment extracts. Since desensitization all the patients can walk among bees with impunity"	"
16	F ?			500 units 250-1,500 units		"
17	M ?			500 "		"
18	M ?			250-1,500 "		"
19	M ?			1,000 " 500-3,000 " 1,000 " 750-2,500 "		"
20	M ?	Vasomotor rhinitis. Also sensitive to grapefruit	Stung from childhood onwards with no effect at first, but later with progressively severe reactions. Recent stings resulted in swollen face, body rash, and "feeling of pins-and-needles in body"	1,000 units 750-3,000 units	Swelling of hands and arms continue, but no longer that "queer feeling" Patient feels that he is better after stings now	Some improvement
21	F 21	Not	Large areas of local erythema with massive swelling as well as general malaise and urticaria	10 units 5-100 units	Moderately severe local reaction with weal, urticaria, and swelling	Not successful

Continued overleaf

Details of 24 Persons Sensitive to Bee Stings Submitted to Desensitization—Contd.

Case No.	Sex and Age	Otherwise Allergic	Reactions from Bee Stings	Whole-bee Extract Skin Sensitivity and Treatment Series	After Desensitization	
					Reactions from Bee Stings	Outcome
22	M 43	Not	Twice stung in the past, with severe anaphylactic reaction each time	10 units 5 and 10 units only	Desensitization in June and July, 1953. Stung again in May, 1956, with bronchospasm and severe anaphylactic shock	Not successful
23	F 48	"	Tachycardia, vomiting, and mental confusion within 2 minutes after a sting	10 units 5-100 units	Stung some months later; had nausea, vomiting, tachycardia, and body pains	"
24	M 52	"	After a sting he became mentally confused, dyspnoeic, intensely red in the face, and developed tachycardia and unconsciousness	1,000 units 500-1,500 units	A sting received 4 months later produced tachycardia, mental confusion, and collapse	"

ordered for that purpose by physicians and are issued in a series of graded strengths referred to as "treatment sets." In this way, 50-60 treatment sets for desensitization purposes were issued annually in 1951-5, 86 sets in 1956, and 115 in 1957. These figures do not, of course, indicate the incidence of serious reactions to bee stings in the country, but merely reflect the spread of knowledge regarding prophylactic desensitization.

In our experimental and clinical studies extracts of whole bees have been employed instead of material from the sting apparatus, because our earlier investigations had shown that in persons sensitive to bee stings intracutaneous testing with extract of whole bee and with extract of sting-venom sac produced similar reactions, indicating, at least, the presence of a common antigen. From a practical point of view, in the large-scale preparation of extracts the removal of venom sacs with stings from large numbers of bees does not seem warranted when the preparation of the equally useful whole-bee extract is so simple.

Progressive skin tolerance to the injection of gradually increasing strengths of whole-bee extracts is the rule, and, as shown below, a parallel state of clinical hyposensitization is similarly attained.

Preparation of Whole-bee Extract

In the preparation of whole-bee extract many hundreds of bees are removed from the hive into a closed container, where they are killed with chloroform. The dead bees are washed in a colander under a flow of cold running water for the removal of honey and debris, and spread out on sheets of blotting-paper for superficial drying. The bodies are weighed and ground in a mortar to a thick paste, which is extracted with a moderate excess of Coca's fluid in the cold for 48 hours, with frequent stirring. The mass is squeezed through a clean, fine double-muslin bag, and the filtrate, collected and preserved with 0.5% phenol, is submitted to paper filtration followed by Seitz filtration. The clear straw-coloured fluid is controlled for sterility, and constitutes the "whole bee" extract.

Standardization of Whole-bee Extract

The "standardization" of the whole-bee extract is arbitrarily based on the total protein nitrogen content of the fluid. The figure thus obtained, and referred to as the "unitage," is taken as the basic "strength" of the particular batch of extract. It is of course no criterion of the allergenic value of the extract, and merely serves as a convenient guide to dilutions which may later have to be made.

Preliminary Skin-testing of the Patient

Skin-testing is regarded as a necessary preliminary to the desensitization process that is to follow. The object is to determine the degree of the patient's sensitivity in "units" to the particular batch of bee-protein extract in use. A "bee-venom skin-test outfit" is issued to physicians, consisting of a series of capillary tubes containing the whole-bee extract in the following graded strengths: 1, 10, 50, 100, 200, 500, 1,000, and 2,000 units per ml.

The skin tests are carried out as follows. With a tuberculin-type syringe, 0.02 ml. of the lowest-strength

extract (1 unit) is injected into the skin of the volar surface of the forearm. Injections are continued in this way with the stronger extracts in the skin test outfit until the occurrence of a local reaction is just apparent—a weal about 1 cm. in diameter with a surrounding area of erythema. No further skin tests are done, but the "unitage" of the extract producing this reaction is noted. All test injections are given by the intracutaneous route in a fresh area of skin each time at intervals of 10 to 15 minutes to allow for the development of any reaction that might occur. In our experience, skin sensitivity is variously shown by sensitive patients to 1-250 units per ml. of fresh whole-bee extract.

A suitable treatment set for desensitization purposes is now prepared on the basis of the skin-test reaction.

Desensitization with Whole-bee Extract

Desensitization is carried out with injections of whole-bee extract by the *intracutaneous* route. This route is advised because the reactions are plainly visible and easily gauged, and dosage can accordingly be suitably regulated. Further, as the amount injected each time is relatively small there is a local reaction only, and, in our experience, no constitutional or other untoward reactions need be anticipated.

The treatment set provided for desensitization purposes consists of a series of dilutions of whole-bee extract of the same batch used for the skin testing in 2-ml. rubber-capped bottles in increasing strengths and commencing at a dilution somewhat less than the reaction-strength noted in the preliminary test.

The intracutaneous injections of each extract dilution are given in doses of 0.05-0.25 ml. at intervals of 2-4 days, with dose increments, when indicated, of 0.05 ml. It is desirable to obtain a weal 1-2 cm. in diameter after each injection. Increase of dosage or use of higher-strength extracts is indicated only when the weal reaction is beginning to diminish in size. It may happen that five, six, or more injections are given from the same extract before proceeding to higher strength.

Example: A positive reaction was obtained in the preliminary skin test with the 10-unit extract. The treatment set is prepared with a series of extracts containing 5, 10, 25, 50, 100, and 250 units per ml. When this course is completed a further series of stronger extracts from 250 units per ml. upwards is issued as indicated. It is desirable to continue desensitization with gradually increasing doses of extract until the patient is receiving injections of extracts of about 1,000 units strength with a local reaction only after each injection. When this final "unitage" of extract is readily tolerated, with the production only of a local weal of 1-2 cm. diameter, maintenance doses of 0.25 ml. each are injected thereafter at gradually increasing intervals until the patient is receiving such an injection once every three to four months. It has not been found necessary to use strengths of extracts higher than 2,000-3,000 units per ml. for effective desensitization. In highly sensitive persons—that is, those reacting to 1-10 units by skin test—it suffices to use a desensitizing extract not stronger than about 1,000 units (corresponding to 1,000-100 times the original skin reaction strength). This final

dosage is reached as a rule in 2-3 months. As in all "desensitization" procedures, it is inevitable that the state of hyposensitization attained will in due course be lowered if not lost. It is for this reason that maintenance doses as described should be continued for as long as thought necessary, especially in bee-keepers and others who cannot avoid contact with bees.

Results of Desensitization

A description has been given (Ordman, 1951) of three persons sensitive to bee stings in whom effective desensitization had been achieved with the use of whole-bee extract administered intracutaneously. Since that time 19 further instances of successful desensitization by this method have come to notice, bringing the total to 22. These 19 cases have emerged from a study of 47 satisfactorily completed reports by physicians who had submitted their bee-sting-sensitive patients to adequate desensitization. In 23 instances the patients had not been further stung by bees, and it is in the remaining 24, where stinging had followed desensitization, that these 19 persons were found to be fully protected (see Table).

It may seem surprising that only 22 authenticated instances of successful desensitization can be quoted when material for prophylactic desensitization had been supplied over the years for some hundreds of persons. The explanation is, firstly, that most people subjected to bee stings in South Africa live in the rural areas and information about them must be obtained by correspondence with their doctors, leading all too often to little or no details about their patients in spite of follow-up letters. Secondly, in the bulk of the replies that do become available the information is vouchsafed that, although the patients had been properly desensitized according to our instructions, they had not subsequently been stung by bees.

Details are given in the Table of the 24 cases which had been submitted to desensitization and about which full reports were obtained. Effects of bee stings before and after such desensitization, results of skin-sensitivity tests, and the desensitization procedure itself are described for each case.

It will be observed that in 19 of these cases (1-19) the results were highly satisfactory, as no reactions, or very mild reactions only, were now occurring in persons who were originally very severely affected by bee stings. In Case 20 "some improvement" only was claimed. In Cases 21, 22, 23, and 24 it was reported that desensitization procedures had produced no benefit at all—that is, reactions after bee stings were now as severe as they had been previously.

Cases 21, 22, 23.—These patients were highly sensitive by skin test to whole-bee extract (10 units). Desensitization was carried out to only 100 units or less instead of to the required 3,000-4,000 units. Desensitization in each of these cases was obviously inadequate, and satisfactory results could not be expected.

Case 24.—For some reason this patient does not appear to have been highly skin-sensitive to whole-bee extract (1,000 units) by test, and desensitization should have gone on with strengths up to 4,000 units or even higher.

It becomes clear from the study of the Table that desensitization with whole-bee extract by the intracutaneous method is a very satisfactory procedure if properly carried out.

Summary and Conclusions

A procedure is described for the prophylactic desensitization of persons sensitive to bee stings by the use of increasing strengths of whole-bee extract administered intracutaneously.

A preliminary intracutaneous skin test is performed to determine the degree of the patient's sensitivity to the extract that is to be used for subsequent desensitization.

Successful desensitization is reported of 19 persons originally highly sensitive to bee stings.

THE POLYSACCHARIDE BEHAVIOUR OF CANCER ANTIGENS

BY

JACK G. MAKARI, M.D., D.T.M.&H., Sc.M.

Research Department, Muhlenberg Hospital, Plainfield, New Jersey, U.S.A.

The high degree of accuracy in the detection of antigens in the sera of patients with carcinoma obtained by use of the Schultz-Dale method has already been reported (Makari, 1955a, 1955b). Recently Burrows (1958) confirmed these results in a thorough investigation of the test. The chemical nature of cancer antigens remains an unsolved problem. However, in studying host-tumour relationships it is extremely important to know the exact nature of these cancer antigens. In an effort to elucidate this problem, Burrows and Neill (1958) have investigated the nature of the immunologically active constituent of serum of patients with carcinoma. They attribute the antigenic activity of such sera in the Schultz-Dale test to the presence of a polypeptide.

The following application of the Schultz-Dale method leads us to believe that cancer antigens behave like polysaccharides and that the activity observed by Burrows and Neill in the polypeptide of the serum is probably due to an associated polysaccharide antigen.

Methods

Details of the Schultz-Dale method, the preparation of antigens, and the testing procedure have been described (Makari, 1955b). Tumour and normal tissues are homogenized in 0.2N saline solution (one part of tissue plus four parts of saline) with a Sorvall homogenizer immersed in cracked ice. This procedure is allowed to continue for 10 minutes with the rheostat set at 115. The homogenate is then centrifuged twice at 3,000 r.p.m. at 5° C. for 10 minutes. The sediment is discarded and the supernatant is further centrifuged for 30 minutes at 10,000 r.p.m. at the same temperature. This sediment is separated and resuspended up to volume with normal saline solution. It is to be noted that this sediment differs from that used in my earlier work (Makari, 1955a, 1955b) in that the latter was obtained between 3,200 and 4,000 r.p.m. The sediment used in the present study—that is, that obtained between 3,000 and 10,000 r.p.m.—was chosen because it was found to have antigens in common with various tumours (Makari, 1958a).

The resuspended sediment and its supernatant comprise the antigens used in this study. They are used both in this form and after treatment by a procedure described by Chang (1953) and Chang, Snyder, and Murray (1953) in their preparation of an erythrocyte-sensitizing substance from rickettsial antigen which was thought to be polysaccharide in nature. In this procedure an equal volume of ether is added, with vigorous shaking for five minutes. It is then left undisturbed, to allow separation into ether and aqueous phases. The aqueous phase is mixed with alkali in the following proportion: nine volumes of the aqueous phase to one volume of 2N NaOH. The mixture is kept in a water-bath at 100° C. for 30 minutes. It is cooled and dialysed for two hours at 0 to 4° C. against several changes of isotonic phosphate saline solution (Na_2HPO_4 , 0.81 g.; KH_2PO_4 , 1.04 g.; NaCl, 6.8 g.; distilled H_2O , 1,000 ml.; pH=6.8). The active material found inside the dialysis bag is then shaken with an equal volume of chloroform for 20 minutes and centrifuged at 2,000 r.p.m. for 15 minutes. In this study the chloroform layer is discarded and the clear aqueous phase containing the sensitizing substances (which are referred to as S.S.) is used. These behave as polysaccharides, being non-dialysable, resistant to boiling and alkalis, and giving a positive Molisch test.

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