SKIN REACTIONS OF PATIENTS AND NORMAL INDIVIDUALS TO PROTEIN EXTRACTS OF STREPTOCOCCI[1]

By CLIFFORD L. DERICK and MARSHALL N. FULTON

(From the Medical Clinic of the Peter Bent Brigham Hospital, Boston)

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During the past few years in conjunction with Swift and Hitchcock at the Hospital of the Rockefeller Institute, one of the authors carried out cutaneous tests with nucleoprotein extracts of various streptococci, the results of which have been published (1) (2). Inasmuch as the greater part of that work was done on patients suffering from rheumatic fever it seemed desirable to determine whether a comparable frequency of positive reactions existed in patients with other diseases and in supposedly normal individuals.

Somewhat similar work was done in 1923 by Bristol (3), who performed tests with whole dried streptococci, and found that in 17 individuals with a history of scarlet fever and 31 without there was a positive reaction in 41 and 61 per cent respectively. Both groups are referred to as "fairly normal persons." Mackenzie and Hanger (4) used an alkaline extract of ground-up organisms, which they termed "intra-cellular antigen," as well as filtrates from plain broth cultures. With these streptococcus derivatives they found a high percentage of positive skin reactions among patients suffering from various diseases but could detect "no relation between the presence or absence of streptococcus allergy and any disease or group of diseases." Some of the strongest reactions observed by them were in presumably normal individuals. Because these authors were unable to demonstrate evidence of free antibodies in the serums of patients either by neutralization tests or by passive transfer in animals, they felt that the allergy or hypersensitiveness represented an acquired reactivity of the cellular tissues as a response to stimulation by bacterial proteins.

Small (5) reported marked general hypersensitiveness in patients

[1] Read by Title before the American Society for Clinical Investigation, May 6, 1929.

with rheumatic fever and arthritis to vaccines and soluble antigens of "Streptococcus cardioarthritidis" injected subcutaneously. This hypersensitive state was manifested by both local and focal reactions. His observations dealt only with the "rheumatic group" of patients.

Birkhaug (6), Kaiser (7), Irvine-Jones (8) and Swift, Wilson and Todd (9) all reported a higher incidence of hypersensitiveness among patients suffering from rheumatic infection than among those with other diseases when tested intracutaneously with filtrates of various strains of streptococci. The last named authors suggested that the reaction is caused by an intracellular toxic substance set free when the organisms autolyze in the culture media rather than by a toxin produced and secreted during their growth. Possibly the incitant in these filtrates is, in fact, of the nature of bacterial nucleoprotein.

The studies most comparable with ours are those of Ando and Ozaki (10) (11), who recently have reported skin tests performed on 860 individuals using a nucleoprotein fraction extracted from a strain of *Streptococcus scarlatinae.* These workers made no note of the association of skin hypersensitiveness with any particular disease condition but showed that there was a gradual increase in the percentage of positive reactors with the increase in age of the individuals tested (See discussion).

METHODS

Cases studied

During the present study 670 individuals were tested. Of these 475 were definitely diseased patients admitted to the medical wards of the Peter Bent Brigham Hospital. When more than one disease was included in the diagnosis, they were classified according to the primary or most important disorder. Forty-six were at The House of of the Good Samaritan all but one of whom were in various stages of convalescence from rheumatic fever or chorea. Ninety-one were either at the Children's or Infants' Hospital; of these 28 were in the general medical wards suffering from various diseases, 39 were infants or premature babies, most of whom presented feeding problems, and 24 had orthopedic conditions but were without a history of recent infection and so were considered as normal controls.[2] In addition 58

[2] We desire to express our thanks to the Staffs of the Children's and Infants' Hospitals and the House of the Good Samaritan for their hearty coöperation.

supposedly normal adults were tested; these included a few patients in whom no evidence of organic disease could be found, members of the resident staff at the Peter Bent Brigham Hospital and students in their final year at the Harvard Medical School.

Preparation of the nucleoproteins

The nucleoproteins used were extracted by Dr. Lancefield at the Hospital of the Rockefeller Institute according to the method which has been published (12). They were precipitated repeatedly until their nitrogen content was constant and stored in the form of dried powder. At least once a month a fresh 0.1 per cent solution of each was made for use at the Hospital of the Rockefeller Institute and a portion was sent to us in cold storage. This was kept subsequently in the ice-box until used. All solutions were cultured both aerobically and anaerobically on liquid and solid media to test for sterility. Dilutions to the required strength were made in normal salt solution immediately prior to performing the skin tests.

Three different nucleoproteins were used in most instances: those from hemolytic streptococcus (S43), green streptococcus (V110A) and ordinary baker's yeast. The latter was used for control purposes. Lancefield (13) has shown that the nucleoprotein fraction of each species of gram positive coccus which she studied, was "immunologically similar within the species." Her study included both the hemolytic and green streptococci. Thus a representative nucleoprotein fraction of these two species of streptococci was used.

Early in the study normal salt solution instead of yeast nucleoprotein was employed for control purposes, but as no positive reactions were encountered, this was discontinued later.

Ten milligrams of these nucleoprotein extracts can be injected intravenously in normal rabbits without eliciting any recognizable reaction. One-tenth milligram when injected intradermally in normal rabbits produces a mild local reaction. This latter dose, however, is many times that which was used in patients reported in this work. We feel therefore that the skin reactions which occurred were not due primarily to any toxic properties of the test materials used.

Method of making and reading the test

At the start 0.01 mgm. of each nucleoprotein dissolved in 0.1 cc. normal salt solution was used as the test dose. Since many of the reactions were unusually marked, it was felt that the results would be more valuable if smaller amounts were injected. Therefore, for most of the work amounts of 0.001 mgm. of hemolytic streptococcus nucleoprotein and 0.003 mgm. of each of the others were used as test doses.

All tests were done in the skin of the forearm except in a few infants who were tested over the anterior surface of the leg. According to the observations of Alexander (14) the skin response to allergens in patients with hay fever varies in intensity in different parts of the body, but the response in the skin of the leg is quite as large as that on the forearm. Therefore these tests in infants are comparable to those done on the other subjects. The injections were made into and not beneath the skin. Tuberculin syringes graduated in 0.01 cc. and with perfectly fitting 26 gauge needles were used to insure accuracy.

Measurements were made at the end of 24 hours in all instances and usually every 24 hours thereafter for at least 3 days with calipers similar to those described by Swift, Wilson and Todd (9). The two greatest diameters at right angles to one another were measured, and the height of all lesions either measured or estimated. After a time one becomes quite accurate in estimating the height of most lesions.

The volume of the lesions was calculated according to the formula used by Derick and Swift (15). Although it is recognized that such results are only approximate, they are, nevertheless, valuable as a basis for comparison. For purposes of presentation (table 1) the volumes of lesions have been transposed to a system of plus values thus, volumes of less than 50 cmm. have been considered negative, 51 to 200 cmm. as $\pm$, 201 to 500 cmm. as $+$, 501 to 800 cmm. as $++$, 801 to 1100 cmm. as $+++$ and all volumes over 1100 cmm. as $++++$. In this scheme we have looked upon all $\pm$ lesions as being doubtful and have classified them as negative. In order to include on a similar basis all patients tested, the volumes of the lesions in the few individuals given the large dose at the start of the work were divided by two. This correction was made, because it was ascertained repeatedly by using both large and small test doses in the same patient

at the same time that 0.01 mgm. of either nucleoprotein extract produced a lesion with a volume twice as great as that which occurred when 0.001 mgm. of the hemolytic streptococcus antigen or 0.003 mgm. of the green variety was used.

Description of the reactions

The type and time of the occurrence of reactions were very similar in all respects to those described by Mackenzie and Hanger (4). A few, occurring early within the space of an hour or two of the time of injection, were of the wheal and erythema type. In practically all instances, however, the reactions reached their maximum intensity after 24 to 30 hours; all readings were made at 24 hour intervals. The

TABLE 1

Code of arbitrarily selected measurements to show the dimensions and volume of lesions

Index	Dimensions of lesions	Volume of lesions
	mm.	*cu. mm.*
0	None to 9 x 13 x 1.0	Less than 50
±	10.5 x 12.5 x 1.0 to 15.0 x 21.0 x 1.5	51 to 200
+	17.0 x 20.0 x 1.5 to 18.5 x 21.5 x 3.0	201 to 500
++	19.5 x 22.5 x 3.0 to 21.0 x 20.0 x 4.5	501 to 800
+++	19.0 x 23.0 x 4.5 to 25.5 x 34.5 x 3.0	801 to 1100
++++	34.5 x 45.5 x 2.0 or 20.0 x 28.0 x 5.0	Over 1100

response which was elicited, varied markedly, ranging all the way from no reaction or a slight erythema without perceptible induration to a large swelling 6 to 8 cm. in diameter and raised 0.5 to 1 cm. at the center. These latter consisted of a hard indurated central area, deep red in color, surrounded by an edematous, pinkish red zone which gradually merged with the adjacent normal skin. In a few of the more intense reactions a vesicle containing turbid fluid formed superficially over the center of the lesion. Rarely light red streaks were observed passing from the more intense lesions up the arm. In two instances there was involvement of the regional lymph nodes as manifested by swelling and tenderness. Whenever present these latter manifestations disappeared within a day or so.

Depending upon their severity, the lesions persisted from a day, when mild, to as many as 14 days when there had been marked infiltra-

tion. Usually the skin at the site of reaction underwent a brownish pigmentation and in a few cases desquamated with a fine branny scale. All lesions eventually were absorbed completely, and at no time was there evidence of central necrosis and sloughing. With two or three exceptions no constitutional symptoms were noted.

TABLE 2

Results of tests in all individuals grouped into 5 year age periods

Age	Number of cases tested	Hemolytic streptococcus nucleoprotein (S 43) Intensity of reaction				Green streptococcus nucleoprotein (V 110A) Intensity of reaction			
		Cases		Percentage		Cases		Percentage	
		− or ±	+ to ++++	− or ±	+ to ++++	− or ±	+ to ++++	− or ±	+ to ++++
years									
0–5	61	58	3	95.1	4.9	60	1	98.4	1.6
6–10	49	24	25	49.0	51.0	33	16	67.4	32.6
11–15	40	16	24	40.0	60.0	28	12	70.0	30.0
16–20	31	15	16	48.4	51.6	22	9	71.0	29.0
21–25	63	21	42	33.3	66.7	43	20	68.2	31.8
26–30	49	20	29	40.8	59.2	33	16	67.4	32.6
31–35	52	16	36	30.8	69.2	39	13	75.0	25.0
36–40	35	9	26	25.7	74.3	27	8	77.1	22.9
41–45	53	26	27	49.1	50.9	43	10	81.0	19.0
46–50	60	29	31	48.3	51.7	52	8	86.6	13.4
51–55	55	23	32	41.8	58.2	46	9	83.6	16.4
56–60	51	21	30	41.2	58.8	43	8	84.3	15.7
60 and over	71	39	32	55.0	45.0	62	9	87.3	12.7
Total.....	670	317	353	47.3	52.7	531	139	79.3	20.7

RESULTS

As noted previously, 670 individuals in all were tested. In each instance nucleoprotein from hemolytic (S43) and green (V110A) streptococci and a control of either normal salt solution or nucleoprotein from baker's yeast were used.

Table 2 represents an analysis of the 670 individuals tested, arranged in five-year-age periods. This table shows the total number of cases tested in each age group and a division of these into negative or positive reactors. The main points to which attention should be called are:

1. That, in the whole group, a much higher proportion (52.7 per cent) reacted positively to the nucleoprotein from the hemolytic streptococcus than to that from the green variety (20.7 per cent).

2. That only very rarely was a reaction observed in an individual under the age of 5 years.

3. That in the groups above the age of 5 years, the percentage of positive reactors varied from 45 to 74 per cent with the hemolytic streptococcus nucleoprotein and from 12 to 33 per cent with the green variety. The figures for the patients between the ages of 6 to 10 and 11 to 15 years are misleading inasmuch as nearly half of the children in each of these age groups were suffering from various forms of rheumatic infection, and among these there was an unusually high proportion of positive reactors. Omitting the rheumatic children from these age groups, the percentages of positive reactors are changed from 51 and 60 per cent to 13 and 34.8 per cent respectively for the hemolytic streptococcus antigen; for the green streptococcus, the percentages are lowered from 32.6 and 30 per cent to 0 and 17.4 per cent respectively. The number of patients with rheumatic fever in any one age group above 15 years was insufficient to be of significant influence on the percentage of positive reactors.

When the positive reactors were further analyzed according to the intensity of their response (not shown in the table), it was found that of the 353 who reacted positively to the hemolytic streptococcus nucleoprotein, considerably over half (209) showed a strongly positive (i.e. +++ or ++++) response in contrast to only 15 strong reactors among the 139 who reacted positively to the green streptococcus antigen.

In a detailed analysis of the cases under 15 years of age (150 in number) we have observed several interesting facts. As shown in table 3 these individuals have been divided into two groups, non-rheumatic and rheumatic. The non-rheumatic group is comprised of 106 individuals. The rheumatic group contains 44 patients all of whom were in some stage of convalescence from rheumatic fever or chorea. Observing first the reactions to the hemolytic streptococcus nucleoprotein we find that out of the 60 non-rheumatic children under the age of 5 years, 58 or 96.6 per cent failed to show any reaction to this antigen. Thus, only 2 positive reactions were observed in the 60 indi-

viduals tested, while in the rheumatic group the one patient tested showed a positive reaction. This one case, of course, has little significance, and due to the infrequency of rheumatic fever at this age, it will be difficult to obtain further data on this point. The correlation between the occurrence of positive skin reactions and rheumatic infection is brought out strikingly in the children over 5 years of age. In the 6 to 10 year age group, of the 23 non-rheumatic children, only

TABLE 3

Results of tests in young individuals

	Age	Number of cases tested	Hemolytic streptococcus nucleoprotein (S 43) Intensity of reaction					Green streptococcus nucleoprotein (V 110A) Intensity of reaction				
			Cases			Percentage		Cases			Percentage	
			− or ±	+ or ++	+++ or ++++	− or ±	+ to ++++	− or ±	+ or ++	+++ or ++++	− or ±	+ to ++++
	years											
Non-rheumatic	0–5	60	58	2	0	96.6	3.4	60	0	0	100.0	0
	6–10	23	20	3	0	87.0	13.0	23	0	0	100.0	0
	11–15	23	15	4	4	65.2	34.8	19	2	2	82.6	17.4
Total.....	.....	106	93	9	4	87.7	12.3	102	2	2	96.2	3.8
Rheumatic	0–5	1	0	1	0	*	*	0	1	0	*	*
	6–10	26	4	11	11	15.4	84.6	10	14	2	38.4	61.6
	11–15	17	1	3	13	5.9	94.1	9	8	0	53.0	47.0
Total.....	.....	44	5	15	24	11.4	88.6	19	23	2	43.2	56.8

* Too few to treat statistically.

3 or 13 per cent reacted positively while in the 26 rheumatic children 22 or 84.6 per cent showed a positive reaction. In the 11 to 15 year age group, there were 8 positive reactors out of 23 (34.8 per cent) in the non-rheumatic children compared to 16 out of 17 (94.1 per cent) in the rheumatic ones. It is noteworthy that among the rheumatic children who had a positive skin reaction, well over half (24 out of 39) were strongly positive (+++ or ++++), whereas there were but 4 strong reactors out of the 13 non-rheumatic children who showed a positive reaction.

An even more striking preponderance of positive tests is seen in an analysis of the results with the green streptococcus antigen. Here none of the non-rheumatic children under 10 years showed anything more than a doubtful reaction and but 17.4 per cent of those from 11 to 15 were positive whereas of the 44 rheumatic children 25 or 56.8 per cent reacted positively.

These figures reveal that the "peak" of positive skin reactors among children tested with our streptococcal antigens is considerably influenced by the presence or absence of rheumatic infection. Omitting the rheumatic individuals from the different age groups, as noted above, we find a gradual rise in the percentage of positive reactors as the children grow older: in these first three age groups from 3.4 per cent to 13 per cent to 34.8 per cent with the hemolytic streptococcus antigen and from 0 to 0 to 17.4 per cent with the green variety. With the rheumatic children the percentages are much higher in each group. This finding of a high incidence of skin hypersensitiveness in patients suffering from rheumatic fever is in keeping with the observations of Swift and his co-workers and others who have used various streptococcus products in their studies, and it emphasizes the important association of such hypersensitiveness with rheumatic infection.

In table 4 data from all the patients, irrespective of age, have been analyzed according to the chief disease from which each suffered. In addition there are three other groups; (no. 10) 82 so-called normal individuals, (no. 21) 28 children, and (no. 22) 39 infants and premature babies. The two latter groups were not sufficiently large to analyze according to disease. The table indicates the number of individuals tested, the intensity of reaction and the proportion of positive and negative reactors in each disease group. The size of the divisions varies considerably, but all are large enough to be of value. The order of arrangement is according to the percentage of positive reactions to the hemolytic streptococcus nucleoprotein. Here, as pointed out from table 2, one finds that there is a much higher proportion of positive reactors to this nucleoprotein than to that from the green streptococcus. This is true for the individual disease groups as well as for the entire series.

A few points respecting several of the divisions of this table are worthy of special mention. In the first place all patients suffering

TABLE 4

Results of tests in all individuals grouped according to disease

	Disease group	Number of cases tested	Hemolytic streptococcus nucleoprotein (S 43) Intensity of reaction								Green streptococcus nucleoprotein (V 110 A) Intensity of reaction							
			Cases						Percentage		Cases						Percentage	
			−	±	+	++	+++	++++	− or ±	+ to ++++	−	±	+	++	+++	++++	− or ±	+ to ++++
1	Rheumatic fever or chorea	59	3	4	10	11	2	29	11.9	88.1	14	15	20	8	2	0	49.2	50.8
2	Liver or gallbladder disease	8	1	0	4	0	0	3	12.5	87.5	3	3	1	1	0	0	75.0	25.0
3	Peptic ulcer	57	7	7	7	4	6	26	24.6	75.4	25	16	8	5	2	1	71.9	28.1
4	Tonsillitis or sinusitis	12	3	0	2	0	2	5	25.0	75.0	7	1	0	2	1	1	66.6	33.4
5	Diabetes	20	3	2	5	4	1	5	25.0	75.0	12	2	5	1	0	0	70.0	30.0
6	Hyperthyroidism or myxedema	12	1	2	1	0	2	6	25.0	75.0	5	5	2	0	0	0	83.3	16.7
7	Arthritis	39	3	8	5	6	6	11	28.2	71.8	23	8	4	1	0	3	79.5	20.5
8	Hypertension	21	5	3	4	0	1	8	38.1	61.9	14	2	4	1	0	0	76.2	23.8
9	Miscellaneous group	54	15	8	5	5	6	15	42.6	57.4	34	8	7	4	1	0	77.8	22.2
10	Normal group	82	25	12	9	3	6	27	45.1	54.9	50	11	20	1	0	0	74.4	25.6
11	Chronic myocarditis	34	12	5	4	6	0	7	50.0	50.0	23	8	2	1	0	0	91.1	8.9
12	Nephritis	30	10	5	3	2	2	8	50.0	50.0	17	3	7	1	1	1	66.6	33.4
13	Asthma and chronic bronchitis	23	6	6	2	3	2	4	52.1	47.9	17	3	3	0	0	0	87.0	13.0
14	Influenza	34	11	8	11	2	0	2	55.8	44.2	24	4	5	1	0	0	82.3	17.7
15	Chronic cardiac valvular disease	29	10	8	6	2	1	2	62.1	37.9	21	3	5	0	0	0	82.7	17.3
16	Syphilis	15	7	3	1	1	0	3	66.6	33.4	12	0	1	0	0	2	80.0	20.0
17	Carcinoma	23	11	4	2	1	1	4	65.2	34.8	20	3	0	0	0	0	100.0	0.0
18	Pneumonia	30	19	3	4	1	1	2	73.4	26.6	24	6	0	0	0	0	100.0	0.0
19	Subacute bacterial endocarditis	4	3	0	1	0	0	0	75.0	25.0	4	0	0	0	0	0	100.0	0.0
20	Tuberculosis	17	9	4	2	0	0	2	76.5	23.5	11	4	2	0	0	0	88.2	11.8
21	Children (under 12 miscellaneous)	28	15	7	4	1	0	1	78.6	21.4	25	2	0	1	0	0	96.4	3.6
22	Infants and premature babies	39	34	5	0	0	0	0	100.0	0.0	34	5	0	0	0	0	100.0	0.0
	Total	670	213	104	92	52	39	170	47.3	52.7	419	112	96	28	7	8	79.3	20.7

from rheumatic fever or chorea irrespective of age were more frequently hypersensitive to the antigens of both hemolytic and green streptococci than were those in any other disease group. Of the 52 patients in this division, who showed a positive reaction to the hemolytic streptococcus nucleoprotein, 29 or 55.8 per cent showed a ++++ response. Secondly, whereas the percentage of positive reactors to this antigen (88.1 per cent) is only slightly higher than that shown in several other disease groups, the percentage of those reacting positively to the green streptococcus nucleoprotein (50.8 per cent) is with four exceptions more than double that shown by any other disease group. These points emphasize, as noted above, the strong tendency in individuals suffering from rheumatic infection to show a high degree of skin hypersensitiveness to these antigens. In contrast to this, of the 29 patients suffering from chronic cardiac valvular disease, only 11 or 37.9 per cent gave a positive reaction to the hemolytic streptococcus antigen and only 3 or 10.3 per cent a strongly positive response. All of these 29 patients had had rheumatic heart disease, but there was no evidence of recent active rheumatic infection, indicating that the high incidence of hypersensitiveness seen in the early course of rheumatic disease does not persist in later years.

Of the four patients with subacute bacterial endocarditis due to *Streptococcus viridans*, only one showed a mildly positive test to the hemolytic streptococcus nucleoprotein and none to the green variety. This is a very small number of patients from which no deduction can be drawn. The finding, however, is in keeping with the observations of Kinsella and Garcia (16), Howell and Corrigan (17) and Swift (18), who have noted a failure of patients suffering from this disease to show skin reactions when tested with living streptococci or their derivatives.

Of considerable interest to us was the high incidence of positive reactors to the hemolytic streptococcus nucleoprotein among the patients with gastric or duodenal ulcer. Three fourths of these patients showed a positive reaction and of these (43 in number) 26 or 60.4 per cent were very strongly positive. It is impossible to draw any definite conclusion from this fact any more than is possible from the similar high incidence in the rheumatic fever group; however, it may be looked on as evidence favoring the conception held by some that peptic ulcer is associated with streptococcus infection. It is

worth noting that the two patients with ulcer reported by Mackenzie and Hanger (4) gave strong reactions to streptococcal antigen.

The proportion and degree of hypersensitiveness shown in the diabetic and thyroid groups were rather unexpected, and no explanation for these findings is offered. The arthritis group included all types of arthritis other than that of rheumatic fever. Various stages of chronicity of the different forms were encountered. There was no very evident correlation between the types of arthritis thought to be streptococcal in origin and their skin hypersensitiveness to the nucleoproteins.

TABLE 5

Results of tests in patients suffering from various forms of nephritis

Type of nephritis	Number of cases tested	Reactions to hemolytic streptococcus nucleoprotein (S 43)—cases			Reactions to green streptococcus nucleoprotein (V 110A)—cases		
		− or ±	+ or ++	+++ or ++++	− or ±	+ or ++	+++ or ++++
Acute	1	0	1	0	1	0	0
Subacute hemorrhagic	10	3	2	5	4	4	2
Chronic without edema	12	8	1	3	10	2	0
Nephrosis	7	4	1	2	5	2	0
Total	30	15	5	10	20	8	2

The miscellaneous group included various diseases such as epilepsy, lead poisoning, mucous colitis and others too few in number to be discussed separately. Our finding that slightly over half of the so-called "normals" were hypersensitive to the hemolytic streptococcus nucleoprotein was not unexpected, since many gave a history of previous attacks of tonsillitis or other infection commonly attributed to the streptococcus. The low incidence of positive reactions in the disease groups known to be due to unrelated microörganisms—tuberculosis, syphilis and pneumonia—again was not unexpected.

Taken as a group, the children, excepting those with rheumatic infection, gave the lowest percentage of positive reactions to the hemolytic streptococcus nucleoprotein (21.4 per cent); only 3.6 per cent gave any positive response to the green variety. There were no

positive reactions to either antigen among the infants and premature babies.

One disease group worthy of special attention is that with the diagnosis of nephritis. This included 30 patients of whom 50 per cent reacted positively to the hemolytic streptococcus nucleoprotein and 33.4 per cent to the green variety. A subdivision of these patients according to the type of nephritis from which they suffered (table 5) shows that not only did more of them react to the hemolytic than to the green streptococcus antigen, but also the intensity of this reaction was definitely more marked. Again, patients with acute or subacute nephritis showed a much higher incidence of reactions than did the chronic cases without edema or those with so-called nephrosis.

In no instance did any of the individuals tested with the nucleoprotein from baker's yeast show what might be considered a positive reaction.

DISCUSSION

The purpose of this study was to ascertain the type and degree of response to an intradermal injection of streptococcus antigens in a large group of general hospital patients. Much work has been done by Swift and his collaborators (1) (2) and by Birkhaug (6), Kaiser (7), Irvine-Jones (8) and others in carrying out similar tests on patients suffering from the "rheumatic series" of diseases. Few of these workers have reported results in any large group of individuals with other diseases than rheumatic fever, though in many instances the control cases have been hospital patients with a variety of ailments. Our studies carried out on 670 hospital patients and normal controls showed that positive results may be obtained in a great variety of disease conditions so that the test has no specific diagnostic value. These results probably are not entirely comparable to those of other workers, since most of them have used as test materials either filtrates of cultures or unpurified extracts of the various streptococci. In this study the nucleoprotein fraction of two types of streptococci was used. This fraction in each instance had been reprecipitated until its nitrogen content was constant, then diluted and treated so that the potency of the test antigen presumably was constant throughout. It should be noted that, although the two streptococcus nucleoproteins used con-

tained approximately the same amount of nitrogen (12.9 per cent), they varied markedly in their ability to elicit reactions. This variability closely paralleled the known virulence of the two species of streptococci from which the nucleoproteins were obtained. Thus, in nearly every instance was the response to the hemolytic streptococcus antigen (S43) greater than that to the green variety (V110A). This is even more striking in view of the fact that the dose of the first was only one-third as large as that of the second.

No satisfactory explanation for this observation is available. Since both antigens were prepared in the same manner, it does not seem likely that one was denatured more than the other during preparation. Whether this is an indication of a higher degree of sensitivity of the tissues to the hemolytic streptococcus antigen, of the greater prevalence of this type of streptococcus as a pathogenic organism, or of some unknown element of greater toxicity in this antigen as compared to the other cannot be said.

Does the age of the individual tested play any part in determining tissue sensitivity to this nucleoprotein fraction of streptococci? If those patients suffering from rheumatic fever, of which there was a large number in the 6 to 10 and 11 to 15 year age groups are eliminated, then it can be stated that under the age of 5 years very few are sensitive, that between the ages of 6 and 15 years there is an increase in frequency of sensitiveness, and above 15 years there is no indication that age plays any significant rôle. These findings are quite in accord with those reported previously by Mackenzie and Hanger (4), who noted a failure on the part of young children to show skin hypersensitiveness to streptococcus products, and to those of Ando and Ozaki (11), who found that only 14.6 per cent of children between the ages of 2 and 5 years showed a positive reaction to streptococcus nucleoprotein, whereas above the age of 15 years 67.8 per cent of those tested reacted positively. All the strong (+++) reactions which these workers observed, were in individuals over 10 years of age. The fact that nearly all of the young patients tested by us failed to give a positive response would suggest that none of them had harbored infections due to streptococci of sufficiently long duration or intensity to allow their tissues to become sensitized to these organisms. Furthermore, this finding that nearly all of the children under 5 years of

age failed to react, whereas between the ages of 15 and 20 years somewhat over half of the individuals tested showed some degree of hypersensitiveness is comparable to the observation of Opie, Landis, McPhedran and Hetherington in their work on tuberculosis (19). These authors reported that in families not harboring a source of contagion only 20 per cent of the children at the age of 5 years showed a positive tuberculin reaction, whereas about 100 per cent in similar families at the age of 20 years gave a positive test.

The question arises as to what relation the occurrence of a positive skin reaction, considered as evidence of hypersensitiveness, bears to disease in any individual. It is impossible to answer this definitely at the present time. The figures in table 4 show that this hypersensitive state is encountered more frequently in some diseases than in others. For instance, the patients suffering from rheumatic fever gave an incidence of 88.1 per cent positive tests as compared to 54.9 per cent in the group of so-called normal individuals tested with the hemolytic streptococcus nucleoprotein. We feel that this high incidence of positive reactors may well be of some significance, though one can go no further than state that it suggests the possible rôle of the streptococcus as an etiological factor in rheumatic infection. There is further interest in the observation that individuals suffering from diseases not infrequently attributed to streptococcal infection such as tonsillitis, sinusitis, arthritis, and acute or subacute nephritis gave a much higher proportion of positive skin reactions to the hemolytic streptococcus antigen than did individuals with diseases known to be due to quite unrelated organisms, namely syphilis, tuberculosis and pneumonia. There are also several disease groups (peptic ulcer, diabetes and thyroid disease), in which about three fourths of the patients were hypersensitive, although the causative factors of these disturbances are unknown, and their relationship to streptococcal infection although suggested has never been proved. The occurrence of a high proportion of positive reactors among patients with acute or subacute nephritis is in keeping with the recently published findings of Hansen-Pruss, Longcope and O'Brien (20). These authors found a high incidence and degree of hypersensitiveness to filtrates of various strains of streptococci among patients suffering from these forms of nephritis. Although the test antigens used in their observations and

ours were not prepared in the same manner, it is possible that theirs contained a considerable amount of nucleoprotein from autolysis during growth or that ours contained a toxic substance. If this is true, then the similar findings have a common significance; namely a hypersensitive state of any or all the tissues of the body to streptococcus derivatives.

Is this hypersensitive state necessarily beneficial to the individual and one to be desired? It is probable that, could an efficient immune state be obtained without concomitant hypersensitiveness, less injury would be inflicted on the tissues at time of reinfection. There is evidence that it is harmful to the living body. As shown by Derick and Swift (15), when rabbits, made hypersensitive by repeated intracutaneous injections of live streptococci, are injected subsequently either into the tissues or intravenously with minimal doses of the same or similar organisms they show respectively either localized fulminating lesions or acute widespread reactions often terminating in death. Control animals, tested in like fashion, show little or no reaction.

Rich and McCordock (21) in their work on tuberculosis believe that hypersensitiveness to tubercle protein may not be necessary for the production of immunity and suggest that probably it would be beneficial to desensitize the body if possible "and so free the tissues from the danger of being destroyed by traces of bacillary products." If such be true, it is not sufficient, as has been suggested previously (2) (22), merely to eradicate foci of infection, when these are found in individuals suffering from such diseases as rheumatic fever, acute nephritis and so forth, but an attempt should be made, if such individuals are found to be hypersensitive, to carry them by suitable vaccination from this to an immune state in which they can respond to a maximum insult with a minimum of tissue reaction. Such work, started in patients with rheumatic fever (23), appears to favor such a conception, but time will determine its ultimate value.

SUMMARY

1. Intracutaneous tests, made with the purified nucleoprotein fraction of a hemolytic and green streptococcus were studied in a group of 670 individuals, most of them patients in the medical wards of a

general hospital. The antigens used were purified till their nitrogen content was constant. A similar protein from baker's yeast was used as a control in part of the work.

2. The hemolytic streptococcus nucleoprotein almost uniformly induced stronger reactions than did the green streptococcus antigen.

3. In individuals over 15 years, age plays no part in determining the presence or absence of sensitiveness to these antigens. In children under 15 years of age there is with increase in age a gradually increasing proportion of positive to negative reactors. This proportion is influenced markedly by the presence or absence in children of rheumatic infection, the percentage of positive reactors among the rheumatic group outnumbering that in the non-rheumatic group by a wide margin.

4. Since positive reactions may be obtained in a great variety of diseases, the test has no specific diagnostic value.

5. The disease from which one suffers at the time of testing appears to bear some relationship to the state of sensitiveness. Whether this is cause and effect or merely an association more apparent than real, the present work does not reveal.

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