THE ASSOCIATION OF STARCH-ACCUMULATING STRAINS OF GROUP A STREPTOCOCCI WITH ACUTE NEPHRITIS AND ACUTE RHEUMATIC FEVER

By NUALA CROWLEY

*Bacteriology Department, Royal Free Hospital School of Medicine,
Royal Free Hospital, London*

With the technical assistance of R. Martin and J. Prescott

(With 3 Figures in the Text)

INTRODUCTION

Certain pathogenic processes following human infection with Group A streptococci, namely, acute rheumatic fever and acute glomerulonephritis, cannot be explained by our present knowledge of the biological activities of either host or parasite. It has often been suggested that rheumatic fever is the result of a reaction following the dissemination, deposition and persistence of an antigen in the tissues. Coons (1952), studying the penetration of bacterial polysaccharides in tissues, found that long persistence was associated with high molecular weight; purified Group A polysaccharide with a molecular weight of about 8000 disappeared after about a week. Strains of Group A streptococci, however, can synthesize another polysaccharide of high molecular weight, namely, starch (Crowley & Jevons, 1955). The starchy substance is formed from maltose and also from maltose polymers like glycogen and amylopectin. It is either a mixture of amylose and amylopectin with an unusually high amylose content, or a single substance which is something between the two (Dr W. J. Whelan, Lister Institute, personal communication). It seems that the molecular weight of the undegraded starch as it exists in the streptococcal cell must be of the order of a million or more.

It seemed possible that this starchy material might provide a high molecular weight substance which, if not itself antigenically significant, might, nevertheless, act as a protective or modifying agent for some other streptococcal product. The incidence of starch-producing strains was studied with this in mind, and this paper reports the results in relation to (i) the production of non-suppurative complications, and (ii) the starch-forming properties of different serotypes.

METHODS

Strains of Group A streptococci

More than 600 strains were collected, the majority being freshly isolated from clinical infections in the year ended 31 March 1958, and received from the Streptococcal Reference Laboratory, Colindale. Four hundred and sixty-nine strains were given a blindfold test, and information relating to source, serotype, clinical infection and sequelae, if any, were supplied later by the Streptococcal Reference

Laboratory. Fifty strains were known to be associated with either rheumatic fever or nephritis, including twelve strains received from Dr R. C. Lancefield, The Hospital of the Rockefeller Institute, isolated from patients of Dr Maclyn McCarty who had developed rheumatic fever, and twenty-two strains isolated from patients with nephritis described by Wilmers, Cunliffe & Williams (1954).

Strains were considered to be associated with the non-suppurative complications if they were either (a) isolated within a few days of onset of infection from patients who later developed the complication, or (b) isolated within a few days of onset of the complication. The collection included some strains, all 'associated with nephritis' which were either isolated more than a week after the onset of symptoms, or from cases where the diagnosis was in question. These strains are in a separate group in Tables 3 and 5.

Medium for detection of starch production

When testing Group A strains for starch production difficulties are encountered because strains show great differences in cultural requirements for starch formation, particularly for a minimal amount of the substrate, maltose, but also for serum. For example, 100 strains were tested on two culture plates, one containing 0.4 % maltose + 8 % serum, and the other containing 0.8 % maltose + 4 % serum. A total of forty-three strains were starch-positive, but only nineteen were positive on both plates. It was possible to test each strain in a wide range of maltose and serum concentrations by using two or more gradient plates (Szybalski & Bryson, 1952). These were prepared as shown in Fig. 1. The agar base was prepared with

Fig. 1. Gradient plates for detection of starch production by Group A streptococci.

Hartley digest broth as supplied by the Park Hospital, Lewisham. Serum was from a pool of normal human serum, and maltose was obtained from L. Light and Co., Colnbrook, Bucks.

Seven strains were streaked in duplicate on 14 cm. Petri dishes as shown in Fig. 2. About 40 % of the strains did not form starch with maltose concentrations of less than 0.8 %, and therefore for a first test, the higher range of maltose

concentrations was the best choice. To find out which strains produced starch from the lowest maltose concentrations, the lower range gradients were the better choice.

Fig. 2. Patterns of starch production by strains of Group A streptococci in varied cultural conditions.

After incubation at 37° C. for a minimal period of 48 hr. the plates were exposed to iodine vapour. The approximate concentrations of maltose and serum in which blue-staining appeared were recorded. The amount of starch in the streaks on the serum gradients was also noted; if more growth was stained than unstained the reading was ++. If more growth was unstained than stained the reading was +, and if only a few colonies or spots were stained the reading was ±/0. Providing that the strains did not produce an extracellular amylase (Crowley, 1954) there was usually little difficulty in distinguishing between strains which had formed relatively large amounts of starch and those which had not.

Recognition of amylase-producing strains

The formation of starch was first noted when studying the effect of human serum on the production of extracellular amylase by Group A strains (Crowley & Jevons, 1955). After growing for 24 hr. in the presence of substrate and 8% or higher concentrations of serum, the strains formed starch, but with the same amount of substrate and lower concentrations of serum, little or no starch was formed but amylase was liberated into the medium. When both substrate (maltose or maltose polymers) and serum were in optimal concentration for starch production, large amounts of starch were formed, which sometimes disappeared from the cells after several days, and the disappearance was attributed to amylase activity. Since the amylase is accompanied by a maltase (author's unpublished observation) both enzymes may contribute to the difficulties of assessing the amount of starch formed by these strains. If strains were amylase-positive the quantity of starch formed was impossible to assess unless the strains were grown

in the precise serum concentration which would prevent the liberation of amylase. All the strains were therefore tested for amylase production on starch plates, containing 1 % soluble starch as described by Crowley (1954).

Quantitative estimation of starch production

Strains were grown in 100 ml. of brain-heart infusion (Difco) containing 10 % human serum. Two-day cultures were centrifuged and the sediment washed once in phosphate buffer at pH 7.4. The cells were then suspended in 40 ml. of buffer and incubated in a water-bath at 37° C. The suspensions were fed with maltose, half-hourly for 8 hr. to give an approximate concentration of 0.8 % during incubation. Using phenol red indicator, the pH was adjusted to 7.2–7.4 with N-NaOH, when necessary. The streptococci were killed by boiling for 10 min. and after centrifuging the sediments were suspended in 10 ml. of distilled water, and shaken overnight with ground glass in a Kahn shaker.

The starch was extracted by a modification of the method of Pucher, Leavenworth & Vickery (1948) for the quantitative estimation of starch in plant tissues. The streptococci were shaken with 3 vol. of 72 % perchloric acid for 30 min. The suspensions were centrifuged, and the supernatant contained the starch in solution which was precipitated by iodine as the starch-iodine complex. Then without further modification of the method of these authors, and using their equation and factors the 'percentage of starch extracted' was obtained by dividing the amount of glucose in the hydrolysate into the optical density of the cell suspension measured in a Unicam spectrophotometer at 6500 Å. With plant tissues it is usual to carry out a second extraction with perchloric acid, and pool the two extracts. Little was gained by a second extraction of streptococcal cell suspensions. A complete extraction of all the starch accumulated by some Group A strains has never yet been achieved in this laboratory, even after six extractions and irrespective of the method used to disintegrate the cells.

Preparation of cell extracts

Strains were grown in 100 ml. broth as described in the previous section, and after centrifuging the streptococci were washed four times in physiological saline, and disrupted with glass beads (Ballotini No. 12) in the Mickle disintegrator. The treatment was continued until the majority of the cells appeared to be disrupted in stained smears. The cell extracts were centrifuged and the supernatant tested for amylase activity by the method of assay previously described by Crowley (1955).

RESULTS

The incidence of starch-producing strains

Sensitivity of the test

Iodine is a highly sensitive indicator which will detect one part of starch in 400,000 parts of other material. This high sensitivity had considerable nuisance value in creating a visual impression that strains were starch producers, when the amount of starch formed in the best conditions for the strains was less than

0.001 % of the dry weight of the cultures. In the range of maltose and serum concentrations shown in Fig. 2 only 21 of 579 strains were completely starch-negative, but 247 produced only insignificant amounts and it would be misleading to call them 'starch producers' when only a few streptococcal cells in the culture were able to utilize maltose in this way.

Table 1. *Conditions in which strains of Group A streptococci form starch*

(The amount of starch accumulated depends on the number of variants in the cultures able to utilize maltose in this way, which may be < 5 to > 40 % of the streptococcal cells, but was always low for the *z* subgroup.)

Subgroup	Minimal maltose concentration	Range of serum concentrations	Time when starch usually detected*	Amylase production	
				Cell free filtrate	Cell extract
<i>x</i>	Low	Wide range	Early	—	+
Strains frequently accumulate starch	0.4–0.6 %	16–0 %	24 hr. onwards	.	.
<i>xy</i>	Variable	Narrow range	Early	+	+
Strains occasionally accumulate starch	0.4 % and higher	16–6 %	24 hr. onwards	.	.
<i>z</i>	High	Low	Late	—	±/0
Strains do not accumulate starch	> 0.8 %	< 2–0 %	> 48 hr. often several days	.	.

* Refers to time in cultures containing serum.

Three kinds of strains

On the basis of requirements for low or high maltose concentrations, for a range of serum concentrations, for the absence of serum and the production of extra-cellular amylase, the strains were divisible into three subgroups *x*, *xy* and *z*, shown in Table 1. The subgroups *x* and *xy* differed very little in their requirements for starch formation, except that *xy* strains did not accumulate any starch in the absence of serum. The *z* strains were very different from either the *x* or the *xy* strains, in requiring twice or three times as much maltose, and forming starch only in the absence of serum and often in very old cultures. In this connexion serum was used in the culture medium not only to promote starch formation by amylase-positive strains but also to ensure good growth and measurable amounts of starch. When *x* and *z* strains were grown without serum on 1 % maltose-agar, prepared with Todd-Hewitt-type infusion broth (Todd & Hewitt, 1932), gross differences in starch production were seen. In 2-day cultures of *x* strains 25 to 50 % of the streptococcal cells contained starch, compared with 2 % or less of the *z* populations. In washed cell suspensions incubated with phosphate buffer + maltose, starch appeared in the streptococcal cells within 30 min., but there was the same disproportion between the two kinds of strain regarding the numbers of cells which formed starch. Though about a third of the streptococci in *x* cultures were capable of utilizing maltose to form starch, none was detectable for 16 hr. or longer when the strains were grown in Todd-Hewitt broth + maltose. The inference was that

the strains utilized maltose to form starch when starved of nitrogen, or possibly when the environment became deficient in a co-enzyme.

Storage of starch by x strains

Table 2 shows that in comparison with the rest, *x* strains readily accumulated starch. The figures for 'percentage of starch extracted' were obtained by the somewhat laborious method described on p. 238, and only representative strains from every batch received were tested in this way. For the results to have any value, the tests must be done in duplicate and repeated several times.

Table 2. *The association of starch-accumulating properties with strains of the x subgroup of Group A streptococci*

Starch production and extraction yields	579 strains			Totals
	<i>x</i> strains	<i>xy</i> strains	<i>z</i> strains	
++ usually yield 0.5–1.0 %	146	29	0	175
+ usually yield 0.25–0.5 %	49	87	0	136
±/0 usually yield 0.25–0 %	95	97	76	268
Totals	290	213	76	579

% starch is related to the total growth (see p. 238).

++, + and ±/0 = rating on gradient plates. Few *z* strains yield more than 0.01 % starch. $P = 0.001$.

Fig. 3. Percentage of starch extracted from cell suspensions of eighteen strains after 8 hr. incubation with maltose at pH 7–7.4. % starch related to total growth. ++, +, ±/0 show gradient plate rating. Amylase-positive strains, broken line shows results when grown in presence of serum.

Fig. 3 shows that the results for eighteen strains, each tested in duplicate on five different occasions, compared well with the gradient plate rating shown at the top of each column. If the cultures were fed with maltose for 16 hr. or longer, the

amount of starch extracted was proportionately increased. Neither in these cultures, nor in continuous cultures fed with maltose for 3 days, have starch-storing streptococci outgrown the rest of the population.

Properties of starchy cultures

Paradoxically, cell extracts of *x* strains which stored up starch contained an enzyme with amylase activity like that of the extracellular enzyme liberated by *xy* strains, yet the stored starch was remarkably stable. Cell suspensions kept at room temperature for a year showed little change in starch content. The lytic factor in filtrates of *Streptomyces albus* (Maxted, 1948) released the Group A polysaccharide from both starchy and non-starchy cultures of the same strain, but the starch remained attached to the cell debris. McCarty (1952) has shown that the bulk of the cell walls of streptococci is formed of Group A polysaccharide, and therefore the failure of the lytic factor to release the starch suggests that it is formed inside the cell wall. After sonic disruption, the bulk of the starch also remained with the cell debris. The difficulty of extraction of the starch was mentioned on p. 238. The present method, using strong perchloric acid, is as drastic as any yet used to isolate and purify a streptococcal cell component; the starch samples thus obtained contain other cell substances in variety.

Relationship to clinical infection

Strains isolated at onset of infection

Table 3 shows 469 strains isolated at the onset of the infection which were subjected to blindfold testing. The results showed an association between starch-accumulating strains and upper respiratory infections known to have been

Table 3. *Strains isolated at onset of infection: association of starch-accumulating strains with development of nephritis and rheumatic fever*

Streptococcal infections	Amount of starch formed by strains			Totals
	++	+	±/0	
Followed by				
(i) acute nephritis	20	1	0	21
(ii) acute rheumatic fever	7	0	0	7
(iii) erythema nodosum	2	2	0	4
(iv) purpura	0	3	0	3
(v) ? nephritis	2	4	1	7
(vi) not known to have developed (i), (ii), (iii), (iv)	90	99	238	427
Totals	121	109	239	469

Acute nephritis (i) $P = 0.001$; acute rheumatic fever (ii) $P = 0.001$.

followed by acute nephritis and by acute rheumatic fever. If all Group A strains were distributed alike in relation to starch-accumulating properties and to the non-suppurative complications, the expected figures for nephritis and rheumatic fever in the ++ column would be about 5 and 2, instead of 20 and 7. Of the

strains in the + + column 25.6 % were associated with non-suppurative complications in comparison with 3.5 % of the strains in the other two columns.

Three serotypes predominated; fifty strains were members of *Type 1*, seventy-one of *Type 4*, and 150 belonged to the T antigen group of closely related specific *Types 5, 11, 12, 27, 44*, but the majority were probably members of *Type 12* (see p. 243).

Strains isolated at the time of the complication

Table 4 shows thirty-four carefully selected strains isolated at, or near, the time of onset of either nephritis or rheumatic fever. Thirty were *x* strains which readily accumulated starch, and four were amylase-positive, of which two were good starch producers, nevertheless. No strain of the *z* subgroup has been associated with

Table 4. *Strains isolated at time of complication: association of starch-accumulating strains with nephritis and rheumatic fever*

Strains from cases		Subgroup	Amount of starch formed			Totals
			+ +	+	±/0	
Acute nephritis	22	<i>x</i>	20	0	0	20
		<i>xy</i>	1	1	0	2
		<i>z</i>	0	0	0	0
Acute rheumatic fever	12	<i>x</i>	9	0	0	9
		<i>xy</i>	2	1	0	3
		<i>z</i>	0	0	0	0

Table 5. *All strains of Group A streptococci tested for starch production*

Streptococcal infections		Amount of starch formed by strains			Totals
		+ +	+	±/0	
Followed by					
(i)	acute nephritis	44	1	0	45
(ii)	acute rheumatic fever	18	1	0	19
(iii)	erythema nodosum	2	2	0	4
(iv)	purpura	0	3	0	3
(v)	? nephritis	16	6	1	23
(vi)	not known to have developed (i), (ii), (iii), (iv)	95	123	267	485
Totals		175	136	268	579

Acute nephritis (i) $P = < 0.001$; acute rheumatic fever (ii) $P = < 0.001$.

either rheumatic fever or nephritis at the present date, nor any strain designated ±/0. Quantitative estimations of starch were made on cultures of all the rheumatic fever strains in Table 4. The four amylase-positive strains appear in Fig. 3.

The results of all strains tested are shown in Table 5, and if all starch-accumulating strains are considered in relation to all sequelae, 45.7 % were followed by non-suppurative complications, compared with 2.8 % of all other strains. The total, however, included sixteen doubtful strains, so that 45.7 % is probably too high a figure for the true incidence of sequelae following infections with starch-accumulating strains. The expected numbers for nephritis and rheumatic fever in the + +

column were fourteen and six, but the observed numbers were forty-four and eighteen. Considering either complication separately, a significantly higher proportion of starch-accumulating strains produced nephritis and rheumatic fever than other strains. The probability of these results occurring by chance was considerably less than 0.001.

Incidence of starch-accumulating strains in relation to serotype

The distribution of starch-accumulating strains in relation to serotype is shown in Table 6. The strains are arranged in T antigen groups of closely related specific types, with predominant serotypes underlined and serotypes which usually produce amylase marked by asterisk. For example, in the group comprising *Types 4, 24*

Table 6. *Serotypes of starch-accumulating strains of Group A streptococci*

Group A streptococci		Amount of starch formed by strains					
		Associated with non-suppurative complications			Not associated with non-suppurative complications		
		++	+	±/0	++	+	±/0
<i>1*</i>	56	3	1	0	3	12	37
<i>2*</i>	30	3	1	0	3	16	7
<i>3†, 13, B3264</i>	29	4	0	0	5	11	9
<i>4†, 24, 26, 29, 46</i>	76	3	3	0	5	27	38
<i>5, 11, 12†, 27, 44</i>	198	52	5	1	41	12	87
<i>6</i>	20	1	0	0	6	0	13
<i>8, 25, Imp. 19</i>	24	1	0	0	8	7	8
<i>9*</i>	16	0	0	0	4	10	2
<i>14</i>	12	0	0	0	4	4	4
<i>15, 17, 19†, 23, 30, 47</i>	19	2	1	0	2	2	12
<i>18</i>	23	5	0	0	3	8	7
<i>22</i>	19	0	0	0	0	2	17
<i>28*</i>	33	0	0	0	8	10	15
Type not identified	24	6	2	0	3	2	11
Totals	579	80	13	1	95	123	267

Predominant serotype (M-precipitin test) in each group of closely related Types is marked †. * = serotype usually amylase-positive. 28 = heterogeneous group of strains with R antigen of Type 28. $P = 0.001$.

26, 29, 46, there were seventy-one strains, all amylase-positive, which were members of Type 4. From time to time amylase-positive strains of any serotype may be isolated but amylase production is usually only a common property of certain serotypes, namely, *Types 1, 2, 4, 9* and strains which contain R antigen (see Type 28). In the group comprising *Types 5, 11, 12, 27, 44*, not all strains were typable by M-precipitin tests but ninety-five strains belonged to Type 12, and ninety-two, by agglutination reactions, were probably members of Type 12.

Of Type 12 strains 47 % were starch accumulators; a greater number than for any other serotype not usually producing amylase.

Unfortunately, no other serotypes were represented by such a large number of

strains, but Table 6 shows that with the exception of *Type 22*, about a third of the strains of other serotypes, not usually producing amylase, may be expected to accumulate starch. For serotypes usually producing amylase, the proportion was 10–20 %, and lowest for *Type 4*. At first glance, these results suggest that starch-accumulating strains are particularly associated with *Type 12*, and it is undeniable that a starch-accumulating *Type 12* strain is endemic here at the present time. If all the strains of the group (*Types 5, 11, 12, 27, 44*) are omitted from the results, leaving more even distribution of serotypes, the findings are still significant, suggesting a primary association between starch-accumulating strains and non-suppurative complications, irrespective of serotype. This is supported by detailed findings about different serotypes, but the results for twenty-four untypable strains merit examination, for here the question of serotype does not arise, and only starch-forming and starch-degrading properties need be considered.

Amylase production and non-suppurative complications

Table 7 shows that 21·85% of amylase-negative strains produced non-suppurative complications as compared with 6·56 % of amylase-positive strains. Of all amylase-negative strains 39·9 % accumulated starch as compared with 13·6 % of all amylase-positive strains. Because amylase production is associated with certain serotypes, the chances of strains of these serotypes producing non-suppurative complications is obviously less than for serotypes not producing amylase, and this suggests a possible explanation for the present association of *Type 12* with non-suppurative complications.

Table 7. *The incidence of amylase-producing strains in relation to non-suppurative complications*

		Amount of starch formed					
		Amylase-negative strains			Amylase-positive strains		
Clinical infections	Numbers	++	+	±/0	++	+	±/0
Associated with non-suppurative complications	94	72	7	1	8	6	0
Not associated with non-suppurative complications	485	74	42	170	21	81	97
Totals	579	146	49	171	29	87	97

If the frequency of different serotypes in this country is considered, and there is little variation from year to year (Public Health Laboratory Service Report, 1957), the most common are *Types 1, 3, 4* and *12*, which accounted for nearly 40 % of all strains. Table 8 shows the detailed results for *Types 1, 4* and *12* in relation to amylase production, and also shows the frequency of these serotypes. *Type 3*, usually amylase negative, is not shown for only ten strains were tested. *Type 12*, usually amylase-negative, is the second most common serotype, while *Types 4* and *1*, usually amylase-positive, held first and third place. If the starch-accumulating, amylase-negative properties are valid markers for the strains most likely to cause

the non-suppurative complications, the chances of *Type 12* strains being so associated are greater than for *Types 4* and *1*. To complete this record, the serotypes of all the strains associated with non-suppurative complications are shown in Table 9.

Table 8. *Starch-synthesizing and starch-degrading properties of the three serotypes most common in England and Wales*

	Number of strains		Amount of starch formed by strains					
			Associated with non-suppurative complications			Not associated with non-suppurative complications		
			++	+	±/0	++	+	±/0
<i>Type 4</i> , frequency 15·3 %	72	{ Amylase - ve	0	0	0	0	1	6
		{ Amylase + ve	3	3	0	7	22	30
<i>Type 12</i> , frequency 10·6 %	95	{ Amylase - ve	45	3	0	16	9	13
		{ Amylase + ve	0	0	0	4	2	2
<i>Type 1</i> , frequency 8·2 %	56	{ Amylase - ve	2	0	0	3	1	4
		{ Amylase + ve	1	1	0	0	11	33

Frequency = figure for survey 1952-56.

Table 9. *Serotypes of strains associated with non-suppurative complications*

Serotype	Acute				Totals
	nephritis	rheumatic fever	Erythema nodosum	Purpura	
<i>1</i>	4	—	—	—	4
<i>2</i>	3	1	—	—	4
<i>3</i>	3	—	—	—	3
<i>13</i>	—	1	—	—	1
<i>4</i>	6	—	—	—	6
<i>5</i>	—	1	—	—	1
<i>12</i>	43	3	1	2	49
<i>5, 11, 12, 27, 44</i>	3	2	3	—	8
<i>6</i>	—	1	—	—	1
<i>8, 25, Imp. 19</i>	—	1	—	—	1
<i>18</i>	2	3	—	—	5
<i>19</i>	—	2	—	1	3
Type not identified	4	4	—	—	8
Totals	68	19	4	3	94

DISCUSSION

Unless all the circumstances in which a Group A streptococcus forms starch are considered, the mere fact of its production reveals little about the strain. Unfortunately, starch production is not an all-or-none phenomenon, and because of the difficulty of extracting all the starch from streptococcal cells, quantitative assay has been unrewarding in providing a sharp definition between starch-accumulating strains and the rest. There are, however, three kinds of strains of Group A streptococci, and all three may be found among members of any one of the serotypes, now prevalent here. One kind, the *x* subgroup, was associated with

nephritis and rheumatic fever, while another kind was occasionally so associated, but the third kind was not. The *x* subgroup were distinguished by their ability to synthesize starch if provided with small amounts of substrate at a time when the streptococci were running short of something in the environment, and they ran short of it at a relatively early stage of culture in medium enriched with serum beyond the needs of the most exacting strains. It was this property, rather than the fact of starch accumulation, which distinguished them from the *z* subgroup, but it was their amylase-negative property which distinguished them from the *xy* subgroup. Experiments are in progress to try to identify the substance which is used up, but in this connexion Bernheimer, Lazarides & Wilson (1957) found that some strains of Group A streptococci elaborated an enzyme which destroyed diphosphopyridinenucleotide (DPN), and had leukotoxic activity. DPNase was frequently produced by *Type 12* strains but also by strains of other serotypes.

According to Griffith (1934) 60 % of streptococcal infections at that time were caused by members of *Types 2* and *3* (each 20 %) and *Types 1* and *4* (each 10 %). He mentioned that albuminuria and haematuria had followed infections with certain strains of *Types 2*, *8* and *9*. Acute rheumatism had followed infections with eleven different serotypes, of which some, *Types 17* and *26*, for example, are now rare (Public Health Laboratory Service Reports, 1957). At present patients with nephritis yield *Type 12* strains more often than would be expected on a chance basis (Rammelkamp & Weaver, 1953; Wilmers, Cunliffe & Williams, 1954) but nephritis has also been found by American workers in association with *Types 4*, *25* and the new *Type 49*, previously known as 'Red Lake' (Updyke, Moore & Conroy, 1955). I have tested the Red Lake strain (by courtesy of Dr Elaine Updyke) and found that it has starch-accumulating properties like those of members of *Type 12* and strains of other serotypes from cases of nephritis.

This study has shown that using starch and amylase production as markers both nephritogenic and rheumatogenic strains resemble each other, irrespective of serotype, though the work was begun with the idea that the starch itself might aid the deposition and persistence of an antigen in the tissues in rheumatic fever. Because of the high recovery rate in acute nephritis the question of a persisting antigen did not seem to arise, and the finding that starch production was a marker for nephritogenic strains was unexpected. There is, at present, no direct proof to support the suggestion that the starchy substance is anything more than a marker. It remains to be seen whether the resemblance of rheumatogenic and nephritogenic strains in this respect is more than superficial, and if so, how the differences in the natural history of the two diseases can be reconciled.

SUMMARY

On the basis of starch synthesis and degradation by the strains three subdivisions of Group A streptococci were made. In defined cultural conditions about a third of the strains accumulated starch, the majority belonging to one subgroup. Of patients from whom starch-accumulating strains were isolated at the onset of infection, the observed numbers who developed either acute nephritis or acute rheumatic fever were more than three times the expected numbers. Of eighty-six strains,

including forty-six members of *Type 12*, and forty belonging to several other serotypes, which were associated with either nephritis or rheumatic fever, more than 90 % were starch-accumulating strains. Though 47 % of all *Type 12* strains accumulated starch, there was a primary association of starch-accumulating strains and the sequelae, irrespective of serotype. On the basis of type-frequency, and the starch synthesizing and degrading activities of the three most common serotypes, *Types 1, 4* and *12*, together with the host factor (for only 25 % of patients infected with starch-accumulating strains developed the sequelae) the chances of *Type 12* incidents are four to one against *Types 1* and *4*. The probability of the findings occurring by chance was considerably less than 0.001.

Technical assistance was paid for by grants from the Royal Free Hospital Endowment Fund, and I thank the Board of Governors of the Hospital. The drawings were made by Mrs Audrey Besterman, medical artist. For all their help and patience I wish to thank Dr R. E. O. Williams, Dr M. Patricia Jevons, Mr W. R. Maxted, Dr P. J. L. Sequeira, Dr W. J. Whelan and Dr Rebecca Lancefield.

REFERENCES

- BERNHEIMER, A. W., LAZARIDES, P. & WILSON, A. T. (1957). Diphosphopyridinenucleotidase as an extra cellular product of streptococcal growth and its possible relationship to leukotoxicity. *J. exp. Med.* **106**, 27.
- COONS, A. H. (1952). The penetration of antigens into connective tissue. *Rheumatic Fever. A Symposium*, p. 217. University of Minnesota Press.
- CROWLEY, N. (1954). On amylolytic strains of *Streptococcus pyogenes*. *J. gen. Microbiol.* **10**, 411.
- CROWLEY, N. (1955). The action of streptococcal amylase in relation to the synthesis of an amylosaccharide by amylolytic strains of *Streptococcus pyogenes*. *J. gen. Microbiol.* **13**, 218.
- CROWLEY, N. & JEVONS, M. P. (1955). The formation of a starch-like polysaccharide from maltose by strains of *Streptococcus pyogenes*. *J. gen. Microbiol.* **13**, 226.
- GRIFFITH, F. (1934). The serological classification of *Streptococcus pyogenes*. *J. Hyg., Camb.*, **34**, 542.
- MAXTED, W. R. (1948). Preparation of streptococcal extracts for Lancefield grouping. *Lancet*, ii, 255.
- MCCARTY, M. (1952). The lysis of Group A streptococci by extracellular enzymes of *Streptomyces albus*. II. Nature of the cellular substrate attacked by the lytic enzymes. *J. exp. Med.* **96**, 569.
- PUCHER, G. W., LEAVENWORTH, C. S. & VICKERY, M. B. (1948). Determination of starch in plant tissues. *Analytic Chemistry*, **20**.
- RAMMELKAMP, C. H. JR. & WEAVER, R. S. (1953). Acute glomerular nephritis. The significance of the variations in the incidence of the disease. *J. Clin. Invest.* **32**, 345.
- REPORT OF PUBLIC HEALTH LABORATORY SERVICE (1957). Serotypes of *Streptococcus pyogenes*: their relative prevalence in England and Wales, 1952-1956. *Month. Bull. of Minist. Hlth Lab. Serv.* **16**, 163.
- SZYBALSKI, W. & BRYSON, V. (1952). Isolation of drug resistant mutants by gradient plate technique. *J. Bact.* **64**, 489.
- TODD, E. W. & HEWITT, L. (1932). A new culture medium for the production of antigenic haemolysin. *J. Path. Bact.* **95**, 873.
- UPDYKE, E. L., MOORE, M. S. & CONROY, E. (1955). Provisional new type of Group A streptococci associated with nephritis. *Science*, **121**, 171.
- WILMERS, M. J., CUNLIFFE, A. C. & WILLIAMS, R. E. O. (1954). Type 12 streptococci associated with acute haemorrhagic nephritis. *Lancet*, ii, 17.