equivalent = 257, calculated 256. A portion of the acid was recrystallized, after which it melted at 56-59°. A mixture with highly purified palmitic acid melting at 60.5-61.5° fused at 57-59°. The polysaccharide recovered from the alkaline solution (CI) resembled the C fraction isolated by the usual process. The properties of the C and CI fractions are summarized in Table I, the data being calculated to the ash-free basis.

TABLE I.

Preparation No.	$[\alpha_D]$	Neutral equivalent	N %	P %	Acetyl %	Basic ash‡ %	Reducing sugars on hydrolysis§ %
520 C	+85°		0.15	0.39	2.7	1.0	93.3
520 CI	+84°	10000	0.05	0.10	1.7	0.0	

‡ As magnesium.

§ Hagedorn-Jensen method, giving 98.3%, calculated as glucose, with an earlier preparation corresponding to CI.

Both fractions reacted at a dilution of 1:2,000,000 with anti-H37 horse serum.[2]

While it is of course possible that the magnesium palmitate originates in substances accompanying the original polysaccharide, rather than as an integral portion of the polysaccharide complex, it is interesting to note that Boivin and his collaborators[3] have reported the isolation of a complex polysaccharide from *B. aertrycke* yielding an unidentified crystalline fatty acid on degradation. Landsteiner and Levene[4] also noted the occurrence of water-insoluble acids in the lipo-polysaccharide Forssman hapten.

8011 C

Bacteriological and Immunological Studies of Acute Glomerulonephritis in New Orleans.

B. C. SEEGAL, D. SEEGAL, E. L. JOST, AND P. CAMERON.

From the Departments of Bacteriology and Medicine, College of Physicians and Surgeons, Columbia University, the Presbyterian Hospital, New York City, and the Department of Medicine, Tulane University, New Orleans.

Previous work[1] has shown that the reported hospital medical admission rate for acute glomerulonephritis is similar in 4 latitude regions of the United States and southern Canada. In contrast,

[3] Boivin *et al.*, *Compt. rend. acad. sci.*, 1934, **198**, 2124.

[4] Landsteiner, K., and Levene, P. A., *J. Immunol.*, 1925, **10**, 731.

[1] Seegal, D., Seegal, B. C., and Jost, E. L., in press, *Am. J. Med. Sc.*

there is a diminished case frequency for scarlet fever[2] and rheumatic fever[3] in the South as compared with the North. Since clinical and laboratory evidence favors the hypothesis that acute glomerulonephritis is chiefly related to a preceding hemolytic streptococcus infection, it appeared peculiar that the diminution in incidence of the hemolytic streptococcus diseases, scarlet fever and rheumatic fever, in the southern latitudes did not also occur in the group of patients with acute nephritis.

In order to gain more accurate information concerning the type of infection predisposing toward acute glomerulonephritis in the South, a comparative study[4] of the hospital records of acute glomerulonephritis was made over a period of years in the following hospitals: The Presbyterian and Babies Hospitals in New York City; the Touro Infirmary, New Orleans; the John Sealy Hospital, Galveston; and the Baylor and Parkland Hospitals, Dallas. From this study it was concluded that the type of infection preceding the attack of acute glomerulonephritis was much the same irrespective of whether the cases occurred in the North or the South. The nature of the infection was that which is usually associated with tissue invasion by the hemolytic streptococcus. The majority of the patients in both groups had had such deep infections as acute cervical lymphadenitis, peritonsillar abscess, otitis media or acute mastoiditis prior to the onset of the acute nephritis. Study of the case histories in the southern hospitals, therefore, seemed to indicate that acute glomerulonephritis in the South, as in the North, resulted most frequently from a preceding hemolytic streptococcus infection. This could not be concluded with certainty since little bacteriological data on the incidence of the hemolytic streptococcus in the throats of healthy individuals and of patients with acute pharyngitis, peritonsillar abscess, lymphadenitis or mastoiditis are available in the cities of the South in which the case histories were studied.

In order to obtain specific data on the presence or absence of the hemolytic streptococcus in infections of the upper respiratory tract preceding or accompanying acute glomerulonephritis in the South, a visit to New Orleans was made in January and February of 1934 by two of us (D. S. and B. C. S.). Through the coöperation of Dr. Vidrine, Dr. Bass, Dr. Musser and Dr. Lemann we were enabled to study throat cultures from patients with acute glomerulonephritis, acute pharyngitis, non-streptococcus diseases, and from medical

2 Schroeder, H. A., and Longacre, A. F. Unpublished work.

3 Seegal, D., and Seegal, B. C., *J.A.M.A.*, 1927, **89**, 11.

4 Seegal, D., Seegal, B. C., and Lyttle, J. F., in press, *J.A.M.A.*

students at Tulane University Medical School. In addition blood was obtained from many of these individuals and the serum was later tested for the presence of the antistreptolysin of Todd. On our earlier visit to New Orleans we sought evidence for the presence of the hemolytic streptococcus in the South by means of testing intradermally a series of normal individuals with the nucleoprotein of the hemolytic streptococcus.

Throat cultures were taken by passing 2 swabs firmly over both tonsillar regions and the posterior pharynx. One of the swabs was then rubbed over about one-eighth of a rabbit blood agar plate* and the organisms further distributed with the platinum loop. The other swab was emulsified in 2 to 4 cc. of physiological saline and a loopful added to 15 cc. of melted agar cooled to 48°C. from which a blood agar plate was poured. After 24 hours' incubation if hemolytic colonies were seen on the plates at least 2 colonies from both the surface and pour plates were picked to another blood agar plate. In the pour plates a search was made for the minute hemolytic streptococci described by Long and Bliss[5] and the plates were kept under observation for 4 days before they were discarded. If the transfers from individual hemolytic colonies proved to be streptococci they were transferred to rabbit blood broth which was examined the next day for hemolysis. A pour blood plate was made from these blood broth tubes. Only the cultures which produced hemolysis in the blood broth and showed typical beta type hemolysis in blood agar pour plates were classified as hemolytic streptococci. Several alpha prime organisms (Brown) were isolated and are under investigation for their possible pathogenic significance, but they are not included in this report.

Antistreptolysin determinations were carried out according to the method of Todd.[6] The "units" of antistreptolysin titer reported here represent the reciprocal of the fraction of a cubic centimeter of the serum required to neutralize a standard amount of the hemotoxin liberated by the Aronson strain of hemolytic streptococcus. Thus a neutralizing dose of 0.01 cc. of serum is equivalent to 100 units of antistreptolysin. Todd considers this value the upper limit of normal in the serums of healthy individuals without a preceding hemolytic streptococcus infection.

* All the culture medium used was prepared in New York and sent South. It was beef muscle infusion medium made up with neopeptone and adjusted to pH 7.6.

5 Long, P. H., and Bliss, E. A., *J. Exp. Med.*, 1934, **60**, 619.

6 Todd, E. W., *J. Exp. Med.*, 1932, **55**, 267, and *Brit. J. Exp. Path.*, 1932, **13**, 248.

Skin tests with the streptococcus nucleoprotein were done in New Orleans, Dallas, and Galveston during the months of May and June, 1933, on a group of patients and on healthy children. The nucleoprotein solution used was supplied to us by Dr. Heidelberger and was derived from *Streptococcus hemolyticus,* strain C203. It was the "K" fraction precipitated by acetic acid and extractable (after removal of less alkaline extracts) between pH 11 and 13.3. The dilution used for the skin tests, after sterilization by passage through a Berkefeld filter, contained 1 mg. N per 100 cc. One-tenth cubic centimeter of this solution, or 0.001 mg. of N, was injected into the skin of the forearm. Readings were made in 24 hours, the presence and extent of erythema, and induration being noted. A reaction, 2 cm. or greater in diameter, associated with palpable induration, was considered a positive test.

Results. Throat Cultures: One hundred and seven throat cultures were taken from a total of 79 individuals in the period January 2nd to February 12, 1934. Studies in 11 patients who had had streptococcus disease are not included in this report. The remaining 68 individuals are divided into groups consisting of acute glomerulonephritis, acute pharyngitis, controls with a history of recent sore throat and controls without a history of recent sore throat. The hemolytic streptococcus was recovered from the throats of 29 of the members of the total group and the minute hemolytic streptococcus from 10. It will be seen from the table that hemolytic streptococci (beta type) were obtained in 8 of 10 cases of acute nephritis, in 9 of 13 cases of acute pharyngitis, in 3 of 12 controls with history of recent sore throat and in 9 of 33 controls without a history of recent sore throat. In addition the minute form of the hemolytic streptococcus described by Long and Bliss was isolated from 10 throats as indicated in the table.

It would appear from this limited series of throat cultures, taken

TABLE I.

Occurrence of Hemolytic Streptococci in the Throats of Control Individuals and Patients with Acute Glomerulonephritis or Acute Pharyngitis in New Orleans.

Disease	No. of patients	No. showing hem. strep. (beta) in throat cult.	No. showing minute hem. strep. (Long and Bliss) in throat cult.
Acute glomerulonephritis	10	8	4
Acute pharyngitis (includes 4 cases of scarlet fever)	13	9	1
Controls *with* history of recent sore throat	12	3	0
Controls *without* history of recent sore throat	33	9	5

during the height of the winter season in New Orleans, that the hemolytic streptococcus was quite readily recoverable from the throats of individuals suffering from acute nephritis and acute pharyngitis. A certain number of normal individuals were also found to carry the hemolytic streptococcus in their throats.

Antistreptolysin titrations were carried out on the serums of 12 patients with acute glomerulonephritis, 55 control patients without recent sore throat, and 37 unselected medical students. Single determinations were made in the control groups. Serial tests were performed in the patients with acute nephritis. The maximum titers in the latter group and the single determinations in the control groups are shown in Table II.

TABLE II.

Maximum antistreptolysin titer in patients with acute glomerulonephritis	Antistreptolysin titer in control patients without recent sore throat				Antistreptolysin titer in 37 unselected medical students, 21 of whom had had mild upper respiratory infect.		
55	10	25	55	71	16	62	111
100	12	25	55	71	16	66	125
111	14	25	55	83	20	71	125
144	16	33	62	100	20	71	125
166	16	33	62	100	25	83	144
200	16	33	62	100	33	83	166
200	16	33	62	100	33	83	166
200	20	33	62	100	50	83	200
500	25	33	62	111	50	83	
						83	
500	25	33	71	125	55	100	
500	25	50	71	125	55	100	
555	25	50	71	166†	55	100	
	25	50	71	250‡	62	100	
	25	50	71		62	100	

† This patient may have had acute glomerulonephritis.
‡ This patient had aestivo-autumnal malaria.

It is evident from these limited observations that the antistreptolysin titer of the control patients without a recent sore throat is similar to that reported by Todd[6] for normal individuals in England and by Coburn and Pauli[7] for subjects without recent hemolytic streptococcus disease in New York City. The higher titers of the medical students in New Orleans are probably explained by the increased exposure of this group to respiratory infection. Twenty-one of the 37 students had recently had an upper respiratory infection.

Nine of the 12 patients with acute glomerulonephritis show serum

[7] Coburn, A. F., and Pauli, R. H., *J. Exp. Med.*, 1932, **56**, 651.

antistreptolysin values which are above the normal value. These figures are indicative of recent infection with hemolytic streptococcus. Similar high titers of this antibody were found in the serums of 20 out of 22 patients with acute glomerulonephritis in New York.[8]

Skin Reactivity to Streptococcus Nucleoprotein in New Orleans, Dallas and Galveston: A total of 152 individuals were tested intracutaneously with 0.1 cc. of a solution of nucleoprotein of the hemolytic streptococcus containing 0.001 mg. N. Fifty-five responded in 24 hours with an area of erythema at least 2 cm. in diameter and palpable induration. The subjects tested were 85 adult patients both negroes and whites with a variety of medical or surgical conditions, and 67 children from 3 to 12 years of age. None of these individuals was known to have had hemolytic streptococcus disease. The number of positive reactions to the nucleoprotein of the hemolytic streptococcus in residents of these southern cities was approximately the same as that found by us in New York when we tested a group of patients on the medical wards of the Presbyterian Hospital with the same lot of nucleoprotein just before our study in the South. Coburn[9] had previously found a similar percentage of positive skin reactions with this streptococcus product in a comparable control group. Schroeder[10] obtained practically identical results in a large group of individuals living at or near the equator (Bangkok, Singapore, Colombo) whom he tested intradermally with the same sample of nucleoprotein in the fall of 1933.

The results of skin tests with nucleoprotein of the hemolytic streptococcus seem to indicate that there is the same degree of skin reactivity to this substance in the South as in New York. The significance of a positive test is open to argument but it suggests acquired sensitization to the hemolytic streptococcus since skin reactions are known to increase in frequency and severity in hemolytic streptococcus disease (Coburn).

Conclusions. This limited bacteriological and immunological search for evidence of the presence or absence of the hemolytic streptococcus in a relatively southern city, where one type of probable hemolytic streptococcus disease (glomerulonephritis) is present in "normal" amount and other types are decreased has shown that

8 Seegal, D., and Lyttle, D., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 211.

9 Coburn, A. F., *The Factor of Infection in the Rheumatic State*, Williams & Wilkins Company, Baltimore, 1931.

10 Schroeder, H. A., unpublished data.

the hemolytic streptococcus can be cultivated from the throats of a majority of individuals with acute nephritis. It has been possible to demonstrate normal carriers for the hemolytic streptococcus. Furthermore, antibody active against the soluble streptolysin (hemotoxin) of *S. hemolyticus* was increased in cases of acute glomerulonephritis. There also was the same degree of skin reactivity to the nucleoprotein of the hemolytic streptococcus in the South as in New York. These bacteriological and immunological findings therefore substantiate the clinical data already reported and give still further evidence that acute glomerulonephritis in the South as in the North is usually the result of a hemolytic streptococcus infection.

No opportunity was available to evaluate the relative frequency of the hemolytic streptococcus in the throats of the population at large in New Orleans as compared with New York nor to determine its seasonal incidence. Information on both these subjects would be interesting and possibly helpful in solving the basic problem which still remains, namely the reason for the "normal" incidence of one type of hemolytic streptococcus disease (acute glomerulonephritis) in the presence of a decreased incidence of other types of hemolytic streptococcus disease (scarlet fever, rheumatic fever) in the South. All strains isolated in New Orleans were brought back to New York and are being compared culturally and immunologically with a series of hemolytic streptococci isolated from the throats of individuals in New York City.

8012 C

Vitamin C and Diphtheria Toxin.

CHARLES K. GREENWALD AND E. HARDE.

From the Bureau of Laboratories, Department of Health, New York City.

In previous work,[1] a relation between Vitamin C and diphtheria toxin was found. Freshly prepared highly labile toxin was used.

In the present work these findings were confirmed and extended using a standardized, stable toxin. In relation to this toxin, we have examined 2 different actions, (a) the effect on guinea pigs of preliminary feeding of excess Vitamin C (cevitamic acid) and of

[1] Harde, E., *C. R. de l'Acad. des Sc.*, 1934, **199**, 618; Harde, E., and Phillipe, M., *C. R. de l'Acad. des Sc.*, 1934, **199**, 738.