localization with no or very slight diffusion and also a simple and direct method for demonstration of this enzyme without the need for a coupling reaction.

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Determination of Anti Group A Streptococcal Polysaccharide Antibodies in Human Sera by an Hemagglutination Technique.* (31967)

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Although there are several excellent tools for serologic diagnosis of Group A streptococcal infection (such as antistreptolysin, antihyaluronidase, antistreptokinase), it is apparent that all current serological reactions are related to the extracellular antigens of Group A streptococcus. With regard to somatic antigens, only the anti M antibodies have been studied to any extent, because of the interest in these type-specific antibodies for the study of immunity. It may be supposed that a reliable technique for determination in human sera of antibodies against the group-specific polysaccharide (C antigen) would be useful in acquiring knowledge of the patterns of Group A streptococcal infection, since it would show an immunologic response to a somatic component common to the various serological types. Several studies dealing with this problem have been published recently (1,2,3,4). The main difficulty is that, if the antigen is in a crude extract, it is mixed with other antigens, so that the specificity of the reaction becomes uncertain; and on the contrary, a highly purified polysaccharide is so inert that an appropriate serological technique is very difficult to set up. We recently devised a technique using a highly purified polysaccharide labelled with ^{14}C(7). This technique seems accurate and reliable but needs expensive equipment, particularly a scintillation meter.

The present paper describes a new method for determination of these antibodies which does not present technical difficulties and seems to give accurate and reliable results.

Material and methods. 1. Hemagglutination reaction. The polysaccharide extracted from the cell wall of Group A streptococcus was

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purified by several precipitations with ethanol and acetone at pH 3.6, and subsequent lyophilization. Purification was continued until the nitrogen content was less than 20 μg of N per mg of dried substance. Elimination of nucleic acids was checked by UV spectrophotometry at 260 mμ, and of amino-acids by chromatography(5).

To 50 mg of purified polysaccharide was added 4 ml of anhydrous dimethylsulfoxide, freshly distilled, 0.25 ml of pyridin, equally anhydrous and freshly distilled, and 0.15 ml of a solution of stearoyl chloride (100 mg per ml in dimethylformamide)(6). This mixture was gently mixed on a magnetic stirrer at room temperature for 2-3 days. The complex was then precipitated by 20 volumes of pure ethanol, or ethanol-acetone (1/1, V/V). The supernatant was discarded, and the precipitate dissolved in 5-10 ml of water. The solution was then purified by chromatography, using Sephadex G 25, medium and a solution of 15 per cent ethanol in water (V/V) as eluent.

The sensitizing solution (0.5 mg of this antigen preparation per ml of distilled water) was stable for months when stored at 4°C. Fresh Group O human erythrocytes were washed and resuspended in saline (2% solution). Five ml of this suspension was mixed with 0.1 ml of the sensitizing solution and incubated at room temperature for 30 minutes, with occasional shaking. Erythrocytes were then centrifuged at 300 $\times$ *g* for 5 minutes, washed twice with saline and resuspended in 5 ml of Sorensen phosphate buffer, 0.15 M, pH 6.8, containing 0.1% human or bovine albumin.

Inactivated sera were diluted by 10-fold dilutions transferring 0.5 ml volumes each time into hemolysis tubes or hemagglutination trays. To each tube containing 0.5 ml of a serial serum dilution beginning with 1/10 and continued in 2-fold increments was added 0.1 ml of the suspension of sensitized erythrocytes. The tubes were read after 6 and 18 hours, at room temperature. The buffer was made up by adding 50.8 ml of a 0.908 g% solution of KH_2PO_4 to 49.2 ml of a 11.88 g% solution of Na_2HPO_4. The results are expressed by the last partial dilution of serum giving a definite agglutination. The reading of the reaction was hindered by hemolysis when (a) the amount of ester was in excess in the polyoside-complex, (b) too much antigen was used for sensitization of erythrocytes or (c) when old erythrocytes were used. Examination is facilitated by a slight shaking of the tubes or trays.

TABLE I. Anti-Group A Polysaccharide Antibodies in Experimental Rabbit Sera.

Rabbit	Immunization	Antibody level*	Antibody-N (μg)
1	No	10	n.d.†
2	No	0‡	n.d.
3	No	20	n.d.
4	No	0	n.d.
5	No	10	n.d.
6	No	0	n.d.
7	Gr. A Strept.	1280	320§
8	"	640	68
9	"	1600	120
10	"	2560	220
11	"	8000	640
12	"	16000	1020
13	Gr. C Strept.	0	n.d.
14	Gr. G Strept.	0	n.d.
15	Bovine serum albumin	0	n.d.

* Expressed by the inverse ratio of the serum partial dilution giving a definite agglutination.
† Not detectable.
‡ No agglutination in the first dilution tube.
§ Rate of antibody-nitrogen per ml of serum against the Group A purified polysaccharide.

2. Sera. The reaction was performed on various rabbit sera (normal, immunized against Group A streptococcus and various antigens), and human, normal and pathologic sera (streptococcal infections and rheumatic fever); they were duplicated after cross-absorption of the sera with various antigens.

Results. Table I gives results observed with 15 rabbit sera. The rate of hemagglutination was between 0 and 1/20 with normal animals, as well as animals immunized with Group C and G streptococci or bovine albumin. However, titers were very high in the sera of rabbits immunized with Group A streptococci (1/640 to 1/16,000). The hemagglutination rate was approximately proportional to the amount of antibody nitrogen.

Table II tabulates results obtained with 185 human sera. On the whole, titers were consistently higher among individuals infected with Group A streptococci than among those considered "normal" (*i.e.*, without streptococcal infection).

TABLE II. Anti-Group A Polysaccharide Antibodies in Human Sera.

No. of cases	Clinical manifestation	Extreme range of antibody levels*	Mean	Mean (after transfer of unit) †	Variance
28	No apparent strep. infection	0–160	64	3.4	.82
62	Rheumatic fever	10–5120	458	6.1	2.0
57	Scarlet fever	10–5120	570	5.5	3.36
38	Other streptococcal infection	10–5120	475	5.8	1.76

* Expressed by the inverse ratio of the last partial dilution giving a definite hemagglutination.

† Transfer of unit for analysis of variance: $10 = 1$; $20 = 2$; $40 = 3$ etc.

The analysis of variance shows that the difference between the first group (no infection) and the 3 others is significant. There is no significant difference between the 3 groups of various clinical manifestations of streptococcal infection.

The specificity of the reaction was controlled on 2 pools of sera (experimental and human) by determining the rate of hemagglutination before and after absorption by Group A streptococcal purified polysaccharide, crude polysaccharide (Lancefield's extract) of Group B, C, F, G and H streptococci, Type III pneumococcal capsular polysaccharide and pneumococcal Cx antigen. From the results in Table III it is apparent that the specificity of the reaction is satisfactory, with the exception of the partial cross-reaction with pneumococcal Cx polysaccharide. This may be due to the hexosamine content of this antigen. However, the presence of C Reactive Protein in the sera did not influence the hemagglutination titer as tested routinely.

Discussion. The polysaccharide-stearide coupling allows the antigen, otherwise quite inert, to coat the erythrocyte membrane. This fixation does not alter the ability of the antigen to combine with antibody, nor does it modify its specificity.

TABLE III. Results of Cross-Absorptions.

		Hemagglutination rate
Anti-Group A rabbit serum	Not absorbed	1 : 1600
Idem	Absorbed with Gr. A polysaccharide	0*
Human serum strept. infection	Not absorbed	1 : 1280
	Absorbed with:	
Idem	Gr. A polysaccharide	0
"	Gr. B "	1 : 1280
"	Gr. C "	1 : 1280
"	Gr. G "	1 : 1280
"	Gr. F "	1 : 1280
"	Gr. H "	1 : 640
"	Pneumococcal type III polysaccharide	1 : 1280
"	Pneumococcal Cx polysaccharide	1 : 320

* No agglutination in the first dilution tube.

The passive hemagglutination technique is technically easy to perform and seems accurate and sensitive. Hemagglutinating antibodies seem to be more abundant in human sera in which titers are proportionately higher than in rabbit experimental sera. Titers found in sera of infected persons are also obviously higher than the "residual" levels of "normal" people.

We presently believe that these antibodies are related to the actual presence of the streptococcus in the infected organism. This hypothesis, however, must be confirmed by more extended studies. This is a necessary prerequisite to evaluate the use of this serological technique for the exploration of Group A streptococcal infection.

Summary. A passive hemagglutination technique, using the purified Group A streptococcal polysaccharide coupled with an ester of stearic acid is described for the titration in experimental and human sera of antibodies against the C antigen of Group A streptococcus. The reaction is easily performed and its accuracy and specificity make it suitable for clinical research.

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Effects of Loading with Tryptophan Metabolites on Metabolism of DL-Tryptophan-7a-C^{14} in Rats.* (31968)

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Previous work with human subjects has demonstrated a relationship between the amount of carrier added to a tracer dose of DL-tryptophan-2-C^{14} and the percent of the C^{14} expired as CO_2. As the level of carrier added to a dose of labeled tryptophan was increased from zero to 2 g, the percent of C^{14} expired as CO_2 in 24 hours increased from 4.5 to 25%(1). The data suggested that given quantities of tryptophan were converted into proteins and enzymes, but that the excess was metabolized to CO_2 probably *via* the glutarate pathway(2,3). In view of this and several reports of *in vitro* and *in vivo* effects of tryptophan metabolites on enzymes related to this pathway, as well as other systems(4,5,6), and because of the increasing evidence that some tryptophan metabolites are carcinogenic (7,8), it was considered of interest to investigate the *in vivo* metabolism of DL-tryptophan-7a-C^{14} as affected by loading doses of L-tryptophan, DL-kynurenine, 3-hydroxyanthranilic acid and quinolinic acid. The loading doses used were equivalent, on a molar basis, to a 2 g load of tryptophan in a 70 kg man, a load which has been used extensively(9), and which has been recommended as a standard test dose for studies of tryptophan metabolism in humans.

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Experimental. An aqueous solution of DL-tryptophan-7a-C^{14} (5.76 μc/mg) containing 1.064 mg per ml was injected intraperitoneally into pairs of rats (400-500 g) at a level of 0.67 ml per kg of body weight. The unlabeled tryptophan, DL-kynurenine sulfate, 3-hydroxyanthranilic acid and quinolinic acid were also injected intraperitoneally at a dosage level of 0.14 mMole per kg of body weight, which would represent on an equimolar basis a 2.0 g dose of L-tryptophan in a 70 kg man. The respiratory CO_2 was collected for 12 hours and the urine for two 12-hour periods. The urine samples were assayed for quinolinic acid (QA)(10) and nicotinic acid(11). By use of carrier techniques these labeled metabolites were isolated from only the first 12-hour urines(12), since the major portion of the excreted radioactivity and metabolite were in this fraction of urine. The urines were not assayed for kynurenine, o-aminohippuric acid, xanthurenic acid, N^1-methylnicotinamide, or N-methyl-2-pyridone-5-carboxamide because of limited amounts of sample, but the carbon-14 labeled components of these metabolites were isolated by carrier techniques.

For the isolation of o-aminohippuric acid, N^1-methylnicotinamide, kynurenine, xanthurenic acid, and N-methyl-2-pyridone-5-carboxamide, the column separation described by McCoy and Chung(13) was used. To 30 percent of the rat urine was added 1.0 mg of the above compounds to allow spectrophotometric