Binding of glycosaminoglycans to the surface of *Treponema pallidum* and subsequent effects on complement interactions between antigen and antibody

T J FITZGERALD,* J N MILLER,† L A REPESH,‡ M RICE,* AND A URQUHART†

From the *Department of Medical Microbiology and Immunology and the ‡Department of Biomedical Anatomy, UMD School of Medicine, Duluth, Minnesota 55812, and the †Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, California 90024, USA

SUMMARY Acidified bovine serum albumin (acid BSA) reacts with glycosaminoglycans to form a precipitate. This reaction was adapted to *Treponema pallidum* to show glycosaminoglycans associated with the surface of the micro-organism. As testicular infection progressed from days 4 to 18, treponemes showed increasing amounts of these surface components. High speed centrifuging effectively removed the glycosaminoglycans, thus indicating that they were loosely bound. The subsequent addition of commercial preparations of hyaluronic acid or chondroitin sulphate resulted in their immediate adherence to the surface of the pathogens *T pallidum* and *T pertenue*, but not to the non-pathogens *T vincenti*, *T denticola*, or *T phagedenis*. The amount adhering to the treponemal surface varied depending on the concentration added. Intradermal inoculation showed that the virulence of *T pallidum* was not altered by the glycosaminoglycans associated with its surface. The coating of treponemes with hyaluronic acid or chondroitin sulphate did not interfere with neutralising antibodies or antibodies found by radioimmunoassay using whole organisms. In contrast, hyaluronic acid or chondroitin sulphate on the treponemal surface did interfere with immobilising antibodies. Results are discussed in terms of the potential role of the treponemal glycosaminoglycans in the infectious process.

Introduction

Freshly isolated *Treponema pallidum* used as antigen in the *T pallidum* immobilisation (TPI), *T pallidum* agglutination (TPA), fluorescent treponemal antibody absorbed (FTA-ABS), and neutralisation tests requires an "ageing" process to maximise reactivity with antibodies. The organism seems to have an outer labile layer that must be altered or removed before seroreactivity.¹⁻⁷ Von Schmerold and Deubner⁸ and Swain⁹ showed a structure like a capsule at the surface of *T pallidum*. This material reacts with both ruthenium red^{10 11} and acidified bovine serum albumin (acid BSA),^{12 13} which suggests that it is hyaluronic acid, chondroitin sulphate, or a

similar glycosaminoglycan (formerly called mucopolysaccharide^{10 12}).

It has been proposed that glycosaminoglycans have an excluded volume that limits penetration of antibodies to bacterial surfaces.¹⁴ The treponemal labile layer responsible for blocking antigen and antibody reactions may partly consist of glycosaminoglycans. These components are highly soluble in water and might possibly gradually dissipate from the treponemal surface during in vitro incubation. Preliminary experiments showed that high speed centrifuging effectively removed the glycosaminoglycans associated with the treponemal surface. The subsequent addition of commercial preparations of hyaluronic acid or chondroitin sulphate resulted in their immediate adherence to the treponemal surface. The purpose of this report was to extend these preliminary findings and to assess whether a coating of glycosaminoglycans interfered with reactions between antigen and antibody.

Address for reprints: Dr T J Fitzgerald, Department of Medical Microbiology and Immunology, University of Minnesota School of Medicine, Duluth, Minnesota 55812, USA

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Materials and methods

TREPONEMES

The Nichols strain of *T pallidum* was maintained by intratesticular passage in Dutch Belt or New Zealand White rabbits. The animals were housed at 18°C to 20°C and given food free of antibiotics and water ad libitum. About 10×10^6 to 30×10^6 treponemes were inoculated per testis. Animals were injected daily with cortisone acetate (Merck Sharpe and Dohme, Westpoint, Pennsylvania, USA) 6 mg/kg body weight starting three days after infection. All experiments except TPI tests were performed with treponemes isolated from rabbits treated with cortisone. Animals were killed on days 8 to 10 by intracardiac injection of pentobarbitol. The testes were removed, rinsed in saline, and extracted aerobically in McCoy's medium containing 1 mmol/l dithiothreitol, 30 mmol/l N-2-hydroxyethylpiperazine-N¹-2-ethanesulphonic acid (HEPES) buffer, and 10% rabbit serum (heated to 56°C for 30 minutes). After 25 minutes, the preparation was centrifuged at $1000 \times g$ for 10 minutes to sediment particulate matter (slow speed centrifuging). In some experiments the supernatant containing the treponemes was then centrifuged at $18\,000 \times g$ for 30 minutes at 4°C (high speed centrifuging). The pelleted organisms were then resuspended in fresh medium. For all experiments except TPI tests, incubation was performed at 37°C in an atmosphere of 5% carbon dioxide, 2.5% oxygen, and 92.5% nitrogen. For TPI tests 5% carbon dioxide, 95% nitrogen was used.

T pertenuis was provided by Dr Ronald Schell of Hahnemann Medical College. The organisms were grown intratesticularly in rabbits not injected with cortisone and they were harvested as described for *T pallidum* after 20 to 30 days. *T vincenti*, *T denticola*, and *T phagedenis* were supplied by Dr Russell C Johnson of the University of Minnesota. These three non-pathogenic species were grown in the modified medium of Livermore and Johnson¹⁵ in an atmosphere of 5% carbon dioxide and 95% nitrogen at 37°C. Organisms were used when they were in the mid-logarithmic growth phase.¹⁶

DISCLOSURE OF GLYCOSAMINOGLYCAN

Hyaluronic acid and chondroitin sulphate react with acid BSA by forming a precipitate.^{17 18} To acidify the BSA, 1 ml of 10% BSA (wt/vol) in physiological saline was added to 0.4 ml of 2 mol/l acetic acid (pH 4). As described previously,^{12 13} about 10μ l treponemal suspension and 10μ l acid BSA were taken up into a capillary tube and mixed by inverting the tube five times. A drop was placed on a slide and studied using dark field microscopy. The presence of precipitate on at least 50 organisms was then

recorded and expressed as a percentage of total organisms.

RABBIT LESIONS

To test neutralisation and virulence, the clipped backs of female animals giving no reactions to rapid plasma reagin (RPR) circle card tests were inoculated intradermally at duplicate sites with 0.1 ml treponemal suspension. During the study period, the animals were clipped when necessary. After the development of typical lesions, samples were taken from them and examined by dark field microscopy for actively motile treponemes.

SERUM SAMPLES

Normal serum was obtained from three to five uninfected animals that gave no reactions to RPR circle card tests. Immune rabbit serum was obtained from eight rabbits that had been infected three to 24 months previously and were shown to be immune by their resistance to challenge infection with 100×10^6 organisms. Serum samples were heat inactivated at 56°C for 30 minutes when necessary.

ANTIBODY TESTS

Each of these tests was performed with treponemes incubated with 250 mg/l hyaluronic acid or chondroitin sulphate, or with medium alone. The TPI test was performed on immune rabbit serum from eight different rabbits; the end point was defined as the highest dilution at which 50% of the treponemes were immobilised.¹⁹ A modified immobilisation test was also used in which the organisms were suspended in the described extraction medium, the source of complement was normal rabbit serum, the test serum concentration was 40%, incubation was performed in 2.5% oxygen, and motility was observed until all preparations were non-motile. The neutralisation test of Bishop and Miller¹ was also slightly modified. The organisms were suspended in extraction medium, the test serum concentration was 50%, and incubation was performed in 2.5% oxygen. Radioimmunoassay was performed as described by Zeltzer *et al*²⁰ and Pepose *et al*²¹ using heated immune rabbit serum as a source of antibodies.

SCANNING ELECTRON MICROSCOPY

Treponemes were incubated with cultured cells derived from rabbit testicular tissue for two hours in Sykes-Moore chambers.²² The cultures were then washed three times with phosphate buffered saline (PBS). This technique of attaching organisms to cells facilitates fixation and processing for electron microscopy.²³ Cetyl pyridinium chloride (CPC) forms a heavy precipitate in the presence of glycosaminoglycans.²⁴ CPC 0.4% was added during fixation with

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glutaraldehyde; controls were fixed in glutaraldehyde without CPC.

CHEMICALS

Hyaluronic acid from human umbilical cord, chondroitin sulphate from whale cartilage, and bovine albumin fraction V were bought from Sigma Co (St Louis, Missouri, USA). All other chemicals were reagent grade.

Results

The addition of acid BSA to freshly harvested *T. pallidum* resulted in variable quantities of precipitate associated with the surface of the organisms. Variations occurred from rabbit to rabbit. With some treponemal preparations almost all organisms were heavily coated; with other preparations half the organisms were coated; with still others, little or no acid BSA precipitate was seen. After organisms were centrifuged at high speed, repeat acid BSA reactions indicated that surface glycosaminoglycans were effectively removed. Identical findings were apparent with *T. pertenuis*; variable quantities of acid BSA positive material were found on the treponemal surface, and high speed centrifuging effectively removed this material.

To investigate whether variations in the amount of glycosaminoglycans were related to the duration of infection, rabbits were infected intratesticularly with *T. pallidum* and killed at various times after infection. Cortisone injections enhance the accumulation of mucoid material within infected tissue,^{6,7,25-27} and 40 animals were therefore injected daily with cortisone and 30 were not. Eight animals injected with cortisone and six not injected were killed each time. Treponemes were extracted from each rabbit separately. Table I summarises the averages of acid BSA reactions after slow speed centrifuging. Small amounts of treponemal glycosaminoglycans were present with and without cortisone four and seven days after

infection. Thereafter, noticeable increases were seen, with slightly higher readings in the animals treated with cortisone. Readings were 38% to 84% without cortisone, and 90% to 95% with cortisone. In general, more precipitate per individual organism was found in preparations from rabbits injected with cortisone.

As an alternative procedure to acid BSA reactions and dark field microscopy, the organisms were exposed to CPC and examined by scanning electron microscopy.²⁴ Treponemes were harvested four or 12 days after infection. The figure shows that surface precipitate was not seen on treponemes after four days (a), but large amounts of precipitate were uniformly distributed along the surfaces of treponemes after 12 days (b). In control preparations not exposed to CPC (c) treponemes on day 12 did not contain detectable precipitate.

High speed centrifuging of treponemes and their resuspension in fresh medium was then used in all subsequent experiments. This step removes both the glycosaminoglycans bound to the treponemes and the soluble glycosaminoglycans present within the testicular extract. *T. pallidum* and *T. pertenuis* were centrifuged at slow speed and then at high speed to remove acid BSA positive material. *T. vincentii*, *T. denticola*, and *T. phagedenis* were also centrifuged at high speed. Organisms were resuspended in medium containing hyaluronic acid and chondroitin sulphate at concentrations of 0, 31, 62, 125, and 250 mg/l, and acid BSA tests were performed immediately. This experiment was repeated six times, and table II shows the average results. Hyaluronic acid and chondroitin sulphate bound to both *T. pallidum* and *T. pertenuis*. Different quantities of glycosaminoglycans were seen, depending on the amount of hyaluronic acid or chondroitin sulphate added to the suspension medium. In 72 hours, some hyaluronic acid or chondroitin sulphate dissipated from the treponemal surface. In contrast, neither hyaluronic acid nor chondroitin sulphate was bound to the surface of any of the three non-pathogenic treponemes.

TABLE I Acid BSA reactions using *T. pallidum* isolated on different days after testicular infection

Days after infection	Mean (SEM)* % treponemes reacting with acid BSA	
	Without cortisone	With cortisone
4	4 (1.0)	7 (0.8)
7	10 (1.9)	14 (1.5)
10	38 (4.7)	
11		95 (2.7)
12	60 (5.1)	
14	84 (6.2)	91 (2.9)
18		90 (3.6)

BSA = bovine serum albumin.

*Standard error of the mean for six rabbits/day of infection without cortisone and eight rabbits/day of infection with cortisone.

TABLE II Adherence of hyaluronic acid (HA) and chondroitin sulphate (CS) to the surface of treponemes

Concentration (mg/l)	Mean (SEM)* % of treponemes reacting with acid BSA			
	<i>Treponema pallidum</i>		<i>Treponema pertenuis</i>	
	HA	CS	HA	CS
0	0 (0)	0 (0)	0 (0.2)	0 (0.1)
31	16 (2.1)	0 (0)	10 (0.8)	25 (3.5)
62	90 (5.4)	42 (4.5)	44 (3.9)	50 (5.6)
125	93 (2.9)	74 (6.5)	67 (9.7)	60 (4.3)
250	100 (1.8)	100 (1.2)	75 (6.3)	80 (8.7)

BSA = bovine serum albumin.

*Standard error of the mean of three experiments.

FIGURE Reactivity of treponemes with cetyl pyridinium chloride (CPC): (a) day 4 non-reactive organisms exposed to CPC; (b) day 12 reactive organisms exposed to CPC; and (c) day 12 organisms as controls, not exposed to CPC. (Arrows indicate non-reactive organisms; arrowheads indicate reactive organisms.)

Rabbit testicular tissue contains relatively large amounts of hyaluronic acid.²⁸ The inability to detect hyaluronic acid bound to treponemes four and seven days after infection may be attributed to "receptors" that may not develop until later in infection. To investigate this possibility, treponemes were isolated from infections at day 4 and centrifuged at high speed. The organisms were incubated with hyaluronic acid or chondroitin sulphate at 0, 31, 62, 125, and 250 mg/l and then assessed for acid BSA reactions. Results closely similar to those in table II were recorded, which indicated that these "younger" organisms also bound glycosaminoglycans.

In the next series of experiments we investigated glycosaminoglycan binding to non-viable organisms. After high speed centrifugation, samples of resuspended organisms were divided in two, and one portion was heat inactivated (at 56°C for 10 minutes). Hyaluronic acid and chondroitin sulphate at concentrations of 0, 31, 62, 125, and 250 mg/l were then added. Heating did not affect the binding of hyaluronic acid or chondroitin sulphate. Similar amounts of each glycosaminoglycan were seen in both the heated and the viable preparations.

We then compared the virulence of treponemal preparations containing variable amounts of hyaluronic acid and chondroitin sulphate associated with the surface of the treponemes. Freshly harvested organisms were centrifuged at high speed to remove endogenous glycosaminoglycans, and numbers were adjusted to 2×10^6 , 2×10^5 , and 2×10^4 organisms/ml. Hyaluronic acid or chondroitin sulphate was added at concentrations of 0, 31, 61, 125, and 250 mg/l to yield a range of surface coating. These preparations were immediately injected intradermally into a total of 240 sites on 24 rabbits. Subsequent incubation periods, progression of lesions, and sites infected out of sites inoculated were recorded. At all three treponemal concentrations, neither hyaluronic acid nor chondroitin sulphate increased or decreased the virulence. In the control, as well as in each of the four hyaluronic acid and four

chondroitin sulphate preparations, the incubation periods, total sites positive, and progression of lesions were closely similar.

Various antibody reactions were then tested to ascertain whether a coating of hyaluronic acid or chondroitin sulphate interfered with antibody binding to treponemal antigens. Neutralisation¹ was tested using two different concentrations of *T. pallidum* in the presence or absence of hyaluronic acid or chondroitin sulphate at 250 mg/l. Table III shows the results after a total of 192 sites on 16 rabbits had been injected intradermally. Comparison of the normal and immune rabbit serum preparations without glycosaminoglycans showed neutralisation. Incubation periods were lengthened and fewer sites developed lesions. Neither hyaluronic acid nor chondroitin sulphate influenced this neutralising effect. Incubation periods, sites positive out of sites injected, and progression of lesions were similar for immune serum preparations exposed to medium alone or to hyaluronic acid or chondroitin sulphate.

We also investigated the effects on antibodies shown by radioimmunoassay.^{20, 21} These procedures required air drying of treponemes followed by fixing in ethanol. It was important to ensure that hyaluronic acid or chondroitin sulphate would coat these fixed organisms. Treponemes centrifuged at high speed and resuspended in fresh medium were air dried on a slide and fixed in ethanol. Hyaluronic acid or chondroitin sulphate 250 mg/l were placed on the slide, and acid BSA tests were performed. Both components coated these fixed organisms. Radioimmunoassay was then performed with fixed treponemes exposed to 250 mg/l hyaluronic acid or chondroitin sulphate, or medium alone, then incubated with heated normal rabbit serum or with heated immune rabbit serum as a source of antibodies. In the controls, which contained medium alone, much higher counts were found in the immune rabbit serum than in the normal rabbit serum wells, indicating the effectiveness of this assay in showing treponemal antigens. Treatment of the fixed

TABLE III Neutralisation by immune rabbit serum (IRS) in presence of hyaluronic acid (HA) and chondroitin sulphate (CS)

Preparation	Concentration of treponemes 10^5 /ml		Concentration of treponemes 10^4 /ml	
	Mean (SEM)* days induration + erythema first appeared	Sites positive/ inoculated	Mean (SEM)* days induration + erythema first appeared	Sites positive/ inoculated
NRS	11.6 (0.8)	16/16	16.8 (2.6)	16/16
NRS-HA	11.0 (0.5)	16/16	16.0 (1.2)	16/16
NRS-CS	11.3 (0.9)	16/16	17.9 (0.9)	16/16
IRS	20.8 (3.7)	16/16	27.1 (1.3)	5/16
IRS-HA	20.5 (1.1)	14/16	25.1 (2.8)	5/16
IRS-CS	21.6 (1.6)	14/16	24.0 (2.7)	3/16

*Standard error of the mean for 16 lesions/group.
NRS = Normal rabbit serum.

organisms with hyaluronic acid or chondroitin sulphate did not affect these antibody reactions. Readings almost identical to those for the two controls were recorded.

TPI tests were performed using organisms harvested from animals not previously exposed to cortisone. As acid BSA positive material was not seen on the surface of these organisms, they were not centrifuged at high speed before testing. Serum samples were prepared from eight different rabbits that had been infected three to 24 months previously. TPI titres were estimated in the presence of hyaluronic acid or chondroitin sulphate 250 mg/l, or medium alone. As shown in table IV, both glycosaminoglycans interfered with immobilisation by immune rabbit serum. Hyaluronic acid decreased TPI titres in each of the eight samples; chondroitin sulphate decreased TPI titres in seven of eight samples. Hyaluronic acid was thus slightly more inhibitory than chondroitin sulphate.

TABLE IV *Effect of hyaluronic acid and chondroitin sulphate on complement dependent immobilisation of T pallidum by immune rabbit serum as measured by the TPI test*

Immune serum samples	Treponema pallidum immobilisation test titre*		
	Control	Hyaluronic acid	Chondroitin sulphate
1	1/821	1/38	1/74
2	1/810	1/257	1/374
3	1/928	1/567	1/679
4	1/748	1/103	1/132
5	1/645	1/161	1/763
6	1/645	1/323	1/387
7	1/821	1/264	1/359
8	1/165	1/103	1/66

*Highest dilution at which 50% of the treponemes were immobilised.

Interpretation of the TPI test is based on the degree of treponemal immobilisation after incubation for 16 hours. In modified immobilisation experiments, motility was seen for longer periods using different concentrations of immune rabbit serum, and using treponemes harvested from rabbits treated with cortisone. Treponemes were incubated with 50% normal rabbit serum, 40% normal and 10% immune rabbit serum, 25% normal and 25% immune rabbit serum, and 50% immune rabbit serum with and without hyaluronic acid 250 ml/l. Table V shows the averages of four separate experiments listing the time required to attain 50% motility (T_{50}). Hyaluronic acid alone did not dramatically affect treponemal motility; in 50% normal rabbit serum without hyaluronic acid the T_{50} was 32 hours, and with hyaluronic acid it was 38 hours (0.21-fold increase). At each concentration of immune rabbit serum

hyaluronic acid interfered with immobilising activity (1.49 to 3.64-fold increases).

TABLE V *Effect of hyaluronic acid on complement dependent immobilisation of T pallidum by immune rabbit serum as measured by modified TPI test*

Concentration (%) of immune serum	Mean (SEM)* h to 50% motility		
	Control	Hyaluronic acid	Increase †
0	32 (6)	38¼ (10)	0.21-fold
10	5½ (¾)	18 (4)	2.40-fold
25	6¾ (2)	16¾ (5¾)	1.49-fold
50	4¾ (1)	21¾ (7¼)	3.64-fold

*Standard error of the mean for four experiments.

†Hyaluronic acid - 1.
control

Immobilising antibodies are initially detectable 30 days after infection.²⁹ We have recently modified the TPI reaction, and have been able to show serum immobilising antibodies as early as 14 days after infection (unpublished observation). The effects of hyaluronic acid 250 mg/l were then assessed in this modified TPI reaction. Three different serum samples were isolated from rabbits 14 days after testicular infection. Table VI summarises the results of three experiments in the presence of complement (unheated) and in the absence of complement (heated); data indicate the time required for treponemal motility to decrease to 50%. Without complement the T_{50} was similar in both the control and in the hyaluronic acid preparations. With complement the T_{50} was 15 hours in the control compared with 36½ hours in the hyaluronic acid preparations (a 2.42-fold increase).

Discussion

The electron micrographs of von Schmerold and Deubner⁸ and Swain⁹ first suggested that *T pallidum* had a structure like a capsule. Further research

TABLE VI *Effect of hyaluronic acid on complement dependent immobilisation of T pallidum by day 14 rabbit serum* as measured by modified TPI test*

Experiment	Hours to 50% motility (T_{50})			
	Control		Hyaluronic acid	
	Unheated	Heated†	Unheated	Heated†
1	14	59	29	67
2	6	134	29	145
3	25	133	51	120
Mean (SEM)‡	15 (5½)	108½ (24¾)	36½ (7½)	110½ (23)

*Isolated 14 days after intratesticular inoculation of 3×10^7 organisms/ml.

†Complement source inactivated at 56°C for 30 minutes.

‡Standard error of the mean for three experiments.

showed glycosaminoglycans associated with its surface: Zeigler *et al* used ruthenium red and transmission electron microscopy,¹¹ Fitzgerald *et al* used ruthenium red and scanning electron microscopy;¹⁰ and Fitzgerald *et al* used acid BSA and dark field microscopy.^{12 13} These observations have been confirmed using CPC as an alternative technique to show the glycosaminoglycans. Each of these techniques gave identical findings of surface variability. Some organisms were heavily coated with a uniform layer, others showed a patchy distribution, and still others did not contain detectable glycosaminoglycans.

Glycosaminoglycans were removed by high speed centrifuging, which indicated that they were loosely bound to the treponemal surface. This confirmed the findings of Alderete and Baseman in which certain rabbit proteins were loosely bound to the surface of treponemes.³⁰ The addition of hyaluronic acid or chondroitin sulphate resulted in their immediate adherence to *T pallidum*. This occurred with both viable and heat inactivated organisms. Adherent glycosaminoglycans gradually dissipated from the treponemal surface over a period of 72 hours. This may have been the result of degradation by treponemal hyaluronidase,^{13 31} or may reflect the relatively high solubility of these compounds in water.

The origin of the glycosaminoglycans associated with treponemes in freshly harvested preparations is unknown. Small amounts were found four and seven days after infection, and much larger amounts more than 10 days after infection. Two possible explanations follow. Firstly *T pallidum* may synthesise a glycosaminoglycan capsule. Numerous other bacteria possess capsules, and in many cases capsular material becomes prominent only during late logarithmic stages of growth in vitro. The capsule of *T pallidum* may become apparent only after extended growth in vivo. Secondly, the glycosaminoglycans may result from passive sticking of rabbit testicular tissue components. The relatively large amounts of hyaluronic acid present within this tissue are tightly bound to proteins,²⁸ and therefore may not be able to bind to the treponemal surfaces. *T pallidum* may possess an enzyme that specifically degrades the rabbit hyaluronic acid and protein complex. As treponemes continued to multiply more hyaluronic acid would be liberated and would eventually uniformly coat treponemal surfaces.

All of the experiments except the TPI reactions (table IV) were performed with treponemes that had been maintained intratesticularly for three years in rabbits treated with cortisone. This procedure may alter the surface composition of *T pallidum*, as suggested by the results in table II. Treponemes from

animals not injected with cortisone during infection showed slightly reduced amounts of glycosaminoglycans associated with their surfaces. In the earlier experiments of Zeigler *et al* ruthenium red positive glycosaminoglycans were shown using treponemes isolated from animals not treated with cortisone.¹¹ The acid BSA technique is relatively crude in showing glycosaminoglycans. Larger quantities associated with the organisms might have been seen using the more refined technique of ruthenium red or CPC and electron microscopy. Most importantly, increasing amounts of glycosaminoglycans are associated with *T pallidum* as infection progresses.

The question remains as to the role of glycosaminoglycans associated with treponemes in the infectious process. These agents do affect host defence mechanisms. Hyaluronic acid inhibits macrophage phagocytosis and lymphocyte mediated cytotoxicity^{32 33}; it also stimulates polymorphonuclear leucocyte phagocytosis, chemotaxis, and random migration, and it suppresses T lymphocyte function.^{34 35} Our findings that pathogenic treponemes bound hyaluronic acid and chondroitin sulphate and non-pathogenic treponemes did not, related binding to pathogenicity. The early events in syphilitic infection, before the appearance of lesions, were not affected by glycosaminoglycans associated with the surface of treponemes. Virulence was unchanged when treponemes were coated with varying amounts of hyaluronic acid or chondroitin sulphate and then injected intradermally. The incubation periods, the total sites developing lesions, and progression of lesions were identical with coated and uncoated organisms.

Treponemal glycosaminoglycans may play a part later in infection after lesions begin to develop and host defences have been sufficiently stimulated. At that time, early appearing antibodies are treponemocidal in conjunction with complement (unpublished observation). Mucoïd material, which partly consists of hyaluronic acid, accumulates within lesions then.^{6 7 25-27} In the study reported here the hyaluronic acid and chondroitin sulphate did not interfere with the antibody reactions independent of complement involving neutralisation or radioimmunoassay (heat inactivated serum). Thus the antibodies for these two reactions were able to penetrate the treponemal coating of glycosaminoglycans. Edwards *et al* showed that the polysaccharide capsule of streptococci, which is hyaluronic acid, inhibited the activation of complement.³⁶ Chang and Boackle showed that hyaluronic acid inhibited complement dependent haemolysis of sensitised red blood cells; they concluded that hyaluronic acid was a potent anticomplementary factor. Experiments are in

progress to ascertain whether a similar mechanism may explain the glycosaminoglycan interference with the complement dependent immobilisation of *T pallidum*.

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