Immunology, 1971, **21**, 1003

A Cross-Reaction Between *Str. pyogenes* and Human Fibroblasts, Endothelial Cells and Astrocytes

D. KINGSTON AND L. E. GLYNN

M.R.C. Rheumatism Unit, Canadian Red Cross Memorial Hospital, Taplow, Maidenhead, Berkshire

(*Received 17th April* 1971)

Summary. Antisera were raised in rabbits to whole streptococci of group A grown in a semi-synthetic medium, containing tryptic digest of casein. Twelve of these sera were tested for their ability to react with a variety of human tissues by the immunofluorescence technique. One serum, raised against a type 6 glossy strain, contained no detectable antibodies to the sarcolemmal/subsarcolemmal region of the myocardium, but gave strong staining of fibroblasts, endothelial cells, smooth muscle and the pia-arachnoid. In addition there was staining of astrocytes, best demonstrated in sections of an astrocytoma, and of the stratum granulosum of the skin. This reaction was prevented by absorption with the homologous organism but not with *Staph. aureus*. In addition the titre of the serum against fibroblasts was not affected by the presence of 10 mg/ml tryptic digest of casein in the diluent. Antisera to two other strains of streptococci (type 6 matt and type 24) gave this staining reaction in addition to the sarcolemmal/subsarcolemmal staining reaction.

INTRODUCTION

The antigenic cross-reaction between human myocardium and at least some strains of *Str. pyogenes* (Kaplan and Meyeserian, 1962) provides an interesting clue to the mechanism by which some streptococcal infections may give rise to rheumatic fever (see reviews by Zabriskie, 1967; Kaplan, 1969). If, however, rheumatic fever is an autoimmune disease provoked by the presence of a human antigenic determinant in the streptococcus, a cross-reaction which concerns an antigen in the myocardium only cannot be a complete explanation of the disease. One has also to explain damage to the endocardium and heart valves, flitting polyarthritis, Sydenham's chorea and erythema marginatum. Therefore in the present study the reaction of a variety of tissues with anti-streptococcal sera was studied by immunofluorescent staining.

This paper describes a cross-reaction involving smooth muscle, fibroblasts, endothelial cells and astrocytes.

MATERIALS AND METHODS

Organisms

The following strains of *Streptococcus pyogenes* were kindly supplied by the Streptococcal Reference Laboratory, Central Public Health Laboratory: type 6 matt (NCTC 8302), type 6 glossy (NCTC 8709), type 24 (NCTC 8305), and type 12. The latter was a known

nephritogenic strain and had the Streptococcal Reference Laboratory number R53/1077. In addition, the Oxford Strain of *Staphylococcus aureus* (NCTC 6571) was obtained from the National Collection of Type Cultures. Stock cultures were prepared by opening the ampoules into cooked-meat medium, incubating, and storing at 4°. These stock cultures were renewed from fresh ampoules approximately every 2–3 months, or if they became contaminated.

Growth medium

The growth medium for the vaccines was Medium 199 (Glaxo Laboratories Ltd.), free of antibiotics and bicarbonate, supplemented with the following materials to the final total concentrations given in parentheses: glucose (11 g/l), tryptic digest of casein (Oxoid tryptone) (20 g/l), phosphate buffer (0·026 molar) and Zn $(CH_3CO_2)_2 .2H_2O$ (1 mg/l). The Medium 199 was supplied as a sterile concentrate, the other solutions sterilized individually by heat. The growth medium was mixed aseptically with no subsequent sterilization. This medium, in common with most of the media used for studies of the cross-reactions between streptococci and mammalian tissues, contained hydrolysed casein and therefore fragments of mammalian protein. Portions of the medium were therefore preserved frozen to examine for the presence of cross-reacting antigens or haptens.

The medium used for growing the absorption suspensions was Oxoid tryptone soya broth.

Vaccines

The streptococci were given two serial subcultures in the special medium before being grown in bulk. It was found necessary to watch the cultures during the incubation as the medium became very acid, and if incubation was prolonged too far the cultures died. The cultures were centrifuged, washed three times with sterile buffered saline, and re-suspended in buffered saline to a tenth of the original volume. This suspension was plated for purity and counted. The vaccine was killed by holding it at 56° for ½ hour and 6 drops inoculated on to blood agar to test for sterility. The vaccines had the following approximate counts: type 6M, 2×10^8; type 6G, 2×10^9, type 12, 10^9; and type 24, 10^9 colony forming units per ml. The type 12 suspension was liable to agglutinate spontaneously and gave erratic counts. The vaccines were stored without preservative at 4° for not longer than 1 month.

Immunization

Adult rabbits were immunized in pairs by injecting 1 ml of the vaccines into the marginal ear vein twice weekly for 6–8 weeks. Nearly all rabbits were re-immunized with a second course after a 2–3 month interval. A number of rabbits died at the start of the second course and subsequently the first few injections of the second course were made under Phenergan cover. The rabbits were bled before and after immunization, the sera separated and stored in aliquots at −20°.

In an attempt to raise antibody against growth medium, two rabbits were immunized according to the following schedule: 0·5 ml of growth medium emulsified with 0·5 ml of incomplete adjuvant (Bacto) was injected into the right thigh of each rabbit; a week later the left thigh was similarly injected, and three weeks later 0·5 ml of growth medium was injected intravenously. After a further 3 weeks the rabbits were bled.

Tissues

Animal tissues were removed immediately after death, small pieces frozen in thin polythene bags in solid CO_2 and alcohol and stored at *c.* −30°. Human tissues were removed at necropsy within 12 hours of death, due to accident or suicide, and treated similarly. The definitive experiments were (with the exception of brain and synovial tissues) carried out on tissues derived from a young apparently healthy adult male of blood group O who died of drowning.

Conjugate

Hylands' goat anti-rabbit γ-globulin was conjugated with fluorescein isothiocyanate according to the procedure of Holborow and Johnson (1967). The conjugates were absorbed once or sometimes twice with an equal volume of packed moistened human heart powder. This absorption, adopted at the suggestion of Dr G. D. Johnson, abolished the tendency of the conjugates to stain heart muscle still present after absorption with spleen powder.

Absorption

Bacterial suspensions for absorption were grown in bulk at the Microbiological Research Establishment, Porton, and supplied as washed packed suspensions. They were stored frozen at −20°. About 7 g were taken, made up to 20 ml with buffered saline and killed by holding at 56° for 1 hour. The suspensions were then broken up in an X-Press (AB Biox, Sweden) at −20° and stored at that temperature. The absorptions were performed by mixing equal volumes of neat serum with the suspension and rotating on a haematology turntable for ½ hour. The supernatant after centrifugation was preserved with sodium azide. The absorptions were controlled by absorbing the sera raised against streptococci with suspensions of *Staph. aureus* as a check against inactivation or non-specific absorption of antibody.

IMMUNOFLUORESCENT STAINING

This was performed substantially as described by Holborow and Johnson (1967). The air-dried 6 μ cryostat sections were reacted (without fixation or prior washing) with the appropriate serum diluted ¼ (except where otherwise stated) for ½ hour, washed for ¼ hour in a Shandon tray, the antiglobulin reagent applied at an appropriate dilution (normally 1/10) for ¾ hour and slides washed for ¾ hour. The control (negative serum) was the pre-immune serum from the same rabbit. The slides were mounted in glycerine containing ¼ of its volume of buffered saline, pH 8·6. They were examined using a Reichert microscope with a ×40 objective, with either a UG1 or a BG12 primary filter and a clear or light yellow-green (GG9) secondary filter respectively. Photographs were taken on Highspeed Ektachrome day or Agfa CT18 using a UG1 primary, clear secondary and a 1–2 minute exposure.

Special techniques for individual tissues

Heart valve. This material, and collagenous material from other sites also, gave a strong green patchy autofluorescence in the absence of serum and conjugate when a BG12 primary filter was used. This was visible with a clear secondary filter, but was very obvious and of a very misleading appearance when a green secondary was used. It is felt that this autofluorescence has occasionally been responsible for claims of staining reactions.

Fortunately Dr G. D. Johnson found this green 'stain' was not given if a UG1 primary filter was used. Therefore all tissues were checked, if not entirely read, with a UG1 filter.

Synovial membrane. These tissues were removed at synovectomy. The initial experiments were carried out on membrane from a patient of unknown blood group with Still's disease. The main experiments were carried out on synovial membrane and associated cartilage removed from a patient of unknown blood group with a haemangioma of the synovium which resulted in villonodular synovitis. It was found that this tissue, and also the chondrocytes in the associated cartilage, had a strong tendency to stain with any sera. This was not given by the conjugate alone, and was therefore due to non-specific absorption of γ-globulins, or to cross-reactions with antibodies commonly present in rabbit sera. It was found that if all the dilutions were carried out in 50 per cent human serum, which was thus present at $2\frac{1}{2}$ times the concentration of rabbit serum (used at 1/5 in these experiments), this non-specific staining was virtually eliminated. It was assumed that this was due to blocking the sites of non-specific absorption with γ-globulins which did not significantly cross-react with rabbit γ-globulins. In addition, since the conjugate was also diluted with 50 per cent human serum, any tendency of the conjugate to react with human globulins would have been blocked. It is suggested that this procedure might be especially useful for rheumatoid synovia since these contain plasma cells producing rheumatoid factor which would react with fixed rabbit γ-globulins. It was adopted for other tissues which gave similar problems, especially brain tissue.

Brain. The first observations were made on brain tissue removed at the same post-mortem as the main tissue set, and therefore of blood group O. Most of the experiments were done on brain tissues from a young boy of unknown blood group killed in a car accident. It was found that brain sections needed to be washed ($\frac{1}{2}$–1 hour in buffered saline at room temperature) for the staining reaction to work. It is not known if this is required for brain tissue in general, or if it was peculiar to the material used. Washing was not required for sections of an astrocytoma (removed at operation from a patient of blood group A and obtained from the Department of Neurosurgery, Radcliffe Infirmary, Oxford).

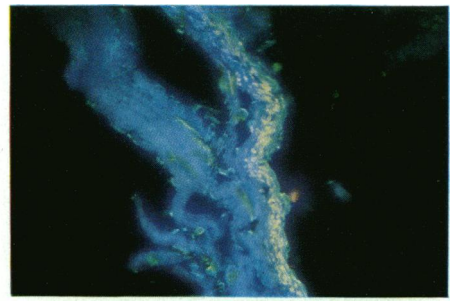
RESULTS

Twelve anti-streptococcal sera were examined for their ability to give immunofluorescent staining of the following tissues: heart muscle, heart valve, liver, kidney, lymph node,

FIG. 1. Mitral valve. Staining of fibroblasts and endothelium. (Serum 13) × 94*.
FIG. 2. Tricuspid valve. Staining of fibroblasts. (Serum 10) × 160*.
FIG. 3. Skin. Staining of epidermis (stratum granulosum; stratum Malpighii, intercellular), fibroblasts. (Serum 13) × 160.*
FIG. 4. Kidney. Staining of glomerular tuft, possibly epithelial basement membrane. (Serum 13) × 160*.
FIG. 5. Brain (cortex). Staining of endothelium and smooth muscle of pial artery; staining of fold of pia-arachnoid. (For low power view see Fig. 10.) (Serum 13) × 160*.
FIG. 6. Brain. Staining of astrocytoma. (Serum 13; dilutions in 50 per cent human serum, conjugate applied at pH 6) × 160*.
FIG. 7. Synovial membrane. Staining of synovial cells and fibroblasts. (Serum 13, dilutions in 50 per cent human serum) × 160*.
FIG. 8. Cartilage. Staining of chondrocytes. (Serum 13, absorbed with *Staph. aureus*, i.e. no specific absorption, dilutions in 50 per cent human serum) × 160*.
FIG. 9. Cartilage. Staining of chondrocytes abolished by absorption with homologous organism. (Serum 13, absorbed with type 6 glossy, dilutions in 50 per cent human serum) × 160*.

* The magnifications given refer to the reproductions in Immunology, not to the virtual image of the microscope with which the photographs were taken.

Endothelial Cells and Astrocytes



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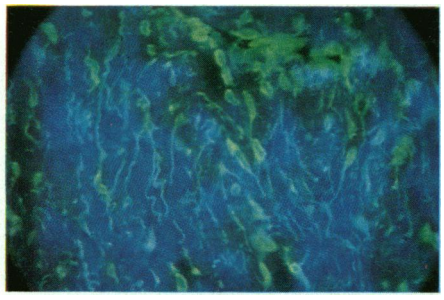

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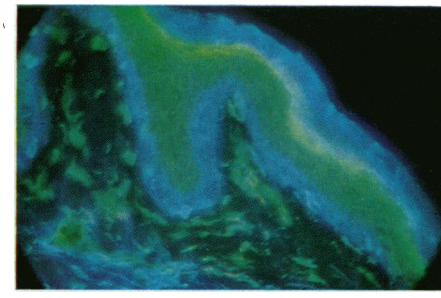

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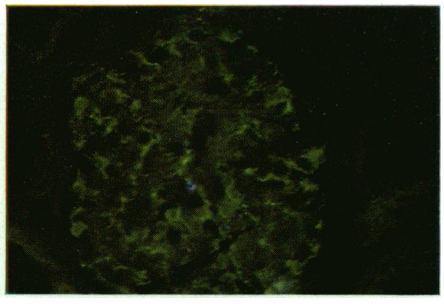

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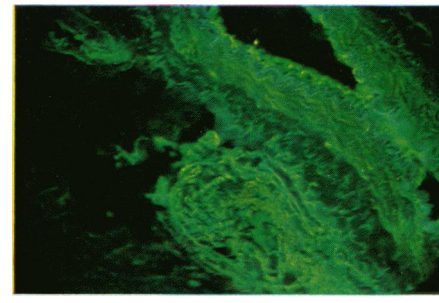

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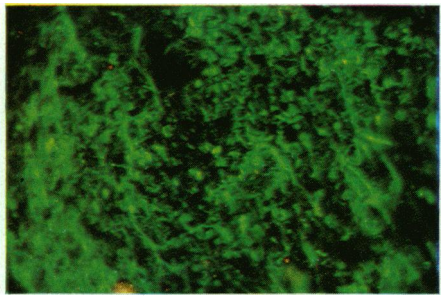

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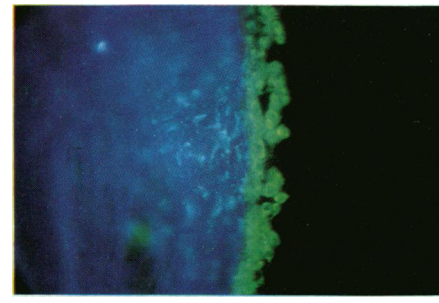

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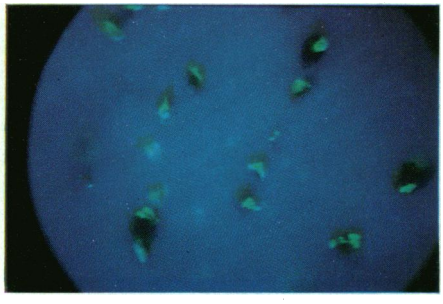

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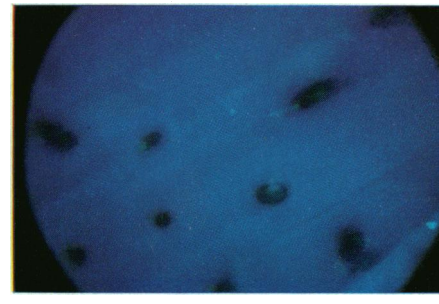

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synovial membrane, cerebral cortex and skin. Strong positive staining was only found with five sera and some of the results with these are summarized in Table 1. Only limited results are available for serum 4, since the animal died on re-immunization and only a preliminary bleed was available. These results showed that there was, in addition to the sarcolemmal/subsarcolemmal staining of the myocardium (Kaplan and Meyeserian, 1962), a further cross-reaction or cross-reactions which involved endothelium and fibroblasts in mitral and tricuspid valves (Figs 1 and 2), stratum granulosum and fibroblasts of the skin (Fig. 3), the glomerular tuft (Fig. 4), endothelium and smooth muscle of arteries, e.g. the pial

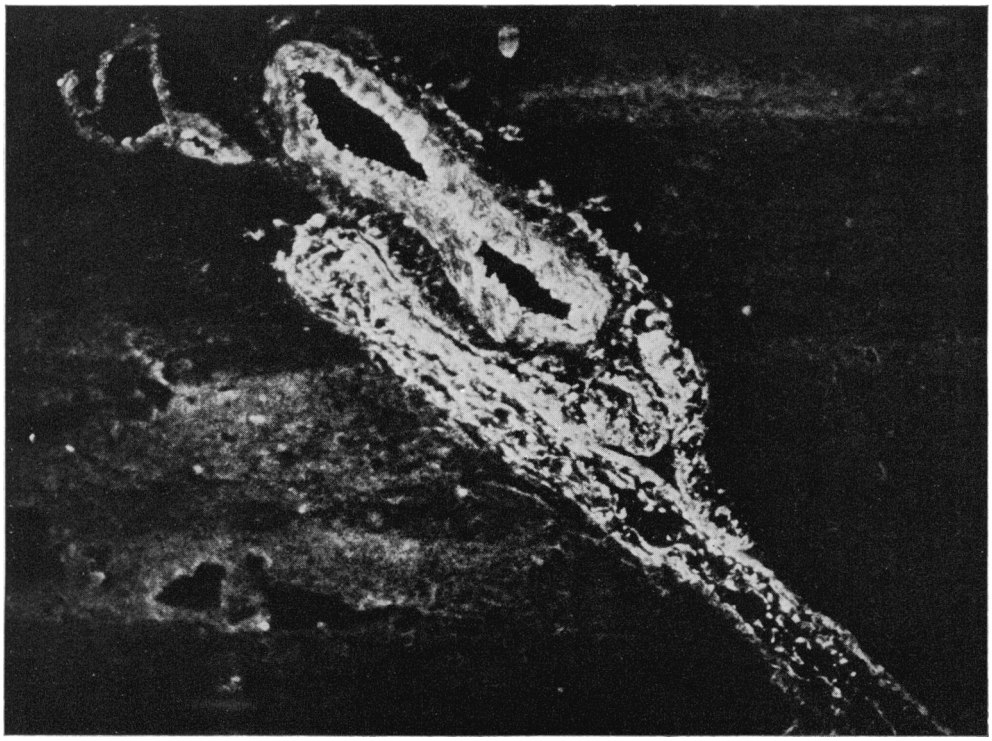

FIG. 10. Brain. Staining of pia-arachnoid and endothelium and smooth muscle of pial artery. (For high power view see Fig. 5.) (Serum 13) × 260. (Magnifications given refer to the reproductions in Immunology, not to the virtual image of the microscope with which the photographs were taken.)

vessels (Fig. 5), capillaries in the brain (Fig. 15), astrocytes (Fig. 6), synovial lining cells (Fig. 7) and chondrocytes (Fig. 8). The fibrillar staining of the pia (Figs 5 and 10) was difficult to identify with any single anatomic structure. Other positive staining reactions included the Kupffer cells (Fig. 11) and epithelium of the biliary ductules (Fig. 12). Serum 13 gave these reactions without any of the sarcolemmal/subsarcolemmal staining, whereas serum 10 gave the latter predominantly and only stained weakly the former group of tissues. In addition, serum 10 and other sera with anti-myocardial antibodies gave reactions with myoepithelial cells (Fig. 13) and the epithelial components of the hair follicles (Fig. 14). To investigate these new reactions further, serum 10 and serum 13 were

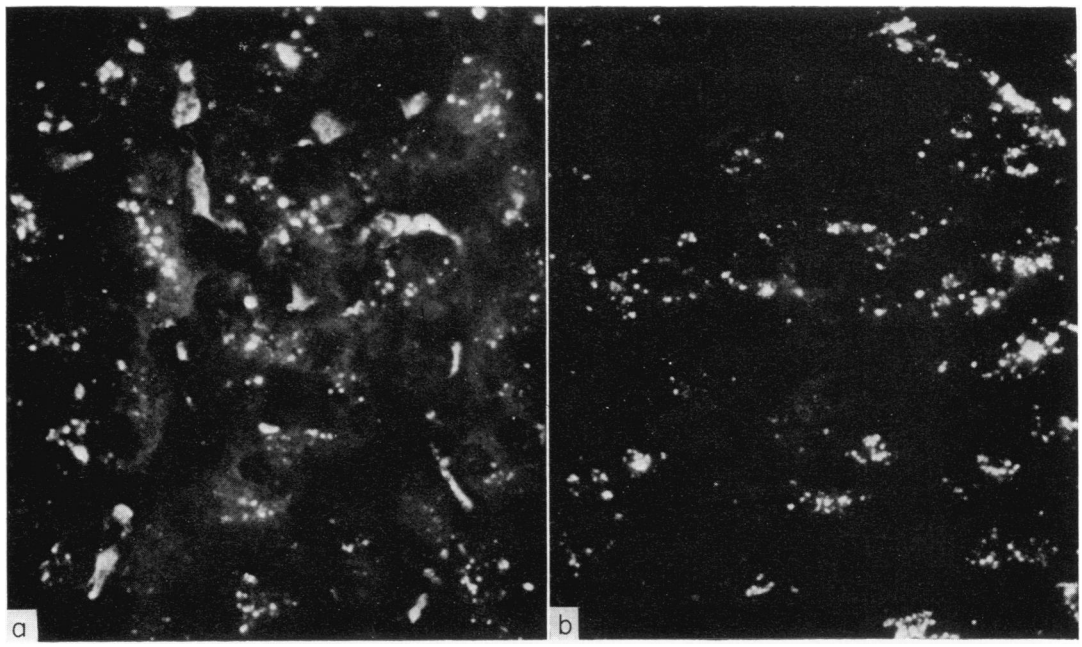

FIG. 11(a). Liver. Staining of Kupffer cells. (Serum 13) × 390. (b) Liver. Control section for (a). (Pre-immune serum from rabbit 13) × 390. (Magnification, see Fig. 10.)

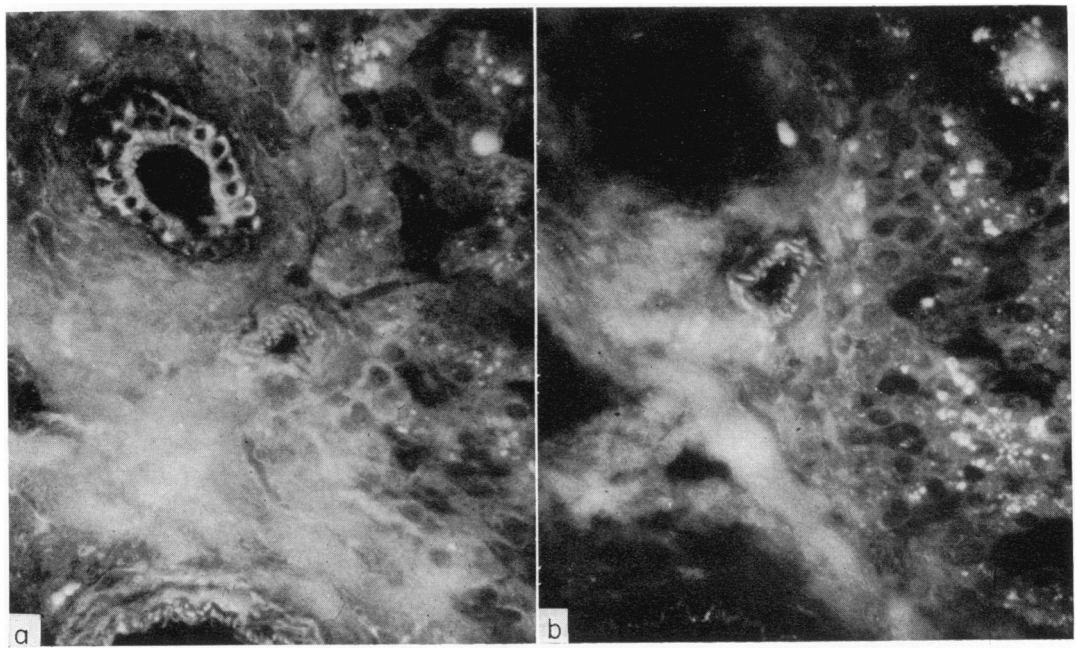

FIG. 12(a). Liver. Staining of epithelium of biliary ductule. (Serum 4) × 390. (b) Liver. Control section for (a). (Pre-immune serum from rabbit 4) × 390. (Magnification, see Fig. 10.)

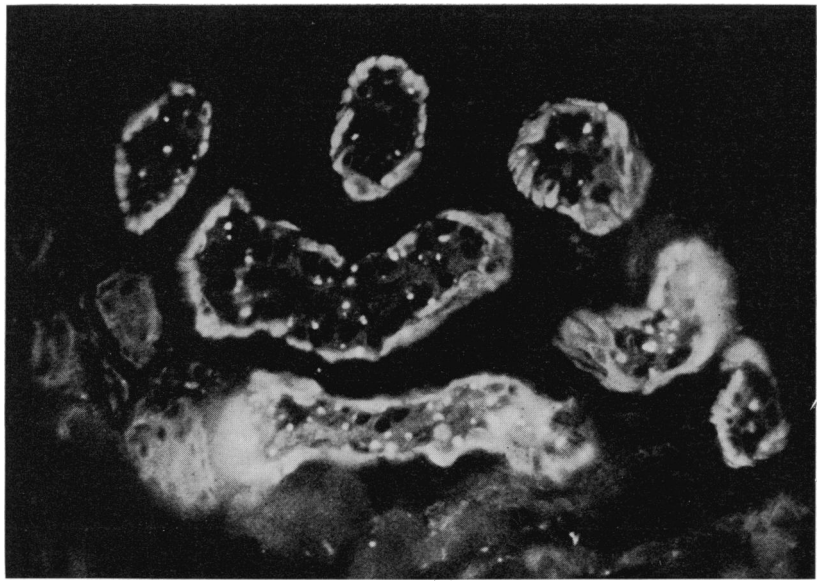

FIG. 13. Skin. Staining of myoepithelial cells of sweat ducts. (Serum 18) × 460. (Magnification, see Fig. 10.)

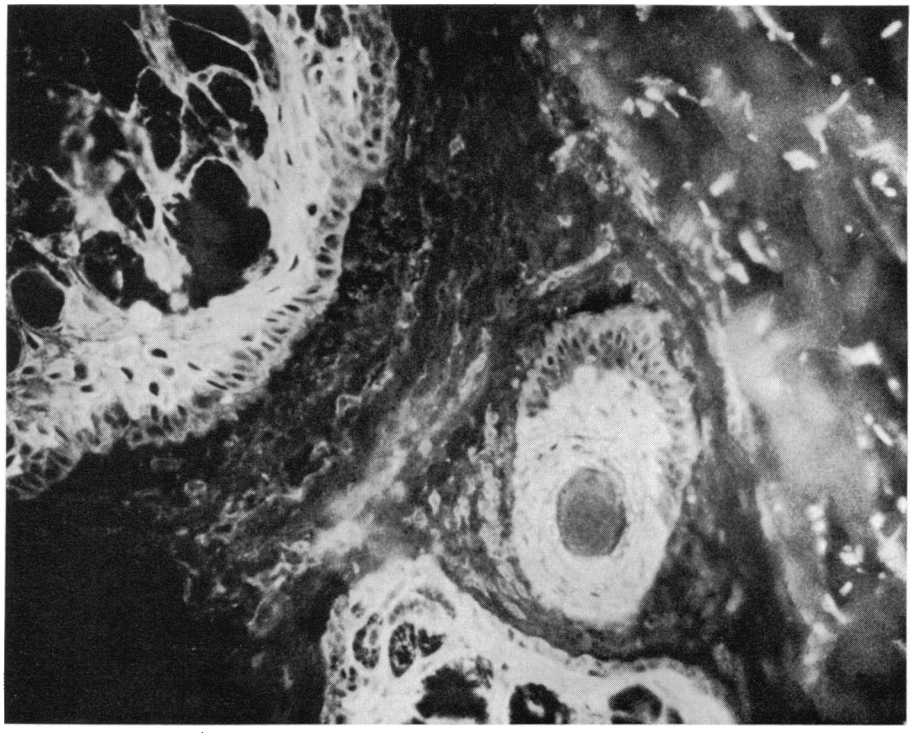

FIG. 14. Skin. Staining of epithelial components of hair follicle. (Serum 18) × 280. (Magnification, see Fig. 10.)

therefore examined in greater detail. The astrocyte staining reaction gave rise to problems and will be discussed separately later.

All the tissues in Table 1 also had the sera from the two rabbits immunized with growth medium applied to them. There was no staining reaction above the pre-immune level. Neither of these sera contained antibodies against tryptone which could be demonstrated by precipitation in capillary tubes or in gel (tryptone at a concentration of 1 per cent, 0·1 per cent and 0·01 per cent). Thus the tryptone is either not very immunogenic or it contains sufficient haptenic components to block a precipitin reaction. It is known that adsorption of substances to streptococci may greatly increase their immunogenicity (Glynn and Holborow, 1952). A further test, reported below, was therefore carried out to eliminate the possibility that the cross-reaction was due to antibodies against growth medium.

TABLE 1

IMMUNOFLUORESCENT STAINING OF HUMAN TISSUES BY ANTI-STREPTOCOCCAL SERA

Rabbit No.	4	10	13	16	18
Streptococcus	24	6 matt	6 glossy	12	24
Type antibodies	+++	+++	(+)	+++	+++
Group antibodies	±	+++	±	−	−
Heart muscle	+++	++	−	−	+++
Sweat myo-epithelial	ND	+++	−	++	+++
Hair follicle	++	++	+	++	+++
Smooth muscle	++	+	++	++	++
Fibroblast[1]	++	+	+++	+	++
Pia-arachnoid	ND	−	+++	ND	+
Endothelium (arteries & heart)	++	+	+++	−	+
Kupffer cells (liver sinusoids)	ND	±	++	−	−
Glomerular tuft	C	C	+++	−	NM
Liver ductules	++	+	++	+	++
Astrocyte	++	±	++	−	ND
Stratum granulosum[2]	ND	+	+++	−	−
Chondrocytes	ND	+	+++	ND	ND
Synovial lining cells	ND	++	+++	ND	ND

None of these sera, or the others tested, gave any detectable reaction with valve substance (as opposed to fibroblasts and endocardium).

[1] Reactions observed in heart valves, skin and synovial membrane. The latter tested with sera 10 and 13 only, in the presence of 50 per cent human serum.

[2] This staining reaction was associated with an intercellular staining reaction in the stratum Malpighii as described for sera from patients with pemphigus vulgaris by Beutner, Rhodes and Holborow (1967).

ND = not done. C = general cytoplasmic staining. NM = nuclear membrane staining. ± very weakly positive; + weakly positive; ++ strongly positive; +++ very strongly positive.

ABSORPTION EXPERIMENTS

Absorption with streptococci

The tissues tested with absorbed sera and in the presence of human serum are shown in Table 2. The pre-immune serum from rabbit 13 was absorbed in a similar manner to the immune sera and applied as a control to check possible effects due to damage to the tissues by bacterial enzymes. No staining reactions were found with the absorbed pre-immune serum. However, immune serum 13 absorbed with type 6 matt showed ballooning of some cells with staining when applied to synovial membrane. The nature of this

reaction is not known and it has been disregarded as it was only found on this tissue and was not given by the serum absorbed with the homologous organism (type 6 glossy). These results show that the cross-reacting antibodies can be removed by absorption with the homologous organism. It is interesting that the sarcolemmal/subsarcolemmal staining reaction given by serum 10 (anti-type 6 matt) was not completely abolished by type 6 glossy even after two absorptions. None of the four rabbits immunized with this latter strain gave heart-reacting antibodies, and it is probable that it is lacking or relatively deficient in the antigen.

TABLE 2

STAINING REACTIONS OF ABSORBED RABBIT SERA

	Tissue	Human serum present	Serum 10 absorbed with			Serum 13 absorbed with		
			6 glossy	6 matt	*Staph. aureus**	6 glossy	6 matt	*Staph. aureus**
Tricuspid valve	Sarcolemmal/ subsarcolemmal	no	+	−	++	−	−	−
	Fibroblast	no	−	−	±	±	−	+++
	Endothelium	no	±	−	+	+	±	+++
Myocardium	Sarcolemmal/ subsarcolemmal	no	±	−	++	−	−	−
	Smooth muscle (artery)	no	−	−	−	±	−	+++
	Endothelium (artery)	no	±	−	±	±	−	+++
Kidney	Glomerular tuft	no				±	±	+++
Cerebral cortex	Pia-arachnoid	yes				−	−	++
	Capillaries (brain substance)	yes				−	−	++
	Smooth muscle (pia arteries)	yes				−	−	++
	Endothelium (pia arteries)	yes				−	−	++
Synovium with patella	Chondrocytes	yes				−	b	+++
	Fibroblasts	yes				−	b	+++
	Synovial cells	yes				−	b	+++

Serum 10 was twice absorbed, serum 13 once.
b = ballooning of cells—impossible to read.
* *Staph. aureus:* Absorption with this organism was included as a control for inactivation of the serum in handling, by dilution, or by non-specific adsorption to bacterial cell walls. The reaction in these columns is thus the equivalent of the unabsorbed serum.

Absorption with tryptone

Since it is important to eliminate the possibility that the observed cross-reactions are due to components in the growth medium, experiments were carried out to see if the fluorescent staining reaction could be inhibited by the presence of tryptone, the only mammalian component of the growth medium. Solutions of tryptone of 10, 1 and 0·1 mg/ml were made up in Oxoid barbitone/acetic acid electrophoresis buffer. It was found that 1 per cent tryptone changed the pH of the buffer from 8·6 to 8·3. Serum 13 was diluted ¼ in these, and also in plain buffer. The diluted serum was held at room temperature for 30 minutes before being applied to cryostat sections of tricuspid valve in the usual way. No variation of intensity of staining (fibroblasts or endothelium) was found. As a further test serum 13 was given two-fold serial dilutions in the buffer and buffer with 1 per cent

tryptone. After preliminary inspection the slides were randomized by a colleague and read blind. The fibroblast staining gave identical readings with or without tryptone, titrating out to a ± reaction at a dilution of 1/20. Thus, if the staining reactions were due to antibodies to components of the tryptone, these components could only be present in very small amounts. It seems highly unlikely that the cross-reaction between fibroblasts and streptococci is due to growth medium.

THE GLIAL CELL STAINING REACTION

Non-specific staining

A staining reaction with astrocytes was given especially by sera 4 and 13. However, the astrocytes in various areas showed very strong non-specific staining. This was especially apparent with the fibres running into the cortex from the pia, but also occurred strongly in patches of brain tissue from different areas. If 50 per cent human serum was used as a diluent, as for synovial membrane, some reduction in non-specific staining occurred; but the application of conjugate which had been twice absorbed with heart powder and diluted in 50 per cent human serum still gave very strong staining in the absence of any preceding serum. An experiment was therefore carried out to find conditions which eliminated or reduced this, based on the idea that it might be due to absorption of the ionized

TABLE 3

EFFECT OF pH ON NON-SPECIFIC STAINING OF GLIAL CELLS WITH CONJUGATE

pH	4·8	5·4	5·7	6·2	6·7	7·3[1]	7·3[2]	7·3[3]	7·3[4]	7·8
Staining	−	±	−	±	+	++	++	++	++	++

[1] Conjugate in buffered saline only.
[2] As ([1]), but preceded by pre-immune serum diluted in buffered saline.
[3] As ([2]), but the pre-immune serum diluted with 1:1 buffered saline and human serum.
[4] As ([1]), but conjugated diluted with 1:1 buffered saline and human serum.

fluorescein molecule. Sections of cortex which showed this staining reaction were washed in buffered saline as usual. A few sections were treated with a pre-immune serum, diluted 1/4 in buffered saline or in 50 per cent human serum, for ½ hour, while the remaining slides were left covered with buffered saline. All the slides were rinsed and then allowed to stand for ¼ hour in saline buffered at pHs from 4·8 to 7·8. Conjugates were applied diluted 1/8 in the appropriate buffer, in one instance containing 50 per cent human serum. The sections were rinsed in buffers at the same pHs before being washed in buffered saline and mounted in buffered glycerine of the standard pHs. The results of the glial cell staining (Table 3) show that under these conditions the non-specific staining is not due to uptake of γ-globulins, nor is it due to a specific reaction between the anti-rabbit globulin conjugate and human tissue components, since it is not blocked by human serum. Since the pH of free fluorescein is about 8, it is probable that the reduction of non-specific staining at pH 6·7 is due to the elimination of the charge on the ionized fluorescein. It is not due to inactivation of the conjugate since strong specific staining of the pia-arachnoid was found at pH 6. Immunofluorescent studies of brain tissue were subsequently carried out as in this experiment with the conjugate applied at pH 6.

The specific staining reaction

Although it is felt that there was specific staining of the astrocytes in brain sections, this was not readily or consistently observed. The definitive study of this reaction was therefore carried out with a well differentiated astrocytoma removed at operation. The material contained a high density of astrocytes whose nature was known by histological examination. This material also showed strong non-specific staining at pH 7·2, but a clear distinction was found when the reaction was carried out at pH 6·0 as described previously. Serum 13 absorbed with either *Staph. aureus* or the homologous strain (types 6 glossy) was applied diluted 1/3 in human serum, the conjugate at 1/8 in 50 per cent human serum. The serum absorbed with *Staph. aureus* gave bright staining, while the serum absorbed with the homologous organism gave very little.

DISCUSSION

There are a number of possible explanations of the observed staining reactions, apart from the existence in the streptococci of an antigen shared with the mammalian tissue. The cross-reacting antibodies might have been autoantibodies to the rabbit's own tissue evoked by the presence of the streptococci (Cavelti, 1947; Linz, Lecocq and Mandelbaum, 1968; Holborow, Schwab and Brown, 1969; c.f. Ali and Oakley, 1967). Alternatively, the tissues might have an affinity for γ-globulins, and the staining reaction become positive owing to their high concentration in the hyperimmune sera. The first of these explanations was eliminated by showing that the antibody could be removed by specific absorption with streptococci. The second was eliminated by showing that only some of the immune sera gave positive staining reactions. Also, the distinction between absorbed and non-absorbed rabbit sera was maintained if the sera were diluted in 50 per cent human serum (Table 2), which would keep the overall γ-globulin levels comparatively constant, assuming that human and rabbit globulins have a similar propensity for non-specific adsorption. Evidence showing that the cross-reaction was not an artefact due to growth medium has already been discussed under Results. Another possible source of misleading cross-reactions is the presence of naturally occurring antibodies. Many rabbits contain naturally occurring anti blood group A antibodies and Forssman antibodies. That these were not important in the present study is shown by (a) the lack of staining with the individual pre-immune sera, (b) the majority of the tissues being of blood group O, and (c) the abolition of staining by absorption with the homologous organism.

No absorptions with human tissue were carried out, and it is therefore not possible to say how many antigen systems are involved. It is clear that as serum 13 gave no sarcolemmal/subsarcolemmal staining, the strong staining reactions associated with that serum (smooth muscle, endothelial cell, fibroblast, etc.) are due to a distinct antigen or antigens.

A number of cross-reactions have been described between mammalian tissues and streptococci, apart from that involving the myocardium. Rapaport, Markowitz and McCluskey (1969) showed that there was an antigen or antigens in the cytoplasmic membranes of *Str. pyogenes* which resulted in the accelerated rejection of skin grafts in guinea-pigs and other animals including rats. Some of the reactions described by us were also examined on rat tissues and found to be present (glomerulus and other endothelial cells, smooth muscle, liver ductule). However, absorption with *Staph. aureus* did not abolish the staining reactions with human tissues (rat tissues not tested), whereas Rapaport, Chase and Solowey (1966) showed that immunization with *Staph. aureus* would cause accelerated rejection of skin grafts in the guinea-pig. Thus, unless there are, in this

respect, strain variations in *Staph. aureus*, or unless *Staph. aureus* can cause non-specific acceleration of graft rejection, these transplantation antigens appear to be distinct from those involved in our staining reactions.

Cross-reactions between *Str. pyogenes* and various connective tissue components have been described: kidney basement membrane (Markowitz and Lange, 1964), heart valve glycoprotein (Goldstein, Halpern and Robert, 1967) and hyaluronate protein (Sandson *et al.*, 1968). Our serum 13 gave good staining of the glomerular tuft and might therefore be thought to be a connective tissue antiserum. As part of a study of the immunology of heart valves, Dr E. Kasp-Grochowska was studying the ability of sera raised against bovine heart valves (BHV) to stain various human tissues. We were therefore able to compare,

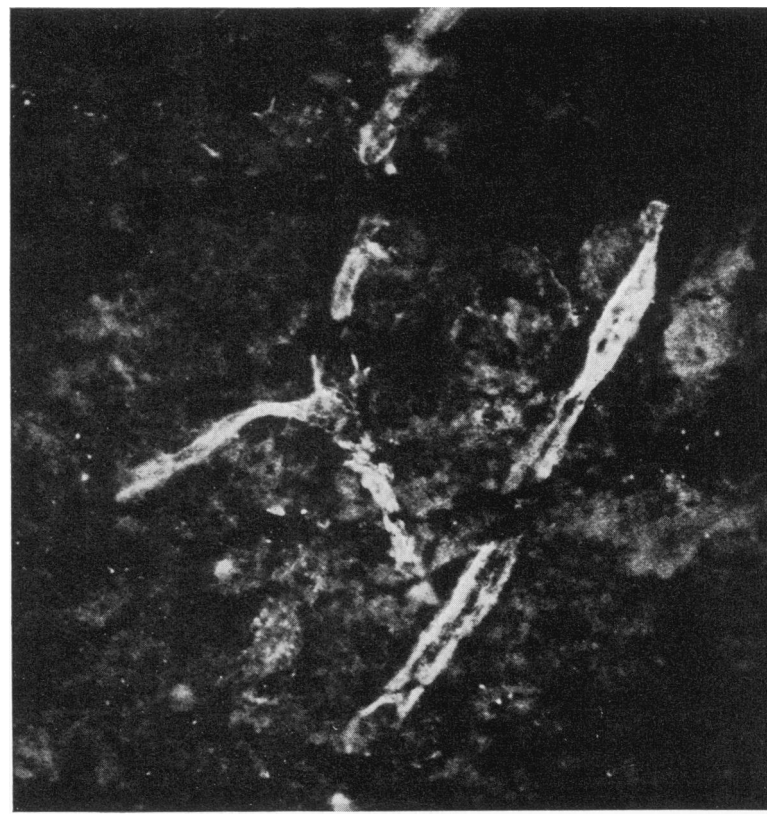

FIG. 15. Brain. Staining of capillaries in the cortex. (Serum 13) × 510. (Magnification, see Fig. 10.)

on sections cut from the same blocks, the connective tissue staining reactions given by an anti-BHV serum and the staining reactions given by serum 13. These were completely different. Although serum 13 gave good staining of the glomerular tuft, the appearance was unlike that of the staining of the basement membrane given by the anti-BHV serum, which, also unlike serum 13, stained Bowman's capsule and the tubular basement membranes. Again unlike serum 13, the anti-BHV serum gave strong staining of reticulin fibres in a lymph node and round the muscle fibres of the myocardium, and also of the basement membrane of the sinuses of the liver. Loewi (1967) found that basement mem-

branes throughout the body reacted with sera raised against chondromucoprotein. Scott (1959) found that, while sera raised against glomerulus and against synovial membrane stained different connective tissues in different proportions, the former stained to some extent renal tubular basement membranes and splenic and hepatic reticulin. Thus it seems clear that serum 13 is not a general connective tissue reagent. The staining of the glomerular tuft given by serum 13 has to some extent a cellular appearance, and since this serum also stains the endothelium of arteries and heart valves it is tempting to think of it as reacting with the endothelial cells of the cardio-vascular system. However, we did not observe staining of the capillaries between the renal tubules. Midgley and Pierce (1963) and Pierce *et al.* (1964) described a staining reaction specific to the basement membranes of epithelial cells. This reaction occurred strongly in the pia-arachnoid and glomerulus, but only weakly with the basement membrane of the renal tubules. There was no reaction with reticulin or collagen. In addition they described strong staining of capillaries in the brain (cf. our Fig. 15), but not elsewhere. Our serum 13 could be reacting with this antigen, but it also reacts with smooth muscle and the endothelial cells of arterioles. We intend to characterize exactly the tissues stained with our sera by using the electron microscope.

Holm (1967) and Holm, Braun and Jönsson (1968) found by precipitin analysis that human kidney contained three antigens that cross-reacted with a nephritogenic strain of streptococcus, and two with a non-nephritogenic strain. This supports our finding that there is a cross-reacting antigen present in non-nephritogenic strains, possibly distinct from that studied by Markowitz and Lange (1964). In addition, these authors used the growth medium of Holm and Falsen (1967), which contains no mammalian products, the only undefined constituents being the small molecular weight components of yeast autolysate. Their work may therefore be taken as a general confirmation of the existence of cross-reacting antigens, since previously the most refined medium contained the small molecular weight components of tryptic digest of casein.

In this study a cross-reaction with fibroblasts, endothelial cells and smooth muscle has been found with sera raised against type 6 glossy, type 6 matt and type 24. Among a few sera examined for another purpose, one raised in a guinea-pig against type 5 had a strong reaction against fibroblasts and smooth muscle (endothelial cells not tested). Thus the cross-reacting antigen or antigens may be widely distributed among streptococci.

As to the pathological significance of these results we can only speculate. It does not escape our notice, however, that we have described cross-reactions with those tissues which are especially affected in rheumatic fever.

ACKNOWLEDGMENTS

We are indebted to Mr J. R. Bartlett, F.R.C.S., Miss C. A. M. Fraser, Dr E. H. Hemsted and Dr E. J. Holborow for assistance in various ways, and to the staff of the histology department for preparing conventionally stained sections.

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