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Binding of Normal Human IgG to Myelin Sheaths, Glia and Neurons

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Summary. The binding of normal human serum, purified IgG and IgG fragments to central nervous tissue was studied by the anti-globulin consumption (AGCT) and immunofluorescence (IF) techniques. In the AGCT, $F(ab')_2$ fragments failed to react, whereas IgG and Fc fragments did so. In IF experiments, the binding was localized to myelin sheaths, glia and neurons; Fab monomers at a protein concentration of 1·3 mg/ml did not react with the tissue, but purified Fc fragments at 0·0625 mg/ml did. The binding is neither tissue- nor species-specific. Lipid and protein extraction procedures indicated that the factor responsible for binding to myelin was basic protein. It was concluded that the binding of normal IgG to central nervous tissue is mediated by the Fc part of the molecule.

INTRODUCTION

The occurrence of antibodies specific to human brain antigens has been described in several studies on sera of patients with multiple sclerosis (for review and references, see Lumsden, 1972). These findings have been interpreted as indications of autoimmune disturbances in human demyelinating disease. However, a recent study (Edgington and Dalessio, 1970) claims that 'antibodies' to myelin exist also in normal human sera. This observation, if confirmed, could imply that an autoimmune state involving myelin exists as a normal phenomenon.

A corresponding problem is met in myasthenia gravis. Sera from patients with this disorder contain antibodies to skeletal muscle. But all sera, also from healthy individuals, contain muscle-binding IgG, which reacts with the tissue, not as antibodies, but by the Fc part of the molecule (Aarli, 1970). On this basis, it was deemed important to examine whether the reaction of normal IgG with myelin is dependent upon similar mechanisms.

This paper therefore presents the results of investigations on the reactivity of normal human serum and IgG fragments therefrom with normal nervous tissue.

MATERIALS AND METHODS

Sera

Normal human sera were obtained from healthy blood donors. Incomplete anti-D serum, the rabbit antisera specific for human IgG (anti-IgG), IgA and IgM, and rabbit

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antiserum to pepsin-digested human IgG (anti-F(ab')$_2$) were the same as used before (Aarli, 1970; Aarli and Tönder, 1970).

Rabbit antisera to human Fab and Fc were purchased from Behringwerke (Marburg Lahn, West Germany). The anti-Fab antiserum was of batch 2193 AT, anti-Fc of batch T 2007 K.

Conjugates

Fluorescein isothiocyanate-labelled rabbit antiserum to human IgG (Batch F 446 F) and goat antiserum to rabbit Ig (batch F458 A) were purchased from Behringwerke.

Treatment of sera

IgG was prepared from normal human serum by QAE–Sephadex column chromatography (Joustra and Lundgren, 1969).

Pepsin digestion was performed as in an earlier report (Aarli, 1970).

Fc fragments of normal human IgG were prepared mainly according to Sanderson and Lanning (1970). After carboxymethylcellulose column chromatography, however, the Fc-containing fractions were pooled and separated from Fab contamination by two repeated electrophoreses on Pevikon (Fahey and McLaughlin, 1963). The final product was concentrated by polyethyleneglycol to a protein concentration of 0·5 mg/ml, as determined by the standard Lowry method. The purity of the Fc was controlled by double diffusion on agar plates and by immunoelectrophoresis, using anti-Fab and anti-Fc (Fig. 1). Immunoelectrophoresis was performed as previously described (Aarli and Tönder, 1970).

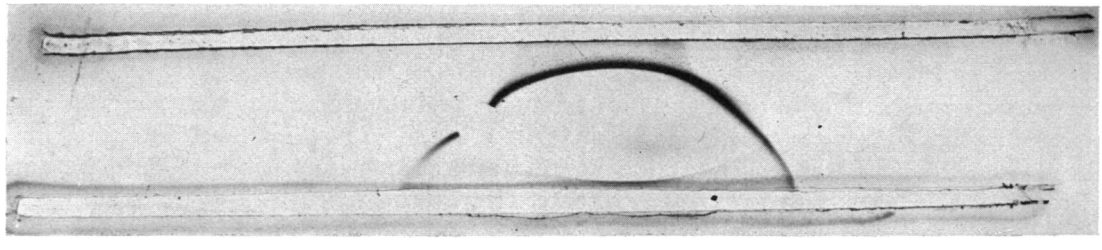

FIG. 1. Immunoelectrophoresis of Fc fragment of normal human serum in present experiment. The anode is to the left. Upper trough: anti-Fc. Lower trough: anti-F(ab')$_2$. At the concentration used here the arc corresponding to Fc is very strong and there is a weak cross-reaction with anti-F(ab')$_2$, owing to the fact that Fc and F(ab')$_2$ have a small amount of heavy chain in common. There is no Fab line: with its different mobility this would appear as a separate arc to the right (cathodic) side of the F(ab')$_2$ arc.

Fab monomers of normal human IgG were prepared as described by Spiegelberg and Weigle (1967), with column chromatography on Whatman DE-52 cellulose of the papain digest, followed by gel filtration of the 0·01 M effluent on a Sephadex G-100 column and rechromatography on DE-52 cellulose. After four absorptions of the effluent with pig liver tissue powder and gel filtration on Sephadex G-100, only Fab was demonstrated and no Fc or undigested IgG.

Elution of IgG from sensitized tissue was performed as previously (Aarli and Tönder, 1970).

Tissues

Normal human brain tissue was obtained from autopsies at the Department of Pathology.

Normal human liver, spleen and skeletal muscle tissues were the same as previously (Aarli and Tönder, 1970).

Purified bovine myelin was prepared by differential ultracentrifugation according to the method of Autilio, Norton and Terry (1964), modified slightly.

For the immunofluorescence experiments, various rat tissues were employed. White rats were killed with diethylether. Specimens of tissue from cerebellum, spinal cord, kidney, gut, liver, spleen, testis and urinary bladder were removed and frozen in liquid nitrogen. Cryostat sections, 8 μm thick, were prepared according to Lumsden (1970). These tissues were stored at −20°. In some experiments, pig liver powder (Wellcome Reagents Ltd, Beckenham, Kent) was employed.

Homogenization of tissues for the antiglobulin consumption test and absorption experiments were performed as by Aarli and Tönder (1970).

Treatment of tissues

For extraction purposes 5 g of homogenized, lyophilized tissue were suspended in 200 ml of chloroform: methanol at a ratio of 2:1, or acetone at 4° for 48 hours. After filtration (Whatman filter paper II), the tissue was washed thoroughly in isotonic, phosphate-buffered saline (PBS), at pH 7·2.

For fixation the sections were immersed in chloroform: methanol at a ratio of 2:1 for 30 minutes at 4° and then allowed to dry for 1 hour in front of a fan. In some experiments, 4 per cent formaldehyde, formol calcium, 96 per cent ethanol, ethanol: diethylether at a ratio of 1:1 or glutaraldehyde were individually employed instead of chloroform: methanol mixture.

For absorption experiments, 50 mg of tissue were suspended in 0·5 ml of serum or IgG fragment solution, incubated for 20 minutes at 37° and overnight at 4°. The sediment was removed from serum by centrifugation (absorbed serum).

The antiglobulin consumption test (AGCT)

This was performed according to Aarli and Tönder (1970).

Immunofluorescence experiments (IF)

These were performed by two methods, called the '2-step' and the '3-step' tests respectively.

For the 2-step test, the sections were incubated with serum or IgG fragment solution at room temperature for 20 minutes. The slides were then rinsed and washed for 30 minutes in PBS with two exchanges of buffer. Thereafter, the sections were immersed in distilled water for 2 minutes and allowed to dry. Conjugate was then added and the sections left at room temperature for 30 minutes. The slides were rinsed with PBS, washed for 30 minutes as above, and the sections were then mounted for IF microscopy.

For the 3-step test, the sections were incubated with serum or IgG fragment solution as above. After washing, anti-Fc or anti-Fab, diluted as indicated, was added. After 30 minutes the sections were washed as above and FITC-labelled goat antiserum to rabbit Ig, diluted from 1:16 to 1:64 as indicated by the preliminary test, was added. Mounting of the sections and IF microscopic equipment were as described by Lumsden (1970). Photomicrography was done using Kodak Tri-X-Pan film.

Histological staining procedures

These were OTAN (for lipids) and modified PAS (for cerebrosides), according to Adams (1965).

Polyacrylamide gel electrophoresis

This was carried out as by Summers, Maizel and Darnell (1965).

RESULTS

ANTIGLOBULIN CONSUMPTION TEST

Binding of normal human IgG to brain tissue

The AGCT was performed with 8 mg of brain tissue incubated with 0·25, 0·5, 1 and 2 ml of normal human serum, respectively. The antiserum was employed at a dilution of 1:32. At this dilution, 8 mg of brain tissue reduced the titre by one step.

The results (Table 1) show that the serum-indicated human brain tissue binds more anti-IgG than does PBS-treated brain tissue. Corresponding results were obtained when purified normal human IgG was employed instead of serum. Consequently, human IgG binds to normal human brain tissue.

TABLE 1

ANTIGLOBULIN CONSUMPTION TEST WITH BRAIN TISSUE AND NORMAL HUMAN SERUM. EFFECT OF INCREASE OF THE SERUM VOLUME

Human brain tissue (8 mg) incubated with:	Titre of antiserum after absorption*	Antiglobulin consumption†
PBS	1024	2
0·25 ml of serum	1024	2
0·5 ml of serum	512	4
1 ml of serum	64	32
2 ml of serum	<32	>64

* Titre of antiserum before absorption=2048. The antiglobulin serum used was anti-IgG at a dilution of 1:32.

† Antiglobulin consumption is expressed as ratio of titres before and after absorption.

Samples of 1 ml of serum from fifteen healthy individuals were tested by this method. The consumption obtained varied between 4 and 32, indicating that the degree of binding may vary from individual to individual.

Factors influencing the binding of IgG to brain tissue

Samples of 8 mg of brain tissue were treated with acetone or with chloroform:methanol at a ratio of 2:1. Other samples were incubated with PBS at 37°, 56° or 100° for 1 hour. After washing, the samples were used for the AGCT with normal human serum. PBS-treated tissue served as control.

After chloroform–methanol extraction of the tissue, followed by thorough PBS washing, the binding of IgG to the residue was minimal. This effect was not observed after acetone

extraction. In order to compensate for loss of tissue during the extraction, the AGCT was performed also with 8 mg of dried, chloroform–methanol-extracted and PBS-washed brain tissue. No significant binding of IgG to the extracted tissue could be demonstrated. Treatment of the tissue with PBS at 37°, 56° or 100° did not influence the results.

Accordingly, chloroform–methanol extraction of brain tissue, followed by prolonged PBS washing (obligatory in the AGCT) removes or destroys the component(s) responsible for the binding of IgG.

The AGCT was performed with normal human serum incubated with human brain tissue and with bovine myelin, purified ultracentrifugally, and the results compared. No significant difference in the results was observed (Table 2). Consequently, part of the IgG binding to brain tissue is to the myelin sheaths.

TABLE 2

ANTIGLOBULIN CONSUMPTION TEST WITH NORMAL HUMAN SERUM INCUBATED WITH HUMAN BRAIN AND WITH BOVINE MYELIN

Tissue (8 mg) incubated with:	Titre of antiserum after incubation with*:	
	Human brain tissue	Bovine myelin
PBS	2048	2048
1 ml of normal human serum	32	32

* Titre of antiserum before absorption = 4096.

IgG binding to various tissues

The AGCT was performed with samples of 1 ml of normal human serum incubated with 8 mg of human brain, liver, spleen and skeletal muscle, respectively. IgG reacted with all tissues. The binding of IgG to spleen and brain tissues was slightly higher than to the other tissues. Samples of 2 ml of normal human serum, diluted 1:2, were then absorbed with 100 mg of pig liver, human skeletal muscle, spleen or brain tissue, respectively. The absorbed sera were tested in the AGCT with brain tissue. Unabsorbed serum of the same batch served as control. A small reduction of the consumption occurred with all the absorbed sera, and repeated absorptions with each of the tissues reduced the consumption significantly.

Consequently, the binding of human IgG to brain is not a tissue-specific binding. Nor is it in any way species-specific, since it is partly removed by pig liver absorption.

Elution experiments

Eluates were prepared from brain tissue treated with normal human serum. In double diffusion on agarose plates, precipitation lines were formed against anti-IgG, but not against anti-IgA or anti-IgM.

AGCT with IgG fragments

IgG from normal human serum was digested by pepsin. After dialysis of the digest against PBS, the protein concentration was 2 mg/ml. The AGCT was performed with samples of 1, 2, 4 and 8 mg of the pepsin digest, with 1 ml of normal human serum and

with PBS. The results, given in Table 3, show that $F(ab')_2$ fragments from normal human IgG did not bind to the brain tissue. The AGCT was then performed with 8 mg of brain tissue incubated with 4 mg of normal human Fc fragments in PBS and with 1 ml of the corresponding normal human serum. Anti-Fc at a dilution of 1:64 served as antiserum. The results demonstrated that purified Fc fragments from normal human IgG reacted with brain tissue (Table 4).

The results of the AGCT experiments are therefore that normal human IgG reacts with normal brain tissue. The reaction is neither tissue- nor species-specific and is mediated by the Fc part of the IgG molecule.

TABLE 3

ANTIGLOBULIN CONSUMPTION TEST WITH BRAIN TISSUE. TITRE OF RABBIT ANTISERUM TO HUMAN $F(ab')_2$ FRAGMENTS BEFORE AND AFTER ABSORPTION WITH BRAIN TISSUE INCUBATED WITH PEPSIN-DIGESTED HUMAN IgG

	Titre	Antiglobulin consumption
Before absorption	4096	
After absorption with tissue treated with:		
PBS	2048	2
1 mg of $F(ab')_2$	2048	2
2 mg of $F(ab')_2$	2048	2
4 mg of $F(ab')_2$	2048	2
8 mg of $F(ab')_2$	2048	2
1 ml of normal human serum	<64	>64

TABLE 4

ANTIGLOBULIN CONSUMPTION TEST WITH BRAIN TISSUE. TITRE OF RABBIT ANTISERUM TO HUMAN Fc FRAGMENTS BEFORE AND AFTER ABSORPTION WITH BRAIN TISSUE INCUBATED WITH 4 mg OF Fc FRAGMENTS AND WITH AUTOLOGOUS SERUM

	Titre	Antiglobulin consumption
Before absorption	2048	
After absorption with tissue treated with:		
PBS	1024	2
4 mg of Fc	128	16
1 ml of normal human serum	<64	>32

IMMUNOFLUORESCENCE TESTS

Effect of various fixation procedures

Experiments showed that the intensity of the fluorescence varies according to the fixation method employed. Table 5 compares the results obtained with routine fixatives commonly employed in immunofluorescence tests of this sort, with the 3-step IF method and using normal human serum diluted 1:2.

TABLE 5

IMMUNOFLUORESCENT STAINING (3-STEP METHOD) OF NERVE CELL BODIES, GLIAL CELLS AND MYELIN SHEATHS BY NORMAL HUMAN IgG. EFFECT OF VARIOUS FIXATION METHODS UPON THE IF RESULTS

	Acetone	Formaldehyde	Chloroform–methanol	Formol calcium	Ethanol (70 per cent)	Absolute ethanol–diethylether	Glutaraldehyde
Nerve cell bodies	+	++	+++	—	—	++	—
Glia	+	+	+++	—	(+)	(+)	—
Myelin	+	+	+++(+)*	—	++	(+)	—
After preceding acetone fixation							
Nerve cell bodies		—	++(+)	—	++	++	—
Glia		—	++(+)	—	+	+	—
Myelin		+	+++	—	(+)	+	—

(+) = Very weak staining. + = Weak staining. ++ = Moderate strong fluorescence. +++ = Brilliant fluorescence.

* The fluorescence in this instance is brilliant, as is the case with the nerve cell bodies, but it is also uniformly consistent throughout all myelin sheaths, whereas nerve cell bodies are not constantly stained. Thus with chloroform–methanol pretreatment, the IF result is qualitatively a predominantly myelin effect.

With acetone fixation, the results were in principle identical with those obtained when using unfixed tissue sections. Both myelin, neurons and glial cells were still stained, although the overall intensity of the fluorescence became generally weaker after acetone fixation. However, the faint green background fluorescence of the grey matter, which was apparent in unfixed sections, was abolished by the acetone fixation, leaving a blue, unstained background. Therefore, the neuronal staining became by contrast rather more prominent in the acetone-fixed sections. With formaldehyde after acetone fixation, neuronal staining totally disappeared, but staining of the white matter remained distinct. Formaldehyde fixation alone resulted in a stronger neuronal staining with a weaker myelin ring pattern of staining.

Ethanol fixation (70 per cent) alone, like acetone–formaldehyde, abolished the neuronal staining, but the myelin ring pattern became much more prominent after this treatment, more so than with acetone–formaldehyde. If the sections were fixed by ethanol–diethyl ether, the neuronal staining was retained, while the myelin ring pattern became more diffuse than in ethanol-treated sections.

With chloroform–methanol-extracted sections, normal human serum produced a brilliant fluorescence alike of neurons, glial cells and myelin sheaths. The pattern of staining was qualitatively unaltered and no structures were stained in these sections which could not be demonstrated in the unfixed sections. However, the intensity of the staining, especially of the white matter, was much stronger than with unfixed sections. The result is that after chloroform-methanol pretreatment, the IF result is qualitatively predominant in the myelin (Fig. 2), since nerve cell bodies are rather not constantly stained although, individually, sometimes brilliantly fluorescent (Fig. 3). As the PBS controls were all negative also with this staining, chloroform–methanol was chosen as standard fixation procedure.

Analysis of the effect of chloroform–methanol upon the sections

Chloroform–methanol-extracted sections failed to stain by the modified PAS and the OTAN methods. Sections, in which the lipids were thus removed, were washed in PBS for 5 minutes. Application of normal human serum now resulted in an intensification of fluorescence. However, when the PBS washing was extended to 30 minutes, the reaction

with IgG was nearly abolished. These results were taken to indicate that the component responsible for the IgG binding is not of lipid nature, and becomes, at least partly, PBS-soluble after removal of lipids from the tissue. To test this, therefore, three hundred sections of rat spinal cord, pre-treated with chloroform–methanol, were extracted by 200

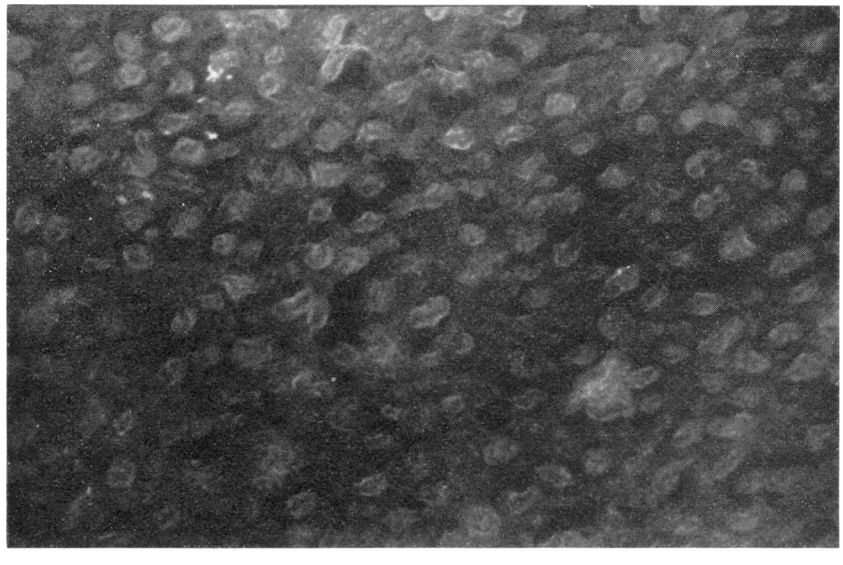

FIG. 2. Binding of normal serum to myelin sheaths in the dorsal column of rat cord. Normal human serum at a dilution of 1:2; rabbit anti-human IgG at a dilution of 1:32; FITC-conjugated goat anti-rabbit Ig at a concentration of 1:16. (This photomicrograph was taken using an exposure time of 40 seconds.) (Magnification ×360.)

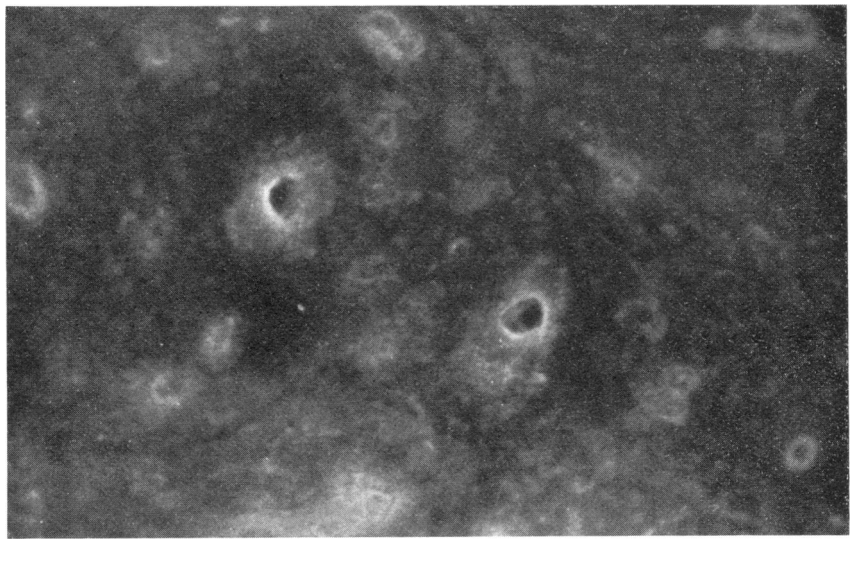

FIG. 3. A similar preparation to that of Fig. 2. Small neurons in the posterior horn show staining of the perikaryon with perinuclear accentuation of immunofluorescence. (This photomicrograph was taken using an exposure time of 40 seconds.) (Magnification ×360.)

ml of PBS for 30 minutes. Thin layer lipid chromatography of the chloroform–methanol extract showed that the lipids present were comparable to those in chloroform–methanol extracts of whole brain, thus verifying the complete, or almost complete, lipid extraction (Fig. 4). The PBS extract was dialysed against distilled water and concentrated by ultrafiltration to approximately 0·5 ml. In polyacrylamide gel electrophoresis, eight bands were produced, of which the distribution of the two most intense corresponded to the basic (fast cathodic) proteins of rat myelin, as compared with standards. The weaker bands were not identified (Fig. 5).

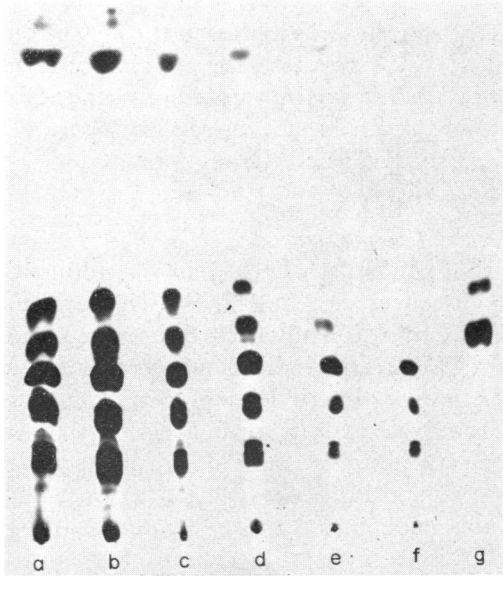

Fig. 4

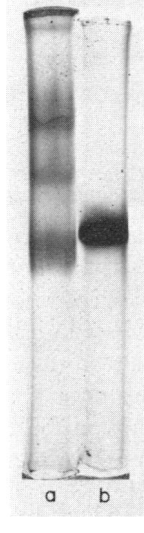

Fig. 5

FIG. 4. Thin-layer chromatography in silica gel (H) developed in chloroform:methanol:ammonia: water (140:50:7:3 v/v) to 3 cm from the top; plate rerun in chloroform:methanol:acetic acid:water (160:20:4:1·5 v/v) to the top of the plate. (a), (c) and (e), chloroform:methanol (2:1) extract of three hundred rat cord sections. (a) Undiluted. (c) 1:5. (e) 1:10. (b), (d) and (f), chloroform:methanol extract of 1 g of bovine brain. (b) Undiluted. (d) 1:5. (f) 1:10. (g), cerebroside standard. The lipid spots for the sections and bovine brain extracts, in ascending order from the origin (at the bottom) represent: phosphatidylserine, phosphatidylinositol, sphingomyelin, choline phosphoglycerides, ethanolamine phosphoglycerides, cerebrosides, cholesterol.

FIG. 5. Polyacrylamide gel electrophoresis of the phosphate-buffered saline extract of delipidized sections. (a) PBS extract diluted 1:20; B, rat basic protein preparation used as a standard (100 μg). Cathode is at the bottom of the figure.

IDENTIFICATION OF THE IgG COMPONENT BINDING TO NORMAL CNS TISSUE

IF pattern with whole serum

Even with unfixed sections of rat spinal cord, staining of myelin, glial cells and neurons was seen. The myelin immunofluorescence was, however, patchy and therefore difficult to evaluate. This was interpreted as due to the high lipid concentration in the white matter. The pattern of immunofluorescence obtained after chloroform–methanol extraction was in principle not different from the staining of the unfixed sections, but the pattern after lipid removal was uniform throughout the whole white matter.

With whole normal human serum (or with purified IgG) applied to chloroform–methanol-extracted rat cord sections, a brilliant fluorescence of the myelin sheaths appeared, as in Fig. 2. In longitudinal sections, the myelin was seen as two distinct parallel fluorescent lines—as, indeed, with the Fc fragment (see below). The neuron bodies were strongly stained, most intensely in the perinuclear area, sparing the nucleus (Fig. 3). In some neurons, a weak staining of the cytoplasm and slightly also in axon hillock and axons was observed.

The third nervous tissue component (irregularly) stained was glia. This immunofluorescence was always nuclear. Since such cells were axonal satellites in chains, they were presumed oligodendroglial.

With the whole sera, the intensity of the staining was higher in the 3-step test than in the 2-step, but the patterns were identical. In the 3-step test, the staining of myelin and glia disappeared at serum dilutions of 1:4–1:8, while the immunofluorescence of neurons was observed with some sera at serum dilutions up to 1:32. Absorption of sera with pig liver, bovine myelin or human brain tissue nearly abolished the reaction.

Immunofluorescence pattern with Fab fragments of normal human IgG

In testing a pathological serum (from multiple sclerosis) it was found that $F(ab')_2$ fragments gave as strong immunofluorescence with anti-Fc as with anti-Fab antisera. Since this positive reaction with anti-Fc serum was likely to be due to the part of the heavy chains which is common to both Fc and $F(ab')_2$, the indications were that to get clear-cut results, the use of Fab monomers rather than of dimeric $F(ab')_2$ fragments were necessary. Fab monomers were thus prepared by repeated column chromatography of papain digests of normal human IgG. In immunoelectrophoresis, only one line appeared, showing electrophoretic mobility corresponding to Fab and no reaction against anti-Fc. In IF tests, using purified Fab monomers, at a concentration of 1·3 mg/ml, anti-Fab at 1:64 dilution, and FITC-conjugated goat antiserum to rabbit immunoglobulin at 1:32, no staining was obtained, either of myelin, glial cells or of neurons.

Immunofluorescence tests with Fc fragments of normal human IgG

Fc fragments were prepared from two different normal human sera. In immunoelectrophoresis, only one line was obtained, with electrophoretic mobility corresponding to Fc. No line was produced against anti-Fab (Fig. 1).

With an Fc fragment solution of 0·5 mg/ml, anti-Fc at a dilution of 1:64 and conjugate as for the Fab tests, brilliant immunofluorescence occurred (Fig. 6). The pattern obtained was indistinguishable from that obtained using normal serum or purified IgG. In transverse sections of rat spinal cord, myelin was seen as distinct rings (Fig. 6) and in longitudinal sections as two parallel lines (Fig. 7). The longitudinal sections also showed chains of glial nuclei parallel to the direction of the myelin (Fig. 7). The neurons were strongly stained, with more intense perinuclear fluorescence, nuclear sparing and moderate staining of the cytoplasm (Fig. 8). While the white matter was uniformly and strongly stained because of the myelin immunofluorescence, the grey matter was generally weaker, but with scattered brilliant single cells, both neuronal and glial (Fig. 9). The staining of myelin, glial cells and especially of the neurons could be seen with Fc diluted up to 0·0625 mg/ml. With sections of rat cerebellum, the staining of grey matter was the most prominent feature, there being a very strong staining of the granular cell layer, and Purkinje cells were also

stained. Myelin was stained too, but owing to the small diameter of the myelin sheaths, the white matter here was not so intensely stained as in the spinal cord.

The reaction of Fc with nervous tissue was abolished by prior absorption of the Fc fragment solution, not only with bovine myelin and human brain tissue, but also with pig liver.

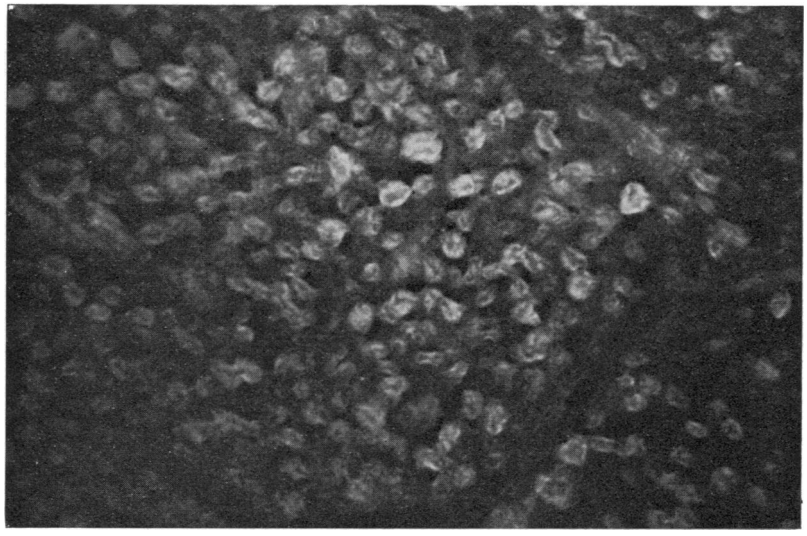

FIG. 6. Fc, from normal human serum, binding to myelin sheaths in cross-section (rat cord dorsal column). Fc, 0·5 mg/ml, rabbit anti-Fc at a dilution of 1:64, FITC-conjugated goat anti-rabbit Ig at a concentration of 1:32. This photomicrograph was taken using an exposure time of 40 seconds. (Magnification ×360.)

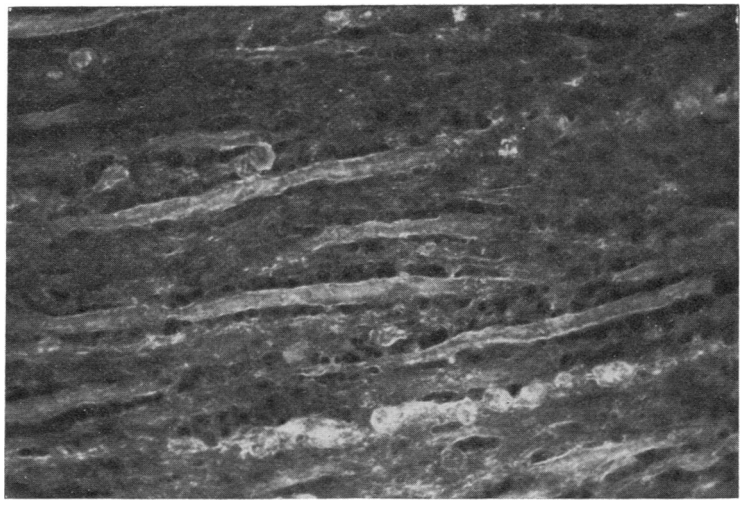

FIG. 7. A similar preparation to that of Fig. 6. The longitudina section shows strong fluorescence of large myelinated fibres. Note the chain of glial cells (possibly oligodendrocytes), also strongly stained. This photomicrograph was taken using an exposure time of 40 seconds. (Magnification ×360.)

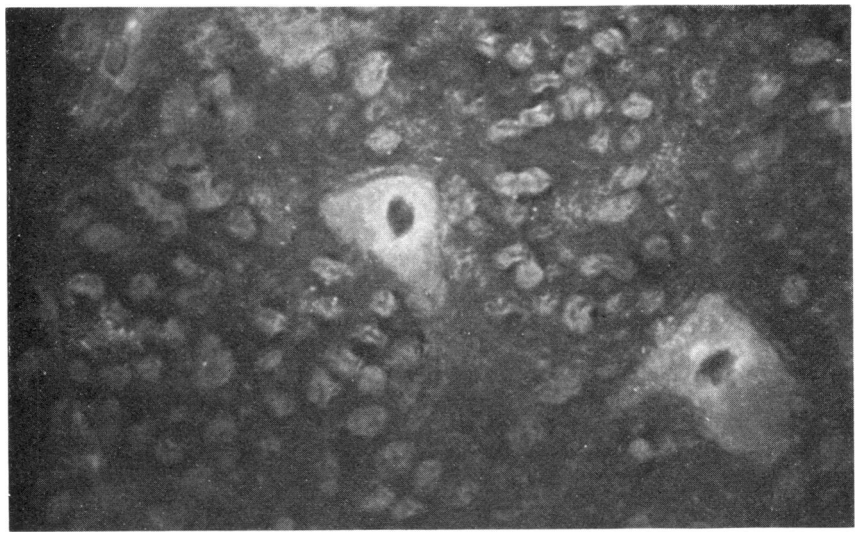

FIG. 8. Normal human serum IgG–Fc reaction with rat chord (reagent titres as in Fig. 6). Two large neurons show a strong fluorescence in the perikaryon; small myelinated fibres were also seen in cross-section. This photomicrograph was taken using an exposure time of 40 seconds. (Magnification × 360.)

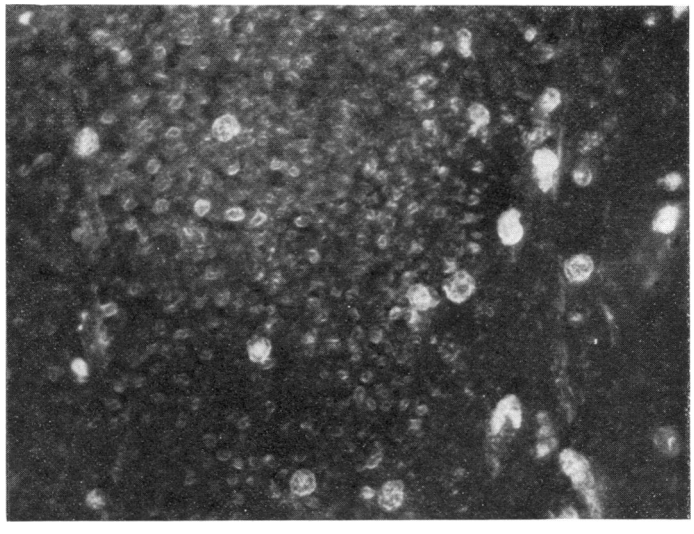

FIG. 9. Cross-section of rat chord treated as in Figs 6 and 8 showing the difference in staining between grey and white matter. In the latter the fluorescence is prominent in the myelin sheaths; a few glial cells are also stained. In the grey matter (right) the background is essentially negative; a few small neurons and glial cells are brightly fluorescent. This photomicrograph was taken using an exposure time of 40 seconds. (Magnification × 196.)

Immunofluorescence tests with Fc fragments and various tissues

Sections were prepared from rat liver, kidney, gut, spleen, testis and urinary bladder. The 3-step IF tests were performed with Fc fragments and both unfixed and chloroform–methanol-extracted sections. With liver a nuclear pattern only was obtained in the hepatocytes, at an Fc concentration of 0·5 mg/ml. There was a very strong reaction with glomeruli in the kidney, while the tubules were not stained. In the gut, smooth muscle and epithelial cells were stained. Lymphocytes and reticulin fibres were the splenic structures which were most strongly stained. There was a strong perinuclear reaction with nuclei of testicular tubules. With the urinary bladder, a strong staining in the core of the folds (lamina propria) and probably of the bladder membrane, was seen.

Compared with the staining of myelin, reactions with these non-nervous tissues were consistently weaker. Next in order to myelin were testis and urinary bladder, with even weaker reactions in liver, kidney, gut and spleen.

DISCUSSION

The results of the present study, employing both AGCT and IF tests, clearly show that normal human IgG reacts with normal central nervous tissue. In this respect, the findings confirm the conclusions of both Allerand and Yahr (1964) and Edgington and Dalessio (1970) as to the affinity of normal human immunoglobulin for nervous tissue.

The morphology of the reaction has not been clarified previously. Allerand and Yahr (1964), using ethanol fixation, observed staining of myelin and glial cells, and not of neurons; but, as we have seen (Table 5) ethanol fixation preferentially abolishes the neuronal reaction. On the other hand, formalin fixation alone, as employed by Beutner, Witebsky, Rose and Gerbasi (1958), gives a strong neuronal reaction, but, as they reported, with combined formalin and acetone extraction the reaction is eliminated.

In the present study, chloroform–methanol extraction of the sections was found to give optimal results. By this method, lipids are efficiently removed from the tissue (Fig. 4). Whilst the immunofluorescence pattern obtained when using unfixed sections is patchy and often difficult to interpret, the staining with delipidized sections, although reacting with the same structures, becomes uniformly even, and therefore preferable for evaluation. With the chloroform–methanol-extracted sections, distinct reactions of IgG with myelin, glia and neurons were observed.

In the AGCT, however, the binding of IgG to chloroform–methanol-extracted brain tissue was greatly reduced. The reason for this apparent discrepancy is presumably the differing extent of PBS washing in the two tests, extensive PBS washing being implicit in the AGCT, while in the IF test, the PBS washing has to be very brief to preserve the immunofluorescence result. If the sections were washed for longer periods of time, the reaction was lost, as was the case with the AGCT. Accordingly it was concluded that the structure(s) responsible for the IgG binding become PBS-soluble after removal of lipids from the tissue. Analysis of the PBS extract on acrylamide electrophoresis showed that several proteins were removed from the tissue during the washing procedure (Fig. 5) and basic protein of myelin was identified as the major component so removed. The other proteins removed were not identified, but probably include nucleohistones.

So far, therefore, our results show that normal human IgG reacts with proteins in myelin

sheaths, neurons and glia and that the active component of the myelin is the highly basic protein which is characteristic of myelin. The nuclear reaction is probably due to nucleohistones. The tissue reactants which account for the non-specific binding of the Fc part of the IgG molecule are therefore probably small protein molecules with high basic charge.

The nature of these reactions has been a matter of dispute. Thus, Allerand and Yahr (1964) regarded the reaction as due to 'an affinity of the gamma-globulin fraction for glial cells and myelin sheaths', a 'non-specific protein interaction' and not an antigen–antibody reaction. Edgington and Dalessio (1970), on the other hand, claimed that the reaction was immunologically specific and by implication specific for myelin and glia. On the contrary, we must now conclude that IgG binding with myelin is through the Fc fragment, and is not a true immunological binding but a non-specific one related to the highly basic protein component of myelin. It is true that Edgington and Dalessio (1970), in addition to whole IgG, tested Fab fragments (at a concentration of 6–8 mg/ml) but they do not report having tested Fc fragments. They may have assumed that since they obtained a positive reaction with Fab fragment this was the sole cause of the immunofluorescence obtained. However, in our experience, the Franklin chromatography procedure used by Edgington and Dalessio does not yield Fab monomers free from whole IgG molecules and the purity of their Fab would be much lower than that obtained by the procedure used (and outlined above) for the present work. Moreover, in our tests, the Fab was tested at a concentration of only 1·3 mg/ml., i.e. at about only one-fifth that used by Edgington and Dalessio, so that the risk of cross-reaction with contaminating Fc-containing IgG is likely to have been high under the conditions of their experiment.

In conclusion, the implications are identical for the two tests, AGCT and IF, for whole serum, IgG and for purified Fab and Fc fractions. Normal human IgG reacts quite strongly with myelin, with satellite glial cells (presumably oligodendroglia) and with nuclear membranes and perikarya of nerve cell bodies. But since it occurs with other tissues, this reaction is not highly tissue-specific. It is dependent upon, and mediated by, the Fc part of the IgG molecule; while the Fab part is not involved in this reaction. Accordingly, the reaction is not an antigen–antibody reaction.

As to the nature of the Fc reaction, our data have revealed that it is by no means diffuse and random, since it is connected with specific proteins both of nervous and other tissues. Removal of lipids facilitates the Fc binding in the nervous tissue sections. Removal of myelin basic protein by PBS washing after lipid extraction almost totally abolishes the reaction with myelin. This finding strongly and clearly indicates that the myelin affinity for IgG is connected, at least in part, with basic protein. However, since Fc fragments bind equally well to cerebellar and peripheral myelin (dorsal roots), this reaction seems to be a universal one for myelin as such, and may therefore be concerned with basic proteins as a group as distinct from the highly CNS-specific basic protein involved in experimental allergic encephalomyelitis and from the different, but equally highly specific, antigen responsible for experimental allergic neuritis. Also, normal human IgG has an affinity not only for nervous tissue, but less strongly for various other tissues, as demonstrated by the absorption experiments. Thus, the IF tests have demonstrated Fc binding to the glomeruli of the kidney, to nuclei in various organs and to structures in the wall of the urinary bladder. Presumably, this binding can similarly be explained by the presence of low molecular weight proteins with similar charged groups in various organs.

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