It is not possible to say whether the liberation of inorganic sulphate that occurs in the living animal is due to the direct hydrolysis of the dipeptide sulphate or of L-serine O[³⁵S]-sulphate liberated as a result of dipeptidase activity. Possibly both routes are involved. Chromatography showed that some of the injected material appears in the urine in an unchanged form, but no free L-serine O[³⁵S]-sulphate was detected although an exhaustive search for this compound was not made. This need not necessarily mean that dipeptidases are without effect on the sulphated dipeptide *in vivo*, since any L-serine O[³⁵S]-sulphate liberated as a result of dipeptidase might well undergo immediate desulphation.

The physiological significance of the desulphation process is still obscure. Sulphated L-serine residues have never been detected in proteins, although the corresponding phosphorylated derivatives are of fairly widespread occurrence.

SUMMARY

1. Potassium glycyl-L-serine O-sulphate and the corresponding ³⁵S-labelled ester were prepared.

2. Partially purified preparations of rat and sheep livers, capable of desulphating L-serine O-sulphate, exhibited feeble activity towards glycyl-L-serine O-sulphate. However, after the intraperitoneal administration of potassium glycyl-L-serine O[³⁵S]-sulphate to rats, about 83 % of the radioactivity of the dose was recovered in the urine. Almost 50 % of this recovered radioactivity was in the form of inorganic [³⁵S]sulphate.

3. Analysis of urines showed the presence of at least four radioactive materials, two of which moved with the mobilities of inorganic [³⁵S]sulphate and glycyl-L-serine O[³⁵S]-sulphate.

4. Faecal preparations were capable of desulphating the ³⁵S-labelled ester. Bile catheter experiments showed that faecal micro-organisms did not contribute appreciably to the formation of inorganic [³⁵S]sulphate found in rat urine after injection of the ester.

5. Crude preparations of rat kidney and rat intestine were capable of cleaving the sulphated peptide to yield glycine and L-serine O-sulphate.

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Polybasic and Polyacidic Substances or Aggregates and the Excitability of Cerebral Tissues, Electrically Stimulated *in vitro*

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Lysine polymers and some basic proteins render cerebral tissues unresponsive to electrical excitation; certain acidic substances of molecular weight about 1000 or more restore excitability (McIlwain, 1959, 1961). The changes in excitability were concluded to be due to the basic peptides hindering the re-establishment of membrane potentials, and this to be dependent on an action of the peptides in diminishing particular ion movements at the tissue

(Hillman, 1961; McIlwain, Woodman & Cummins, 1961); each of these actions is antagonized by the acidic substances. Substances of both categories become attached to the tissues whose excitability they alter (Thomson & McIlwain, 1961; Balakrishnan & McIlwain, 1961).

Many simple acidic substances do not restore excitability, but the few substances known to restore it are very disparate in structure. They in-

clude cerebral-ganglioside components and suramin, an aromatic hexasulphonic acid that is trypanocidal. Further understanding of their action could come by exploring structural requirements for restoring excitability, and this information has been sought in the present study. Advantage has been taken of associated investigations (D. B. Gammack, J. H. Perrin & L. Saunders, unpublished work) to examine a number of cerebral lipids in aqueous suspensions of known micellar size.

EXPERIMENTAL

Tissue preparation, incubation, and analysis. A guinea pig was struck on the back of the neck, exsanguinated, the brain removed, and slices of cerebral cortex were cut with a blade and recessed glass guide which were wet with glycylglycine-glucose medium (McIlwain & Rodnight, 1962). This medium contained (mm): NaCl, 124; KCl, 5; KH_2PO_4 , 1.24; MgSO_4 , 1.3; CaCl_2 , 2.8; glycylglycine, 30; glucose, 10. It was adjusted to pH 7.4 by NaOH and was saturated with oxygen. Slices were floated from the cutting blade or guide to a shallow dish of the medium, and trimmed to fit the silver-grid electrodes of McIlwain & Joanny (1963). Slices were then lifted on the rider of a torsion balance, drained from adhering medium by repeated contact with a glass surface, weighed, floated into the electrodes, and with them fitted to manometric electrode-vessels A (McIlwain & Rodnight, 1962). These were conical, of about 15 ml. capacity, and contained 3.5 ml. of incubation fluid in their main compartment. A centre well contained 0.15 ml. of 5N-NaOH and received a filter-paper wick immediately before placing the vessels on their manometers.

Typical experiments included six slices of approx. 60 mg. wet wt., all within electrodes. At chosen times during the experiment, electrical pulses of alternating polarity were applied from a condenser-pulse generator (McIlwain & Rodnight, 1962) to some or all of the vessels, at peak potential of 10V, time-constant 0.4 msec. and frequency of 100/sec. (For choice of these characteristics, see McIlwain, 1954.) Manometric readings of gas pressure were taken at 5 min. intervals, the oxygen uptake/g. of tissue was calculated and expressed graphically, and respiratory rates were obtained.

Methods for examining effects of added substances on the tissue's response to stimulation. (a) Substances were examined for their effects on normal tissue by incorporating them in the 3.5 ml. of glycylglycine incubation medium in four or five of the six vessels of a manometric experiment.

(b) Substances were examined as antagonists to inhibitory polypeptides by the following procedure (for choice of conditions, see below). Four or five vessels of a manometric experiment contained, in the glycylglycine medium before the tissue was added to it, 150 μg . of clupeine sulphate/ml. The manometric experiment was commenced in normal fashion by shaking the mounted vessels at 37.5°, but after 18 min. of incubation all vessels were removed from the thermostat and from their manometers and the clupeine-containing fluid was sucked away thoroughly with a Pasteur pipette. Glycylglycine medium (1.5 ml.) was added, swirled round the vessel and electrode, and also removed, this operation taking 30–40 sec. with each vessel.

Glycylglycine medium (3.5 ml.), with or without the substance being tested as antagonist, was then added. The ground-glass joint of the vessel was freed from aqueous fluid by filter paper, regreased and refitted to its manometer, and the vessel contents were again equilibrated with oxygen. The tissue's respiratory rate was measured during successive periods of 30–45 min., without and with the application of pulses.

Substances. Clupeine sulphate and salmine sulphate were from L. Light and Co., Colnbrook, Bucks.; the former contained 24.7% of N and 6.36% of S. Ganglioside preparations: curves G_1 and G_0 of Fig. 1 were obtained with material containing 26.5% of *N*-acetylneuraminic acid (McIlwain, 1961); similarly prepared material (Booth, 1962) was used for the acid-treatment which yielded preparations AG1 and AG2. Preparation GT3D (made according to the method of Trams & Lauter, 1962) was a gift from Dr L. S. Wolfe, Montreal Neurological Institute, and contained approx. 30% of *N*-acetylneuraminic acid.

Partially hydrolysed gangliosides were prepared and analysed by Dr D. B. Gammack and Mr D. A. Booth as follows. A solution (10 mg./ml.) of a preparation of gangliosides from ox brain with 27.8% (w/w) of *N*-acetylneuraminic acid was heated at 80° for 1 hr. in 0.10N- H_2SO_4 to yield material containing 6.2% (w/w) of *N*-acetylneuraminic acid (AG1). A similar solution heated under the same conditions for 2 hr. yielded material containing 1.6% (w/w) in one case, and in the other 3.4% (w/w), of *N*-acetylneuraminic acid (AG2). Preparation AG1 contained predominantly ganglioside species with one *N*-acetylneuraminic acid residue/sphingosine molecule by thin-layer chromatography (Gammack, 1963); preparation AG2 consisted of only traces of three *N*-acetylneuraminic acid-containing species and a large component which did not move on the chromatogram and which gave no Bial orcinol reaction. Before inclusion in the metabolic media, AG1 and AG2 were dialysed exhaustively against distilled water at 0° to remove H_2SO_4 . *N*-Acetylneuraminic acid contents were determined by analysis by the Bial orcinol reaction.

Gangliosides, before acid-treatment, readily yielded aqueous sols. Sols of the other lipids (see Fig. 1 and Table 2) were prepared by ultrasonic treatment, as described by Saunders, Perrin & Gammack (1962). The equiv.wt. of phosphatidylserine was taken from the data of Baer & Maurukas (1955) as 792, and of the sulphatides from the data of Lees, Folch, Sloane-Stanley & Carr (1959) as 970.

Poly-D-glutamic acid was kindly given by Professor H. N. Rydon, being preparation H230B of Hanby & Rydon (1946) from the capsular substance of *Bacillus anthracis* (mol.wt. 21000; 160 residues; equiv.wt. by titration 145), and concluded to be linked largely through the α -carboxyl groups of the glutamic acid residues. Poly-L-glutamic acid was from Mann Laboratories (mol.wt. 50000; 388 residues; equiv.wt. taken to be 129). Chondroitin sulphate A and chondroitin sulphate C were kindly given by Dr A. G. Lloyd, being fractionated as described by Meyer, Davidson, Linker & Hoffman (1956), Dodgson, Lloyd & Spencer (1957) and Dodgson & Lloyd (1961); the equiv.wt., 675, was calculated from a sulphate content of 14.2%. Suramin and the analogue (II) were given by Dr A. Spinks, Imperial Chemical Industries Ltd., Pharmaceuticals Division; equiv.wts. were calculated to be 238 and 233 respectively.

RESULTS

Inhibition of excitability by protamines, and the test system adopted for studying restoration

Incubation of cerebral tissues with clupeine-containing media for 18 min. greatly diminished their response to excitation (Expts. *A* in Table 1). This was not dependent on the continued presence of clupeine in the fluid medium, for after 18 min. the medium was replaced by one without clupeine. The conditions of Expts. *A* (Table 1) were selected on the basis of previous investigations (McIlwain, 1959, 1961); before their adoption in the present study, comparison was made with tissues exposed to clupeine for a longer period, and also to salmine (Expts. *B* and *D* in Table 1). These changed conditions did not give better test systems but showed that the conditions adopted were not narrowly based. An attempt was made to diminish variability in the degree of inhibition caused by clupeine in Expts. *A* (Table 1) by preincubating all tissues of an experiment in one batch of fluid (Expts. *C* in Table 1). Variability was not, however, diminished, and the conditions of Expts. *A* were adopted as a test system for studying restoration of excitability.

In the first experiments which employed the conditions of Expts. *A* (Table 1), variation in response among slices was observed to be greater in the presence of protamine (with or without an added agent) than in its absence. This could be ascribed to a difference in behaviour to protamine of slices cut first from the outer convexity of the cortex ('outer slices') and those cut subsequently ('inner slices'). Exposure to protamine under the same conditions gave for outer slices a response of 130 ± 6 (4 experiments) and for inner slices 118 ± 7

(10 experiments). In the presence of a number of added substances under conditions otherwise similar, outer slices gave responses which were $13 \pm 7\%$ (9 experiments) greater than those with the inner slices. An instance is quoted in Fig. 1. In subsequent experiments inner slices were almost always used.

Substances restoring excitability

Sphingolipids. Ganglioside preparations were the most effective of the substances previously examined (McIlwain, 1961) in restoring excitability to cerebral tissues, and this was also the case during the present examination. Fig. 1 (*a*) shows similar activity in two ganglioside preparations, of which 0.15–0.6 mg./ml. had maximal effect. In exploring the structural basis for ganglioside action, advantage was taken of the ready removal of the *N*-acetylneuraminic moiety by mild acid-treatment (Svennerholm, 1957, 1958; Klenk & Gielen, 1961; Booth, 1962). The heating with 0.1 *N*-sulphuric acid for 2 hr. which produced the preparations *AG2* (see the Experimental section) left them with 52 and 110 μ moles of *N*-acetylneuraminic acid/g., or 5–10 % of that of the original ganglioside. The product *AG2* was, however, without activity in restoring excitability when present in concentrations 20 times those in which the original ganglioside acted (Fig. 1*a*). The shorter acid-treatment which yielded *AG1* (Fig. 1) left 24 % of the *N*-acetylneuraminic acid of the starting material, and also an appreciable proportion of its activity as a restoring agent.

Examination of two other sphingolipids also indicated the importance of a strongly acidic group in restoring tissue excitability (Fig. 1*b*). Sphingomyelin, which is a diester of phosphoric acid, was without effect in concentrations many

Table 1. *Protamines as inhibitors of the response of cerebral tissues to electrical stimulation*

The protamine sulphates (0.15 mg./ml.) used were clupeine sulphate in Expts. *A*, *B* and *C* and salmine sulphate in Expts. *D*. Slices of guinea-pig cerebral cortex were trimmed and weighed, and in Expts. *A*, *B* and *D* each slice was fitted to an electrode immediately after weighing; the electrodes were placed in separate manometric vessels containing 3.5 ml. of glycylglycine-glucose medium either with or without clupeine sulphate (0.15 mg./ml.). In Expts. *C* the slices were trimmed, and six were placed together in a 150 ml. manometric vessel with 21 ml. of the medium with clupeine sulphate. After preincubation of slices in Expts. *A*, *B* and *D*, media were removed from the vessels and replaced by fresh glycylglycine medium; incubation without and with stimulation followed, as described in the Experimental section. After preincubation of slices in Expts. *C*, the contents of the flask were poured into a shallow dish, and the slices were drained, weighed and fitted to electrodes which were then placed in individual electrode vessels and then treated as in Expts. *A* and *B*.

Group of Expts.; no. of values in each group	Conditions of preincubation	Respiratory rate (μ moles/g. wet wt./hr.)				Respiratory rate with pulses (% of rate without pulses)	
		No pulses		With pulses		No protamine	With protamine
		No protamine	With protamine	No protamine	With protamine		
<i>A</i> ; 12	Individual vessels, 18 min.	60 $\pm$ 3.5	58.5 $\pm$ 5.1	112 $\pm$ 5.2	70.3 $\pm$ 7.0	186 $\pm$ 6	120 $\pm$ 9
<i>B</i> ; 6	Individual vessels, 28 min.	61 $\pm$ 4	54 $\pm$ 6.2	108 $\pm$ 5.6	59.5 $\pm$ 5.8	165 $\pm$ 8	109 $\pm$ 15
<i>C</i> ; 6	Collectively, 18 min.	—	54 $\pm$ 4	—	62.5 $\pm$ 5.9	—	116 $\pm$ 11
<i>D</i> ; 3	Individual vessels, 18 min.	59	58	114	64	193	110

times those in which gangliosides acted; but a sulphatide preparation (cerebroside monosulphuric ester) caused appreciable restoration of excitability when added in concentrations 2–10 times those in which gangliosides acted. The maximal restoration observed with sulphatides was not as great as with gangliosides. A suspension of stearic acid was without effect (Table 2).

Phospholipids. The three other phospholipids examined (Table 2) gave results parallel to those of

the sphingolipids. Lecithin, phosphatidylethanolamine, phosphatidylserine and sphingomyelin are all diesters of phosphoric acid, but of them only phosphatidylserine, with the additional acidic grouping of the amino acid, restored response on excitation to over 50 %. The restoration achieved with phosphatidylserine (Table 2) approached that given by the sulphatides rather than by the gangliosides, though it was reached with lower concentrations (by wt.) of the lipid. Lecithin and phosphatidylethanolamine gave responses between 30 and 40 %.

Other substances. The substances described above are all normal constituents of cerebral tissues, and knowledge of activity in carboxylic acids and sulphonic acids of relatively large molecular size is extended by the other substances of Table 2. The synthetic hexasulphonic acid, suramin, was active (Thomson & McIlwain, 1961), and in the present experiments restored the response to values approaching 50 %. Its action is now seen to be shared by an analogue (II). This lacks the methyl groups and the trypanocidal activities of suramin (Spinks, 1948), but was found to carry approximately the same activity in restoring excitability. L-Glutamic acid was without action in this respect, but the polymer of some 390 L-glutamic residues yielded responses of over 50 %, and this value was approached by the polymer of D-glutamic acid. Chondroitin sulphate A and chondroitin sulphate C (Dodgson & Lloyd, 1961) acted to similar extents. The two differ in a number of physical properties but are similar in chain configuration and charge relationships (Mathews, 1959); chondroitin sulphate A probably exists in cerebral tissues (Szabo & Roboz-Einstein, 1962) but the preparation employed was derived from cartilage.

Other observations

In the test system employed, the tissue being examined was exposed to the two agents, protamine and an antagonist, successively. This was judged necessary from previous observations that agents which restored activity would, if added to protamine alone, form complexes with it and prevent its attachment to the tissue (McIlwain, 1961; Thomson & McIlwain, 1961). Not all agents forming complexes with protamine were, however, effective in restoring excitability to protamine-treated tissue. Among the substances now studied, the following gave precipitates with protamine (0.5 mg./ml. in the glycylglycine medium) when added in concentrations in which they were effective in restoring excitability: the sulphatide preparation, phosphatidylserine, the polyglutamic acids, the chondroitin sulphates, suramin and the suramin analogue (II). A precipitate was not observed with the acid-treated ganglioside AG2.

Fig. 1. Sphingolipids and the respiratory response of protamine-treated tissues to electrical excitation. Experiments were carried out with slices of guinea-pig cerebral cortex as described in Expts. A in Tables 1 and 2. The values quoted for respiratory response correspond to those of the last column of Table 1 and of Table 2, and are mean values of three results, except that when s.d. values (indicated by vertical lines) are given they represent four to eight results. Respiratory rates of the tissues, and their responses in the absence of protamine, were also determined and were within the values quoted in Tables 1 and 2. The substances added were examined for effect on respiration and respiratory response in the absence of protamine but were without action in these respects. G₁, Ganglioside specimen examined with inner slices of cortex; G₀, with outer slices. AG1, Ganglioside specimen treated with acid for 1 hr. (see the text); AG2, for 2 hr. Sul, Sulphatide. Sph, Sphingomyelin.

Table 2. *Ability of added substances in restoring response to cerebral tissues*

Slices of guinea-pig cerebral cortex were treated with clupeine sulphate as described in Expts. A in Table 1, except that after preincubation added media contained the substances listed below. Values for respiratory rates in μ moles of O_2 /g. of tissue/hr. were close to those of Table 1 and the percentages quoted below correspond to the last two columns of Table 1; further details are given in the Experimental section.

Substance added	Concn. of added substance (mg./ml.)	Respiratory rate with pulses (% of rate without pulses)	
		No protamine	With protamine
Lecithin	0	188	121, 122
	0.3	—	120, 131
	1	180	134, 128
	3	—	133, 138
Phosphatidylethanolamine	0	181	118
	0.3	—	136
	1	179	120
Phosphatidylserine	0	182	116, 125
	0.1	—	141, 153
	0.5	—	152, 155
	1.5	169	144
Stearic acid	0	190	117, 112
	3	183	113, 116
Poly-D-glutamic acid	0	190	126 $\pm$ 7 (4)
	0.28	—	136
	0.86	191	145, 149
	2.5	—	155
Poly-L-glutamic acid	0	181	120, 126
	0.1	—	128, 124
	0.3	—	140, 146
	1	—	142
Chondroitin sulphate A	0	173	117, 121
	0.3	—	120, 121
	1	170	136, 149
Chondroitin sulphate C	0.3	—	130, 142
	1	—	145
Suramin	0	182	121 $\pm$ 6 (4)
	0.1	—	125, 109
	0.3	—	135
	1	190	145, 148
	2.9	—	139
Suramin analogue (II)	0	186	116
	0.3	—	140
	1	176	126, 146

DISCUSSION

Gangliosides and protamine were previously concluded to affect tissue excitability through combination as acids and bases with tissue constituents (Thomson & McIlwain, 1961; McIlwain *et al.* 1961). The present observations support this and enable the interactions effective in restoring excitability to be specified in more detail.

(1) Under conditions close to those of the present experiments, cerebral tissues incubated with clupeine gained 6.1 mg. of the protamine/g. of tissue, which represents some 7.2 or 22 μ equiv./g. according to the basicity judged relevant (McIlwain, 1961). The findings summarized in Table 3 show that each of the agents which restores excitability is acidic and requires to be added in

quantities similar to or greater than these amounts. If it is assumed that the basic groups of the protamine are partly neutralized through attachment to the tissue, this implies that the agent restoring excitability is restoring a preponderance of acidic groupings to the tissue. With gangliosides and suramin, restoration of excitability was shown previously to be accompanied by restoration of potassium assimilation to the tissue, and with the present substances also their acidic groups appear likely to determine tissue excitability by facilitating cation movement (McIlwain *et al.* 1961; McIlwain, 1963).

(2) The restoring agents are polyacidic, either as molecules or as aggregates. This is evident with the polyglutamic acids, the chondroitin sulphates, suramin and the suramin analogue. With the lipids

the property is conferred by micellar aggregation (D. B. Gammack, J. H. Perrin & L. Saunders, unpublished work). The polyacidic character can be understood to be necessary if the basic groups added to the tissue by the protamine are to be replaced by acidic groups while leaving protamine attached to the tissue, as indicated in Fig. 2. Continued attachment of protamine in the presence of gangliosides and of suramin has been observed (Thomson & McIlwain, 1961). A monoacidic substance that did not form aggregates could do no more than neutralize the basic groups of the protamine, and this presumably is inadequate for restoration of excitability. Neither *N*-acetylneuraminic acid, the acidic moiety of the gangliosides, nor *L*-glutamic acid is effective in restoring tissues rendered inexcitable by keeping them in cold media under specific conditions. Under these conditions the histones of the tissue are lost from the cell nuclei and act as endogenous inhibitors. The inhibited tissue that results is considered equivalent to the protamine-treated tissue of the present experiments (Marks & McIlwain, 1959; McIlwain, 1959, 1961; Wolfe & McIlwain, 1961). The site of the interactions adumbrated in Fig. 2 is presumably the outer cell membrane or the endoplasmic reticulum, as basic proteins are preferentially attached to microsomal material (see preceding references and McIlwain, 1963). This outer part of the cell may thus have some resemblance to the fixed-charge systems of Ling (1962).

Ganglioside preparations not only form polyacidic aggregates, but, moreover, contain components with more than one *N*-acetylneuraminic acid residue/molecule; a trisialoganglioside has been reported by Wolfe & Lowden (1963). The firmest combination with protamine has been found (Booth, 1962) in the ganglioside fractions richest in neuraminic acid. It is relevant here that the acid-treatment which yielded preparations AG1 and AG2 from gangliosides brought about greater diminution in their ability to restore excitability than in their content of *N*-acetylneuraminic acid. An acid degradation of disialogangliosides through monosialo derivatives has been reported (Kuhn *et al.* 1960). The acid-treated gangliosides remained non-diffusible and thus, like the gangliosides, they existed in aqueous solutions as aggregates of large particle weight. Non-acidic aggregates did not, therefore, act in restoring excitability.

(3) Adequate acidity is needed in restoring agents, and this is provided by carboxylic or sulphuric anions; the phosphoric diesters are less effective.

(4) Structural requirements, beyond these minima, do not appear to be exacting when judged by the criterion of restoration of some 50 % of the tissue's respiratory response. Gangliosides, how-

ever, remain the substances of which the smallest amounts give the greatest recovery of excitability. As their action is related to ion movements across the cell membrane, their potency may be in part dependent on the many hydrophilic groups contributed to the molecule by its several carbohydrate residues. A single galactose residue is present also

Table 3. *Quantities of acidic substances restoring excitability to protamine-treated tissues*

The concentrations restoring activity are approximate only; they are derived from Fig. 1 and Table 2, and are the concentrations needed to raise respiratory response to 50 % or, in the few instances when this is not reached, to the greatest extent observed. The values of the third column are derived from those of the second column, from the knowledge that, during the experiments quoted, tissues of mean fresh wt. 60 mg. were suspended in 3.5 ml. of fluid. The equivalent weights of the substances concerned are derived as described in the Experimental section.

Preparation	Concentration restoring excitability (mg./ml.)	Amount restoring activity (μ equiv./g. of fresh tissue)
Ganglioside	0.2	8
Sulphatide	1	60
Phosphatidylserine	0.5	37
Chondroitin sulphate	1	87
Poly-D-glutamic acid	2.5	1000
Poly-L-glutamic acid	1.0	450
Suramin	0.5	122

Fig. 2. Hypothetical relationship between acidic groups of tissue components (A), the basic polypeptides (B) which inhibit excitability, and acidic agents (C) which restore excitability. The basic polypeptide is drawn in the fully extended form which it is likely to occupy through mutual repulsion of its basic groups (see Katchalsky, Danon & Nevo, 1959). A polyacidic restoring agent is illustrated above, and an aggregate below.

in the cerebroside and sulphatide molecules. In the chondroitin sulphates several are present, but these substances carry no lipophilic properties and their molecules may be judged less adjustable to the conformation of the tissue or the basic component of Fig. 2 than is a polyacidic aggregate. The minimal concentration of gangliosides found to give near-maximal restoration of excitability is close to the critical micelle concentration (Gammack, 1963), and is thus a concentration at which ganglioside molecules may arrive individually at the protamine-treated tissue and aggregate there in fashions partly dependent on the spacing of the basic groups of the protamine. Comparable phenomena have been postulated in relation to the metachromatic properties of the gangliosides (Harris & Saifer, 1960; Harris, Saifer & Weintraub, 1961; McIlwain, 1963). Interactions such as those of Fig. 2 imply the inhibiting and restoring agent to extend some 50–100 Å from the acidic groups of the tissue component. This appears feasible; even minimal estimates indicate a 150–200 Å spacing between the outer membranes of adjacent cerebral cells or cell-processes (Gray, 1959; Luse, 1962).

SUMMARY

1. Cerebral-cortical tissues lost their normal excitability on incubation in protamine-containing media, rinsing and examination in a second medium similar to the first but without protamine.

2. Excitability could be restored by adding ganglioside preparations to the second medium; this property of the gangliosides was lost on mild acid-treatment which removed their *N*-acetyl-neuraminic acid residue.

3. Phosphatidylserine and sulphatide preparations also restored excitability, though for a given effect they were required in greater concentrations than were the gangliosides. Their restoring activity was attributed to the polyacidic aggregates existing in their aqueous solutions; phosphatidylethanolamine and lecithin showed lesser activity.

4. Chondroitin sulphates, suramin and polyglutamic acids (descending order of activity) also restored excitability.

5. The effective agents complied with the following general description: they were polyacidic or existed in aqueous solution as polyacidic aggregates, and were required in quantities equivalent to or greater than the basic groups added to the tissue by the protamine with which it had been incubated.

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