ISOLATION OF BRAIN STRANDIN, A NEW TYPE OF LARGE MOLECULE TISSUE COMPONENT*

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This paper reports the isolation from brain tissue of a substance to which the name of strandin has been given for descriptive purposes. This term has been chosen because, when dried from aqueous solution, strandin has the property of forming long strands that show perfect orientation under polarized light. As isolated from brain by any one of the three procedures described below, strandin is an electrophoretically homogeneous compound. By ultracentrifuge studies, it is shown to have a main component with a minimal molecular weight of 250,000. It is soluble in water and chloroform and is extracted quantitatively from the tissue with chloroform-methanol mixtures (1). Chemically, it can be classified as a lipide, since among its constituents are found fatty acids and sphingosine or a sphingosine-like substance. However, many of its properties are quite different from those of a typical lipide.

Strandin is found in gray matter in relatively large concentration; i.e., 6 to 7 mg. per gm. of wet tissue. It is found in white matter at one-tenth its concentration in gray matter and in brain tumors in concentrations larger than in white matter and smaller than in gray matter. In other tissues that have been studied, namely, heart, skeletal muscle, uterus, lung, liver and kidney, strandin is found in very small amounts; i.e., <0.01 per cent.

The first evidence obtained for the presence of strandin in brain tissue was the observation that brain lipides, freed of proteolipide protein (1) by drying under adequate conditions, and of non-lipide contaminants by dialysis, contained more NH₃-N (after acid hydrolysis) than could be expected from their cephalin content. In a search for the lipide or lipides that were the source of this non-cephalin NH₃-N (after acid hydrolysis), it was found that a fraction of brain lipides that was available as a by-product in the preparation of cephalin was especially rich in it. From

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this material, by a procedure described below (Procedure A), a fraction was separated in low yield which contained 1.0 per cent NH$_2$-N (after acid hydrolysis) and which appeared to be a lipide different from any of the known lipides. This isolation has already been reported in a preliminary note (2). This new lipide was found to be freely soluble in water in true solution and also to contain as a constituent a chromogenic radical that darkened on treatment with 6 N HCl at 100° for a short period of time. This latter observation became the basis of a method for the quantitative estimation of the new lipide fraction. This method proved to be a valuable tool for following the fate of the new lipide in pilot fractionations that were carried out in an attempt to develop satisfactory procedures for its preparation from brain tissue. It made possible the eventual development of two procedures for preparing strandin in good yields.

One of these, Procedure B, follows for the most part the general lines of classical methods for fractionation of lipides in the sense that it depends on the solubilities of strandin in various solvents. The other procedure (partition-dialysis) is based on the facts that strandin is freely soluble in water and that it is undialyzable. A chloroform-methanol extract (1) from gray matter is dialyzed against frequently renewed water. An aqueous layer that forms inside the bag is collected and dried. The residue obtained shows the typical strandin strands. It is identical with that obtained by the other two procedures, with one minor exception, noted below.

From the study of its physical properties, strandin appears to be either a pure substance in different states of aggregation, or a mixture of closely related compounds. All preparations studied have been found to be homogeneous in the electrophoretic field in which they act as negatively charged molecules over the range pH 8.6 to 1.38 in which electrophoretic patterns have been obtained. Ultracentrifuge analysis, for which we are deeply indebted to Professor J. L. Oncley of the Harvard Medical School, has shown strandin to contain two components, one with a sedimentation constant of s 11 and a second one with a sedimentation constant of s 16. The first one is the most abundant and constitutes four-fifths of preparations of strandin obtained by the partition-dialysis method. Since the partial specific volume of strandin has been found to be 0.78, if it is assumed that the main component of s 11 has a molecule of spherical shape, the calculated molecular weight is about 250,000. This is a minimal value; if strandin has a molecule of asymmetrical shape, the value obtained would be much larger. That the molecule is not very asymmetrical in shape is indicated by the fact that strandin solutions do not show any marked birefringence of flow.
Attempts to check the molecular weight of strandin by osmotic pressure measurements, for which we are indebted to Dr. J. Bourdillon of the Division of Laboratories and Research of the New York State Department of Health, have shown the material to be unsuitable for reliable measurements, but such values as have been obtained are consistent with the idea that strandin preparations are of a very large molecular size.

Results from the study of the chemistry of strandin can be summarized as follows: Strandin prepared by Procedures A and B contains 2.6 per cent N, <0.2 per cent P, and <0.2 per cent S. Among its constituents, five different radicals have been recognized: a water-soluble primary amine, which is combined to the rest of the molecule through its NH₂ group and which may be glucosamine; a sphingosine or a sphingosine-like substance; a fatty acid; a carbohydrate, which is not glucosamine; and a chromogenic group, which darkens on treatment with 6 N HCl at 100° for a short period of time.

Strandin prepared by the partition-dialysis method appears to be identical with that obtained by the other methods, with the exception that it contains a small amount of amino acids in combination (0.2 to 0.4 per cent α-amino acid N after acid hydrolysis; none before hydrolysis). These combined amino acids may represent a small amount of protein impurities, or may be due to the fact that there are two closely allied strandins, one having a primary amine as a constituent and the other having an amino acid. The relationship between these two hypothetical strandins would be similar to that between phosphatidyl ethanolamine and phosphatidyl serine.

It is possible that all major strandin constituents have been recognized. If it is assumed that the primary amine is glucosamine and that the water-insoluble nitrogen (after acid hydrolysis) is sphingosine, the sum of these two residues, plus carbohydrate, plus fatty acids, accounts for over 70 per cent of the weight of strandin. The balance would include chromogenic material and phosphatides and sulfatides present as contaminants.

The foregoing information on the chemistry of strandin shows that a new compound or group of compounds is being dealt with. Its low phosphorus and sulfur content shows it to be neither a phosphatide nor a sulfatide. The presence of a constituent water-soluble primary amine shows that it is not a cerebroside. That strandin is different from gangliosides (3) has been established by an analysis of strandin for neuraminic acid by the method of Klenk and Langerbeins (4) which shows strandin to contain at the most 1.5 per cent neuraminic acid as compared with 21 to 24 per cent neuraminic acid in gangliosides.

Essentially no other progress has been made towards establishing the structure of strandin. Repeated attempts to isolate some of its different
constituents as pure substances have failed. A reason for this failure may
be that strandin is a very complex molecule and that each of the five dif-
ferent constituent radicals that have been recognized is not a single sub-
stance but a mixture of closely related compounds.

Strandin cannot be extracted from gray matter or from brain tumor
tissue with 5 per cent aqueous trichloroacetic acid. Since strandin itself
is freely soluble in such solutions and strandin added to the tissue can be
recovered quantitatively by extraction of the tissue with trichloroacetic
acid, it is necessary to conclude that, in tissue, strandin is bound to some
other component, presumably a protein.

EXPERIMENTAL

Analytical Methods—Most of the methods used have been enumerated
elsewhere (5).

Quantitative Estimation of Strandin—A sample containing between 0.1
and 2 mg. of strandin is placed in a 15 cc. centrifuge tube. If the sample
is an aliquot of extract, the solvents are removed as described elsewhere
(6). 0.5 cc. of water is added and the tube let stand until the residue has
formed an emulsion in the water. To the emulsion is added 0.5 cc. of
concentrated HCl, and after mixing by twirling, the tube is placed in a
boiling water bath for exactly 15 minutes. It is removed from the bath,
cooled under the tap, and 5 cc. of acetone are added to its contents. The
contents are stirred thoroughly, and the tube is stoppered with a cork
stopper and centrifuged for 10 minutes at 2500 r.p.m. The transparent
supernatant solution is decanted into a colorimeter tube of 18 mm. diam-
eter and light transmittance read at a wave-length of 475 mm in the junior
Coleman spectrophotometer which has been set to 100 per cent transmitt-
ance against an adequate blank. The amount of strandin present in the
sample is read from a standard curve.

Reliability of Method—The procedure described above was arrived at
after a study of the effect of the length of time of heating on the intensity
of the color produced with different amounts of strandin. It was found
that the intensity of color reached a maximum on heating for 15 minutes,
no significant increase in color intensity developing on longer time of heat-
ing, and that, for concentrations of strandin between 0.1 and 2.5 mg. per
cc. of 6 N HCl, the intensity of color produced was proportional to the
concentration of strandin according to Beer's law.

The specificity of the method for strandin has been established by deter-
mining the amount of color produced by 15 mg. samples of each of the
following substances: serine, ethanolamine, choline, inositol, glycerol, gly-
cerophosphoric acid, oleic, palmitic, stearic, and linoleic acids, galactose,
glucose, ribose, sphingosine, cholesterol, phosphatidyl serine, phosphatidyl
ethanolamine, lecithin, total ether-soluble brain lipides, cerebrosides, etc. In all cases, the color produced was less than the amount produced by 0.15 mg. of strandin. The specificity of the method was also established by determining the amount of color produced by known amounts of strandin added to 15 mg. samples of the substances enumerated above. It has been found that the amount of color produced is the same in the presence, or in the absence, of any one of those substances.

In summary, the method described yields reliable data on any lipide mixture containing more than 1 per cent of the new lipide. Duplicate estimations check usually within ±1 per cent.

 Procedure A for Preparation of Strandin

The starting material is a lipide fraction which is obtained as a by-product from the preparation of cephalin by a method described in detail elsewhere (7). Brain tissue is extracted in succession twice with acetone, once with ethanol, and twice with petroleum ether. The petroleum ether extracts are combined, the solvent removed by vacuum distillation, the residue suspended in ether, and the ether suspension placed in the ice box until a clear supernatant separates. The ether-insoluble residue is collected by centrifugation. It is dissolved in 10 parts by weight of chloroform; the solution is diluted 3-fold with methanol, allowed to stand at 22° overnight, and filtered. The filtrate is concentrated to half its volume. To it is added an equal volume of methanol and the solution let stand at 22° for 24 hours. The precipitate that forms is collected on a Büchner funnel and dissolved in a chloroform-methanol mixture, 4:6 by volume, in the proportion of 10 cc. of solvent mixture per gm. of material. The solution is placed at −10° for 24 hours. A precipitate that forms is dissolved in the same solvent mixture at the same concentration as above and the solution placed at −10° for 24 hours. The precipitate that forms is treated once more in the same manner. The precipitate finally obtained is dissolved in 30 parts by weight of acetic acid at 60° and the solution allowed to stand for 24 hours at 22°. A precipitate forms which is removed by centrifugation. To the supernatant are added 6 volumes of acetone and the mixed solution is allowed to stand at 22° for 24 hours. The precipitate that forms is collected by centrifugation, washed twice with acetone, and dried. It represents about 3 per cent of the starting material; i.e., about 0.3 mg. per gm. of wet weight of brain tissue. The material obtained is a birefringent hard white powder. It is soluble in chloroform and water and insoluble in ether, methanol, ethanol, and acetone. When an aqueous solution is dried in a vacuum desiccator, the residue consists of a mass of strands with a glass wool-like appearance.
Isolation of Strandin from Brain

Procedure B for Preparation of Strandin

The procedure is run at 4° unless otherwise stated. Cattle brains obtained at the slaughter-house are freed of membranes. Gray matter is obtained by gross dissection. This dissection need not yield pure gray matter, but tissue that can be described as being predominantly gray matter. 30 gm. portions of tissue are extracted with 600 cc. of chloroform-methanol mixture, 2:1 by volume, in a Waring blender, which is run for 2 minutes. The extract is filtered. The filtrate is taken to dryness by vacuum distillation of the solvents and the residue is emulsified in about 2 cc. of water per gm. of starting tissue. The emulsion is placed in cellophane bags and dialyzed against distilled water for 3 days, the outside water being changed twice daily. After dialysis, the emulsion is lyophilized. To the residue in the lyophilizing flask is added a volume of ethyl ether corresponding to 1 cc. of solvent per gm. of starting tissue. The flask is warmed to bring the ether to the boiling point. The container is allowed to cool, stoppered with a cork stopper, and placed in the ice box overnight. A clear ether supernatant can be decanted without difficulty, except for a small portion which is separated from the ether-insoluble residue by centrifugation at 2500 r.p.m. for 30 minutes. As much as possible of the resulting clear ether supernatant is removed with a siphon and the ether-insoluble material is freed of residual ether by gently blowing a current of nitrogen over its surface. The removal of ether does not need to be complete. To the ether-insoluble material is then added an amount of chloroform-methanol mixture, 2:1 by volume, corresponding to 0.25 cc. of solvent mixture per gm. of starting tissue. The solvent mixture is brought to the boiling point, and methanol is added slowly to the solution in the proportion of 0.17 cc. per gm. of starting tissue. The addition of methanol should take about 30 seconds. The container is allowed to cool, stoppered with a cork stopper, and placed at -10° for 24 hours. At the end of the 24 hour period, the insoluble material is collected over a Büchner funnel, precooled to -10°. The insoluble material is transferred back to the same container. The addition of chloroform-methanol mixture, 2:1 by volume, followed by bringing the solvent to boiling, addition of methanol, etc., as described above, is repeated. The insoluble material on the Büchner funnel is transferred to a boiling flask and is extracted for 2 hours under a reflux with boiling chloroform-methanol mixture, 2:1 by volume, in the proportion of 0.25 cc. of solvent mixture per gm. of starting tissue. The hot mixture is then filtered through a Büchner funnel.

The filtrate is dried by vacuum evaporation of the solvents. The residue is transferred to a glass-stoppered cylinder and to it is added chloroform in the proportion of 0.09 cc. per gm. of starting tissue. Part of the
chloroform can be used to insure the quantitative transfer of the residue. Usually, the residue goes completely into solution in the chloroform. To the solution are then added in succession methanol and ethanol in the proportions of 0.06 and 0.03 cc. per gm., respectively, of starting tissue. The solvents are mixed by inverting the stoppered cylinder, care being taken to relieve the pressure by removing the stopper after each inversion of the cylinder, since the mixing of the three solvents used results in a slight heating of the mixture. To the solution is finally added water in the proportion of 0.06 cc. of water per gm. of starting tissue. In summary, chloroform, methanol, ethanol, and water are used in the relative proportions by volume of 3:2:1:2. The cylinder is inverted about 200 times. A milky mixture results, which on standing overnight resolves slowly into two transparent phases of about equal volume. The upper phase is collected by means of a siphon, concentrated by vacuum distillation, and finally dried in a vacuum desiccator. The residue shows some of the strands typical of strandin. It amounts to about 2 per cent of all the solutes in the original tissue extracted; i.e., 1.2 to 1.4 gm. per kilo of gray matter. It is still only 75 to 85 per cent pure as compared to the purest preparations that have been obtained.

To purify it further, the material is dissolved in 20 parts of acetic acid by weight at 60°. The procedure followed is then exactly as described under Procedure A. The yield of strandin is about 0.9 gm. per kilo of starting tissue.

**Preparation of Brain Strandin by Partition-Dialysis Method**

The procedure is run at 4° unless otherwise stated. A chloroform-methanol extract of gray matter is prepared as in Procedure B. 250 cc. aliquots of the extracts are placed in 70 cm. lengths of cellophane dialysis tubing of 28 mm. diameter, and the bags are submersed vertically in water and let stand for 3 days. There should be at least 5 times as much water as extract. The outside water is changed twice daily. As water enters the bag, the contents become milky. After a few hours the contents start clearing simultaneously at the top and at the bottom of the bag. Both clear zones progress slowly towards each other and after 24 hours the contents consist of a transparent water phase in its upper half, a transparent chloroform phase in its lower half, and a fluff at the interphase. After 3 days the bags are removed from the water and the water phase collected and concentrated by vacuum distillation. When the volume of the solu-

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1 Under the conditions described, cellophane yields a dark oily material in amounts of about 0.4 mg. per sq. cm. of surface of the cellophane. This material is water-soluble and freely dialyzable. It is removed completely from the system by changing the outside water repeatedly.
tion is only a few cc., it is transferred to a cellophane bag and dialyzed for 3 more days against 100-fold its volume of water, the water being changed twice daily. Finally, the solution inside the bag is collected and dried in a vacuum desiccator.

A glass wool-like residue is obtained with the characteristic long strands of strandin. The yield of strandin is 3 to 3.5 gm. per kilo of gray matter.

Properties of Strandin—Unless specifically stated, the following applies to strandin prepared by any of the three methods described. Strandin is freely soluble in water, in chloroform, sparingly soluble in acetic acid, and insoluble in ether and acetone. When obtained from solutions in organic solvents, either by drying or by precipitation, it is a hard white powder, which in the polarizing microscope can be seen to consist of markedly birefringent microscopic strands. When obtained by drying its water solution, strandin invariably forms long glimmering strands (Fig. 1) which are strongly birefringent and show perfect orientation when viewed with polarized light (Fig. 2).

Electrophoretic Study of Strandin—Electrophoresis patterns of strandin have been obtained in 0.1 M veronal buffer at pH 8.6, in 0.1 M acetate buffer at pH 4.7, in 0.1 M citrate buffer at pH 2.0, and in 0.1 M HCl-glycine buffer at pH 1.38. In all cases, single sharp ascending and descending boundaries have been obtained (Fig. 3). At all these pH values used, strandin behaves like a negatively charged molecule. Values for mobility measurements at pH 8.6 are $4.69 \times 10^{-3}$ cm$^2$ per volt per second and at pH 1.38, $6.03 \times 10^{-4}$ cm$^2$ per volt per second.
Ultracentrifuge Analysis of Strandin—For this work, the partial specific volume of strandin was estimated on three different concentrations of strandin: 2, 1, and 0.5 per cent. Values from 0.77 to 0.79 per cent were obtained. No special precautions were taken to attain high accuracy. Therefore, the average value of 0.78 per cent given here is considered an approximation to the true value. The ultracentrifuge analysis was carried out by Professor J. L. Oncley. Results with three different preparations are given in Table I. It can be seen that, while not completely homogeneous, preparations obtained by the partition-dialysis method contain a

Table I

Results of Ultracentrifuge Analysis of Strandin Preparations

<table>
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<tr>
<th>Method of strandin preparation</th>
<th>Components, per cent of strandin</th>
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<tbody>
<tr>
<td></td>
<td>Solvent</td>
</tr>
<tr>
<td>Procedure A</td>
<td>0.10 M NaCl buffered</td>
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<tr>
<td></td>
<td>with phosphate (pH 7.0-7.1)</td>
</tr>
<tr>
<td>Partition-dialysis</td>
<td>Same as above</td>
</tr>
<tr>
<td></td>
<td>0.15 M NaCl unbuffered, pH 4.7</td>
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main component which comprises four-fifths of the substance. While the two different components observed may indeed represent two different substances, they may also be one single substance in different states of aggregation. The calculated molecular weight for the main component, $s_{11}, \bar{V} = 0.78$, would be about 250,000 for a spherical molecule. This appears to be the minimal possible value for the molecular weight of strandin.

**Chemistry of Strandin**—Strandin prepared by Procedures A and B had the following per cent composition: C 50, N 2.6, P <0.2, S <0.2, NH$_2$-N <0.1, $\alpha$-amino acid N <0.1, NH$_2$-N (after acid hydrolysis) 1.15 (all of it water-soluble), $\alpha$-amino acid N (after acid hydrolysis) <0.1, carbohydrate (as galactose) 20 to 21, water-soluble N (after acid hydrolysis) 1.3, water-insoluble N (after acid hydrolysis) 1.3, and neuraminic acid <1.5 per cent.

The only difference between strandin prepared by the partition-dialysis method and that by Procedures A and B is that the former contains combined amino acids: $\alpha$-amino acid N, after hydrolysis, 0.2 to 0.4 per cent, before hydrolysis, <0.02 per cent. The possible significance of this finding has already been discussed.

**Nature of Nitrogen in Strandin**—On acid hydrolysis with 6 N HCl at 100$^\circ$, nitrogen in strandin separates into two equal parts, half of it being water-insoluble (and chloroform-soluble) and half water-soluble. The water-insoluble nitrogen must represent sphingosine or a sphingosine-like substance. The water-soluble nitrogen is 90 per cent NH$_2$-N, which reacts quantitatively with nitrous acid in about 3 minutes (8). By the method of Elson and Morgan (9) for estimation of free glucosamine, the water-soluble fraction from acid hydrolysates of strandin shows a glucosamine content that accounts for all NH$_2$-N present. This suggests that the amine may be glucosamine, but constitutes no final proof.

**Nature of Carbohydrate Group in Strandin**—The carbohydrate content of 20 to 21 per cent of strandin, given by reaction with orcinol, is not changed after acid hydrolysis with 6 N HCl at 100$^\circ$ for several hours or after alkaline hydrolysis with 2 N NaOH at 100$^\circ$ for 2 hours, the only length of time of hydrolysis that has been tested. This proves that if the carbohydrate is galactose it must be combined in strandin as a galactoside, since free galactose is completely destroyed by alkali treatment as described. Since amino sugars in general (10), and glucosamine in particular, do not give any color on reaction with orcinol, the possibility that the carbohydrate group might be identical with the NH$_2$-N-bearing group is also eliminated. Finally, that the carbohydrate is not identical with the chromogenic group is shown by the following experiment. Strandin is treated with 6 N HCl at 100$^\circ$ for 15 minutes, which results in full develop-
ment of the brown color from the chromogenic group. After cooling, the brown-colored solution is filtered and the filtrate treated in succession with equal volumes of amyl alcohol and ethyl ether. An almost colorless solution is obtained which is found to contain all of the carbohydrate present in the original strandin.

**Fatty Acids in Strandin**—Fatty acids can be separated from the products of alkaline or acid hydrolysis of strandin as follows: Strandin is hydrolyzed with 6 N HCl at 100° for 2 hours. The hydrolysate is allowed to cool and an equal volume of chloroform is added to it. The two phases are mixed by adequate stirring. After separation of the phases by centrifugation, the dark chloroform solution is collected. It is washed twice with water and taken to dryness. A crystalline residue of fine needles, heavily contaminated with a dark amorphous material, is obtained. The dark material is the product of destruction of the chromogenic group by the action of the acid. The residue is leached with acetone. Most of the amorphous dark material remains undissolved. The acetone solution is allowed to stand overnight at 0°. Some dark amorphous material precipitates. The clear supernatant is allowed to dry by evaporation of the acetone at room temperature. A residue of fine needles is obtained which represents 25 per cent of the starting amount of strandin. Neutral equivalent, 310 to 320 for different preparations.

The isolation of fatty acids from strandin after alkaline hydrolysis proved to be a technically difficult problem. No fatty acids were obtained when strandin was saponified with 10 per cent aqueous NaOH at 100° overnight, and the product acidified to pH 2 and extracted with ethyl ether and petroleum ether. Chloroform could not be used because it formed an inseparable emulsion with the product. Fatty acids were finally separated by extracting repeatedly the acidified saponification product with twice its volume of acetone. After four such treatments, all of the water from the product had passed into the acetone, leaving behind a residue of sodium chloride. The combined acetone extracts were dried, the residue leached with acetone, and the solution dried. The residue, which consisted of fine needles and some dark material, was leached with acetone, and the solution dried. The residue of fine needles represented about 15 per cent of the weight of the starting strandin. Neutral equivalent, 310.

The crystals of fatty acid obtained either by alkaline or by acid hydrolysis contained small concentrations of N, 0.4 to 0.8 per cent. In one case in which nitrogen-free needles were obtained, the neutral equivalent value was 306. These needles melted sharply at 55°.

**Nature of Chromogenic Group in Strandin**—The only evidence for the presence of such a group is the production of brown color when strandin
is heated with 6 N HCl at 100°. That the production of this color is attributable to a specific constituent and not to the whole strandin molecule is shown by the fact that, after alkaline hydrolysis with 2 N NaOH at 100° overnight, the hydrolysate, on being treated with 6 N HCl at 100° for 15 minutes, produces the same intensity of brown color as the intact strandin. That this chromogenic residue is distinct from the other four residues described is shown by the fact that it is the only one to be destroyed by short time acid hydrolysis.

Free Acid Groups in Strandin—As isolated by the methods described here, strandin contains small amounts of inorganic cations. These can be removed as follows: Strandin is dissolved in 0.1 N HCl, and the solution dialyzed exhaustively against cold distilled water and dried. A residue consisting of typical strandin strands is obtained. Neutral equivalent to pH 8.2, 1500. The acid groups in strandin must be carboxyl groups, since the amounts of P and S present in strandin are not enough to provide more than a small fraction of the titratable acidic groups present.

SUMMARY

1. From brain tissue a substance of high molecular weight has been isolated, to which the name of strandin has been given because, on drying from aqueous solutions, it forms strands which show good orientation under polarized light. It is freely soluble in water and chloroform. It is extracted from tissue with a 2:1 chloroform-methanol mixture by volume. It is especially abundant in gray matter.

2. Strandin contains 2.6 per cent N, <0.2 per cent P, and <0.2 per cent S. It contains <1.5 per cent neuraminic acid. Among its constituents are fatty acids, sphingosine or a sphingosine-like substance, carbohydrate, a primary amine which is combined in strandin through its NH₂ group, and a chromogenic group. The latter is destroyed quantitatively with production of brown color on heating with 6 N HCl at 100° for 15 minutes. This has been used for the development of a method for the estimation of strandin.

3. Strandin has been prepared by three different methods. Essentially identical products have been obtained. They are all electrophoretically homogeneous and in the ultracentrifuge they show a main component (80 per cent), with a minimal possible molecular weight of 250,000.

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