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The Distribution of Gangliosides in Subcellular Fractions of Guinea-Pig Cerebral Cortex

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Gangliosides are acidic, acid-sensitive, non-dialysable, water-soluble lipids containing *N*-acetylneuraminic acid, hexosamine, hexoses and ceramides which in the brain occur mainly in grey matter. The aim of the present investigation was to study the localization of gangliosides in the cerebral cortex by following their distribution in subcellular fractions, neuraminic acid content being used as marker. This investigation followed from the findings of McIlwain (1960*a*, *b*, 1961) that gangliosides from ox brain, mucopolysaccharides and mucoproteins containing neuraminic acid restore the respiratory response to electrical stimulation in cerebral-cortex slices in which this response had been abolished by cold pretreatment of the tissue or by protamines, histones or synthetic poly-L-lysine. The lack of response to electrical pulses in slices pretreated in cold media appears to be due to a migration of the tissue's histone from the nucleus and its combination with acidic, extranuclear sites (Wolfe & McIlwain, 1961). McIlwain (1960*a*, 1961) suggested that the inhibitory action of basic proteins is due to combination with tissue gangliosides which may be involved normally in the processes of excitation or recovery. These results were discussed with Dr W. E. van Heyningen, who had tentatively identified gangliosides as receptors of tetanus toxin in neural tissues (van Heyningen, 1959).

A short account of this work has already appeared (Wolfe, 1960).

EXPERIMENTAL

Preparation of homogenates

Guinea pigs were stunned by a blow on the neck, the throat was cut for bleeding to death and the brain removed. The cerebral hemispheres were removed from the brain stem; by opening the ventricles and by dissection with a blunt instrument the layers of subcortical white matter were removed. The cerebral-cortical tissue was then weighed on a torsion balance and placed in ice-cold 0·32M-sucrose adjusted to pH 7·0 (with NaOH). A medium/tissue (v/w) ratio of 10 was used. Homogenization was carried out in a precooled, model 10 Emanuel–Chaikoff hydraulic tissue homogenizer (Microchemical Specialties Co.) with an orifice of 25μ after preliminary brief dispersion in a glass homogenizer with a polythene plunger.

Preparation of subcellular fractions

Method 1: *differential centrifuging.* The fractionation of the 0·32M-sucrose homogenates was carried out in a no. 40 rotor of a Spinco model L preparative ultracentrifuge. All operations were conducted at 0–4° and the separated fractions were examined with a phase-contrast microscope. The following fractions were prepared: (1) The residue sedimenting after 10 min. at 800 *g* (R1) consisting mainly of nuclei, blood vessels and myelin fragments. (2) The residue sedimenting after 10 min. at 5000 *g* (R2) consisting mainly of mitochondria but also containing small glial nuclei and whorls of myelin. (3) The residue sedimenting after 10 min. at 20 000 *g* (R3) consisting of small mitochondria and large microsomal particles. (4) The residue sedimenting after 95 min. at 105 400 *g* (R4) consisting of the microsomal and post-microsomal particles. When examined under the high power of the phase-contrast microscope this fraction appears as a shimmering mass of indistinct particles. (5) The supernatant (S1). Fractions R1 and R2 were washed twice and R3 once by resuspension in 10 vol. of 0·32M-sucrose and centrifuging. The washings were added to the next fraction. After each centrifuging the supernatant fractions were removed with a Pasteur pipette. Particular care was necessary in removing the supernatant (S1) from the microsomal fraction (R4) to avoid disturbing the smallest particles on the surface of the pellet.

Method 2: *differential centrifuging and subfractionation in density gradients.* The separation of the primary subcellular fractions and further subfractionation of the 'crude mitochondrial' fraction was by equilibrium centrifuging in sucrose density gradients. The following fractions were prepared: (1) The residue sedimenting after 10 min. at 800 *g* (P1). (2) The residue sedimenting after 20 min. at 22 500 *g* (P2). (3) The residue sedimenting after 95 min. at 105 400 *g* (P3). (4) The supernatant (S2).

The P2 fraction was separated into subfractions by layering the pellet resuspended in 10 ml. of 0·32M-sucrose over a density gradient consisting of 10 ml. of 0·8M-sucrose and 10 ml. of 1·2M-sucrose and centrifuging in the SW25·1 rotor of the Spinco ultracentrifuge, at 53 500 *g* for 140 min. Three distinct layers were apparent after centrifuging. (1) A layer (P2A) of white material between the 0·32 and 0·8M-sucrose layers. (2) A layer (P2B) of greyish-white material between the 0·8 and 1·2M-sucrose layers. (3) A tan-coloured residue (P2C) beneath the 1·2M-sucrose layer. The layers were separated by means of a Pasteur

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pipette with the tip bent at right angles to the body of the pipette. The separated layers were brought to approx. 0·3 M-sucrose and centrifuged at 105 400 *g* for 20 min.

Determination of gangliosides

Extraction. The particulate fractions prepared as described above were extracted with cold (4–5°) $CHCl_3$–methanol (2:1, v/v) and subjected to solvent partition as described by Folch, Lees & Sloane-Stanley (1957), the choice of some conditions being those of Long & Staples (1959). The procedure used is outlined in Fig. 1. The supernatant fractions at stage 3 were evaporated to dryness at 60° in a rotary evaporator and the residue was extracted with $CHCl_3$–methanol. Most of the sucrose in the particulate fractions remained in the residue after the second extraction with $CHCl_3$–methanol. The remainder of the sucrose was removed by dialysis. Dialysis was carried out for 16 hr. in 0·25 in. diam. Visking tubing (Viscose Development Co., London) in cylindrical jars (approx. 17 l. capacity) with two changes of water. The water was agitated with a Vibromix stirrer (Shandon Scientific Co. Ltd., London). After dialysis, the sacs were emptied and rinsed and the contents made to a standard volume.

Estimation of neuraminic acid. Measured quantities of the dialysed extracts of the subcellular fractions were evaporated to dryness and determinations of the neuraminic acid content were carried out with Bial's orcinol–$FeCl_3$–HCl reagent according to the procedure of Böhm, Dauber & Baumeister (1954) as modified by Long & Staples (1959). Synthetic *N*-acetylneuraminic acid was used as standard.

Other procedures and materials

Nitrogen in the various subcellular fractions was estimated by the usual micro-Kjeldahl procedure with selenium oxide–$CuSO_4$ mixture as catalyst.

Ultrasonic disruption. In experiments using ultrasonic-disruption procedures a Mullard–MSE ultrasonic disintegrator (60 w, 20 kcyc./sec.) was used. (We are indebted to Professor C. H. Gray for the use of this apparatus.) The suspensions were placed in a thick-walled glass tube supported in an ice bath. The stainless-steel stub, 19 mm. diam., was immersed 4 mm. below the surface.

Materials. All chemicals and solvents used were of A.R. grade. Protamine (clupein sulphate) and lysozyme chloride were obtained from L. Light and Co. Ltd. Calf-thymus histone was prepared according to the procedure of Luck, Rasmussen, Satake & Tsvetikov (1958). The following materials were gifts: a preparation of ox-brain ganglioside, *N*-acetylneuraminic acid content 26·5%, from Professor H. McIlwain; synthetic *N*-acetylneuraminic acid from Miss Patricia Carroll; egg lysolecithin from Professor R. H. S. Thompson.

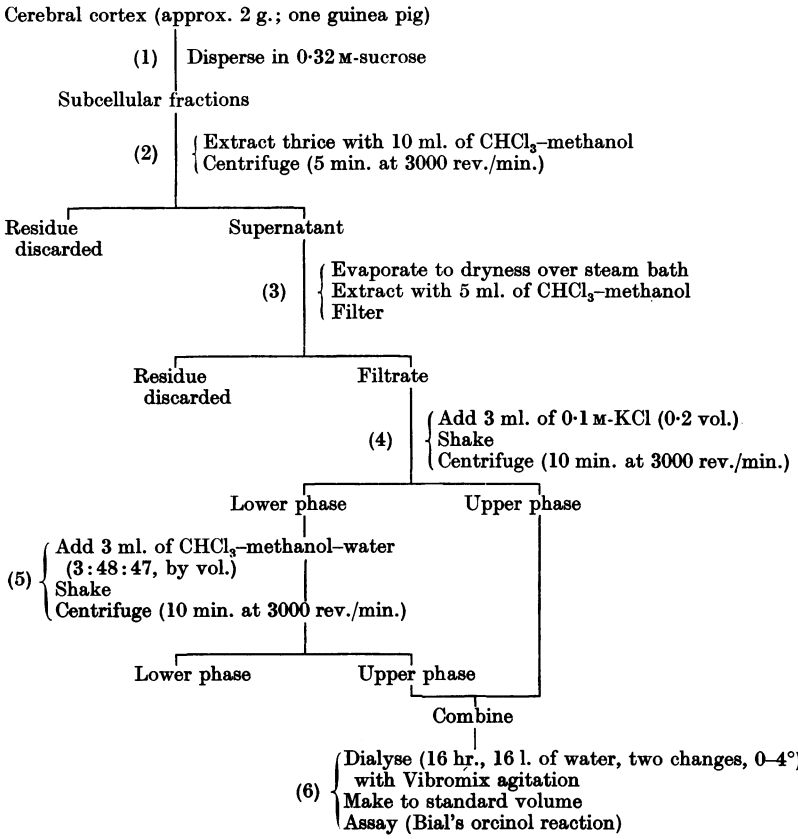

Fig. 1. Procedure for the extraction of gangliosides from subcellular fractions.

RESULTS

Occurrence of gangliosides in particulate form

When gangliosides were extracted from the particulate and supernatant fractions obtained after centrifuging homogenates of guinea-pig cerebral cortex at 105 400*g* for 30 min., 22 % of the gangliosides occurred in the supernatant fraction. However, if the time of centrifuging was increased to 95 min. (i.e. an integrated field of about 10 000 000*g*-min.) no gangliosides were obtained from the supernatant fractions (Table 1). Therefore under these latter conditions gangliosides apparently occur entirely in particulate fractions. From the results shown in Table 1 it is concluded that some gangliosides occur bound to the very smallest, post-microsomal fragments of cerebral-cortex homogenates. Particular care was required in removing the supernatant fractions from the pellets so that the delicate uppermost material was not disturbed. On occasions when this was disturbed accidentically, gangliosides were recovered from the supernatant fraction.

The values obtained for the total ganglioside content of homogenates are in good agreement with those obtained by Long & Staples (1959) for the grey matter of the rat and by Dr S. Balakrishnan in this laboratory for the grey matter of the guinea pig.

Recovery of added gangliosides. From the results shown in Table 2 it is clear that if an aqueous solution of ox-brain ganglioside is added to homogenates 94 % of it is recovered in the total particulate fraction. When the nuclear fraction was analysed 87 % of the added gangliosides were recovered. From these results it appears that soluble gangliosides added to homogenates of cerebral cortex form an insoluble complex with some component of the nuclear fraction. Evidence presented below suggests that these components are the nucleohistones.

Distribution of gangliosides in subcellular fractions

Fractions prepared by differential centrifuging only. The ganglioside contents of subcellular fractions prepared by Method 1 (see the Methods section) are shown in Table 3. It is shown that the microsomal fraction (R 4) contains the greatest amount of gangliosides and the mitochondrial fraction (R 2) the least. Unwashed nuclear fractions were found to contain up to 110 μg. of *N*-acetylneuraminic acid/g. of fresh tissue in the extracts. However, this could be decreased by approximately 50 % with two washings with 0·32 M-sucrose. Washing up to four times did not further lower the ganglioside content.

Subcellular fractions were prepared by Method 2 (see the Methods section) without further sub-

Table 1. *Occurrence of gangliosides in particulate fractions*

Procedures for the extraction of gangliosides and the determination of *N*-acetylneuraminic acid are described in the Methods section. The values are converted into μmoles by dividing by 309 (mol.wt. of *N*-acetylneuraminic acid).

Fraction	Centrifugal force: *g*	Centrifugal force: min.	*N*-Acetylneuraminic acid (μg./g. fresh wt. of tissue) (mean ± S.E.M.)
Residue	105 400	30	312 ± 16 (3)
Supernatant	—	—	88 ± 9 (3)
		Total	400 ± 11 (1·28 ± 0·04 μmoles)
Residue	105 400	95	476 ± 87 (8)
Supernatant	—	—	0 (8)
		Total	476 ± 87 (1·54 ± 0·28 μmoles)

Table 2. *Recovery of gangliosides added to homogenates of guinea-pig cerebral cortex*

The 0·32 M-sucrose homogenates were divided into two equal portions and to one portion 400 μg. of ox-brain gangliosides (containing 106 μg. of *N*-acetylneuraminic acid) in 1 ml. of 0·32 M-sucrose was added and to the other portion 1 ml. of 0·32 M-sucrose. The portions were then centrifuged at 105 400*g* for 95 min. or the nuclear fraction was separated as outlined in the Methods section.

Fraction	Centrifugal force: *g* (av.)	Centrifugal force: min.	*N*-Acetylneuraminic acid (μg./g. fresh wt. of tissue) (mean ± S.E.M.): Gangliosides added	*N*-Acetylneuraminic acid (μg./g. fresh wt. of tissue) (mean ± S.E.M.): No gangliosides added	Mean recovery (%)
Total particulate	105 400	95	527 ± 48 (3)	427 ± 50 (3)	94
Supernatant	—	—	0	0	—
Nuclear	800	10	157 ± 8 (3)	65 ± 10 (3)	87
Ganglioside only in 0·32 M-sucrose	105 400	95	None sedimented		

fractionation of the P2 fraction in a density gradient. The ganglioside content of these fractions is shown in Table 4. It is seen that the P2 fraction contains the greatest amount of gangliosides. Thus an increase in the centrifugal force from 200 000*g*-min. used in the separation of fraction R3 (Table 3) to 450 000*g*-min. used in the separation of fraction P2 (Table 4) results in the sedimenting of particles that contain considerable quantities of gangliosides.

Fractions prepared by differential centrifuging and subfractionation in a density gradient. Subcellular fractions were prepared by Method 2 (see the Methods section) and the P2 fraction was further subfractionated in a sucrose density gradient. The gangliosides present in these fractions are shown in Table 5. The P2 fraction contained the greatest amount of gangliosides. Of the subfractions the middle layer (P2B), consisting of particles intermediate in density between 0·8 and 1·2 M-sucrose, contained 56·5% of the gangliosides of the P2 fraction.

Although the P2B fraction contains the greatest amount of gangliosides expressed as μg. of *N*-acetylneuraminic acid/g. fresh wt. of tissue, if the results are expressed in μg. of *N*-acetylneuraminic acid/mg. of nitrogen the microsomal fraction, P3, contains the highest concentration of gangliosides.

Table 3. *Distribution of gangliosides in subcellular fractions of guinea-pig cerebral cortex*

Fractions were prepared by Method 1 as outlined in the Methods section. The ganglioside contents are expressed as μg. of *N*-acetylneuraminic acid in the dialysed, aqueous-partitioned $CHCl_3$–methanol extracts of the fractions.

Fraction	Centrifugal force: *g*	Centrifugal force: min.	*N*-Acetylneuraminic acid (μg./g. fresh wt. of tissue)	*N*-Acetylneuraminic acid: % of total
R1	800	10	52	11·4
R2	5 000	10	16	3·5
R3	20 000	10	82	17·8
R4	105 400	95	309	67·3
S1	—	—	0	0
		Total	459 (1·48 μmoles)	

Table 4. *Distribution of gangliosides in subcellular fractions of guinea-pig cerebral cortex*

The primary fractions were prepared by Method 2 as outlined in the Methods section. Other details were as described in Fig. 3.

Fraction	Centrifugal force: *g*	Centrifugal force: min.	*N*-Acetylneuraminic acid (μg./g. fresh wt. of tissue)	*N*-Acetylneuraminic acid: % of total
P1	800	10	40	9·0
P2	22 500	20	268	60·9
P3	105 400	95	139	31·1
S2	—	—	0	0
		Total	447 (1·45 μmoles)	

Table 5. *Distribution of gangliosides in subcellular fractions of guinea-pig cerebral cortex*

The primary fractions were prepared by Method 2 as outlined in the Methods section. The P2 fraction was layered over a sucrose density gradient. The P2A fraction contains particles in the upper layer, the P2B contains particles from the middle layer and the P2C contains particles passing through the 1·2 M-sucrose layer.

Fraction	*N*-Acetylneuraminic acid (μg./g. fresh wt. of tissue)	*N*-Acetylneuraminic acid: % of total	Nitrogen (mg./g. fresh wt. of tissue)	*N*-Acetylneuraminic acid (μg./mg. of nitrogen)
P1	53	9·2	3·0	17
P2A	140	24·4	2·5	56
P2B	231	40·2	4·7	50
P2C	38	6·6	1·8	21
P3	112	19·6	1·3	86
S2	0	0	4·8	0
Total	574 (1·87 μmoles)		18·1	—

Distribution of gangliosides in fractions after ultrasonic disruption

Ultrasonic treatment (for 10 min.) of the suspended particles remaining after the removal of the nuclear fraction caused a shift in the *N*-acetylneuraminic acid-containing material from the P2 to the P3 fractions with a small amount appearing in the supernatant fraction (Tables 6*A* and *B*). Therefore ultrasonic treatment disrupts particles normally sedimenting in the P2 fraction so that they now sediment in the microsomal fraction P3. It does not solubilize gangliosides to any appreciable extent. Considerable solubilization of other constituents does occur, as shown by the increase in nitrogen content of the supernatant fraction. These results indicate that the ganglioside-containing fragments in the P2 fraction, of which the greater proportion are found in the P2B layer (Table 5), are further broken by ultrasonic treatment into smaller fragments which are, however, still sedimentable by centrifuging.

Ultrasonic treatment of homogenates in which the nuclear fraction had not been removed considerably lowered the amounts of gangliosides obtained from the total particulate fraction (Table 6*C*). Ultrasonic irradiation causes the disruption of nuclei and leads to the degradation of nucleoproteins (see Medawar & Zubay, 1959). It is possible that the basic substances released from nuclei after ultrasonic irradiation have combined with the gangliosides associated with other subcellular particles in the homogenate and prevented their extraction by chloroform–methanol (S. Balakrishnan & H. McIlwain, in preparation).

Effect of various treatments of the homogenate on the extraction of gangliosides

Calcium chloride. The addition of calcium chloride (5 mM) to homogenates, followed by their separation into subcellular fractions, caused most of the tissue gangliosides to sediment with the nuclear fraction (P1). This is most likely to be due to clumping and agglutination of cytoplasmic particulates in the presence of bivalent ions.

Incubation and dialysis. Incubation of 0·32 M-sucrose homogenates of cerebral cortex in a stoppered vessel for 90 min. at 37·5° and then separation of the homogenate into particulate and supernatant fractions caused a 50 % loss of gangliosides. Dialysis of the homogenate against 16 l. of distilled water for 4 hr. caused an even greater loss of gangliosides (Table 7). Prolonged dialysis for 16 hr. resulted in complete loss of neuraminic acid-containing materials from both particulate and supernatant fractions.

Lysozyme and lysolecithin. Incubation of homogenates with 2 mg. of lysozyme chloride for 30 min. at 37·5° caused no loss or change in the distribution of gangliosides. However, incubation with high concentrations of the cytolytic reagent, egg lysolecithin, caused a considerable loss of the extractable gangliosides from the total particulate fraction without a corresponding increase in the supernatant fraction (Table 7).

Trypsin. Digestion of homogenates with 2 mg. of crystalline trypsin had little effect on the content or distribution of gangliosides (Table 7).

Sodium deoxycholate. Sodium deoxycholate, 0·5 %, in 0·01 M-phosphate buffer, pH 7·4, lowered

Table 6. *Distribution of gangliosides in subcellular fractions of guinea-pig cerebral cortex after ultrasonic irradiation*

The fractions were prepared by Method 2 as outlined in the Methods section. (*A*) Ultrasonic treatment (for 10 min.) of the homogenate after the separation of the nuclear fraction. (*B*) No ultrasonic treatment. (*C*) Ultrasonic treatment of the homogenate, nuclei were not removed and the particulate and supernatant fractions were then separated by centrifuging at 105 400*g* for 95 min.

	Fraction	Nitrogen (mg./g. fresh wt. of tissue)	*N*-Acetylneuraminic acid (μg./g. fresh wt. of tissue)
(*A*)	P1	2·4	39
	P2	3·9	88
	P3	4·8	239
	S2	6·4	14
	Total	17·5	380 (1·23 μmoles)
(*B*)	P1	2·4	39
	P2	9·7	230
	P3	1·6	94
	S2	4·2	0
	Total	17·9	363 (1·18 μmoles)
(*C*)	Total particulate	—	106
	S2	—	0
		Total	106 (0·34 μmole)

Table 7. *Gangliosides in particulate and supernatant fractions after various treatments*

In these experiments 10 ml. of 0·32 M-sucrose homogenate of guinea-pig cerebral cortex containing 1 g. of tissue was subjected to a variety of treatments and separated into a total particulate and supernatant fraction by centrifuging at 105 400*g* for 95 min. The gangliosides were extracted and determined as outlined in the Methods section.

Treatment	*N*-Acetylneuraminic acid (μmoles/g. fresh wt. of tissue)	
	Particulate	Supernatant
None	1·54±0·28 (8)	0 (8)
Incubated (90 min., 37·5°)	0·79	0
Dialysed (4 hr., water)	0·37	0
Dialysed (16 hr., water)	0	0
Lysozyme (2 mg.; 30 min., 37·5°)	1·55	0
Lysolecithin (2 mg.; 30 min., 37·5°)	1·22	0
Lysolecithin (30 mg.; 30 min., 37·5°)	0·60	0
Trypsin (2 mg.; 3 hr., 37·5°)	1·20	0·06
Sodium deoxycholate (0·5 %, pH 7·4)	0·60	0·03
EDTA (0·1 M, sodium salt)	1·19	0·34
Clupein sulphate (1 mg.)	0	0
Calf-thymus histone (1 mg.)	0	0

Table 8. *Affinity of subcellular fractions of guinea-pig cerebral cortex for basic proteins compared with their ganglioside content*

Subcellular fractions were prepared by Method 2 as outlined in the Methods section. The P 1 fraction was washed three times and the P 2 A, P 2 B, P 2 C and P 3 fractions were washed once with 0·14 M-NaCl and then resuspended in 0·14 M-NaCl and centrifuged at 105 400*g* for 30 min. Quantities (200 mg.) of each pellet were weighed into 2 ml. polythene Spinco tubes and 2 ml. of a solution of protamine (0·5 mg./ml.) or calf-thymus histone (1·0 mg./ml.) prepared in 0·01 M-phosphate buffer, pH 7·4, was added. The tubes were shaken for 5 min. to resuspend the particles and then centrifuged at 105 400*g* for 30 min. Portions (1 ml.) of the supernatants from the tubes were hydrolysed with 6 N-HCl and the arginine content was determined by the Sakaguchi reaction (see Wolfe & McIlwain, 1961). The results are expressed in μg. of arginine remaining in the supernatant and compared with protamine or histone solutions without addition of subcellular particles. Results for *N*-acetylneuraminic acid content of extracts of pellets are derived from Table 5.

Fraction	Moist wt. of pellet (mg./g. fresh wt. of tissue)	*N*-Acetylneuraminic acid			Arginine content (μg./ml.) of supernatant after addition of basic protein to pellet	
		μg./g. fresh wt. of tissue	μg./mg. of nitrogen	μg./200 mg. moist wt. of pellet	Protamine	Histone
P 1	505	53	17	21	195	115
P 2 A	100	140	56	280	138	95
P 2 B	230	231	50	199	63	55
P 2 C	94	38	21	81	131	139
P 3	100	112	86	224	30	30
Protamine alone	—	—	—	—	217	—
Histone alone	—	—	—	—	—	146

the total gangliosides extractable with no gain of gangliosides in the supernatant fraction (Table 7).

Ethylenediaminetetra-acetic acid. No effect of ethylenediaminetetra-acetic acid (EDTA) on the total amount of gangliosides was found. However, 22 % of the total now occurred in the supernatant fraction. This is believed to be due to the greater difficulty in separating the supernatant fraction from the light, postmicrosomal membranes in the presence of EDTA. These postmicrosomal membranes have been shown to contain gangliosides (Table 1).

Basic proteins. When the protamine, clupein sulphate or calf-thymus histone was added to homogenates no gangliosides could be extracted subsequently from either the particulate or the supernatant fractions (Table 7).

Affinity of subcellular particles for basic proteins

The microsomal fraction has been shown to contain particles that form insoluble complexes with basic proteins (Wolfe & McIlwain, 1961). McIlwain (1960*a*, 1961) has produced evidence suggesting that extracted gangliosides form water-soluble, firmly bound complexes with basic proteins. In the tissue, however, gangliosides are present in water-insoluble cellular constituents and are rendered soluble only by certain extraction procedures. In Table 8 the removal of basic proteins from solution as insoluble complexes by

subcellular fractions is compared with the ganglioside content of these fractions. Fractions P2B and P3 show most affinity for basic proteins and also contain a high concentration of *N*-acetylneuraminic acid in the chloroform–methanol extracts. However, fraction P2A, although representing only 24% of the total tissue gangliosides, contains in a 200 mg. portion of the moist pellet a high concentration of gangliosides. This fraction does not show a corresponding affinity for basic proteins.

DISCUSSION

The experiments demonstrate that gangliosides are found mainly in the microsomal and postmicrosomal fractions of cerebral-cortex homogenates (Tables 1 and 3). However, when fractions were separated under conditions similar to those described by Hebb & Whittaker (1958), a large part of the gangliosides appeared in fraction P2 (Table 4). The centrifugal force used to separate this fraction (i.e. 22 500*g* for 20 min.) is considerably greater than is usually used for the separation of brain-mitochondrial fractions (see Brody & Bain, 1952; Hanzon & Toschi, 1959). Consequently fraction P2 contains particles which under conventional sedimentation procedures would occur in the microsomal fraction. When the particles of the P2 fraction were separated on the basis of density differences (Table 5) gangliosides were found mostly in particles of intermediate density (the P2B layer) and in least amount in the densest particles (the P2C layer). Studies of these fractions with the electron microscope (Whittaker, 1959; Gray & Whittaker, 1960) have shown that the P2A layer contains disrupted myelin fragments, the P2B layer contains pinched-off nerve endings, torn off during homogenizing, and the P2C layer contains mitochondria. The P2B fraction contains the synaptic vesicles enveloped within membranes, forming larger particles which appear identical with the terminal boutons seen in sections of cerebral cortex on the dendrites of neurones (Gray, 1959). It is therefore probable that gangliosides occur in fractions that contain fragments of cell membranes, particularly of dendrites. Parallel with this, the acidic mucopolysaccharides of bacteria containing muramic acid (3-*O*-α-carboxyethyl-D-glucosamine), a substance closely related to neuraminic acid, occur entirely within cell membranes (Strange & Kent, 1959; O'Brien, Glick & Zilliken, 1960).

It is now known that much of the microsomal fraction arises through fragmentation of the ergastoplasm during homogenizing and contains the endoplasmic reticulum and the ribonucleoprotein particles (Palay & Palade, 1955; Palade & Siekevitz, 1956; Porter, 1957). However, other cytological entities do occur. It is probable that the gangliosides occurring in the postmicrosomal particles are associated with the delicate fragments of membranes unattached to other subcellular constituents. It is unlikely that gangliosides are associated with the ribonucleoprotein particles in the microsomal fractions since EDTA in high concentrations has little effect on their distribution. Bivalent cations are required to maintain the integrity of these particles and chelating agents such as EDTA make them soluble (see Roberts, 1958).

The studies of McIlwain (1960*a*, *b*, 1961) have shown that ox-brain gangliosides combine with basic proteins. Harris & Saifer (1960*a*) subsequently noted that the metachromasia of strandin is suppressed by protamine sulphate, and these authors also have demonstrated a direct interaction between strandin and protamine by a turbidimetric procedure, finding it dependent on the ionic environment (Harris & Saifer, 1960*b*). The present investigation has also indicated an interaction between basic proteins and gangliosides. However, the presence of other acidic substances in subcellular fractions which may also combine with basic proteins must be considered. It should be noted that subfraction P2A, which apparently contains mainly myelin fragments, also contains gangliosides, and yet this fraction does not show a corresponding affinity for basic proteins (Table 8). At present this remains an anomaly. Klenk (1947) claimed that white matter contains negligible amounts of gangliosides, but Long & Staples (1959) reported a ganglioside content of white matter of about half that of grey matter for the rat brain. It is possible that the gangliosides associated with myelin elements may occur in less reactive combinations than do grey-matter gangliosides.

SUMMARY

1. Gangliosides occurred mainly in the microsomal fraction of homogenates of guinea-pig cerebral cortex separated by usual differential-centrifuging techniques. Within the microsomal fraction gangliosides were associated with two groups of particles differing considerably in sedimenting properties.

2. Ganglioside-rich particles were found in a subcellular fraction separating as a layer between 0·8 and 1·2 M-sucrose after equilibrium centrifuging in a density gradient.

3. Ox-brain gangliosides added to homogenates were recovered almost completely from the nuclear fraction.

4. Ultrasonic treatment of homogenates after removal of the nuclear fraction caused a shift in gangliosides to subcellular fractions containing

smaller particles but did not solubilize them. Much less gangliosides were extracted after ultrasonic treatment of homogenates containing nuclei.

5. The effect of added agents and various treatments to homogenates on the quantity of gangliosides extracted from particulate and supernatant fractions was studied.

6. Addition of protamine or histone completely prevented the extraction of gangliosides.

7. A comparison of the affinity of subcellular fractions for basic proteins with the content of gangliosides was made. The fractions containing most of the tissue gangliosides showed the greatest affinity for basic proteins except a fraction rich in myelin fragments.

8. The probable localization of gangliosides in cell membranes is discussed.

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Oestriol Metabolism by Rat- and Rabbit-Liver Slices

ISOLATION OF 2-METHOXYOESTRIOL AND 2-HYDROXYOESTRIOL

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When Marrian, Loke, Watson & Panattoni (1957) isolated 16α-hydroxyoestrone (3:16α-dihydroxyoestra-1:3:5-trien-17-one) from pregnancy urine, they suggested that this compound might be the intermediate in the conversion of oestrone (3-hydroxyoestra-1:3:5-trien-17-one) into oestriol (oestra-1:3:5-triene-3:16α:17β-triol). To test this suggestion, experiments were started to see if liver preparations could convert 16α-hydroxyoestrone into oestriol. Rat-liver slices could readily bring about this reduction but during the experiments the observation was made that the yield of oestriol was dependent on the gas phase above the incubation medium. When incubation was under air the yield of oestriol was 10% but increased to 22% on carrying out the incubation under nitrogen (R. J. B. King & G. F. Marrian, unpublished results). A number of other workers have found that oestrogen metabolism by rat-liver preparations is influenced by the gas phase used during the incubation (Ryan & Engel, 1953; Szego, 1953; Riegel & Mueller, 1954).

A number of new oestrogen derivatives have been isolated from biological material in recent years. These include 6′α′-hydroxyoestradiol-17β (oestra-1:3:5-triene-3:6′α′-17β-triol) and 6-oxo-oestradiol-17β (3:17β-dihydroxyoestra-1:3:5-trien-6-one)

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