Incorporation of Tritiated Water (HTO) and Organically Bound Tritium (OBT) into Phospholipids and Gangliosides of Rat Brain

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Three generations of rats were long-term exposed to HTO in drinking water at activity of 37.0 kBq/ml or to food containing OBT at activity of 48.1 kBq/g. The rats consumed tritiated water and tritiated food ad libitum. In the experiment the \( F_1 \) and \( F_2 \) generation of rats were exposed continuously from conception to the 21-st or 120-th day of age and rats of \( F_3 \) generation during 22 days of their intrauterine life. It was found that the amount of tritium incorporated into the major rat brain phospholipids and gangliosides after administration of tritiated food was higher than after administration of tritiated water. Tritium activity in all studied phospholipids and gangliosides was the highest in 21-day-old rats exposed during both pregnancy and lactation.

INTRODUCTION

Earlier studies of the incorporation of tritiated water and organically bound tritium from food into organic compounds of brain\(^1,2\) showed that OBT accumulates into amino acids, proteins, nucleic acids and lipids to a higher degree than HTO. However, the lipid fractions responsible for this accumulation have not yet been identified.

The aim of the present study was to examine tritium incorporation into the brain phospholipids and gangliosides of rats exposed for three generations to tritiated water and to organically bound tritium in food.

MATERIALS AND METHODS

The study was performed on two groups of Wistar rats, one of which was exposed to HTO (HTO-group) and the other was given food containing OBT (T-food group). The tritium activity of tritiated water was 37.0 kBq/ml and of tritiated food 48.1 kBq/g. Tritiated food used in the experiment was a mixture of tritiated rabbit meat and powdered standard chow. The method of preparation of tritiated meat has been described in detail by Pietrzak-Flis et al.\(^3\)

The tritiated meat was composed of 76.1 ± 1.4 per cent weight of proteins, 1.1 ± 0.1 per cent of nucleic acid and 22.8 ± 0.9 per cent of lipids. Rabbit meat proteins contained 49.9
± 0.5 per cent of total tritium activity of the dry meat, nucleic acid 19.2 ± 2.3 per cent and lipids 27.4 ± 2.9 per cent. The distribution of tritium among amino acids of proteins contained in food was not uniform (Table 1). The highest tritium content was observed in phenylalanine, tyrosine, methionine and cysteine. Small quantities of tritium were found in tryptophan, lysine and proline. All other amino acids of rabbit meat proteins did not contain radioactivity.

Table 1. Distribution of tritium activity among amino acid residues of rabbit proteins contained in tritiated food: results of quantitative analysis after two-dimensional thin-layer chromatography.

| Amino acid       | Tritium activity expressed as per cent of total amino acids radioactivity (mean ± S.D.) |
|------------------|----------------------------------------------------------------------------------------|
| Lysine           | 1.1 ± 0.3                                                                               |
| Histidine        | 0                                                                                       |
| Arginine         | 0                                                                                       |
| Aspartic acid    | trace                                                                                   |
| Glutamic acid    | trace                                                                                   |
| Proline          | 0.9 ± 0.2                                                                               |
| Glycine          | 0                                                                                       |
| Serine           | 0                                                                                       |
| Threonine        | 0                                                                                       |
| Cysteine         | 9.4 ± 0.8                                                                               |
| Tyrosine         | 28.4 ± 1.1                                                                              |
| Alanine          | 0                                                                                       |
| Valine           | 0                                                                                       |
| Leucine          | 0                                                                                       |
| Isoleucine       | 0                                                                                       |
| Methionine       | 17.5 ± 0.9                                                                              |
| Phenylalanine    | 39.8 ± 1.3                                                                              |
| Tryptophan       | 3.2 ± 0.5                                                                               |

Details of TLC of amino acids were presented in the previous paper (2).

The exposure of three-month-old parent females (P₀) began three weeks before mating with nonexposed males of the same strain, and continued at the same tritium regimen up to delivery of F₃ generation. All P₀ females delivered naturally, on the third day postnatally the pups were weighed and the litters were normalised to eight pups per dam. On the 21-st day the pups were weaned. At weaning one male and one female of each litter were killed and their brains were taken for analysis. The remaining F₁ female rats at the age of three months were mated with nonlitter-mate males of the same group. All F₁ dams delivered naturally and the litters were normalised to eight pups per dam on the 3-rd day postnatally. The F₂ pups were weaned on day 21 and one female and one male of each litter and the F₁ dams were sacrificed. At the age of about three months the F₂ females were mated with nonlitter-mate
males of the same group. At full term the F₃ litters were delivered by Caesarian section from F₂ dams which had commenced natural delivery and the term fetuses were dissected. The time schedule of experiment is given in scheme 1.

The whole brains of dissected rats were immediately removed, weighed and frozen. The

**Scheme 1. The time schedule of experiment.**

| START OF EXPOSURE OF P₀ FEMALES | 21 d |
|---------------------------------|------|
| MATING                          | 22 d |
| BIRTH OF F₁                    | 21 d |
| WEANING                         |      |
| Section of some 21-day-old pups of the F₁ generation |
| MATING                          | 22 d |
| BIRTH OF F₂                    | 21 d |
| WEANING                         |      |
| Section of all F₁ females and some of 21-day-old pups of the F₂ generation |
| MATING                          | 22 d |
| BIRTH OF F₃ BY CAESARIAN SECTION |      |
| Section of all F₂ females and all the term fetuses of the F₃ generation |
duration of the total experiment, from the treatment of female rats P0 for a period of three
weeks before mating for assignation of equilibrium of tritium activity in body water to delivery
by Cæsarian section of F3 generation, was 277 days.

Each brain of the dissected animals of the F1 and F2 generations was homogenized,
lyophylized, equilibrated with water and lyophylized again to remove the exchangeable tritium
from brain tissues. The brains of F3 fetuses were pooled for each litter, twice lyophylized to
remove the exchangeable tritium and used for further analysis.

Lipids from the total homogenate of dry brain tissue were extracted with chloroform:
methanol:water (16:8:1 v/v) according to procedure of Folch et al. The extract was then
partitioned into an methanolic-aqueous phase, containing gangliosides and an chloroform
phase, containing other lipids together with phospholipids, by addition of 0.15 M KCl aqueous
solution as described by Folch et al. 4)

The methanolic-aqueous phase was dialysed for 48 hr against water at 4°C and then
concentrated by lyophylization. The lyophylized sample was dissolved in minimum of methanol,
filtered and the gangliosides precipitated by the addition of two volumes of diethyl ether. This
precipitate was redisssolved in a minimum warm methanol and the gangliosides crystallized
by cooling the solution. The crystalline product was dissolved in a chloroform:methanol (2:1
v/v) to attain a concentration of approximately 1 mg/ml. This solution was fractionated by
thin-layer chromatography on 20 x 20 cm precoated silica gel plates (thickness 0.25 mm)
type DC-Fertigplatten Kieselgel 60 from Merck A. G. (Darmstadt, Germany), using chloroform:
methanol:2.5 N amonia (60:35:8 v/v) as developing systems. A mixture of gangliosides
obtained from Sigma (St. Louis, Mo., U.S.A.) served as reference substances. Visualization of
gangliosides was performed by using resorcinol-HCl reagent or iodine vapour. Colorimetric
determination of individual gangliosides, scraped from TLC plates, was carried out by the
resorcinol method of Svennerholm 7) as modified by Miettinen and Takki-Luukkainen 9).
For determination of tritium activity in the rat brain gangliosides the silica gel layers containing
individual gangliosides were scraped and suspended in 15 ml of toluene-PPO-POPOP with 4%
Cab-O-Sil. All radioactivity measurements were carried out by liquid scintillation spectrometer
(Model 3315 Tri-Carb Packard Inst. Co.) with an efficiency of 25%.

The chloroform phase was concentrated in vacuo to volume about 0.2 ml and the phospho-
lipids were precipitated by addition of 5 ml of acetone and 0.1 ml of 10% MgCl2·6H2O in
methanol. The precipitate was dissolved with chloroform:methanol (2:1 v/v) to attain a
concentration of approximately 10 mg/ml. This solution was fractionated by two-step thin-
layer chromatography on plates 20 x 20 cm coated with a layer (0.5 mm on adjustable
Desaga applicator) of adsorbent prepared from 30 g Silica Gel G (Merck A. G.) and 65 ml of
water. The chromatograms were first developed in chloroform:methanol:30% amonia:water
(140:50:7:3 v/v) and after drying in the mixture of chloroform:methanol:acetic acid:water
(160:20:4:1.5 v/v) 11). The spots were detected by iodine vapour and by ammonium molybdate-
perchloric acid reagent. Phospholipids fraction were identified by comparison with reference
compounds obtained from Sigma and by ninhydrin reagent for free-amino group of phos-
phatidylserine and phosphatidylethanolamine 12). Phosphorus determinations in fractions
revealed with iodine was carried out by the method of Bartlet 9) after elution of phospholipids
from adsorbent according to De Bohner et al. The lipid-bound phosphorus were determined after digestion with 60% perchloric acid. For determination the tritium activity in rat brain phospholipids, phospholipid spots were cut out, suspended in 15 ml of toluene-PPO-POPOP with 4% Cab-O-Sil and measured in a liquid scintillation spectrometer with a counting efficiency of 25%.

RESULTS

Five distinct phospholipid classes: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and sphingomyelin were well separated from the total brain phospholipids mixture of 21-day-old rats of F1 and F2 generation, 120-day-old F1 females after lactation, F2 females after Caesarian section and the F3 term fetuses. The changes with age in phospholipid composition of whole brain for F1, F2 and F3 generation of rats, obtained by lipid-bound phosphorus determination in fractions isolated after two-step TLC are given in Table 2.

It should be pointed out that the applied separation method was selected to obtain complete resolution of the complex phospholipids mixture but, it did not measure the content of different phospholipids with precision. The phospholipid composition obtained in this study may, therefore, deviate from composition obtained by more specific techniques.

The phospholipid composition of brain for F1 and F2 rats did not differ significantly (Student's t-test). No differences were also noted between brain phospholipid composition of rats of the HTO and T-food group.

The specific activities of the different phospholipids are given in Tables 3 and 4. Specific activities are calculated using the number of hydrogen atoms in the particular phospholipid classes. The statistical analysis of data by Student's t-test for the HTO-group and for the T-food group indicates that specific tritium activities in individual phospholipids of the F1 and F2 generations in each tritium treatment group did not differ significantly. However, the

| Fraction of phospholipids* | The term fetuses (14) | 21-day-old (17) | 120-day-old (34) |
|---------------------------|----------------------|-----------------|------------------|
| Pch                       | 5.25 ± 0.12          | 5.07 ± 0.24     | 5.64 ± 0.15      |
| Pea                       | 3.24 ± 0.30          | 4.30 ± 0.02     | 6.19 ± 0.21      |
| Ps                        | 1.43 ± 0.13          | 1.87 ± 0.09     | 3.17 ± 0.16      |
| Sph                       | 0.36 ± 0.02          | 0.90 ± 0.08     | 1.85 ± 0.12      |
| Pi                        | 0.39 ± 0.02          | 0.37 ± 0.05     | 0.51 ± 0.03      |

*Pch = phosphatidylcholine, Pea = phosphatidylethanolamine, Ps = phosphatidylserine, Sph = sphingomyelin, Pi = phosphatidylinositol.

Figures in brackets indicate number of pooled brains.

Results are given as mean ± S.D. of six replicate analysis of total phospholipids mixtures.
Table 3. Specific activities (kBq/mole of hydrogen) of major brain phospholipids and gangliosides in rats of different age continuously exposed to tritiated water.

| Fraction of lipids | 21-day-old | 120-day-old | The term fetuses |
|-------------------|------------|-------------|-----------------|
|                   | F₁         | F₂          | F₁             | F₂             | F₃             |
| Pch*              | 29.32 ± 2.38 | 32.07 ± 3.09 | 15.17 ± 1.25   | 14.57 ± 2.03   | 21.51 ± 2.45 |
| Pea*              | 28.83 ± 3.42 | 29.97 ± 3.05 | 12.09 ± 1.08   | 11.16 ± 0.94   | 14.24 ± 1.56 |
| Ps*               | 26.44 ± 3.61 | 28.82 ± 3.24 | 9.58 ± 0.82    | 9.38 ± 1.00    | 12.61 ± 1.78 |
| Sph*              | 10.33 ± 1.53 | 11.87 ± 2.01 | 6.17 ± 0.98    | 5.40 ± 0.86    | 7.84 ± 0.94 |
| PI*               | 12.37 ± 1.39 | 13.74 ± 1.23 | 1.22 ± 0.09    | 1.10 ± 0.12    | 1.58 ± 0.10 |
| GMI               | 30.72 ± 2.14 | 34.68 ± 3.01 | 16.64 ± 1.08   | 16.18 ± 1.90   | 19.02 ± 1.34 |
| GD₁a              | 35.43 ± 3.61 | 36.36 ± 2.45 | 18.49 ± 1.34   | 17.56 ± 1.21   | 23.37 ± 2.32 |
| GD₁b              | 36.60 ± 4.29 | 37.52 ± 3.58 | 19.03 ± 2.05   | 18.09 ± 2.03   | 23.77 ± 2.32 |
| GT₁b              | 38.37 ± 4.04 | 40.62 ± 4.62 | 23.41 ± 2.32   | 21.38 ± 2.45   | 26.17 ± 2.32 |

*Abbreviations as in Table 1.
Figures in brackets indicate number of pooled brains.
Values represent the mean ± S.D. from ten determinations by TLC procedure.

Table 4. Specific activities (kBq/mole of hydrogen) of major brain phospholipids and gangliosides in rats of different age continuously exposed to tritiated food.

| Fraction of lipids | 21-day-old | 120-day-old | The term fetuses |
|-------------------|------------|-------------|-----------------|
|                   | F₁         | F₂          | F₁             | F₂             | F₃             |
| Pch*              | 83.38 ± 7.02 | 76.96 ± 4.93 | 33.37 ± 2.45   | 33.51 ± 3.01   | 38.93 ± 1.57 |
| Pea*              | 76.27 ± 4.64 | 73.43 ± 3.28 | 26.35 ± 2.04   | 25.77 ± 2.85   | 28.48 ± 2.06 |
| Ps*               | 74.93 ± 7.25 | 70.03 ± 8.54 | 21.08 ± 1.59   | 21.38 ± 2.00   | 23.14 ± 1.90 |
| Sph*              | 30.03 ± 2.10 | 28.25 ± 3.28 | 13.02 ± 1.87   | 12.63 ± 1.29   | 14.19 ± 2.08 |
| PI*               | 35.72 ± 3.01 | 32.97 ± 2.46 | 2.73 ± 0.21    | 2.54 ± 0.34    | 2.81 ± 0.19 |
| GMI               | 76.80 ± 4.64 | 86.70 ± 5.42 | 36.14 ± 1.21   | 37.21 ± 2.06   | 39.94 ± 3.29 |
| GD₁a              | 87.87 ± 5.01 | 91.99 ± 7.25 | 44.00 ± 3.84   | 40.03 ± 2.85   | 46.44 ± 2.78 |
| GD₁b              | 87.84 ± 4.38 | 93.80 ± 8.04 | 45.67 ± 4.09   | 42.87 ± 4.64   | 49.49 ± 2.04 |
| GT₁b              | 96.34 ± 7.25 | 101.14 ± 9.17 | 55.72 ± 5.41   | 50.17 ± 3.02   | 59.46 ± 3.02 |

*Abbreviations as in Table 1.
Figures in brackets indicate number of pooled brains.
Values represent the mean ± S.D. from ten determinations by TLC procedure.

Specific activities of these phospholipids for the F₃ term fetuses, for the 21-day-old F₁ and F₂ rats and for 12-day-old F₁ and F₂ rats of both exposed groups differ significantly. The highest tritium incorporation into brain phospholipids was observed in the 21-day-old F₁ and F₂ rats.
and the lowest in the 120-day-old F1 and F2 females. The level of tritium in brain phospholipids of F3 term fetuses was still higher than in their mothers (F2 females).

As can be seen in Tables 3 and 4 the incorporation of ingested tritium from tritiated water into brain phospholipids differ from that observed after tritiated food. For all studied phospholipid classes incorporation of organically bound tritium in food was significantly higher, according to Student’s t-test, than incorporation of tritiated water.

The greatest differences in incorporation of tritium from food and from tritiated water was observed between phosphatidylcholine and others. Phosphatidylcholine accumulated much more tritium than other phospholipids during prenatal life. However, the latter accumulated relatively more tritium during postnatal life. Phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine display high specific activities for all studied rats. The specific activities of sphingomyelins and particularly phospholipids containing inositol were low.

Four major gangliosides of rat brain: GM₁, GD₁a, GD₁b and GT₁b (the ganglioside nomenclature system of Svennerholm14) is used) were well separated by TLC from the complex brain ganglioside mixtures of 21-day-old rats of F1 and F2 generation, 120-day-old F1 females after lactation, F2 females after Caesarian section and the F3 term fetuses. The changes with age in ganglioside composition of rats, obtained by ganglioside-sialic acid determination in fraction isolated after TLC, are given in Table 5. The ganglioside composition of brain for F1 and F2 generation did not differ significantly (Student’ t-test). No difference was also observed between brain ganglioside composition of rats of the HTO- and T-food groups.

The distribution of organically bound tritium among individual gangliosides in both tritium treatment groups of animals and their specific activities are given in Tables 3 and 4. Specific activities are calculated using the number of hydrogen atoms in the particular ganglioside classes. The statistical analysis of data by Student’s t-test for the HTO-group and for T-food group indicates that specific tritium activities in individual gangliosides of the F1 and F2 generations did not differ significantly. However, the specific activities of these gangliosides for the F3 term fetuses, for the 21- and 120-day-old rats of both exposed groups differ significantly. The highest tritium incorporation into brain gangliosides was observed in the 21-day-old F1 and F2 rats and the lowest in the 120-day-old F1 and F2 females. The level of tritium in four major

Table 5. Concentration (mg/100 mg of dry brain tissue) of the brain gangliosides assayed in rats of different age: results of quantitative analysis after thin-layer chromatography.

| Fraction of gangliosides* | The term fetuses (14) | 21-day-old (17) | 120-day-old (34) |
|----------------------------|----------------------|-----------------|------------------|
| GM₁                        | 0.099 ± 0.007        | 0.136 ± 0.009   | 0.179 ± 0.010    |
| GD₁a                       | 0.340 ± 0.020        | 0.373 ± 0.015   | 0.462 ± 0.025    |
| GD₁b                       | 0.128 ± 0.012        | 0.152 ± 0.010   | 0.193 ± 0.008    |
| GT₁b                       | 0.267 ± 0.030        | 0.299 ± 0.016   | 0.377 ± 0.009    |

*Gangliosides are named according to Svennerholm (14).
Figures in brackets indicate number of pooled brains.
Results are given as mean ± S.D. of six replicate analysis of total gangliosides mixtures.
brain gangliosides of F3 term fetuses was higher than in their mothers (F2 females).

Similarly as for phospholipids, the incorporation of ingested tritium from tritiated food was significantly higher (Student's t-test) from that observed after tritiated water.

The highest specific activities of tritium are found in trisialogangliosides GT1b of both tritium exposed groups and the lowest in monosialogangliosides GM1.

Except fetal choline phospholipids, all major gangliosides of rat brain display relatively high specific activities than other phospholipids of fetal, young and adult rat brains.

**DISCUSSION**

In this experiment firmly bound tritium was determined in two types of lipids: phospholipids and gangliosides, isolated from whole brains of three generations of rats exposed to HTO and T-food.

Tritiated food used for animal feeding was a mixture of organically bound tritium in proteins, lipids and nucleic acids. In these proteins tritium was bound with residues of lysine, proline, cysteine, tyrosine, methionine, phenylalanine and tryptophan. But the distribution of this radionuclide among these amino acids was not uniform. Amino acids essential to the rat brain e.g. phenylalanine, tyrosine and methionine contained about 85.7 per cent of the whole protein tritium, while in non essential amino acids 14.6 per cent of total tritium activity was found.

In the experiment the F1 and F2 generation of rats were exposed continuously from conception to the 21-st or 120-th day of age and rats of F3 generation during 22 days of their intrauterine life.

The obtained results indicate that the tritium derived from tritiated constituents of food entered into the all examined lipid compounds of brain to a greater extent than from aqueous form of tritium. The higher concentration of tritium in brain gangliosides and phospholipids after administration of OBT in food than after administration of the equivalent amount of tritium in HTO might indicate, that tritiated essential amino acids, carbohydrate, fatty acids, minerals and vitamins, derived from diet, are directly utilized by brain for synthesis of these tritium labelled lipids. Therefore, not tritiated water but tritiated organic constituents of food were a more effective donors of tritium for brain gangliosides and phospholipids.

The differences in tritium incorporation into particular phospholipids and gangliosides reflect the corresponding metabolic activities of these lipid classes in brain and their ability to incorporate one of both forms of tritium.

The differences in incorporation of both forms of tritium into brain phospholipids and gangliosides of F3 term fetuses, 21-day-old F1 and F2 rats and 120-day-old F1 and F2 females, may probably result from differences in tritiated precursors utilized for building up these lipids. The slightly greater accumulation of tritium in all studied brain phospholipids and gangliosides of the F3 term fetuses, than in the lipids of their mothers (F2 females) suggest, that chemical substances, required by brain for synthesis of these lipids, derived indirectly through the degradative and biosynthetic processing of tritiated maternal or their own tissues were more effective tritium donors than tritiated water or tritiated substances, derived directly from the
diet. On the other hand, this effect may be due to the fact that brain phospholipids and gangliosides are formed, to a large extent, just during fetal life and immediately after birth and then considered to be relatively stable from the metabolic point of view.

The observation, that tritium activity of phospholipids and gangliosides was highest in 21-day-old animals of F, and F2, who have been contaminated during first 21 days of life mainly from milk of their mothers, might suggest that OBT of certain constituents of milk was utilized to a greater extent for the synthesis of studied brain lipids than tritium from HTO or OBT from organic substances contained in food. This result confirms observation of Rochalska et al. for mice, which had been fed tritiated milk powder.

In all generations the highest tritium are found in phosphatidylcholine, in tri-, di- and monosialogangliosides, in phosphatidylethanolamine and phosphatidylserine. The lowest specific activity of tritium are shown in phosphatidylinositol and sphingomyelins.

It should be pointed out that results in Tables 3 and 4 yield information on the distribution of organically bound tritium among phospholipids and gangliosides present in the whole brain and do not give any evidence on the tritium deposition in gangliosides and phospholipids isolated from the different regions and structures of brain. A assessment of the hazard of such lipid-bound tritium in the brain is difficult, since the author ignores the exact anatomical and cellular sites of tritium deposition and their relation to potentially radiosensitive structures of brain.

However results of these study confirm earlier observations that the amount of tritium incorporated into rat brain lipids after administration of tritiated food is higher than after administration of tritiated water and is the highest in rats contaminated mainly from milk of their mothers. As well as milk appears to be a particularly effective precursor for certain phospholipids and gangliosides and this fact should be considered for assessing the risk from tritiated food in the developing organism.

REFERENCES

1. Z. Major (1980) Incorporation of tritium into organic compounds of brain. Int. J. Radiat. Biol., 37: 455-458.
2. M. Kowalska (1985) Incorporation of tritiated water (HTO) or organically bound tritium (OBT) into amino acids of rat brain proteins. J. Radiat. Res., 25: 99-108.
3. Z. Pietrzak-Flis, I. Radwan, Z. Major and M. Kowalska (1982) Tritium incorporation in rats chronically exposed to tritiated food or tritiated water for three successive generations. J. Radiat. Res., 22: 434-442.
4. J. Folch, M. Less and G. H. Sloane-Stanley (1957) A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-509.
5. R. J. Penick, M. H. Meisler and R. M. McCluer (1966) Thin-layer chromatographic studies of human brain gangliosides. Biochim. Biophys. Acta, 116: 279-287.
6. R. K. Yu and K. Iqbal (1979) Sialosylgalactosyl ceramide as a specific marker for human myelin and oligodendroglial perikarya: gangliosides of human myelin, oligodendroglia and neurons. J. Neurochem., 32: 293-300.
7. L. Svennerholm (1957) Quantitative estimation of sialic acids. II. A colorimetric resorcinol-hydrochloric acid method. Biochim. Biophys. Acta, 24: 604-611.
8. J. C. Dittmer and M. A. Wells (1969) Quantitative and qualitative analysis of lipids and lipid components. In Methods in Enzymology. Vol. XIV. Academic Press, New York, 482-530.

9. T. Miettinen and I. T. Takki-Luukkainen (1959) Use of butylacetate in determination of sialic acid. Acta Chem. Scand., 13: 856-858.

10. M. Kats (1972) Techniques in lipidology. In Laboratory Techniques in Biochemistry and Molecular Biology. Vol. 3. North Holland Publishing Company, Amsterdam, p. 393.

11. N. M. Neskovic, D. M. Kostic (1968) Quantitative analysis of rat liver phospholipids by a two-step thin-layer chromatographic procedure. J. Chromatog., 35: 297-300.

12. V. P. Skipsky, R. F. Peterson and M. Barclay (1962) Separation of phosphatidyl ethanolamine, phosphatidyl serine and other phospholipids by thin-layer chromatography. J. Lipid. Res., 3: 467-470.

13. L. S. DeBohner, E. F. Soto and T. De Cohan (1965) Quantitative analysis of phospholipids by thin-layer chromatography. J. Chromatog., 17: 513-519.

14. L. Svennerholm (1963) Chromatographic separation of human brain gangliosides. J. Neurochem., 10: 613-623.

15. M. Rochalska, R. Van Bruwaene, G. B. Gerber and R. Kirchmann (1982) Organically bound tritium and its distribution in mice fed organically labeled milk powder. Annales de l'Associat. Belge de Radio-protect., 7: 345-352.