Sphingomyelin Synthesis in Niemann-Pick Disease*

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LIPID LEVELS in the viscera of patients with Niemann-Pick disease are extraordinarily elevated. Although this increase involves the monoaminophosphatides and cholesterol as well, it is the surfeit of sphingomyelin which has consistently occupied the major attention of investigators. Because of these increased levels and also because of the enlargement of the organ, the spleen from such a patient may contain as much as 200 times the normal absolute amount of sphingomyelin. A list of possible mechanisms for this abnormality has been presented previously from this laboratory.[1] One possibility is that an acceleration of the rates of synthesis for the key lipids occurs in these patients and the studies herein were designed to explore this hypothesis.

For the purpose of the project, it was accepted that the residue of alkali-stable and acid-stable phosphatide from a washed lipid extract could be collected and taken to represent "sphingomyelin." It was further assumed that the radiophosphorus activity of this residue would be the result of biologic synthetic incorporation of the proffered inorganic P^{32} into sphingomyelin in a definite relation to native production of this lipid by the tissue under discussion. No attempts were made to investigate specific pathways or acceptance of labeled precursors other than the simple phosphate.

Few reports exist which give information on rates of synthesis of sphingomyelin relative to that of the total phospholipids. Hunter[2] stated that in twenty-four hours the specific activity of sphingomyelin in the livers of cats fed inorganic P^{32} was only about one-eighth that of the activity of lecithin. Hunter and Levy[3] reported that after subcutaneous injection the rate of incorporation of inorganic P^{32} into rat liver sphingomyelin was about 50 to 65 per cent that of the total phospholipids. Rates of incorporation into spleen were more nearly equal although at lower levels than the liver. Zilversmit et al.[4] showed in experiments with dogs that specific activities of sphingomyelin in liver ran considerably behind those of lecithin.

METHODS AND MATERIALS

The lipid analytic methods employed were the same as those previously described.[1] Portions of extract were dried on planchets for radioactivity counting (end window GM counter, with scaler). For the *in vitro* studies 1 mm. slices of tissue (average weight 100 to 150 mg.) were suspended in 25 ml. of borate-buffered (pH 7.4) Krebs-Ringer solution with added glucose, similar to the technic described by Popjak,[5] in a Dubnoff shaking incubator at 37°c. The human material was obtained at laparotomies being performed for the usual indications, with the special cooperation of the surgical and pathology departments to aid in the promptness of incubation.

Patient S. S., with Niemann-Pick disease, was presented as "Patient 18" in the earlier review.[1] Her spleen was found to contain 6.32 per cent of the fresh weight as sphingo-

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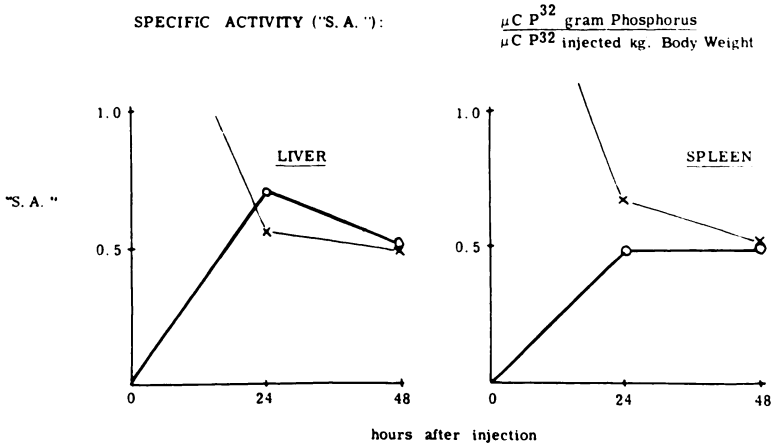

FIG. 1. *In vivo* demonstration of phospholipid synthesis. Intraperitoneal injection of inorganic P^{32} (2 to 4 μc.) in twelve normal adult rats that were sacrificed at twenty-four and forty-eight hours. Specific activity of inorganic and lipid phosphorus expressed in terms of dosage of isotope. O—O = lipid P^{32}; X—X = inorganic P^{32}.

myelin. Patient J. K., a forty month old child, had a very similar clinical picture with hepatosplenomegaly, pulmonary changes, mildly lipemic serum and normal intellectual development (the spleen yielded 6.29 per cent sphingomyelin). Patient P. M. is the younger sibling of Patient 11 in the previous report, and showed severe neurologic symptoms. Patient A. B. had mild organ enlargement and a lighter tissue abnormality (spleen 1.33 per cent sphingomyelin).

RESULTS

The *in vivo* experiments with normal adult rats (Fig. 1) indicated that the labeling of liver phospholipid reaches a maximum at or before twenty-four hours after injection of the tracer while the spleen shows a slower rate and lower general level. An average of 2.9 per cent of the injected isotope is found in the lipid phosphorus of the whole liver at twenty-four hours, falling to 2.3 per cent by forty-eight hours; in the whole spleen these figures are 0.078 per cent at twenty-four hours and 0.093 per cent at forty-eight hours. The specific activity of the sphingomyelin phosphorus reached in these tissues (Fig. 2) approaches that of the total lipid phosphorus, although sphingomyelin appears to be slower to reach maximal utilization of the inorganic P^{32}.

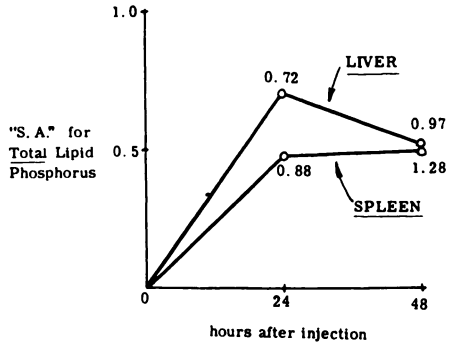

FIG. 2. Same experiments as shown in Figure 1, listing comparative data for sphingomyelin phosphorus. Numbers show ratio of sphingomyelin phosphorus specific activity to the specific activity of total lipid phosphorus at that point.

The *in vitro* (tissue slice) studies (Tables I and II) again showed that the hepatic activity in total phospholipid synthesis is greater than that of the spleen. With the animal work only about five minutes passed between removal of the specimen from the body and its establishment in the metabolic shaking incubator. In the human material this interval was usually several times as long which may account for lower absolute levels of specific activity. Sphingomyelin appears to be synthesized at relatively poorer rates by tissue slices, perhaps reflecting the unnatural circumstances or the short incubation time (Table III).

When specific activity is expressed in terms of grams of lipid phosphorus (as in the *in vivo*

TABLE I

Measurement of *In Vitro* Phospholipid Synthesis—Liver Slices*

Material Studied	Phospholipid Content (% of fresh wt.)	Specific Activity	
		mμc. lipid P^{32} / gram tissue	μc. lipid P^{32} / gram lipid P
Rats (2 animals)	2.00–2.17	16.8–17.2	19.8–21.0
Average		*17.0*	*20.4*
Human subjects			
General:			
M. T. (F, 1 yr., conv. hepatitis)	1.93	6.5	7.4
R. C. (M, 5 mo., conv. hepatitis)	1.68	4.6	7.0
K. S. (M, 6 yr., Gaucher's dis.)	2.77	8.5	9.5
Average		*6.5*	*8.0*
Niemann-Pick disease:			
J. K. (M, 3 yr.)	9.65	3.6	1.5
S. S. (F, 11 yr.)	14.6	20.0	4.1

* Incubated in borate-buffered Krebs-Ringer solution with 4 μC of inorganic P^{32} added; four hour data.

TABLE II

Measurement of *In Vitro* Phospholipid Synthesis—Spleen Slices*

Material Studied	Phospholipid Content (% of fresh wt.)	Specific Activity	
		mμc. lipid P^{32} / gram tissue	μc. lipid P^{32} / gram lipid P
Rats (4 animals)	1.18–1.36	1.5–9.1	3.6–19.2
Average		*6.8*	*13.9*
Human subjects			
General:			
M. T. (F, 1 yr., conv. hepatitis)	1.72	2.7	3.9
E. D. (F, 11 yr., spherocytosis)	1.25	4.9	8.2
C. W. (M, 10 yr., I.T.P.)	1.34	6.6	11.4
S. R. (F, 4 yr., Gaucher's dis.)	1.91	7.0	11.1
C. R. (F, 6 yr., Gaucher's dis.)	1.87	3.4	6.0
K. S. (M, 6 yr., Gaucher's dis.)	2.03	5.5	7.7
Average		*5.0*	*8.7*
Niemann-Pick disease:			
J. K. (M, 3 yr.)	9.50	3.8	1.4
S. S. (F, 11 yr.)	8.13	20.6	5.5

* Incubated in borate-buffered Krebs-Ringer solution with 4 μC of inorganic P^{32} added; four hour data.

work), the liver showed levels for sphingomyelin about two thirds those of the total phospholipids and the spleen about one half. If expressed per unit of tissue weight, the ratio is decreased in proportion to the tissue level of sphingomyelin; and both liver and spleen then show about 10 per cent as much sphingomyelin activity as total phospholipid.

When handling the material from the patients with Niemann-Pick disease, a critical problem arises regarding selection of units for specific activity because of the great residuum

TABLE III
Relative Sphingomyelin Specific Activity*
$\left(\frac{\text{Sphingomyelin } P^{32}}{\text{Total Lipid } P^{32}}\right)$

Based on:	mμc. lipid P^{32}/ gram tissue	μc. lipid P^{32}/ gram lipid P
I. Liver slices		
Rat XIV	0.12	1.14
Rat XVI	0.01	0.09
R. C. (M, 5 mo., conv. hepatitis)	0.19	0.79
K. S. (M, 6 yr., Gaucher's dis.)	0.10	0.70
Average	*0.10*	*0.68*
Niemann-Pick disease:		
J. K. (M, 3 yr.)	0.04	0.07
S. S. (F, 11 yr.)	0.07	0.12
Average	*0.06*	*0.10*
II. Spleen slices		
Rat XIV	0.17	0.55
Rat XVI	0.13	0.43
E. D. (F, 11 yr., spherocytosis)	0.14	0.50
C. W. (M, 10 yr., I.T.P.)	0.06	0.23
S. R. (F, 4 yr., Gaucher's dis.)	0.13	0.47
C. R. (F, 6 yr., Gaucher's dis.)	0.16	0.58
K. S. (M, 6 yr., Gaucher's dis.)	0.11	0.46
Average	*0.13*	*0.46*
Niemann-Pick disease:		
J. K. (M, 3 yr.)	0.07	0.09
S. S. (F, 11 yr.)	0.07	0.10
Average	*0.07*	*0.10*
III. White blood cell suspensions		
B. (normal)	0.29	0.56
Niemann-Pick disease:		
P. M. (F, 2 yr.)	0.14	0.29
A. B. (M, 7 yr.)	0.13	0.37

* Sphingomyelin *in vitro* synthesis data, from the same experiments as presented in Tables I and II. Results given as ratio of sphingomyelin phosphorus radioactivity to that of total lipid phosphorus.

of lipid in these tissues. Utilization of radioactivity per gram of tissue rather than per gram of lipid phosphorus seems more valid, since one would assume that synthesis should be continuing in the organ in spite of the previously collected lipid. On this basis the sphingomyelin synthesis in the material from patients proved to be unremarkable. Specific activity (mμc. of sphingomyelin P^{32}/gm. of tissue) ranged from 0.2 to 2.7 in liver slices of the controls (four hours), with results of 1.3 to 1.5 in the liver of a patient with Niemann-Pick disease. Spleens of controls yielded specific activities of 0.4 to 1.4 and spleen slices from patients with Niemann-Pick disease showed a specific activity of 0.2 to 1.4. The comparative data are given in Table III, for sphingomyelin P^{32}/total lipid P^{32}, showing that it is not possible to demonstrate an increased synthesis of sphingomyelin in this system. Suspensions of washed white blood cells from two other patients with Niemann-Pick disease were also tested, with similar results (Table III).

SUMMARY

A study has been made to investigate the rate at which synthesis of sphingomyelin utilizes tracer inorganic phosphate, with particular attention to the comparison with the whole phospholipid area. *In vivo* rates (twenty-four and forty-eight hours, rats) show sphingomyelin to be somewhat more slowly synthesized, but the ultimate specific activity approaches that of the total phospholipids. With tissue slices, followed for four hours, sphingomyelin production appears to be proceeding at an average of one half to two thirds the rate for the total phospholipids. Liver and spleen slices (and white blood cell suspensions) from patients with Niemann-Pick disease have been tested for *in vitro* lipid synthesis, but the reporting of results is handicapped by the presence of a large pool of previously formed lipid. Specific activity measurements (expressed in terms of unit weight of tissue) do not show evidence for increased synthesis of sphingomyelin in Niemann-Pick disease.

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