Incorporation of P^{32}-Labeled Orthophosphate into Tissue Phospholipids of Intact Animals

Summary

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THE DIFFERENT phospholipids of a given tissue differ strongly in their respective rates of incorporation of P^{32}-labeled orthophosphate.[1-3] The studies summarized herein yielded information concerning the behavior of the plasmalogens; the phosphorus metabolism of the plasmalogens had not been investigated hitherto owing to the lack of suitable analytic methods.

ANALYTICAL PROCEDURE

A procedure for the determination of the specific radioactivity of P^{32}-labeled plasmalogens was developed on the basis of the selective and quantitative conversion of phosphatidyl compounds, plasmalogens and sphingophospholipids to water-soluble phosphorus compounds as described by Schmidt et al.[4] In this technic, the separation of lipid phosphorus from the water-soluble P-compounds formed during the specific degradation steps is accomplished by precipitation of the former with ammonium sulfate at high concentration, followed by phosphorus analysis of the aqueous filtrates. Owing to their high salt content, such filtrates are not suitable for measurements of radioactivity. The original technic was adapted for application to studies of incorporation by using the purified lipid precipitates rather than the aqueous filtrates for determinating the specific radioactivities.

RESULTS

Representative figures for the relative specific radioactivities of the total phospholipids and of the water-soluble phosphorus compounds of tissues of rats are given in Table I. Each animal was killed three hours after receiving an injection of P^{32}-labeled orthophosphate. Each value for relative specific radioactivity is the quotient between the specific radioactivity of the indicated phosphorus fraction and that of the specific radioactivity of the total phospholipid phosphorus of the liver.

Representative figures for the ratios between the specific radioactivities of the respective individual phospholipids and those of the total phospholipids in some tissues of rabbits are given in Table II. Each rabbit was killed three hours after receiving an injection of P^{32}-labeled orthophosphate.

CONCLUSIONS

In agreement with earlier results of other investigators,[5] it was found that orthophosphate is incorporated into the phospholipids of the heart much faster than into those of the skeletal muscles (Table I). Possibly this difference is a direct consequence of the simi-

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TABLE I

Relative Values of the Specific Radioactivities of Lipid and Acid Soluble P Fractions of Rat Tissues after Injection of P^{32}-Labeled Orthophosphate

P-Fraction	Relative Specific Radioactivity				
	Liver	Heart (L.V.)	Skeletal Muscle	Brain	Plasma
Total lipid P	1	0.12	0.025	0.008	0.14
Acid-soluble P	3.0	2.6	0.32	0.1	2.6

TABLE II

Ratios Between the Specific Activity (S.A.) Values of Individual Phospholipid Fractions and Total Phospholipids of Rabbit Tissues after Injection of P^{32}-Labeled Orthophosphate*

Tissue	(Total) Lipid-P	Phosphatidyl-P	Plasmalogen-P	Sphingolipid-P
Heart	100	115	13	5
Skeletal muscle	100	112	20	11.6

* S.A. of individual lipid-P fraction:S.A. of total lipid-P × 100.

larly striking difference observed between the rates of P^{32} incorporation into the acid-soluble P-fractions of these two types of tissues[6] rather than an indication of a more rapid metabolic utilization of phospholipids in the heart.

In the heart and skeletal muscles of rats and rabbits, the rates of P^{32} incorporation into the plasmalogens are considerably slower than those of P^{32} incorporation into the phosphatidyl compounds.

REFERENCES

1. CHARGAFF, E. The relative speed of formation of lecithin and cephalin in the body. *J. Biol. Chem.*, 128: 587, 1939.
2. HEVESY, G. and HAHN, L. Turnover of lecithin, cephalin and sphingomyelin. *Kgl. Danske Videnskab. Selskab, Biol. Medd.*, 15: 1 (No. 5), 1940.
3. DAWSON, R. M. C. A hydrolytic procedure for the identification and estimation of individual phospholipides in biological samples. *Biochem. J.*, 75: 45, 1960
4. SCHMIDT, G., OTTENSTEIN, B., SPENCER, W. A., KECK, K., BLIETZ, R., PAPAS, J., PORTER, D., LEVIN, M. L. and THANNHAUSER, S. J. The partition of tissue phospholipides by phosphorus analysis. *Am. J. Dis. Child.*, 97: 691, 1959.
5. ENTENMAN, C., RUBEN, S., PERLMAN, L., LORENZ, F. W. and CHAIKOFF, I. L. Radioactive phosphorus as an indicator of phospholipide metabolism. III. The conversion of phosphorus to lipide phosphorus in the tissues of the laying and nonlaying bird. *J. Biol. Chem.*, 124: 795, 1938.
6. FLECKENSTEIN, A., JANKE, J. and GERLACH, E. in collaboration with DEUTICKE, B. Concentration and turnover of the energy-rich phosphates of the heart according to studies with paper chromatography and radiophosphorus. *Klin. Wchnschr.*, 37: 451, 1959.

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