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Entrance of Radioactive Phosphorus into Sphingomyelin of Various Tissues of Cat.

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Measurement of the rate of uptake of radioactive phosphorus by the total phospholipid fraction of various organs has been carried out by many investigators.[1] A few reports have appeared in which lecithin and cephalin have been separated for the determination of radioactivity in the individual phospholipids.[2] This report is concerned with the measurement of the rate of entrance of radioactive phosphorus into the phospholipid sphingomyelin. Such data should give an indication of the part which sphingomyelin plays in total phospholipid phosphorus metabolism and of what its rôle in the body may be. Since this work was undertaken, one report of a similar measurement has appeared. Chargaff, *et al.*,[2] measured the activity in the sphingomyelin fraction of the brain of rats and found the phosphorus turnover for sphingomyelin to be about the same as for brain cephalin.

In order to be able to obtain sufficient sphingomyelin, the cat was chosen for the experimental animal. Seven young female animals were used. They were killed at 1, 2, 4, 6, and 8 days after receiving by stomach tube 5 to 10 mg of radioactive phosphorus in the form of a solution of Na_2HPO_4.

The fresh tissues (brain, kidney, heart, spleen, lung, intestinal mucosa, liver, and skeletal muscle) were ground with sand and extracted with alcohol, twice with 3:1 alcohol-ether, and twice with 1:1 chloroform-methanol. The extracts were combined and the solvent removed under diminished pressure. The lipids were dissolved in petroleum ether containing a little chloroform and any residue centrifuged out. Sphingomyelin, in itself rather insoluble in petroleum ether, is carried into solution at room temperature by the large amount of other lipids present.[3] It was isolated by a micromodification of the method published by Thannhauser, *et al.*[4] This modification, worked

[1] Artom, C., Sarzana, G., Perrier, C., Santangelo, M., and Segre, E., *Arch. internat. physiol.*, 1937, **45**, 32; Artom, C., Sarzana, G., and Segre, E., *Arch. internat. physiol.*, 1938, **47**, 245; Perlman, I., Ruben, S., and Chaikoff, I. L., *J. Biol. Chem.*, 1937, **122**, 169; Chargaff, E., *J. Biol. Chem.*, 1939, **128**, 587.

[2] Chargaff, E., *J. Biol. Chem.*, 1939, **128**, 587; Chargaff, E., Olson, K. B., and Partington, P. F., *J. Biol. Chem.*, 1940, **134**, 505.

[3] Haven, F. L., personal communication; Taylor, J. D., personal communication.

out in this laboratory for these determinations, is almost identical with that published recently by Erickson, Avrin, Teague, and Williams[5] and need not be further described. The phosphorus analyses were performed by the method of Kuttner and Lichtenstein.[6] The radioactivity measurements were made according to the methods in general use in this laboratory.[7]

The radioactive phosphorus content of the sphingomyelin reaches a maximum within 8 days in all tissues except the brain and the skeletal muscle. In the latter 2 tissues the uptake of radioactive phosphorus by sphingomyelin is very slow and continues for more than 8 days. In the liver, lung, and intestinal mucosa the maximum is reached at 2 days after feeding. The maximum appears to come at about the same time, perhaps a little later, in the kidney, spleen, and heart. Expressed as percent of administered dose per mg of sphingomyelin phosphorus the maxima (averages from the values on 2 animals in each case) are as follows: liver 0.021, lung 0.017, intestinal mucosa 0.016, kidney 0.012, spleen 0.012, and cardiac muscle 0.0065. At the end of 8 days the value for skeletal muscle is about 0.0012 and for brain 0.0008.

Preliminary data on the liver indicate that the activity of the total phospholipid fraction reaches a maximum during the first day. This maximum is 0.17% of the dose of radioactive phosphorus fed and is about 8 times as great as that for sphingomyelin.

Conclusions. Radioactive phosphorus is incorporated into sphingomyelin to the greatest extent in the liver, the lung, and the intestinal mucosa. With the exception of the lung the tissues distribute themselves in approximately the same order for sphingomyelin activity as for total phospholipid activity. The lung is relatively higher in the case of sphingomyelin. Radioactivity in sphingomyelin reaches a maximum at about two days in most tissues. In the case of liver the total phospholipid fraction appears to be many times more active than sphingomyelin, indicating that this phospholipid, though possessing a certain definite activity, plays only a small part in phospholipid phosphorus metabolism in this organ.

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[4] Thannhauser, S. J., and Setz, P., *J. Biol. Chem.*, 1936, **116**, 533; Thannhauser, S. J., Benotti, J., and Reinstein, H., *J. Biol. Chem.*, 1939, **129**, 709.

[5] Erickson, B. N., Avrin, I., Teague, D. M., and Williams, H. H., *J. Biol. Chem.*, 1940, **135**, 671.

[6] Kuttner, T., and Lichtenstein, L., *J. Biol. Chem.*, 1930, **86**, 671.

[7] Bale, W., Haven, F., and LeFevre, M., *Rev. Scient. Instruments*, 1939, **10**, 193.