

FORMATION OF PHOSPHOLIPIDES IN TISSUES

sponsored by the Atomic Energy Commission, Division of Biology and Medicine under contract No. AT-(40-1)-288, Title II, with the Bowman Gray School of Medicine of Wake Forest College.

Project leader: Dr. Camillo Artom

The present report covers the work done from July 1, 1949 through March 15, 1950. This work is a continuation of previous work which was summarized in the final report to the Office of Naval Research. Copy of this report, dated September 1, 1949 was sent to the Division of Biology and Medicine of the Atomic Energy Commission.

A. Formation of phospholipides in the liver of intact animals.

As in previous experiments, the formation of phospholipides has been studied in the liver of animals killed at a given time after the administration of isotopic inorganic phosphate. Additional experiments were made in the past month using rats of various age groups and maintained on diets with various protein levels. To some of these diets guanidoacetic acid or diethanolamine was added, in an attempt to further inhibit the synthesis of certain phospholipide fractions. Analytical and isotopic determinations of both inorganic and lipide phosphorus were made in the liver. On the basis of the large number of data obtained in these and previous experiments, it became possible to compare the various methods of expressing the rates of formation of phospholipides in the liver ("percentage of the isotopic dose injected", "specific activity", "relative specific activity", "total radioactivity/specific activity of the inorganic phosphorus"). The statistical elaboration of the data has not yet been completed. However, it seems likely that the values calculated by the last method closely represent the amounts of phospholipides synthesized in a given time by the liver of the animals. When such amounts are referred to the same weight of animal, the values are remarkably constant, in spite of considerable variations in the age and diet of the animals, and even when functional and probably also anatomical alterations are detectable in the liver. Accordingly, on the basis of previous and present findings the following general concepts might be suggested: a) The synthesis of phospholipides proceeds unimpaired, even when the dietary supply of their N-containing precursors is considerably reduced and other deficiency symptoms (such as fatty infiltration of the liver) are visible. Apparently the formation of phospholipides in the liver takes a high degree of priority in the processes of the intermediary metabolism, including those involving the transfer or synthesis of methyl groups; b) The rate of phospholipide synthesis in the liver is not considerably affected by conditions which presumably reduce the number of the active cells or their functional capacity; and c) Since in intact animals the synthesis of phospholipides proceeds to a rate not far from normal even under very adverse conditions, one should assume that the liver has a very extensive functional reserve for this process.

It is expected that more time will be required for the complete elaboration of the data, before a complete paper on the subject can be published.

B. Formation of phospholipides in surviving slices of the liver.

Previous experiments in which the synthesis of phospholipides has been studied in surviving slices of the liver of rats under various nutritional conditions have been continued. In some experiments the total phospholipides have been fractionated into choline- and non-choline-containing and the formation of these two groups of phospholipides followed comparatively. The amounts of isotopic phospholipides synthesized by one gram of tissue during a 4 hour period of incubation with radioactive phosphate

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has been taken as a basis for comparison. Results of the more recent experiments show that a fasting even of short duration, causes a detectable decrease in phospholipide formation. The finding of a much more marked decrease in this process, when the animals had been previously maintained on diets low in protein, has been confirmed. The recent data also further corroborate the concept that this decrease is due to the low intake of dietary proteins rather than to a deficient supply of choline or of its precursors. No difference was noted in the rate of formation of choline- or non-choline-containing phospholipides by the isolated tissue.

The results of the experiments with tissue slices may be compared with those of the experiments in intact animal. As mentioned above, the formation of phospholipides in the liver of intact animals is independent to a large extent from the composition of the diet, whereas phospholipide synthesis in the isolated tissue is greatly affected by the nutritional state of the animal. It is tentatively suggested that in the isolated tissue the phospholipide synthesis occurs at a maximum speed, whereas in the intact animal, regulatory mechanisms are operating to keep the rate of phospholipide formation at a level adequate to the needs of the body, such a level being in ordinary conditions much below the maximum functional capacity of the tissue.

The action of various substances (choline, ethanolamine, betaine) added in vitro to the surviving slices of liver has been also studied further and most of our previous findings have been confirmed. Further experiments are in progress, in which other substances (e.g. diethanolamine, thiourea, etc.) are added in vitro to the isolated tissue.

A preliminary report of the experiments on the isolated liver tissue will be presented next April at the meetings of the Federation of the American Biological Societies in Atlantic City, N. J.

C. Lipide composition of liver mitochondria.

Under proper conditions the synthesis of phospholipides does occur even with liver homogenates. When these homogenates are fractionated by differential centrifugation, the large granules fraction (resulting essentially of mitochondria) appears very active in this respect. Since a large proportion of lipides is present in the mitochondria it was thought worthwhile to make a detailed study of the lipide composition of these structures.

Six preparations of "large granules" separated from rat liver by differential centrifugation, have been analyzed. Complete extraction of the lipides seems to be difficult. Total lipides extracted from the trichloroacetic precipitate averaged 24% of the dry weight. The remaining part consisted essentially of proteins and nucleoprotides and a smaller proportion of substances soluble in trichloroacetic acid. The average composition of the lipides was as follows: 78% phospholipides, 4.5% cholesterol (half or more of it, as free cholesterol), 18% of neutral fat and other lipides. The tentative distribution of the individual phospholipides was: lecithin 43%, sphingomyelin 7%, non-choline-containing phospholipides 50%. Most of these were phosphatidyl ethanolamine, but a non-negligible fraction could not be justified as ethanolamine- or serine-containing phospholipides. With the exception of one preparation, very little excess of N over P was found in the lipide extracts. However, the possibility of an incomplete extraction of cerebrosides, if present, can not be dismissed. In conclusion, mitochondria have a characteristically high proportion of lipides, especially phospholipides, but their composition does not appear to be essentially different from that of the lipides extracted from the whole unfractionated liver.

The results of this investigation were reported at the January meeting of the Southeastern Section of the Society for Experimental Biology and Medicine, Durham, N. C.

In the future experiments it is planned to study in more detail the in vitro synthesis of phospholipides under the action of mitochondria from the liver of rats in various nutritional states.

D. Incorporation of C¹⁴ labeled choline into the phospholipides.

These experiments have been hampered by difficulties in the synthesis of isotopic choline. Dr. Arthur Roe of the University of North Carolina has now developed for us a method of synthesis which seems both convenient and efficient. We are about to use the synthesized material in experiments on isolated liver slices. We are planning to run also parallel experiments with other labeled compounds which are, or may be intermediary terms in the formation or degradation of choline (such as betaine, dimethylglycine, and dimethylethanolamine). At our suggestion, Dr. Roe is studying methods for the synthesis of these compounds. It is hoped that in the course of our experiments some indications will also be obtained concerning the mechanism of other biological methylations, besides those directly involved in the synthesis of choline.

The following papers based on the results obtained in the previous and present experiments of this project have been published:

THE ACTION OF AN ANALOGUE OF ETHANOLAMINE (DIETHANOLAMINE) ON THE FORMATION OF LIVER PHOSPHOLIPIDES, *Journal of Biological Chemistry*, Vol. 180, No. 2, Page 495, September, 1949. (Complete report).

LIPIDE PHOSPHORYLATION IN THE LIVER AS RELATED TO THE DIETARY SUPPLY OF METHYL DONORS AND METHYL ACCEPTORS, Abstracts of the First International Congress of Biochemistry, Cambridge, England, August 1949, Page 22; *Journal of the Elisha Mitchell Scientific Society*, Vol. 65, No. 2, December 1949. (Preliminary report).

LIPIDE PHOSPHORYLATION IN THE LIVER OF RATS FED THIOUREA AND RELATED COMPOUNDS, *Journal of the Elisha Mitchell Scientific Society*, Vol. 65, No. 2, December 1949. (Preliminary report).

The following preliminary reports have been or are on the program of scientific meetings:

THE LIPIDE COMPOSITION OF THE MITOCHONDRIA FROM RAT LIVER, Society of Experimental Biology and Medicine, Southeastern Section, Durham, N. C., January 1950.

LIPIDE PHOSPHORYLATION BY SURVIVING SLICES OF THE LIVER OF RATS ON VARIOUS DIETS, Federation of the American Biological Societies, Atlantic City, N. J., April 1950.

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Summary of the work from July 1, 1949 through March 15, 1950 on the project described as

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In intact animals, the formation of phospholipides proceeds unimpaired even under very adverse conditions, such as a dietary deficiency of choline or choline precursors. This suggests that phospholipide formation in the liver takes a high priority in the processes of the intermediary metabolism. On the other hand, in isolated liver slices phospholipide synthesis is closely dependent upon the previous dietary regime and is reduced by a decreased intake of dietary proteins. It is suggested that in the isolated liver the formation of phospholipides proceeds at a maximum rate, whereas in the intact animals this process is kept at a level adequate to the needs of the animal, such a level being, in ordinary conditions, much below the functional capacity of the tissue.

A study of the synthesis of phospholipides by isolated liver mitochondria has been started. As a preparatory step for such a study, a detailed analysis of the lipide composition of mitochondria has been made.

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