

The Mode of Action of Lipotropic Agents

PROOF OF THE *IN VIVO* INCORPORATION OF TRIETHYL- β -HYDROXYETHYL-AMMONIUM HYDROXIDE INTO THE PHOSPHOLIPID MOLECULE

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Several theories have been proposed to account for the lipotropic action of choline, betaine, methionine, inositol and other compounds which exert similar effects on the deposition of lipids in the liver. The discovery (Hershey, 1930; Hershey & Soskin, 1931) reported from the laboratory of one of us (C. H. B.) that lecithin prevents the accumulation of excessive amounts of fat in the liver of depancreatized dogs seemed to offer support for the hypothesis, originally advanced by Leathes, that fatty acid transport involves incorporation of these compounds into the phospholipid molecule. The demonstration by Best & Huntsman (1932) that choline is the lipotropically active constituent of the lecithin molecule did not alter the main concept. If choline cannot be synthesized by mammals, or if its rate of synthesis is not equal to all requirements, the amount available would probably be the limiting factor in the formation of new lecithin molecules. Thus the viewpoint has been adopted that ingested choline exerts its lipotropic effect, in part at least, by stimulating synthesis of lecithin. Using radioactive phosphorus as a tracer element, a more rapid turnover of liver phospholipids was shown to follow ingestion of choline (Perlman & Chaikoff, 1939) and the relatively rapid incorporation of dietary choline into the phospholipid molecule has been established (Stetten, 1941). Welch & Landau (1942) have shown that arsenocholine (an analogue of choline containing arsenic in place of nitrogen), which is lipotropic, also enters the lecithin molecule. The fact that inositol, which possesses lipotropic properties (Gavin & McHenry, 1941), is also a constituent of phospholipids (Anderson, 1930; Folch & Woolley, 1942) is of considerable interest to those who adopt the viewpoint that a lipotropic agent exerts its effect by virtue of promoting phospholipid synthesis. Methionine and betaine probably act by supplying labile methyl groups for choline synthesis.

About 10 years ago, before tracer elements were available, Channon & Smith (1936) first proposed testing the hypothesis that choline exerts its lipotropic action through promoting synthesis of lecithin. They wrote: 'Light should be thrown on this question if a base could be found which possessed an action on liver fat similar to that of choline and

which could be shown to be present in the liver . . . by isolation of a suitably characteristic derivative. Such a base might be found to have been incorporated in a new phosphatide molecule in which it had replaced the choline of lecithin'. Channon & Smith (1936) described the synthesis of the ethyl homologue of choline (triethyl- β -hydroxyethylammonium hydroxide) and discovered its strong lipotropic activity. The subsequent attempt by Channon, Platt, Loach & Smith (1937) to detect the compound in liver phospholipids, after feeding it to rats for 20 days, was unsuccessful. They utilized the lesser solubility of the chloroaurate of the triethyl homologue in their search for it in the hydrolyzed liver phospholipids. Channon *et al.* (1937) stated at the end of an experimental paragraph, 'The absence of triethyl choline chloroaurate was thus established'. This wording was unfortunate since all that was actually proven was that the compound sought for was either absent or present in amounts too small to be detected by the procedure used. This failure to establish the presence of the triethyl homologue in the liver phospholipids has acted as an impediment to the general acceptance of the hypothesis that the lipotropic action of choline involves reactions which utilize the intact molecule.

The writers of this communication felt, as did Channon *et al.* (1937), that ' . . . it is a particularly attractive hypothesis . . . that choline should exercise its action by enabling lecithin synthesis to occur' and in view of the work of Welch & Landau (1942) it was considered desirable to reinvestigate the ingenious attempt of Channon and his colleagues to use the triethyl homologue of choline as a tracer substance. The procedure used by Channon *et al.* (1937), which would not disclose the presence of less than 20% of the homologue when mixed with choline, did not seem to us adequate for the task in hand. We were thus led to reinvestigate the problem with new analytical and fractionation procedures.

When it was shown (McArthur, Lang & Lucas, 1945) that the *cholineless* mutant (34486) of the bread mould *Neurospora crassa* was unable to utilize the triethyl homologue of choline (cf. Horowitz, Bonner & Houlahan, 1945), and that there was a significant difference between the enneaidide assay

authorities believe that the lipotropic effect may be explained by the observation that choline accelerates the turnover of the phosphorus in liver phospholipids. The assumption has been made that choline similarly promotes an accelerated turnover of all constituents of the phospholipid molecule. Experimental evidence for any increased rate of turnover of fatty acids in liver phospholipids under the influence of dietary choline, is not yet available, however, and hence a completely satisfactory explanation of lipotropic action must await further investigation.

There seems to be some confusion in the minds of certain recent writers on the subject of lipotropic action as to whether the labile methyl groups or choline *per se* is the ultimate lipotropic factor. The evidence is accumulating that the intact choline molecule is the effective lipotropic substance. Compounds such as betaine and methionine appear to exert their lipotropic effect by contributing labile methyl groups either for the synthesis or sparing of choline. The experiments of Welch & Landau (1942) have established that arsenocholine, which is lipotropic and does not possess labile methyl groups, when fed, is incorporated *in toto* into the phospholipid molecule. In the case of this choline analogue, it is the intact molecule and not a labile methyl group which exerts the biological effect.

The present experiments with the triethyl homologue of choline, which also is lipotropic and lacks labile methyl groups, supply another example of a lipotropically active substance which is utilized intact in the synthesis of new phospholipid molecules. The arguments advanced by Welch & Landau (1942) in support of the hypothesis that the intact choline molecule is the active lipotropic substance are substantiated by the findings reported above.

Several explanations may be offered for the failure of Channon *et al.* (1937) to detect the triethyl homologue of choline in the liver phospholipids of their experimental animals. The basal diet fed by

them was so low in protein (5% casein) that it probably did not permit metabolic processes to function normally. At this low protein intake, the quaternary ammonium chloride may have exerted a more toxic action than it did in the present experiments. Whatever the explanation, it has been possible to feed six times the daily dose used by them, and to give it for twice as long a period of time, without any greater weight loss or mortality than they observed. Furthermore, improvements in micro-analytical methods during the past 10 years have probably contributed to the present successful accomplishment of the project initiated by Channon and his colleagues. The establishment of the fact that the lipotropically active triethyl homologue of choline is biologically incorporated into the molecule of liver phospholipids, removes one of the impediments to general acceptance of the hypothesis that the lipotropic action of choline involves reactions which utilize the intact molecule.

SUMMARY

1. A significant difference between the micro-chemical and microbiological assays for choline in the hydrolysates of the phospholipids isolated from the livers of rats fed triethyl- β -hydroxyethyl-ammonium chloride, suggested that appreciable amounts of an unnatural base had been incorporated into the liver phospholipids. Fractionation procedures were devised which led to chemical proof that the triethyl homologue of choline had been incorporated into the phospholipids.

2. This finding provides further proof that the lipotropic effect of choline is associated with the intact molecule rather than its methyl groups.

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