dosage of choline chloride. In Fig. 1 B the actual percentage of liver fat in the test animals, without reference to the value in the controls, is plotted against choline chloride dosage.

The curves indicate that at the higher dosage levels the effect of increasing the concentration of choline chloride in the diet is slight and only at levels of less than 0.05%, under the conditions described, is there a marked correlation between concentration and action. It would appear evident that in assaying the activity of any lipotropic substance it is important to use relatively small doses in order to obtain the most significant relation between the size of the dose and the lipotropic action produced.

It will be appreciated that under any conditions the amount of "extra fat" actually "transported" or otherwise disposed of due to the influence of added choline chloride is relatively small. A 20 g mouse under the experimental conditions will deposit only about 0.15 ± 0.05 g of "extra fat" in the liver in the absence of added choline chloride, although consuming nearly 5 g of fat during the 6-day test period. From the figures it is evident that the smaller the dose of choline chloride the greater is its relative efficiency in preventing the deposition of a portion of this small amount of fat, that is, more fat is "transported" per molecule of choline chloride.

Summary. A 6-day method for the assay of lipotropic substances in the mouse is described. It is shown that a significant relation between the concentration of choline chloride added to a low choline-high fat diet and the lipotropic effect produced exists only at levels approaching the minimum effective dose, under the experimental conditions described.

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Lipotropic Action of Certain Compounds Related to Choline Chloride.*

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The hypothesis that choline chloride functions lipotropically by

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virtue of supplying an essential component of lecithin and possibly other lipid complexes appears particularly worthy of consideration. In this connection many compounds related to choline chloride have been subjected to examination, using the mouse method described in the previous paper. Two of these compounds, the arsenic analogue of betaine hydrochloride and α-methyl-β-phenylcholine chloride have apparently not been prepared hitherto; the preparation of these and other compounds will be described in a later publication. It is desired to list the results obtained with the various substances but to reserve the larger part of the interpretation at present.

TABLE I.

Compound	Formula	Lipotropic Activity
(1) Choline Chloride	$(CH_3)_3N \cdot CH_2CH_2OH$ (Cl)	+
(2) Betaine Aldehyde Chloride*‡	$(CH_3)_3N \cdot CH_2CHO$ (Cl)	+
(3) Betaine Hydrochloride	$(CH_3)_3N \cdot CH_2COOH$ (Cl)	+
(4) Phosphocholine Chloride‡	$(CH_3)_3P \cdot CH_2CH_2OH$ (Cl)	+
(5) Phosphobetaine Hydrochloride‡	$(CH_3)_3P \cdot CH_2COOH$ (Cl)	—
(6) Arsenocholine Chloride†	$(CH_3)_3As \cdot CH_2CH_2OH$ (Cl)	+
(7) Arsenobetaine Hydrochloride‡	$(CH_3)_3As \cdot CH_2COOH$ (Cl)	—
(8) β-Methylcholine Chloride*	$(CH_3)_3N \cdot CH_2CH(CH_3)OH$ (Cl)	—
(9) α-Methyl-β-phenylcholine Chloride‡	$(CH_3)_3N \cdot CH(CH_3)CH(C_6H_5)OH$ (Cl)	?
(10) Ethyl ether-β-methylcholine Chloride*	$(CH_3)_3N \cdot CH_2CH(CH_3)O \cdot C_2H_5$ (Cl)	—
(11) Betaine Aldehyde Acetal Chloride*‡	$(CH_3)_3N \cdot CH_2CH(OC_2H_5)_2$ (Cl)	+
(12) α-Methylbetaine Hydrochloride‡	$(CH_3)_3N \cdot CH(CH_3)COOH$ (Cl)	+
(13) Calcium Phosphorylcholine Chloride‡	$(CH_3)_3N \cdot CH_2CH_2O \cdot PO_3Ca$ (Cl)	+
(14) Trimethylamine Oxide Hydrochloride‡	$(CH_3)_3N \cdot OH$ (Cl)	—

* Compounds or precursors generously supplied by Merck and Co., Inc.
† Generously supplied by Hoffmann-LaRoche, Inc.
‡ Prepared by the authors.

The writers believe that statements concerning the precise activity of various compounds in terms of choline chloride are unwise unless a large number of experiments have been done in that dosage range where the relation between size of dose and lipotropic effect is most significant. Preliminary observations indicate, however, that N-betaine aldehyde chloride, N-betaine hydrochloride, and the P and As analogues of choline chloride are more than one-half as active as choline chloride.

It will be noted that the P and As betaines are not lipotropic in action. This indicates that the P and As analogues of choline chloride act *per se* (or as the corresponding aldehydes) and suggests, therefore, that N-betaine hydrochloride acts by virtue of its conversion by the organism into choline chloride (or the corresponding aldehyde) and not *per se*.

The testing of the As analogue of betaine aldehyde chloride would appear to be the next step in the investigation of the mechanism of action of choline chloride. The synthesis of this compound will be attempted in the near future. Other lines of investigation now in progress have suggested, however, that the oxidation of choline chloride to betaine aldehyde and betaine[1] which occurs in the liver and kidney of certain species (rat;[2] mouse and dog, recent observations of the writers) is probably not directly concerned with the mechanism by which choline chloride and its related compounds influence the deposition of fat in the liver.

The hypothesis that choline chloride is phosphorylated and utilized in the synthesis of lecithin is supported by the finding that the phosphoric acid ester of choline chloride is unaffected by liver phosphatases and therefore protected from oxidation in the liver. Further, the observations previously described (Welch[3]) concerning the appearance of arsenic in lecithin isolated from rats fed arsenocholine chloride have been confirmed in mice. Semi-quantitative spectrographic analysis of lecithin isolated from mice fed the arsenic analogue for a 6-day period indicates that arsenic is present corresponding roughly to 2% "arsenolecithin." Fractionation and chemical examination of the isolated lecithin are now under way. No arsenic has been found spectrographically in phospholipids of control animals, with or without the addition to the diet of arsenic pentoxide; however, other arsenic compounds will be examined in this regard.

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[3] Welch, A. DeM., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 107.