degree of ketonuria should occur in both instances. Since a decreased ketonuria occurs in adrenalectomized animals and since ketogenesis apparently is undisturbed, ketolysis can not be regarded as the factor responsible for the decreased ketonuria.

A more plausible explanation for the discrepancy between the ketone body excretion and the blood ketone level is that following the removal of the adrenal glands, the renal threshold for ketone bodies is markedly increased. Thus, even with identical blood ketone levels the adrenalectomized animal will excrete less ketone bodies than will the normal. This is in accord with the observations of many investigators who have demonstrated that adrenal insufficiency is characterized by marked abnormalities in renal function,[6] and hence it is not surprising that these renal disturbances manifest themselves by an inability of the kidney to maintain its normally low threshold for ketone bodies.

Our data suggest the conclusion that the adrenal gland *per se* is not essential for the ketogenic activity of extracts of the anterior pituitary gland and that an increase in the renal threshold for ketone bodies is responsible for the decreased ketonuria observed in the adrenalectomized animals treated with A.P.E. However, this does not exclude the possibility that in late stages of adrenal insufficiency secondary changes in liver function ensue, with a consequent decrease in ketogenesis. These and other studies will be published in greater detail elsewhere.

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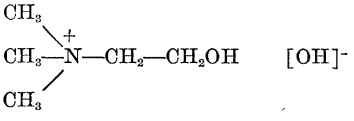
THE CHEMICAL NATURE AND NOMENCLATURE OF CHOLINE DERIVATIVES

CONSIDERABLE confusion exists as regards the nomenclature and understanding of the chemical nature of compounds of the choline type. Choline is an organic homologue of ammonium hydroxide, but the name suggests an amine. Perhaps because of this inappropriate



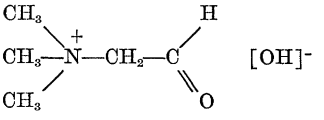
name it is not generally appreciated that choline, like tetramethylammonium hydroxide, is a very strong base, comparable with the caustic alkalies. Like sodium hydroxide, choline reacts with hydrochloric acid to form a chloride, a term which should be used in preference to "hydrochloride." Like sodium chloride, in aqueous solution the chloride salt of choline is neutral in reaction and dissociates into cations and anions. Strictly speaking, choline salts should no more be termed "choline" than should sodium chloride be referred to as "sodium," or ammonium chloride as "ammonium." The term "choline hydrochloride" is incompatible with the structure of this "onium" compound and the use of such terminology (by research workers and manufacturers) contributes to the erroneous idea that the chloride salt contains loosely bound hydrochloric acid which should be neutralized before biological use. It is obvious that these comments apply equally forcibly to the various derivatives of choline such as acetylcholine, "mecholyl," etc.

Ordinary nitrogenous compounds containing tri-(co)valent nitrogen form "salts" with hydrochloric acid by the addition of the hydrogen ion through a covalent link (shared electron doublet), making the molecule a cation and enabling it to hold the chloride ion by electrovalency. Such "salts" are termed hydrochlorides, although the nomenclature appears to have no justification except that of common usage, for "salts" with other than simple halogen acids are not so designated, for example, codeine phosphate, strychnine nitrate, etc.

In compounds of the choline type the four-covalent-one-electro-valent state is constant, an anion being ever present, whether it be hydroxide, chloride or bicarbonate. Substances of this type are classified as onium compounds and the names of the salts are derived in the same manner as are those of sodium hydroxide; for example, choline chloride, trimethyl-β-hydroxyethylarsonium bromide, tetramethylphosphonium sulfate, etc.

The importance of the charged nitrogen atom is evidenced not only by derivatives of choline, but also by such biologically significant compounds as vitamin B_1 and the nicotinic acid amide portion of the coenzyme system.

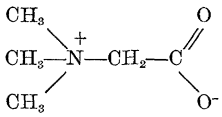
The nomenclature of the biological oxidation products of choline is unfortunate. Betaine aldehyde, the primary product of oxidation, has a particularly undesirable name, since it implies that the compound pos-



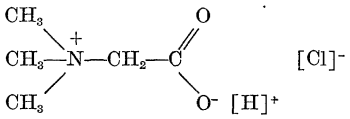
sesses the properties of a betaine, which is not the case. In aqueous solution betaine aldehyde and its salts are electrolytes, dissociating into cations and anions.

The name "betaine" (pronounced *bē′ tȧ in*) is generically applied to a large group of compounds having a zwitterion structure similar to that of the compound termed betaine specifically, the latter being the secondary oxidation product of choline.

[6] A. Grollman, "The Adrenals," pp. 170 and 180. Williams and Wilkins Company, Baltimore, 1936.



In the case of betaine the hydrochloric acid salt is usually referred to as betaine hydrochloride, and with a great deal more justification than in the case of the chloride salt of choline or of the so-called hydrochlorides of alkaloids, etc. Inspection of the zwitterionic nature of the hydrochloric acid salt of betaine



indicates that the hydrochloric acid is loosely bound; in fact the hydrochloric acid may be titrated as free acid. Pure crystalline betaine hydrochloride may well be used as a stable source of standard hydrochloric acid; doubtless other stable, crystalline salts of betaine might be used as standards for other acids.

The present confusing nomenclature might be avoided by the use of the cumbersome, but adequately descriptive, chemical names, or by the introduction of a more logical system of common names, preferably based on the familiar term "choline" as the root. Since the names which have been proposed so far fail to indicate the onium structure of the compounds, Professor Austin M. Patterson has suggested (in a personal communication) the name *cholinium* for the choline cation; this would permit the use of the terms cholinium hydroxide, cholinium chloride, acetylcholinium bromide, etc., for the salts and derivatives. In such a system betaine aldehyde might become aldocholinium hydroxide, a name which indicates its properties, and betaine aldehyde chloride, aldo-cholinium chloride, a term which suggests its chemical and physiological relationships. The root name *cholonium* would appear theoretically more sound, but might be accepted less readily, since the relation to the term now in common usage is less easily recognized. A satisfactory name for betaine itself is not easily found, its carboxy group being difficult to indicate (carboxycholinium, according to the international system, would indicate the replacement of a hydrogen of the choline cation by a carboxyl group); betainium is the only root name which would appear applicable, and this is far from satisfactory.

The writer will welcome suggestions and comments on this or other systems of nomenclature for the choline compounds. Professor Patterson has suggested that if the workers in this field should arrive at definite proposals regarding terminology, these proposals might be presented to the American committee on biochemical and organic nomenclature of the International Union.

The author wishes to express his appreciation for the advice and suggestions of Professor Austin M. Patterson, Professor C. F. Cori, Profesor H. T. Graham and Dr. T. H. Jukes.

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BIOLOGICAL DETERMINATION OF VITAMIN B_1 (THIAMIN) IN RHIZOBIUM TRIFOLII

WHILE studying growth factor requirements of the nodule bacteria, it became desirable to determine the ability of the organisms to synthesize vitamin B_1. Since only minute amounts of cellular material were conveniently available, feeding experiments were impractical. The situation required a rapid biological assay, applicable to small quantities of ordinary culture media.

For this purpose, a quantitative method was developed, which is an application of Knight's[1, 2] demonstration that, under suitable conditions, the growth of *Staphylococcus aureus* is proportional to the amount of vitamin B_1 present in the medium. The base medium employed for the estimation of vitamin B_1 is similar to that of Knight[1, 2] and Fildes,[3] but with the substitution of casein hydrolysate for gelatine hydrolysate or known amino acids:

Acid hydrolyzed casein	20 ml (equivalent to 0.4 gm Merck's casein)
Dipotassium phosphate	0.5 gm
Glucose	0.3 gm
Cysteine, HCl	2.0 mgm
Nicotinic acid	0.02 mgm

pH–7.0
Distilled water to 50 ml

Five ml amounts of this medium are tubed, and sufficient distilled water added so that together with the addition of the test material, the final volume is 10 ml. The medium is autoclaved one hour at 15 pounds, vitamin B_1 additions being made under aseptic conditions after sterilization.

A trace of growth from a nutrient agar culture is suspended in 10 ml water (approximately 100,000 bacteria per ml), one drop of which is used for inoculation. No growth occurs in the base medium. However, as shown in Fig. 1, in the presence of as little as .00005 micrograms per ml of vitamin B_1, detectable growth results, the amount of growth increasing rapidly with higher concentrations of the vitamin to .001 micrograms per ml. Growth is determined after

[1] B. C. J. G. Knight, *Brit. Jour. Expt. Path.*, 16: 315–326, 1935.
[2] B. C. J. G. Knight, *Biochem. Jour.*, 31: 731–737, 1937.
[3] P. Fildes, G. M. Richardson, B. C. J. G. Knight and C. P. Gladstone, *Brit. Jour. Expt. Path.*, 17: 481–484, 1936.