"We have made it a rule not to use for the test any milk that exceeded an acidity of 0.15 per cent calculated as lactic acid and we have abandoned altogether any idea of adjusting milk that is more acid by adding sufficient alkali. If the milk is not naturally at the proper acidity, we do not use it.

"I am decidedly of the opinion that in the next revision of the National Formulary adjustment of the milk by the addition of alkali should be omitted but that preferably a test for acidity should be included and milk that runs more than 0.15 per cent acidity as lactic acid should not be used.

"Of course, with a reference standard for direct comparison it does not make so much difference if the acidity varies somewhat and the adoption of such a standard would help to eliminate the errors that may occur due to variations in sources of supply of milk."

Laboratory No. 4 states,

"These tests indicate that, compared with the laboratory test last year, this rennin standard has decreased slightly in activity."

Laboratory No. 5 concludes that the standard rennin "curdles the milk in slightly less time than we reported last year." This laboratory also makes the suggestion that the titration for acidity can be made more sensitive, if five drops of the milk being titrated be added to 5 cc. of water. The pink color shows up better. It is customary to use 50 cc. of the milk, to which 0.5 cc. of phenolphthalein solution has been added. After the preliminary titration a second one should be made using just a little less than expected amount of alkali.

Laboratory No. 6 remarks that this year's results "are very close to those which we reported last year."

This year's work confirms the previous conclusion that the National Formulary Fifth Edition Method is unsatisfactory and unreliable, and that a standard rennin would overcome the difficulties inherent in the present method. The work adds another year's cumulative evidence for the excellent keeping quality of the A. D. M. A. reference rennin and its suitability as a standard in case a method based upon the use of a standard is adopted in the National Formulary Sixth Edition.

RECOMMENDATIONS.

It is recommended that,

1. The study of the keeping quality of the A. D. M. A. reference rennin be continued.
2. On behalf of the American Drug Manufacturers' Association, a revised method for the assay of rennin be submitted to the National Formulary Sixth Edition Revision Committee. Such method will make use of a standard rennin and will omit adjustment of the acidity of the milk.

In conclusion, I want to express my very sincere appreciation to the members of the committee for their splendid collaboration during these many years. It is hoped that their efforts will result in improved methods of assay in the forthcoming editions of the United States Pharmacopœia and of the National Formulary.

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W. H. BLOME,	F. W. HEYL,
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DETERMINATION OF TAUROCHOLIC ACID IN BILE SALTS.*

F. E. WILLSON.

The bile contains as its chief constituents, taurocholic acid and glycocholic acid. These generally occur as the sodium salts and are not to be found in the pancreatic juice or in any of

* Presented at the Twenty-Second Annual Meeting of the American Drug Manufacturers' Association held at Hot Springs, Virginia, May 8–11, 1933.

the normal animal secretions other than the bile. Their function in the bile is to emulsify fats and assist in the absorption of fatty acids. In this way they are material aids to digestion. These acids may be present in the bile in varying proportions depending upon the animal source and the general metabolism of the animal. Besides these two acids there may be present other analogous substances such as taurocholeic acid and glycocholeic acid. It is generally agreed these are present in very small amounts, if at all, and are therefore worthy of little consideration.

The problem of obtaining a satisfactory method for the determination of the relative amounts of taurocholic acid and glycocholic acid has been a matter of investigation for a number of years. At first, the chief interest in this respect was the determination of the two acids in bile, itself, so that the results might be used in conjunction with certain clinical observations. When these two important constituents of the bile were made available commercially in the form generally known as bile salts the demand for methods of determinination become even more a matter of interest and investigation.

The methods for the determination of the taurocholic acid content of bile and mixtures of bile salts are based principally upon its sulphur content since taurocholic acid contains sulphur while glycocholic acid does not. Some of the earlier investigators attempted to use a method based upon a difference in solubility of the two acids with a subsequent amino nitrogen determination. Foster and Hooper (1) suggested such a method in which the taurocholic acid fraction was hydrolyzed with sodium hydroxide into taurine and cholic acid. The amino acid content of the taurine was then determined by the Van Slyke method. A later method (2) used a similar line of procedure but besides determining the amino nitrogen of the taurine, the sulphur content was also determined and the two used in conjunction in calculating results. The same investigators elaborated on this method in a later communication (3) and suggested that the Asboth-Dunning method for determining the sulphur content be used. Many other methods are to be found by reference to the literature but in principle most of them follow along the same lines. Since most of these earlier methods were for the determination of taurocholic acid in the bile, itself, there was a tendency to separate it from the glycocholic acid by means of solvents. These methods gave unsatisfactory results because the differences in solubilities made use of did not entirely exist. Also, Hammersten showed that in many samples of bile there were present certain ethereal sulphates and sulpholipins that possessed the same solubilities as did taurocholic acid. Therefore, any method using the sulphur content for calculating the amount of taurocholic acid in bile would give erroneous results if these substances were present. In the commercial product, marketed as bile salts, we are not confronted with this problem since the methods of preparation practically exclude the ethereal sulphates and sulpholipins. Therefore our chief problem from a commercial standpoint is to obtain some satisfactory method for determining sulphur in organic combination in a product of this kind. From this determination the taurocholic acid content then can be calculated.

Several years ago the Sub-Committee on Digestive Ferments and Glandular Products of the American Drug Manufacturers' Association adopted for study the bile salts. Dr. Kirk submitted methods (4) for determining both glycocholic acid and taurocholic acid. The method for determining taurocholic acid was based upon a sulphur determination using the Hoffman-Gortner method (5). On giving this method a trial in a number of different laboratories it was found that extremely divergent results were obtained. The taurocholic acid content of the particular sample under investigation was found to range anywhere from 30 per cent to 49 per cent. This indicated that the method was unsuitable for analytical purposes. This wide range of results appeared to us as being due to the fact that complete oxidation of the sulphur was not insured. The method had a further disadvantage in that it required a considerable length of time to carry it out. This is a point that must be considered in routine analytical work.

Since there were two serious objections to the Hoffman-Gortner method applied to bile salts it seemed that there should be some other available method for use. For that reason a search was made for methods and the ones that showed promise of meeting the two requirements were given a trial. The method which gave consistent results and at the same time was rapid in manipulation was one employing the Parr Sulphur Bomb. Following is an outline of the method devised for its use as applied to bile salts:

Procedure.—A 1-Gm. sample of bile salts is weighed into the ignition cup of a Parr Sulphur Bomb. One Gm. of potassium chlorate and 10 to 15 Gm. of sodium peroxide, followed by 0.2

Gm. of benzoic acid, are added. After covering the ignition cup with the top equipped with the ignition wire the whole is sealed in the bomb by means of the screw-cap. The bomb is then well shaken to insure uniform mixing of the contents. The contents are then ignited in the usual manner using an electric current. After ignition the bomb is cooled, the ignition cup together with the separated top is placed in a 600-cc. beaker. About 250 cc. of distilled water is then added and the fusion mixture brought into solution with the aid of heat. After complete solution the ignition cup and top are removed, washing well with distilled water. The solution is then carefully acidified with hydrochloric acid. After an acid reaction has been reached the solution is filtered and the sulphur determined in the filtrate preferably by the gravimetric method using barium chloride solution. The amount of sulphur found multiplied by 16.07 gives the amount of taurocholic acid present in the sample.

If the gravimetric method is used the sulphur is in combination as barium sulphate and must be calculated from the weight of the precipitate obtained. The conversion factor 16.07 is obtained by considering taurocholic acid to have the formula $C_{26}H_{45}NSO_7$ with a molecular weight of 515.45.

The reagents used in this method must necessarily be first tested for sulphur and found to be sulphur free.

Determinations by this method may be carried out rapidly. The preparation and ignition of the sample requires only about 20 minutes while the sulphur determination ordinarily requires about 2 hours. This is a considerable advantage over the Hoffman-Gortner method which generally requires several days to carry to completion.

The proposed method also has the added advantage of carrying the oxidation to completion. This probably can be best illustrated by comparison of results obtained by the Hoffman-Gornter method and this Parr-Bomb method.

	Hoffman-Gortner Method.	Parr-Bomb Method.
Determination No. 1.....	32.90% taurocholic acid	38.92% taurocholic acid
Determination No. 2.....	33.89% taurocholic acid	41.22% taurocholic acid
Determination No. 3.....	37.89% taurocholic acid	42.59% taurocholic acid
Determination No. 4.....	33.22% taurocholic acid	42.33% taurocholic acid

It will be observed from the above results that lower results are obtained by the Hoffman-Gortner method. This would be indicative that complete oxidation of the sulphur is not reached. With the Parr-Bomb method there was some variance in results but when it is considered that the conversion factor from sulphates to taurocholic acid is high (16.07) these results would be expected.

The Parr-Bomb method has been used with considerable success in this laboratory since its submission to the A. D. M. A. The following table contains the results of the analyses of four different samples of bile salts which are representative of results that may be expected:

Sample No. 1.....	I.	38.04% taurocholic acid
	II.	40.37% taurocholic acid
	III.	38.84% taurocholic acid
Sample No. 2.....	I.	40.73% taurocholic acid
	II.	41.36% taurocholic acid
	III.	40.89% taurocholic acid
Sample No. 3.....	I.	47.19% taurocholic acid
	II.	47.58% taurocholic acid
	III.	46.53% taurocholic acid
Sample No. 4.....	I.	47.23% taurocholic acid
	II.	47.69% taurocholic acid
	III.	47.76% taurocholic acid

In working with bile salts, we believe the Parr-Bomb method will give more accurate and reliable results for the sulphur content than any of the other available methods. Consequently a truer evaluation of taurocholic acid will be obtained. If the method were applied to bile a special preparation of the sample would have to be first carried out in order to exclude any of the other sulphur-containing substances, other than taurocholic acid, being present.

LITERATURE CITED.

(1) Foster and Hooper, *J. Biol. Chem.* (1919), 355–366.
(2) Rosenthal and Falkenhausen, *Arch. exptl. Path. Pharmakol.*, 98 (1923), 321–338.
(3) Rosenthal and Falkenhausen, *Klin. Wochschr.*, 2 (1923), 1111–1114.
(4) Proceedings of the American Drug Manufacturers' Association (1929).
(5) Hoffman and Gortner, *J. A. C. S.*, 45 (1923), 1033.

ASSOCIATION BUSINESS

AD INTERIM BUSINESS OF THE COUNCIL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION, 1932–1933.

Office of the Secretary, 10 West Chase St., Baltimore, Md.

LETTER NO. 10.

July 26, 1933.

To the Members of the Council:

70. Tentative General Program for the Eighty-First Annual Meeting. Motion No. 20 (Council Letter No. 9, page 663) has been carried and the tentative general program is approved. Local Secretary Stanley and his associates have been advised and copies of the program have been sent to the pharmaceutical press.

71. Authorizing Dr. Hilton to Sign Checks in the Absence of Treasurer Holton. Motion No. 21 (Council Letter No. 9, page 663) has been carried and the approved banks of deposit have been advised of the authority granted to S. L. Hilton in the absence of C. W. Holton.

72. Research Award. Motion No. 22 (Council Letter No. 9, page 664) has been carried and Dr. Husa has been advised.

73. Contract for Printing and Distributing the Year Book, Volumes 20 and 21. Motion No. 23 (Council Letter No. 9, page 664) has been carried and the contract for printing and binding the YEAR BOOK, Volumes 20 and 21, in one binding has been awarded to the Lord Baltimore Press, Baltimore, Md.

74. Applicants for Membership. Motion No. 24 (Council Letter No. 9, page 664) has been carried and applicants for membership numbered 161 to 173, inclusive, have been elected to membership.

75. Contract for Printing and Binding the National Formulary VI. Recently Chairman DuMez issued the following bulletin to the members of the Committee on Publications:

"After consultation with Chairman Gathercoal of the Committee on National Formulary as to the progress of revision and in view of the possibility of increases in cost, invitations were sent out recently to the firms which might be interested to submit bids on the cost of printing and binding the N. F. VI.

"Bids were received from the J. B. Lippincott Company, Philadelphia, Pa., the Lord Baltimore Press, Baltimore, Md., and the Mack Printing Company, Easton, Pa. The estimates of these three firms were carefully checked and that submitted by the Mack Printing Company was found to be the most favorable. This bid will result in decided reduction in the cost of the book over that of the N. F. V. It is estimated that there will be a saving of between $5000 and $6000 on the first series of 25,000 copies. Approximately 45,000 copies of the N. F. V have been printed and if the sale of the N. F. VI compares, there will be a total saving of from $10,000 to $12,000. Of course, the amount cannot be definitely stated as the contract calls for an adjustment for labor and materials on the basis of existing conditions, at the time later series may be printed.

"Inasmuch as the Mack Printing Company handled the work satisfactorily for the N. F. V, and as this firm has now had the experience of printing one edition of the National Formulary, and as their bid is the most favorable, it is suggested that it be recommended to the Council of the AMERICAN PHARMACEUTICAL ASSOCIATION that the contract for printing and binding the N. V. VI be awarded to the Mack Printing Company."

The following communication has just been received from Chairman DuMez:

"A majority of the members on the Publication Committee have voted in favor of the