

A CRITICAL STUDY OF METHODS OF ASSAY
OF
CHLORAL HYDRATE.

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CHLORAL HYDRATE.

BY
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INTRODUCTION.

INTRODUCTION.

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So much has been written during the last twenty or thirty years about the quantitative determination of chloral hydrate, and so many methods and modifications of existing methods have been put forward, some with considerable merit, and others open to more or less serious objections, that a general survey of the subject was considered desirable.

On account of the extensiveness of a complete study of every known method, the work has been, of necessity, made rather cursory in certain places, and more exhaustive in others. Where a unanimity of opinion exists among investigators, regarding some phase of the work, the writer has perhaps been satisfied with a few results, if these appeared to bear out that opinion. Other phases which appeared somewhat more contentious, were gone into more fully, and if the writer's findings were of a rather opposite nature to that of another investigator, the work was discussed at some length, the theory gone into carefully, and, if necessary, statements in the literature brought to bear, as supporting evidence.

Certain methods were reviewed and criticised merely on the general principles involved, the originator's own figures being used, without much corrob-

orative work being done.

Points regarding the technique of the methods, manipulative difficulties, etc., are discussed where they are worthy of mention.

Certain methods which appeared to give erroneous results, were examined to find the reason for the errors, and if possible, to correct them. One was found to be capable of correction by a slight change, the error in another was due to a secondary reaction which it was not found possible to eliminate, and so on.

Rather particular attention was paid to a method for estimating chloral hydrate in organs and tissues, the details of which appeared in an authoritative book. Quite recently, in a very late edition of the same book, the method is reproduced exactly as in the earlier one. This method is attacked severely, and in view of its fundamental weakness, and of the numerous statements of other investigators regarding this point, it is surprising that it appears in the same form in the recent edition. The method of estimation has been modified by the writer in such a way as to give results much more nearly correct and less liable to criticism.

One of the main purposes in undertaking this particular study, was to try to develop the method which was most adapted for the determination of chloral hydrate in organs and tissues, so that this phase of the work was studied a little more in detail.

The older methods which depend, in general, on the liberation and measurement of chloroform from chloral hydrate, are obviously unsuitable for its detection in possibly quite small amounts in the animal organism, and these methods, therefore, are merely mentioned. Another reason for not studying these methods is that they are inherently approximate, and are not used at the present time where exactness is required.

Other methods, although perhaps not entirely suited for the determination in bulky aqueous distillates from organs, have been studied to some extent, nevertheless.

Finally, a series of tests was arranged, using for all of them, one carefully prepared solution. The various methods were followed, using this solution, and the results may be considered to be strictly comparative. The tests were made within as short a time as was found convenient, the solution was well corked in an amber bottle and kept in the refrigerator during the course of these experiments.

The results of this series of tests is recorded elsewhere in this report.

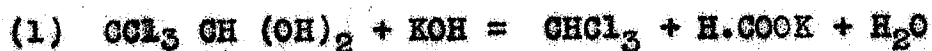
**THEORETICAL
CONSIDERATIONS.**

THEORETICAL CONSIDERATIONS.

Before reviewing the actual methods of analysis, it will be well to discuss in a general way the fundamental reactions on which the methods are based, so that ^{Certain} inherent difficulties may be properly understood at the outset.

Chloral itself is the trichloraldehyde of acetic acid ($\text{CCl}_3 \cdot \text{CHO}$) and chloral hydrate ($\text{CCl}_3 \cdot \text{CH}(\text{OH})_2$) is a hydrated form of this substance, although it appears not to be an ordinary hydrate. It is one of the few substances having two hydroxyl groups attached to one carbon atom. It combines with Sodium bisulphite, ammonia, hydrocyanic acid and acetic anhydride, and reduces an ammoniacal solution of silver.

One particular property of this substance is used as a basis for its estimation. It is acted on very readily by alkalis, giving as reaction products chloroform and alkaline formate according to the following equation;-



The method based on this reaction may be divided into those which depend on a measurement of the volume of chloroform obtained and a subsequent calculation to the amount of chloral hydrate, and those which depend on a determination of the formic acid produced, either by titration of the alkali used up in neutralizing the acid, or by the reduction of mercuric chloride solution.

This reaction as represented by equation (1) appears at first sight to be quite simple, and indeed if the reaction

stopped with the products named above, no difficulty would be encountered. The fact is, however, that a slower secondary reaction is set up between the chloroform and a further quantity of alkali which in the cold appears to be represented mainly as follows,



carbon monoxide being given off and alkaline chloride being formed.

A further complication arises if the alkaline mixture is heated, for, under these conditions a still larger quantity of alkali is used up in neutralizing a second portion of formic acid produced according to the equation



Thiele & Dent¹ have shown that 66% of the chloroform at boiling temperature, is capable of conversion to formate according to this equation. Werner² also shows the possibility of the production of formate from CHCl_3 .

A knowledge of the conditions under which these reactions proceed most favorably, is therefore necessary to a study of methods based upon them. In this connection Enklaar³ says that the rate of decomposition of chloral hydrate by bases, is proportional to the excess of base used over that necessary to form the substance $\text{CCl}_3(\text{CH}(\text{OK})\text{OH})$, and in a second paper⁴ explains what he considers to be the mechanism of the reaction.

A method of determining the formic acid produced during this change, by measuring the volume of alkali used up in neutralizing it, is in common use and is discussed in the next section. Also a similar determination, using the method of reduction of mercuric chloride is given.

VOLUMETRIC DETERMINATION OF FORMIC ACID
LIBERATED ACCORDING TO EQUATION (1)

This method is quite rapid, and if certain precautions are observed it is sufficiently accurate for all ordinary purposes. Substances capable of acting on the alkali should be absent. The liquid should on no account be heated, and the alkali must not remain any longer in the reacting mixture than is necessary to complete equation (1). The alkali should not be too concentrated.

Various modifications suggested by different authors, and given more in detail in the next section, are simply attempts to fulfil the above conditions.

It seems however, that there is always a little doubt as to whether reaction (1) has gone quite to completion, and whether reaction (2) has become appreciable. Self's⁵ suggestion to estimate the amount of chloride after neutralization, and make the necessary correction, seems to be the only practicable way of overcoming the difficulty. It appears to work very well at ordinary temperatures, and certainly gives a definite measure of the progress of reaction (2). Even apply-

applying this correction, the results are erroneous, however, if the mixture is boiled, as under these conditions, a portion of the alkali is used up in neutralizing a further quantity of formic acid produced according to equation (3).

METHOD USING THE REDUCTION OF
MERCURIC CHLORIDE.

The formic acid in the last method was estimated by determining how much standard alkali was used up in its neutralization. The formic acid may also be determined by its reducing action on mercuric chloride, the reduced calomel being filtered and weighed. The method was due originally to Scala⁶, and being gravimetric is capable of giving more accurate results than the previous one. A further point in its favour is that in cold solutions even after long standing with alkali, no more than the merest traces of formate are given according to equation (3).

When proceeding by this method therefore, the alkali may be allowed to remain in the cold chloral hydrate solution until all reasonable doubt as to the completion of reaction (1) is dispelled, and the liquid then made very slightly acid, and the calomel precipitated, as will be described later. This method takes more time than the last one, but it is open to less objections.

Fewer precautions need to be observed, and these are less of a theoretical, than of a technical nature, such as

ensuring the complete precipitation of the calomel.

On the whole, this method is quite satisfactory, if it be remembered that the alkaline liquid must not be heated. In dealing with large distillates, which must be reduced to smaller bulk before precipitating the calomel, they must obviously be exactly neutral during evaporation. If acid, formic acid from reaction (1) will be lost, if alkaline, the chloroform will be attacked, giving an excessively large amount of formic acid. (See equation 3).

A special method of treatment is outlined in the next section to deal with bulky distillates containing chloral hydrate.

A method due to Archangelsky⁷ assumes the formation of chloride but not of formic acid, from the action of alkali upon chloroform in hot solution. He therefore boils the alkaline liquid down to small bulk, and then precipitates the calomel. Elsewhere in this report it will be shown that greatly exaggerated and uncertain values result if this procedure is followed. The modified treatment which is suggested in this paper gives results which are consistent, never too high, and are quite close to the theoretical value.

METHOD USING THE REDUCTION OF MERCURIC CHLORIDE
AFTER DECOMPOSITION BY MAGNESIUM OXIDE.

A method has been suggested in which the reaction expressed by equation (1) is carried out by refluxing the chloral hydrate solution with magnesium oxide. The cleavage of chloral hydrate by this means has been found possible, and, owing to the weaker basic properties, and greater insolubility of the alkali, it has been considered that possibly the interference of the reactions expressed by equations (2) and (3) might, by the use of this alkali be eliminated.

It has been found by several investigators that chloroform is acted on, only to a very slight extent by magnesium ^{oxide} with the formation of a trace of magnesium chloride, but, what seems rather remarkable is that a considerable amount of chloride is formed (about 5% of the original substance) when chloral hydrate itself is boiled with magnesium oxide, so that for our purpose we must consider that equation (2) is quite an appreciable factor in the possible decomposition of the liberated chloroform.

Let us now consider the reaction which we have designated as Reaction (3). Tests made by the author with chloroform and magnesium oxide, for the possible presence of formate were definitely negative. This would suggest therefore, a good method of determination, to reflux with MgO , filter

off or dissolve the MgO remaining at the end of the reaction, and then estimate the formate as usual. In fact, such a method appears in a standard work on drugs. It was thought, however, that by analogy with the formation of chloride from chloral hydrate but not from chloroform, that whereas in the case of pure chloroform, no formate was produced, there might be a possibility of the chloroform being attacked in this way when produced from chloral hydrate. Experiments to test this theory were rather surprising. Instead of results, higher than would be accounted for by the normal amount of formic acid, or at least equal to them, a lower result was in every case recorded. The average value was about 6% too low. Tests were made to ensure the complete decomposition of the chloral hydrate, to guard against loss from the reflux condenser etc., different amounts of MgO were taken, and the refluxing carried on for different lengths of time. It was ^{finally} decided that magnesium formate must be slightly unstable, and two German chemists, in fact, state this to be the case with Mg Formate, and that of the heavy metals, mercury copper etc.

While discussing these theoretical considerations, it might be well to make a short passing reference to the method of Kippenberger ⁵ since he uses magnesium oxide.

The method is essentially one of boiling the organs and tissues with magnesium oxide and distilling off the chloroform produced, which is then collected and determined.

After observing that a considerable amount of the liberated chloroform was converted to chloride according to the equation,



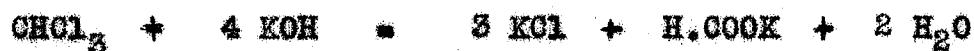
and was, consequently, held back in the distillation flask, he arranged an absorption apparatus to measure the amount of carbon monoxide liberated according to the above equation, thus correcting for any loss of chloroform due to this cause. Theoretically, this would seem to be all that would be required to obtain a perfect result. The amount of chloride obtained, compared with his yield of carbon monoxide however, seems to indicate that the above equation may not be the correct one. Furthermore, if Mg Formate decomposes, giving CO and CO₂, part, at any rate, of the CO measured by Kippenberger⁸ may be from this source and not from the chloroform.

From these considerations then it would appear that magnesium oxide, heated with chloral hydrate under a reflux, is not a satisfactory way of obtaining the equivalent amount of formic acid, and the determinations of the liberated chloroform is attended with some difficulty as indicated in the method just described. It occurred to the author that perhaps if the conversion were done in the cold, in a stoppered bottle, say overnight, the correct amount of formic acid would result. Experiments indicated that this seemed likely, - in fact a much better result was obtained by this

method than by refluxing, but probably a partial decomposition takes place at the boiling temperature at which the reduction to calomel is carried on, as the result is still a little low.

METHOD OF CONVERSION TO CHLORIDE,
BY CAUSTIC ALKALI IN ALCOHOL.

Since the time of Dumas it has been known that boiling alcoholic potash attacks chloroform according to the Equation;-



Since chloral is readily converted, under the circumstances of the test, to chloroform, chloral will also undergo this change.

As this reaction is accompanied by the formation of certain volatile substances including organic chlorides, such as carbonyl chloride, according to Prunier and Messler⁹, the complete conversion of all the chloroform to KCl by heating under a reflux condenser, is unlikely. In fact, A.V.Harcourt¹⁰ states that results depending on this reaction are a few per cent too low. Nieloux¹¹ gives the values 1.5 to 2.0 % low as a constant error. M.Berthelot¹² states that all the chlorine does not combine with the potassium. My own findings indicate that an error of several per cent results if the conversion is done under a reflux con-

denser. By conducting the test in a pressure bottle, however, quite good results were obtained.

For the treatment of a large amount of liquid, several objections are obvious, using either method. A large amount of alcohol must be added, which greatly increases the bulk of liquid. No idea of using a pressure bottle can be entertained for large amounts of liquid, and the refluxing method gives, as before mentioned, low results. A further objection arose in the authors experience. The glass of the pressure flask was attacked, causing a precipitate which had to be removed by filtration before precipitation of the chloride with silver nitrate could be made. Wallis¹³ found this to be the case and stated that Puckner¹⁴ noticed the result by titration of the alkali, to be lower than that by precipitation of the chloride, and stated that this was due to the action of the alkali on the glass of the bottle.

My results were obtained by the gravimetric method, and after filtration of the interfering material, and conducting a blank determination, no difficulty was experienced in getting fairly good results. It might be stated at this point, that considerable chloride is dissolved from the glass, and a blank value of about 7 mgms. of AgCl was obtained. P. Self⁵ also found considerable chloride from the glass, when examining this method. The same author found a pressure of from $3\frac{1}{2}$ to 4 atmospheres in bottles during the tests, and consid-

erable risk of explosion is involved under these conditions.

BY CONVERSION TO CHLORIDE BY
ZINC DUST AND ACID.

This method was originated by Self⁵ in about 1907, after noticing in Watt's Dictionary of Chemistry, (Vol ii p.4) a statement that chloral hydrate, when boiled with Zinc dust is decomposed giving Zinc Chloride and Oxychloride. He suggests doing as stated, then determining the chloride formed.

The method, performed as described by Self⁵ gave results about 15 % low, in the hands of the author, but this was found to be due merely to the time of reaction suggested by Self, being a little too short. Further, he adds acetic acid after the boiling, allows it to stand for two minutes, and then filters off the excess of zinc. There seems no object in conducting the test this way, for in 2 minutes there is manifestly little hydrogen liberated. I found that the test was more satisfactory if the acid was added at the beginning and the boiling was carried on for a longer period than twenty minutes.

Since Zinc dust sometimes contains small amounts of chloride, as pointed out by Self, it is well to conduct a blank determination. The method is quick and clean, there is no etching of the glass and no scaly precipitate occurs as in the alkaline conversion.

Furthermore the results are more exact, at any rate than those done with alkali under a reflux condenser.

IODOMETRIC METHODS.

A method suggested in 1903 by Erwin Rupp¹⁵, and one by I.M.Kolthoff¹⁶ depend on the oxidizing action of a solution of hypiodous acid, produced by mixing iodine solution and alkali. The action may be represented as follows:-



It was later suggested that this method gave good results only if the iodine were added to the chloral hydrate first, the alkali being added afterwards.

The action of alkali and chloral hydrate has been already indicated- Equation (1), earlier in this report. The action is quite rapid, and the first products, at any rate, are alkaline formate and chloroform. This reaction appears to be an interfering secondary reaction in the iodometric method, and is the probable reason for its failure to give exact results if the alkali is not added last.

From certain results obtained in a study of this method, however, it would seem that even if the alkali is added last, this interaction between it and the chloral hydrate is not wholly prevented, using the

concentrations indicated by Rupp¹⁵. About 97 % was the best value obtained by the writer, following the method exactly as described. Wallis¹³ claimed to get no better result than 97 % either.

I found, further, that the manner of adding the alkali affected the results. Pouring in the stated amount of alkali from a pipette, without stirring during the addition, gave a result of about 95 %. By pouring in the alkali drop by drop, with constant shaking, an average value of 98.8 % was obtained.

It seemed, then, that if this secondary reaction could be reduced to a minimum, or, if possible, entirely eliminated, still better results might be obtained. The most likely way, was to dilute the alkali considerably and then make the addition very slowly drop by drop with constant shaking. A value averaging 99.5 % was obtained when this was done, which was about the same value as that obtained by the other methods. It was therefore considered that the results could not, probably, be improved any more, and in a later section the recommendation is made that this procedure be adopted when following this method.

The suitability of this method for bulky distillates was tried out by taking a very ^{much} diluted sample of chloral hydrate. By taking the precautions indicated, a good result was obtained.

In the procedure recommended by Kolthoff¹⁶ the alkali is twice as strong, even, as that suggested

by Rupp¹⁵ and, furthermore, a larger quantity is used so that the possibility of an erroneous result by this method is considerably greater. In fact, results from 62 down to 36 % were obtained in actual ~~xxxxxxx~~ tests.

In the following section, the various methods will be described in some detail, and work done by others, and suggestions made by them will be included, along with my own findings stated in a general way.

**DISCUSSION
OF
METHODS.**

DISCUSSION OF METHODS.

Early Methods

Early investigators used mainly methods in which chloroform or chloral was liberated, and directly estimated, but owing to the difficulty of measuring very small volumes with accuracy, such methods have been replaced to a large extent by methods capable of greater accuracy.

Some of these earlier methods and their originators are given below:-

Müller¹⁷ uses cold KOH for the decomposition of the chloral, Wood¹⁸ boils with lime, and measures the chloroform in the distillate. Paul¹⁹ and Umney²⁰ both heat with ammonia and measure the liberated chloroform. Versmann²¹ adds strong sulphuric acid and measures the liberated chloral.

None of these are very accurate, and indeed, for quite small amounts or for dilute solutions, such methods would be useless.

Volumetric Methods.

Meyer and Haffter²² were amongst the first to use a volumetric method but no mention is made by them of the possible sources of error which were mentioned in the previous section. They use Normal Soda and remark that "Tenth normal alkali does not react with sufficient readiness to make the use of it available."

This volumetric method, was, however, an advance over the earlier ones, and appeared in the old British Pharmacopoeia. (Prior to the 1916 edition). The method set down therein, is essentially as follows;-

If 4 gms. of chloral hydrate be heated with 30 ccs. of normal sodium hydroxide, no more than 6 ccs. of normal sulphuric acid should be required to neutralize the remaining soda.

It was found, however, that this method gave very unsatisfactory results, and Hinrichs²³ has the following to say about its uncertain results;- By warming until all odor of chloroform has disappeared, results 180 to 200 % of theoretical are obtained, while under certain other conditions, three times the theoretical amount may be obtained. He suggests using half-normal alkali and completing the reaction within two minutes at the ordinary temperature. He adds, further, that the results are a little too high, owing to the slight acidity of all samples of chloral hydrate in water.

The B.P. Method was omitted from the next issue (1916) but no satisfactory method was substituted.

Hinrich's method is a distinct advance over the other, as any error in his method, is, at any rate within a few per cent, whereas in the older method, it was liable to be very much greater than that. Hinrich's method has, however, been criticised, chiefly on the ground that during very hot summer weather,

results are obtained which are too high, owing to the partial decomposition of the liberated chloroform under the conditions laid down by him.

Self⁵ has suggested a method, or rather a correction to apply to the above method. A chlorine determination is made after neutralization with sulphuric acid, and by this means, any chloroform which has been converted to chloride is allowed for. Very consistent results appear to be obtained by this method, even under quite wide variations of time and temperature.

Self⁵ draws what I would consider an unfair comparison, however, to bring out the merits of his method. For example, he conducts two tests at 104°F, allowing them to stand 10 minutes with alkali, and states that by Hinrich's method an error of 3-4 % creeps in. In another case the alkali was allowed to be in contact with the reacting substances for 2 hours. Hinrich's method states that the reaction should be stopped at the end of 2 minutes, and these cases quoted by Self, which nearly approach this figure give results within 0.4 % of his own, this difference being due to the acidity of the chloral hydrate. I found the error due to free acid in the sample to be about 0.4 %, and Self states it to be about this amount.

It is certain, however, that a volumetric method depending on the arresting of the reaction on the chloroform before it has progressed to an

appreciable extent, will always be a little arbitrary and uncertain. Self's⁵ method has the merit of being applicable under any conditions of time, and within certain limits, of temperature also, and is useful in the examination of unknown mixtures. If boiled, however, the chlorine correction alone fails to give a correct value on account of the formation of sodium formate.

On boiling a sample, I obtained a value, using Self's chlorine correction, of 101.96% for a specimen which was actually about 99.5% pure. At the ordinary temperature and standing with alkali for 12 hours, I obtained a very good value, however.

Other investigators have varied the time and strength of the alkali to meet the situation.

Garnier²⁴ suggests the use of tenth normal caustic potash for 15 - 20 minutes at 15° C. or less.

Bourdet²⁵ claims that there is considerable error in the French Codex method unless the alkali is cold and free from carbonic acid. He suggests using half normal alkali, and reduces the time to 15 minutes.

Alcock and Thomas²⁶ suggest shaking 4 gms. in a stoppered bottle with 30 ccs. Normal caustic soda for a few minutes without heat and then titrating.

Sargeant²⁷ suggests warming for 5 minutes at 50° C. before titrating back.

François²⁸ uses sodium hydroxide and reduces the time to 1 minute.

Andron³⁰ allows the alkali to act for ten mins.

Quite recently, Francois²⁹ has criticised Andron's ten minute contact, stating that it may lead to decomposition of the chloroform produced.

I found my results quite satisfactory at 71° F. and at 92° F., if 3 gms were treated with a little Ca or BaCO₃, washed on a filter to about 40 ccs. volume, added 25 ccs. Normal soda for 2 minutes to the neutral filtrate, and then titrated with Normal HCl, using azolitmin indicator. At 63° and at 59° F. the results were from $\frac{1}{2}$ to 1% low.

It would seem that a satisfactory method could be worked out, for the purpose of the B. P., using one stated temperature. In hot climates it should be possible to regulate the temperature of a test such as this, by some artificial means.

Owing to the fact that this method is not well adapted to the measurement of comparatively small amounts, and in large dilution, not very much work was done in reviewing the particular suggestions made by Francois²⁸, Andron³⁰, etc. Normal or half normal alkali would still be too strong to get accurate readings, if, say, a decigramme or less, of chloral hydrate were being determined. One decigramme requires for its neutralization, only about $1\frac{1}{2}$ ccs. of half normal alkali. Thus an error of one drop of the latter would represent a difference of about 5% in the value obtained for the determination of chloral hydrate, under these conditions.

Garnier's²⁴ method, using tenth normal alkali

would be better as regards this particular source of error, but if the chloral hydrate is in fairly dilute solution, that is, if a large bulk of liquid is being treated, it is difficult, with an ordinary quantity of alkali of this strength, to get a sufficiently high concentration as to complete the required change. This method is therefore valueless for the above stated purpose.

A little work was done by the writer to see just how quickly the first reaction was completed under certain sets of circumstances, and at what rate the second reaction was progressing under the same circumstances. This was really done in connection with the writer's modification of the method of Archangelsky⁷, but since the volumetric method is based on this very point, it is briefly mentioned here.

25 ccs. of 1% chloral hydrate was treated with 10 ccs. N-Soda for different periods of time, At 2secs. the reaction was about 93% completed. At 5 secs. the reaction was just about complete. At 2 min. the value obtained was about 2 or 3% too high.

By diluting very much more than this, a solution was obtained which reacted slowly enough to make it possible to plot a curve showing the rate of change undergone by the chloral hydrate. (Curve marked I.)

The correct value, under these latter conditions, was obtained at a 4 minutes interval, and at 10 and 45 minutes, the value had not measurably inc-

-reased.

The second reaction under these conditions is therefore quite slow, and the difficulty of the high results observed by so many investigators would seem to be overcome, if the conversion were conducted at a much higher dilution. The above change, completed, as stated, in about 4 - 10 mins. at 25° C. had an initial concentration of alkali equal to only 0.02 Normal. This corresponds to 10 ccs. of Normal alkali, added to the chloral hydrate dissolved in 500 ccs. of water.

If larger amounts of chloral hydrate were used as would be necessary for an accurate assay, probably more alkali would be necessary to complete the reaction in the above time, and a more rapid secondary reaction might take place under these conditions. The results obtained, however, might well repay any study along the lines suggested. The law indicated by Enklaar³ governing the rate of the first reaction might be considered in this connection.

Before leaving this method of determining the formic acid by volumetric means, mention should be made of some similar experiments performed by Wallis¹³. This investigator separated the chloroform produced on the addition of alkali, by a steam distillation, then made acid, steam distilled the acid mixture and titrated the distillate. As would be expected from what has gone before, a high result was obtained, and it was necessary to collect a large amount of distillate to

obtain all the formic acid. He next tried removing the chloroform with two successive shakings with petroleum spirit, and a subsequent steam distillation of the aqueous layer, previously acidified. This time his result was much better, but still not exact. A further experiment was tried, similar to the first one, but using calcium hydroxide. A high result was obtained here also, showing, incidentally, that lime attacks chloroform at the boiling temperature in an analogous way to that of the caustic alkalies, although not to the same degree.

The results of Wallis¹³ are given here as tending to support the evidence as to the further action of alkali on the chloroform initially produced.

Chloral Hydrate used Gms.	Amount Solv't water. ccs.	Method.	Purity indicated %.
.8036	10	Added excess N-NaOH, steam distilled off CHCl_3 , acidified, dist. off formic acid. Titrated distillate.	126.6
.8036	10	Excess N/2 NaOH. Shook out twice with petrol. spirit. Acidif. aqueous layer, steam dist. and titrated distillate.	101.5
.8220	10	Excess of $\text{Ca}(\text{OH})_2$, boiled off CHCl_3 , acidif. steam dist. and titrated distillate.	104.03

Method using the Reduction of Mercuric Chloride

This method was used originally by Scala⁶ and consists essentially in adding acetic acid to the formate solution, which after being made neutral or faintly acid, is treated with an equal volume of saturated mercuric chloride solution. The whole is then heated on the boiling water bath for several hours, and the precipitated calomel filtered on a Gooch crucible, dried at 100° C. cooled and weighed. The weight of calomel multiplied by 0.3503 gives the equivalent amount of chloral hydrate.

The above is an official method in use by the Association of Official Agricultural Chemists of America, for the determination of formic acid, but is slightly modified as follows:-

The mercuric chloride solution is made up with sodium chloride. 100 gms mercuric chloride and 150 gms of sodium chloride are dissolved in water to a litre. A fifty per cent solution of sodium acetate is also prepared. To the solution under examination (150 ccs or less.) 10 ccs. of the sodium acetate are added, 2 ccs. of 10% HCl and 25 ccs of the mercuric chloride reagent. The whole is then immersed in a boiling water bath for 2 hours to completely precipitate the mercurous chloride which should not exceed 1.5 gms. If it does a smaller aliquot should be taken.

This method has the advantage, lacking in the volumetric method described, of measuring only

the formic acid, and not the total acidity. Since it is much simpler to prevent Reaction (3) than Reaction (2), many precautions necessary in the previous volumetric methods may now be almost entirely disregarded. For example, as mentioned in the previous section, a longer time may be given for the complete conversion of the chloral hydrate into chloroform and formic acid, and as long as the mixture is not heated, no appreciable error is introduced. It has a disadvantage, in common with the last method, of being rather unsuitable for bulky distillates, but this difficulty is more easily overcome in this method. Further details of the treatment of such liquids is given in the next section.

No particular references were found in the literature regarding this method, but one or two of the writer's observations may be mentioned here. The A.O.A.C. method already stated indicates that the precipitation should be performed by immersion for 2 hours in a boiling water bath. If ~~set~~ on the bath in the ordinary way, it is my experience that the precipitation may not be complete at the end of 2 hours, and, indeed, may not be quite certain ^f after 4 hours. At any rate, after wasting considerable time at the beginning of the study, through incomplete precipitations, I made quite certain by allowing them to stand the whole day (about 8 hours).

Another point which should be observed,

and if not, may introduce rather serious errors, is that of covering the beaker in which the precipitation is being conducted, with a clock-glass.

The A.O.A.C. method is done in slightly acid solution, and since the reaction with the mercuric chloride is not instantaneous, some formic acid may escape before being acted upon. In point of fact, a closed vessel fitted with a reflux would be the most correct way to conduct the test. The difference between this and the last mentioned method is so slight, however, as to be hardly worth the extra trouble, and seems to be within the experimental error, anyway.

The above points were observed when making determinations of formic acid from chloral hydrate, as discussed in the next section.

Archangelsky's⁷ Method

A method of estimating the amount of chloral hydrate in animal tissues and organs, suggested by Archangelsky⁷ is discussed here because the actual determination of chloral is based on the principles involved in the last section. The method, as outlined by Archangelsky, and which appears in the 1928 edition of Autenrieth's Book "The Detection of Poisons and Powerful Drugs" is, briefly, as follows:-

The finely cut organs, reduced to a thin liquid, are steam distilled with an equal weight of 20% phosphoric acid for from 12 to 20 hours.

The distillate is treated with 30 - 50 ccs. of Normal caustic soda and the whole evaporated down to 30 - 50 ccs. This is then neutralized with acetic acid, and an equal amount of saturated mercuric chloride added. Several hours on the boiling water bath precipitates an amount of calomel equivalent to the formic acid present.

The calomel is filtered off, dried, weighed and calculated to chloral hydrate by using the factor 0.3503.

The mechanism of the cleavage of chloral hydrate by alkali into chloroform and formic acid has been discussed at some length in the preceding pages, and the precautions necessary to prevent the formation of a further quantity of formic acid according to Equation (3) have been stated. Since the method about to be discussed takes no account of these changes, it is interesting to note what difference in results is obtained by following the method exactly as stated, and by using a modification which overcomes the objections.

As pointed out by Thiele and Dent¹, chloroform and alkali react to form formic acid. According to these authors, amounts of chloroform ranging from 21.14 to 36.14% are convertible to formic acid between the temperatures of 25° C. and 100° C.

The conditions are made favourable, it is true, by stirring and by continuing the action for several

hours, but nevertheless, it is definitely shown that formic acid results from the interaction. The writer confirmed this point, at any rate, as regards the boiling temperature. The conditions of Archangelsky's method are such that the chloroform produced is heated with alkali, at any rate until what remains is removed by boiling, and it would be expected, therefore, that an abnormally high result would be obtained from the use of this method.

It might also be expected that with different amounts of chloral, a different concentration of alkali would remain after the first action was over, and thus some results might be higher than others. If a distillate were heated very, very gradually, a different value again might be recorded, as the chloroform would be driven out of the solution while the temperature was yet low enough to prevent very much formic acid from being produced, and a value close to the theoretical one might in this case be obtained.

These variations were actually obtained by the writer. Three values obtained by using .25 gm. chloral, were found to be about 150% of the true value. One determination using 2 gms. of chloral hydrate gave a value of about 107% of the correct amount, while a similar test in which the solution was, after warming, allowed to cool during the night, and was subsequently evaporated, gave a value about equal to the theoretical one. Two earlier results had given 117.3 and 115.8%

Thus besides almost invariably giving high results, the method also gives very uncertain results.

A modification of the method to overcome these objections, was based on the fact that the distillate, after conversion to chloroform and formic acid must be exactly neutral during its evaporation to low bulk. If made acid, formic acid will be lost; if alkaline, the objections mentioned above will be encountered. The modification consists then, in allowing 30 - 50 ccs. of normal alkali to remain in the distillate or in the separate portions of distillate, only for about 10 - 15 minutes, in the cold, with occasional stirring, normal acid then being run into the solution until neutral using litmus (azolitin). About 3 drops of N- acid in excess are now added, and about a decigramme of precipitated CaCO_3 is added. After stirring for some time, the litmus indicator will gradually change color, and the neutralized liquid may then be filtered and evaporated to small bulk. When cold, one or two ccs. of 10% HCl are added to dissolve any small amount of carbonate, and the whole washed into a graduated flask, from which aliquot parts may be taken for the precipitation.

This method gave, for chloral hydrate of about 99.5% purity, results of 99.04 and 98.72%.

By omitting the filtration of the excess CaCO_3 , and simply evaporating, a result of 98.36% was obtained.

Any objection which might be made, that the preliminary treatment with alkali might increase the formic acid content, was overcome by making up a mixture of formic acid and chloroform occurring in 0.2 gms of chloral hydrate, adding alkali and treating exactly as indicated. The value of the formic acid present was first determined in the absence of the chloroform. The result when the chloroform was present was 0.1% lower than the blank value, No increase was observed, therefore,

Results using this method, then, although a trifle low, are more consistent than those obtained by the method without modification.

Another point which was investigated in regard to the method of Archangelsky, was the length of time necessary to remove all but the merest traces of chloral hydrate from the tissues, by steam distillation. It was thought that the time given, 12 - 20 hours, seemed almost unnecessarily long.

A steam distillation was first tried, using a simple solution of 4 gms. chloral hydrate in a litre of liquid made acid according to Archangelsky's instructions. The receiver was changed when each 100 ccs. of distillate had come over, in order to trace the rate of recovery of the chloral hydrate. The titration method of estimating the recovered chloral, prevented a long series being obtained, and owing to the difficulties with this method which have already been discus-

-sed, the results may be considered rather approximate.

A complete series was next obtained by taking 2 gms. chloral hydrate and distilling as before, but determining the chloral by the modified method just discussed. By the time three and a half litres was distilled over, no weighable amount remained behind, and the total recovery was 98.10%, the substance being about 99.5% pure. Considering the fact that there were nineteen separate determinations and the possibility of as many attendant losses, this recovery was considered fairly good.

A distillation was now made using sausage meat ground thoroughly with chloral hydrate solution. The recovery seemed a little slower in this case, and four and a half litres was distilled. The last 500 ccs. of distillate contained only 3 mlgms. so the distillation was discontinued. The total recovery was found to be 96.6%.

The results of these two distillations are indicated in the form of a graph, the curves being designated as Curve III and Curve IV. (Page 61a)

It is doubtful if a much longer distillation would have added more than a part of a per cent. to the above, and the fact is that a little must always remain behind, no matter how long the distillation is carried on. Unless a very exact result be required, therefore, it would seem that the above time of distillation is sufficient for most purposes.

Another distillation using 4 gms. of chloral hydrate in a litre of liquid, was made, taking off each 500 ccs. up to 4 litres, and treating it by the modified method indicated. The whole was then evaporated down, and washed into a graduated flask. an aliquot portion was then taken for the precipitation. The completeness of the removal of chloral hydrate was confirmed by treating a further quantity of 100 ccs. of distillate with $HgCl_2$, after conversion with alkali, for several hours on the water bath. No sign of a precipitate was observed. It may be stated then, that in this test, no measurable amount of chloral was left behind. A total recovery of 5.918 gms. was obtained corresponding to a value of 96.0%, which agrees pretty well with the first distillation, which gave 98.1%.

An objection may be raised in regard to the suitability of a method of distillation and reduction of mercuric chloride, for the establishing of the presence of chloral hydrate in organs etc. It might be argued that certain volatile bodies present in animal tissues, especially when decomposed, might easily reduce mercuric chloride and give an erroneous result. An experiment performed by the writer, showed that even fresh meat gave a few migs. of material (not crystalline) which would be weighed as $HgCl$. There was this difference, however, in that case the precipitation was conducted in a portion of the distillate without any previous treatment.

It will be noted that in the modified method, the neutralized solution is boiled down to a very low volume, so that all the volatile bodies except those of an acidic nature would disappear, only the acidic substances being held back as salts. Of the fatty acids, only the first member, formic acid, has the power of reducing mercuric chloride, so that the possibility of any considerable error after being subjected to the proposed treatment, is very slight indeed in the opinion of the writer.

In connection with the estimation of chloral hydrate in urine, or alimentary organs, Dragendorff and Tiesenhausen³¹ state that an extraction may be made directly with ether, but that if blood is present, distillation is recommended. The sensitivity of various tests for chloral hydrate is also given, that for formic acid, which is the test used in the above method, being given as 1/9000 gramme.

Method using reduction of Mercuric chloride after decomposition by Magnesium Oxide.

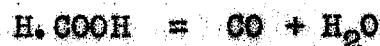
In Fuller's Book "Chemistry and Analysis of Drugs and Medicines" appears the method of determining the formic acid produced by the treatment of chloral hydrate with MgO. This was discussed at some length in a preceding section, and it was shown that the amount of formic acid is always lower than the theoretical amount. It was suggested that the

magnesium formate is partly decomposed at the boiling temperature. H.Kunz-Krause and P.Manicke³² state that this is so, and sum up their report as follows;-

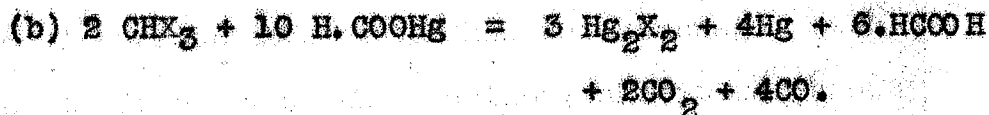
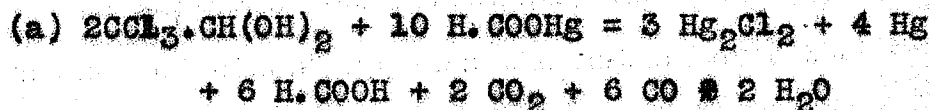
"MgO and the oxides of Hg, Zn and Cu react with
 "chloral hydrate in a similar manner to that of
 "the alkaline and alkaline earth metals insofar
 "as chloroform results, independently of the
 "chemical character of the metallic ion involv-
 "ed. There is this difference, however, in that
 "the formic acid simultaneously formed, remains
 "as formate in the case of alkali and alkaline-
 "earth hydroxides, while with MgO and the oxides
 "of the heavy metals, decomposition of the form-
 "ate into CO and CO₂ takes place."

Equations using Hg. indicate the kind of reactions which go on.

Principal reaction.



Secondary reactions.



This would seem to dispose of the possibility of an easy method of determining formic acid by the use of MgO, at any rate at the boiling temperature.

It has been mentioned that the writer obtained much better results by standing overnight, in the cold, instead of refluxing, but that the loss which is still in evidence, might be due to the partial decomposition of the formic acid at the boiling temperature at which the reduction is carried out.

At any rate, performing the tests as described above, I obtained results about 6% low when refluxed, and 2 - 3% low when done in the cold.

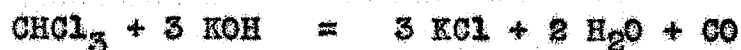
Method of Kippenberger⁸.

This method is really the alternative method to the one just described. In that, the formic acid was determined, after cleavage by MgO; in this, the chloroform, similarly produced, is determined.

It was shown by Kippenberger⁸ that chloroform itself was only acted upon by MgO to a very slight extent. This, I found by my own experiments to be the case. Rosenthaler and Reis³³ state that it is not acted upon, but that when chloral hydrate is similarly treated, the latter is decomposed chiefly into chloroform and formic acid, a secondary reaction at the same time taking place, whereby CO & MgCl₂ are formed. This was found also by Kippenberger,

who considers the loss of chloroform by this reaction so serious as to work out a means of correcting for it.

For certain obvious reasons, a determination of the chloride remaining in the distillation flask at the end of the experiment is hardly practicable when dealing with organs, tissues etc. so Kippenberger estimates the amount of CO evolved according to the equation;-



which theoretically should take account of any chloroform decomposed in the above manner. His purpose in evolving this method, is to find one suitable for forensic purposes. He wishes to overcome the loss of the amount of chloroform undergoing the above change.

Unfortunately, his recovery is quite low, and is not improved very much by the correction mentioned.

The method is briefly as follows;-

The material is tested with litmus, and made slightly acid. MgO is then added and the whole distilled in the ordinary way into a Woulff's bottle. The second neck of this bottle is connected to a wash-bottle containing HCl and then to a ten-tube Lunge absorption tube containing palladium chloride which is kept warm by means of a bath of warm water. Air is aspirated through the apparatus until the chloroform is all over, and the CO has been absorbed into the palladium chloride. Any liquid chloroform collected in the Woulff's bottle is separated in a funnel, and

weighed directly. The aqueous liquid containing dissolved chloroform is treated by Vortmann's method, a procedure using KMnO_4 and Iodine. To these two quantities is added the amount of chloroform corresponding to the reduced palladium. The following results given by Kippenberger indicate the values obtained.

(1) Chloral Hyd. used.	(2) Liq. CHCl_3 <u>Calc.as</u>	(3) Dis'lvd CHCl_3 Chloral	(4) CO Hyd.	(5) Per cent. recov- ered.	(6) Left in dist'n flask.	(7) Per cent unaccounted for.
5.0	3.8006	0.2601	0.0115	31.4	10.9	7.7
10.0	8.9151	0.2522	0.0415	92.1	4.5	3.4
10.0	9.0507	0.4312	0.0328	95.1	2.1	2.8

The volume of liquid decreases from 300 ccs, in the first to 200 ccs in the second, and the last has only 120 ccs.

From a study of the above figures, several things will be noticed. A considerable loss which is unaccounted for is shown in Column 7. This, it is explained, is by loss of chloroform from the use of rubber connections. Another loss is the considerable quantity of chloroform which is found at the termination of the test, in the distillation flask. (Column 6.) These two amounts reduce the percentage of recovered material considerably, as will be seen from Column 5, and the greater the volume of liquid present at the beginning, the greater the loss, it would seem. With, say, a litre of liquid, as was used by the writer in studying the

method of Archangelsky, the loss would most likely be greater. A very interesting point, however, is in connection with the ^{small} amount of palladium recovered.

In an earlier experiment, in which Kippenberger refluxed 5 gms of chloral hydrate in 100 ccs. of water with 10 gms of MgO, the amount of chloride left in the flask corresponded to a decomposition of 0.2384 gms of chloral hydrate, converted into chloride. If this amount be compared with the values in Column 4, of the table, it will be noticed that there is a very great difference. The amount of chloral hydrate, calculated from the recovered palladium is only a small fraction of what would be expected from the known amount of chloride formed in the single experiment. How can this difference be explained? It might be interesting to compare the chloride left in a given distillation with the amount of CO evolved during the same distillation, to see if the given equation actually represents the reactions involved. It may not be as simple as suggested by Kippenberger. The equations given by Kunz-Krause³² at any rate suggest that it may be fairly complex. A correct interpretation of the reactions involved would probably explain the values given in Column 7 of the table, as unaccounted for. A rather serious objection to the measurement of the CO evolved, as a measure of the chloroform decomposed to chloride, arises if the work of the German chemists Kunz-Krause and Manicke³² be consid-

ered in this connection. They have shown, as indicated a little earlier, that magnesium formate partly decomposes into CO and CO₂. Thus a part of the absorbed CO in Kippenberger's experiments may have been from this source and not from the chloroform at all, since Mg formate is present in the distillation flask, and may be decomposed as indicated.

The method has a value for forensic purposes, in that chloroform is actually recovered and weighed as such, but the percentage recovery might be improved to some extent. Until a more definite relationship is established between the CO evolved and the amount of chloroform decomposed into chloride, the whole method rests on rather insecure foundations, in the opinion of the writer.

Method of conversion to chloride by Caustic Alkali in Alcohol.

This method has been used by a considerable number of chemists, perhaps because it is also a method of determining chloroform.

The theory of the conversion by alkali to chloride has been indicated, and many of the references in the literature are to variations in technique, or to applications to the analysis of some particular drug preparation or similar substance. For example, L.de St. Martin³⁵ uses the method to determine chloroform. He suggests adding a definite volume of alcoholic KOH, and

after the conversion to titrate the excess of KOH, and also to determine the chlorine.

Pückner¹⁴ uses a pressure bottle, but otherwise it is similar to the last.

Futt³⁶ determines the chloroform in cough syrups by the use of a pressure flask.

Sasse³⁷ hydrolyses chloroform to chloride, and then titrates the chloride.

Murray³⁸ and Moraw³⁹ of the A.O.A.C. studied and finally approved a pressure bottle method for the determination of chloral hydrate and chloroform in drug products.

Kolthoff¹⁶ applies the same principles but uses a sealed tube for the purpose.

Willgerodt⁴⁰, in determining chloroform in liniment uses a pressure bottle

Dowzard⁴¹ also uses the method of heating with alcoholic alkali, to determine the chloroform in lozenges.

Russwurm⁴² studies the amount of chloroform in organs by making an acid and then an alkaline distillation, combining the distillates, diluting with alcohol and refluxing with alcoholic KOH. The chlorine is then estimated by titration.

Nieloux¹¹ determines CHCl_3 in amounts from 0.1 to 0.004 gms., by heating for 1 hour in a reflux 60 ccs. of alcohol containing the chloroform with 10 ccs of 10% alcoholic KOH, then estimating

the chloride. This was the method, in general, used by the writer in this work.

Wallis¹³ in trying to work out a satisfactory method of determination, decided from a consideration of the work of some French workers, Chancel & Parmentier⁴³ who had investigated the decomposition of chloroform, to use a closed vessel with alcoholic caustic soda for three hours.

This method is stated exactly, since it was used by the writer when making this type of conversion in a bottle under pressure.

0.1 gms of chloral hydrate in 10 ccs alcohol is treated with 10 ccs of Normal aqueous soda in a small glass bottle. A rubber stopper is tied securely into the neck of the bottle, which is heated for 3 hours in the water bath. On cooling and opening the bottle, the chlorine content of the liquid is determined by precipitating with AgNO_3 from nitric acid solution, or by titration.

Wallis found the best results were obtained when the alcoholic alkali was equal to about 50% by volume of alcohol, and with this strength, he obtains a series of results; - 99.47---99.3--- 99.4---99.3.

My own results were obtained in a round bottomed Jena glass flask with rather small neck which was easily corked and tied. The results gave a mean value of 99.63%.

Method of Examination of Organs by Bettink and Van den Driessen Mareeuw⁴⁴.

This method was noticed when perusing the literature of the subject. It is of interest since these writers state that the methods of Archangelsky⁷ and of Kippenberger⁸ for the estimation of chloral hydrate give divergent values. From the discussion of these two methods given already, it seems only to be expected that a considerable difference should occur.

The writers then go on to give a method of repeated extractions with alcohol, the chloride naturally present is removed by a little AgNO_3 , the excess of AgNO_3 is removed with MgO , and the whole is then refluxed for 5 hours with KOH free from chlorides. The chloride from the decomposition of the chloral is then determined.

The low values obtained by this method have already been discussed.

Conversion to Chloride by Zinc dust and Acid.

This method, as given by Self⁵ is as follows:-

0.5 gms of chloral hydrate in 60 ccs. of water is treated under a reflux condenser for 20 mins. with 2 gms of zinc dust. 10 ccs. of acetic acid are now added, and after 2 mins. the clear liquid is filtered from the zinc, and the chloride

determined.

By carrying out the determination exactly as stated above, the correct values could not be obtained. By liberating nascent hydrogen throughout $1\frac{1}{2}$ hours, however, by adding the acid at first, the reaction was found to be complete.

My purpose in making a comparative study was to compare the results obtained by the various methods, but also to include results which were quite certain to have been completed. A sufficiently long time was given for the reaction therefore, in order to accomplish this end. It is just possible that with the acid added at the beginning, a twenty minute period of refluxing might be sufficient.

My exact results are given in the Chapter on Experimental Work.

Iodometric Methods.

Rupp's Method¹⁵ is briefly stated as follows:-

Take 10 ccs of 1% chloral hydrate solution and place for 10 mins. in a stoppered bottle with a mixture of 25 ccs. N/10 Iodine solution, with 2.5 ccs of N/KOH solution. Then dilute with 50 ccs. water, add 5 ccs. HCl (Sp. Grav. 1.125) and titrate the liberated Iodine with N/10 Thiosulphate. 1 cc. Iodine = 0.00827 gms. chloral hydrate.

Rupp⁴⁵ states later, that the Iodine must be added first and the alkali last, and the low results obtained by the writer and by Wallis¹³ have been mentioned. By a suitable modification, quite accurate results were obtainable.

The modification consists in adding to the mixture of chloral hydrate and Iodine, instead of 2.5 ccs of Normal KOH, 25 ccs of Tenth normal KOH or NaOH, with the precaution of adding it rather gradually, with constant shaking. After 10 mins. continue as suggested by Rupp.

Results averaging 99.49 were obtained.

The method of Kolthoff¹⁶ is carried out as follows:-

To 25 ccs. N/10 Iodine solution, add 10 ccs. of 2N Caustic soda and 10 ccs. of N/10 soln. of the chloral hydrate. Stopper and stand 1 hr. Acidify with dil. HCl and titrate the excess of Iodine with Thiosulphate solution.

Adding these in the order named, a result of only 36.4% was obtained by the writer.

Modifying this by adding the alkali last results of 59.1 and 62.4% were obtained. The reason for the uncertain results is probably the same as in the method of Rupp, so that the subject need not be further discussed.

Comparison of Results by Various methods.

An experiment to make a comparison between the results obtained by the various methods, was performed, using an accurately prepared 1% solution for all the tests.

For the volumetric test, only one author's method was suitable, that of Garnier²⁴ using tenth-normal alkali. The methods using normal or half-normal alkali on a 1% solution of chloral hydrate, would obviously be incapable of giving results of the same order of accuracy, as would the other methods tried, since 1 cc. N-alkali = 0.1655 gms. chloral hydrate. Garnier's method had a better chance of giving an accurate result, but even with this, it was thought that if 25 ccs. of chloral solution were used instead of 10 ccs. and about a proportionate amount of alkali was used, that a more accurate result would be obtained. The temperature was kept at 13 - 14°C. for 20 mins. and values of 98.7% - 98.7% were obtained. It would seem that either a larger amount of alkali, a stronger solution, or a higher temperature, should be used in order to complete the reaction.

Better results were obtained by using, as before, a neutralised solution of chloral hydrate, but treating with 30 ccs. tenth normal alkali for ten mins. at room temperature. Results of 99.3 - 99.3 were obtained by this method.

The determination of the formic acid by reduction of HgCl_2 , after a conversion with 10 ccs. of N-soda for 3 - 4 mins., gave values of 99.4 - 99.4%, and another one at 20 - 25 mins. gave the same value.

A similar result, after conversion with 50 ccs. of tenth normal alkali for 20 - 25 mins. at a temp. of about 25° (not, as in Garnier's method at 15°) gave a result of 99.4 also. It would seem as though the conversion were complete therefore, and the above figures may be taken as the values obtainable by this method, when the conversion is properly completed at ordinary temperatures.

After conversion by refluxing with HgO , the results were low, when subsequently treated with HgCl_2 . This has been discussed pretty completely in the previous sections of this report. About six results, all obtained under slightly different conditions were, every one found to be low, before using the special solution used in the comparative study. The results obtained in this special study were 92.88 and 92.40%. These results, along with the earlier values and the exact conditions of the various tests, are given in the section dealing with the experimental work.

The high and uncertain results obtained by the method of Archangelsky⁷, which needs no further discussion, are exemplified by the following results:- On 2 gms. - 107% when boiled quickly, 99.73%

when warmed, allowed to cool overnight and then evaporated down. On 0.25 gms heated naturally on a boiling water bath - 150.5% - 148.5% - 131.0%. were the values obtained.

The modification which I suggested for dealing with bulky solutions of chloral hydrate, for use in conjunction with the steam distillation used by Archangelsky, gave on the special solution, values of 99.04% - 98.72%.

By the method of refluxing with alcoholic alkali, which is reputed to give low results, I obtained values several per cent low. They were 96.23 and 95.31% respectively.

Heating with alcoholic alkali in a closed bottle according to the method of Wallis¹³, the following results were obtained. 99.54 - 99.84 - 99.77 - 99.38%, or a mean value of 99.63%. The importance of using gas tight bottles is illustrated by the fact that, at first two bottles were used with rather wide necks, and whose corks leaked slightly. Results of 98.38 and 98.61 were obtained with these.

The treatment of Chloral hydrate solution with Zinc dust, according to the method of Self⁵ gave low results; - 84.6 - 85.91%, but by putting in the acid at the beginning of the test and allowing it to proceed for 1½ hrs, results of 99.90 - 99.95 - 99.75 were obt-

ained.

Of the iodometric methods, that by Kolthoff¹⁶ gave very low and uncertain results; - 36.4 - 59.1 - 62.4%, the lowest being obtained when the materials were mixed in the order stated by Kolthoff. The other two were obtained by changing the order.

The method due to Rupp¹⁵ gave at first, results from 94.8 to 97.7%, but later by adding the alkali slowly drop by drop, results of 98.25 and 98.45 were obtained. By using the modified treatment with tenth normal alkali and constant stirring, values of 99.49 - 99.49 were obtained.

The various results in this series are given in tabular form under Experimental Work.

By leaving off values which we have reason to think are incorrect because of some theoretical difficulty, or because of the obvious incompleteness of the reaction, we obtain a mean value of about 99.5%. Keeping this figure in mind, we may examine the table, and see which methods tend to give results below, and which above this value. The columns chosen for the determination of this mean value were No's 1, 3, 9, 11 & 13.

**EXPERIMENTAL
WORK.**

EXPERIMENTAL WORK

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Preliminary

A statement in Thorpe's Dictionary of Chemistry, credited to Dumas, states that "boiling KOH does not affect chloroform".

This was found not to be the case, before reading the statements of other investigators. Chloroform was boiled with 10 ccs. N/10 alkali. Only 8.0 ccs of N/10 acid were required to neutralise the remaining alkali, and a dense ppt. of AgCl was obtained on adding silver nitrate.

Volumetric

Action of alkali on chloral hydrate under varying methods of treatment showed the following:-

With N/10 soda in cold for 10 mins.	- 99.3%
" " " " " overnight.	-110 %
With N-Soda in cold overnight.	-138 %
" " " boiled for some time.	-205 %

The method was the usual one of titrating back with standard acid, and the usual calculation of assuming the acidity to be all formic.

Volumetric. (contd.)Correction for chloride.In cold.

Took 25 ccs. 1% chloral hydrate solution, added
30 ccs. tenth normal alkali, and stood at room
temp. for 12 hours.
Back titrated with H_2SO_4 and determined chloride.

Alkali consumed = 15.59 ccs N/10
Chlorine equiv. = .56 ccs N/10

15.03 ccs x .1655 x 4
= 99.50%

Compare with mean value of 99.5 obtained
from the comparative table.

By boiling

Repeated the above, but brought slowly just
to boiling, then cooled and titrated.

Alkali consumed = 19.1 ccs N/10
Chlorine equiv. = 3.79 N/10

15.31 ccs equiv. to
101.96%

Self's⁵ chlorine correction good at
ordinary temps. - valueless at boiling.

Error due to acidity of Chloral Hydrate.

3 gms of chloral hydrate dissolved in water, and treated with a little CaCO_3 and filtered. This treated for 2 mins with 25 ccs. N-soda, and then titrated with acid.

A similar test was made on a portion which had not been neutralised. The volume of liquid being made the same as the first.

Untreated-----	99.85%
Neutralized-----	99.41%

To test Hinrich's²³ method at various temperatures.

The above test was made at room temp.
This was repeated at $33\frac{1}{2}^{\circ}$ - $17\frac{1}{2}^{\circ}$ and at 15°C .

At $33\frac{1}{2}^{\circ}$..	99.52
At $21\frac{1}{2}$ -----	99.41
At $17\frac{1}{2}$ -----	99.03
At 15-----	98.97

Hinrich's method gives good results at 21°C or above, but seems incomplete below this.

Rate of Decomposition of Chloral Hydrate by Alkali.1st Expt.

Took 25 ccs. 1% solution - Added 10 ccs N-Alkali for varying periods of time. Then titrated with acid.

At 2 secs.-----	93.0%
At 5 secs.-----	about correct value
At 2 mins.-----	several % high.

Rate of Decomp. (contd.)Expt. 2.

Repeated the last, diluting chloral solution so that normality at the beginning would be one quarter of that of the previous test.

At 5 secs the reaction was 65% completed.

This action was still too rapid to be able to plot a curve.

Expt. 3

Diluted still further to one quarter the normality of the last. Initial normality here is about 0.02 N.

At 5 secs.....	26.48%	converted
15 "	46.34	
30 "	76.12	
45 "	81.44	
1 min.....	89.36	
4 mins.....	99.30	
10 mins.....	99.30	
45 mins.....	99.30	
3 hrs.....	107.92	
12 hrs.....	129.08	

Curve I.

Purpose was to plot a curve so that the course of the reactions, both (1) and (2) could be seen.

Above results are rather approximate as N-soda was used.

Expt. 4

Similar to the last but three times as much alkali was used.

At 4 - 5 mins...22°C.....	99.44%
30 mins...22°C.....	107.44%
30 mins...32°C.....	110.76%

Considerable error now at 30 mins.

Rate of Decomp (contd.)Expt. 5. Using tenth normal alkali.

25 ccs. 1% solution chloral hydrate, neutralised, and filtered. Added 30 ccs. tenth normal alkali.

At 2½ mins.....	25°C99.9%	
5 mins.....	"100.1%	
10 mins.....	"100.2%	<u>CURVE II</u>
15 mins.....	"100.2%	
2 hrs.....	"101.0%	
4 hrs.....	"102.8%	

The first readings were not taken early enough to complete the series, but the rate is shown pretty well.

To find the action of boiling Na_2CO_3 on CHCl_3 .

According to statements by Degres⁴⁶ it was thought that alkaline carbonates would not attack chloroform as does caustic alkali.

Expt. 0.5 ccs CHCl_3 and 20 ccs water were refluxed with 10 ccs. Normal Sodium Carbonate for 1 hr.

Result. A heavy ppt. of AgCl was obtained with AgNO_3 , and 26 mgms of HgCl_2 were obtained on reducing HgCl_2 .

This only corresponds to a decomposition of about 0.8% of the chloroform taken. The error would probably be too large to permit its use for splitting up chloral hydrate, however.

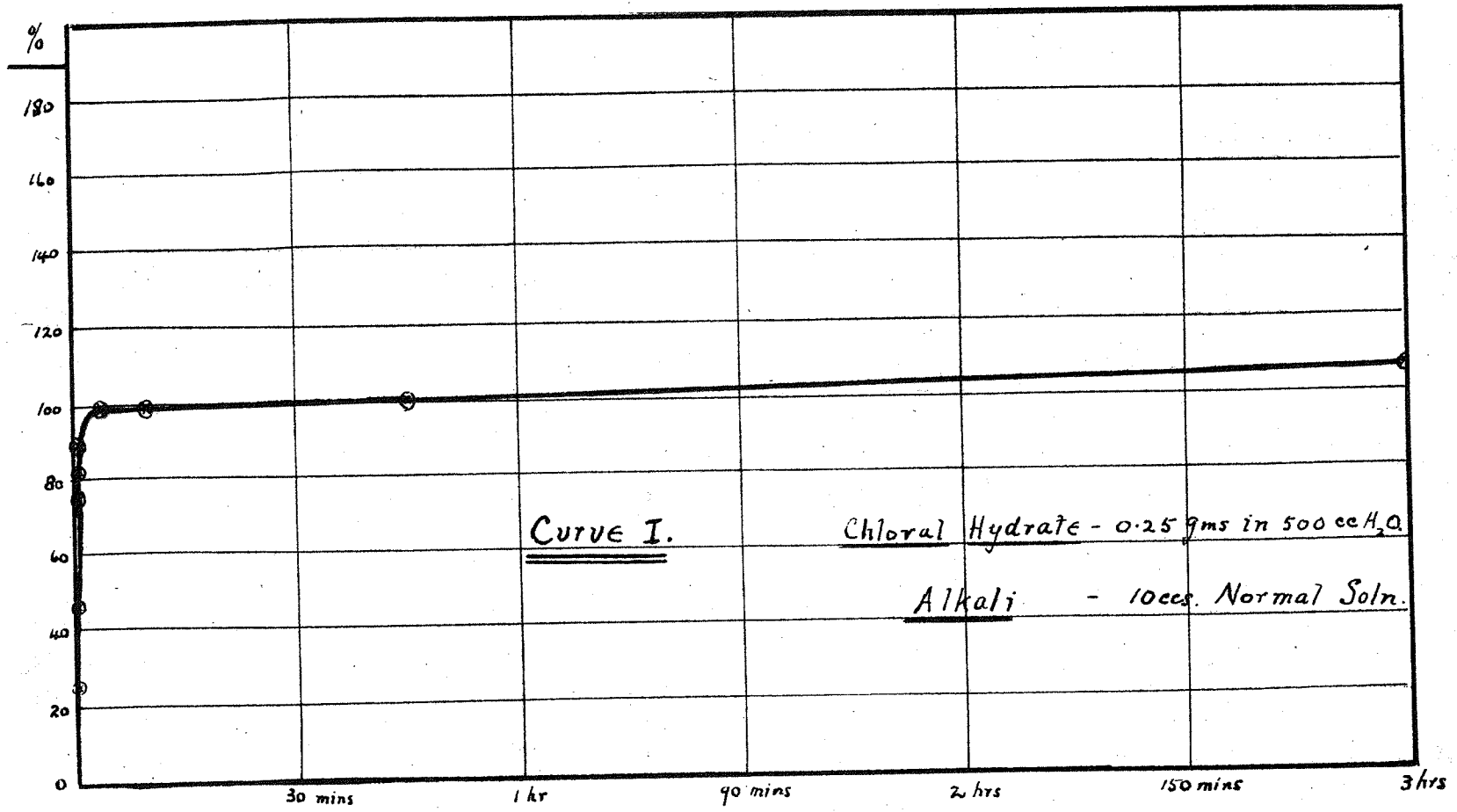
Volumetric. Comparative Series

Expt.

25 ccs. special solution. 30 ccs. N/10 alkali added, after neutralising with CaCO_3 . After 10 mins, titrated back.

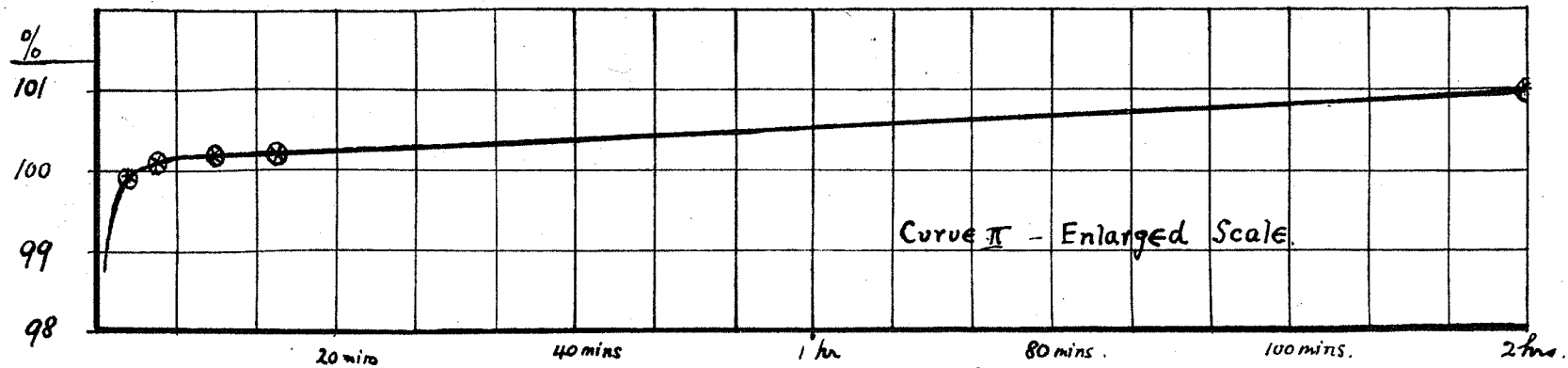
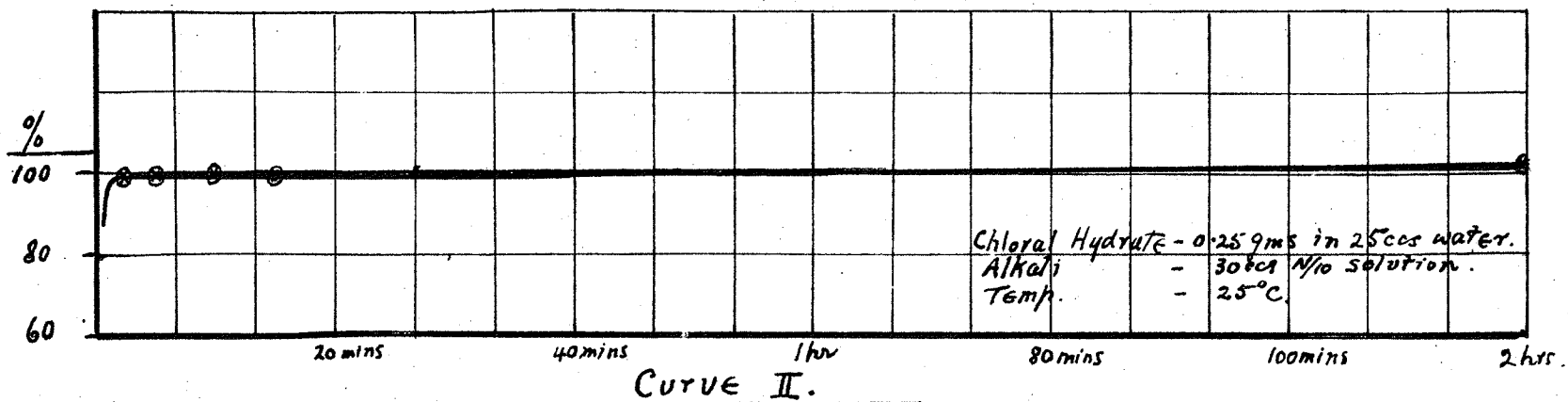
Results. 99.3%
99.3%.

Rec'd here



Curve showing Decomposition of Chloral Hydrate.

(55a)



Rate of Change - Using N/10 Alkali.

Volumetric-Comparative series (contd.)Expt. 2. Garnier's Method.

25 ccs special solution treated with a slight excess of alkali (18.6 ccs. of N/10) for 20 mins. at 13 - 14° C. The reaction appears incomplete.

Results - 98.70%
98.70%.

Method of Reduction of Mercuric chloride
after decomposition of Chloral by NaOH.

Expt. To find out if long contact between alkali and chloral results in higher values, by this method.

- (1) 25 ccs of 1% chloral hydrate solution and 30 ccs. N/10 alkali were allowed to stand 12 hrs. at room temp. Formic acid then determined.

This was repeated leaving alkali only for a short time in contact with chloral.

Result. The difference between the above was no greater than is obtained between duplicate determinations when using this method.

- (2) Repeated the above expt. but used the concentrations stated in the modified Archangelsky's method, and used an artificial mixture of formic acid and chloroform.

Result. The amount of formic acid found agreed with the amount taken, showing that no increase had taken place.

HgCl₂ method - Comparative seriesExpt. Reduction method but used different methods of conversion with NaOH.

- (1) 25 ccs. special solution, with 10 ccs of N - alkali for 3 - 4 mins, Made slightly acid and precipitated.

Results. - 99.36%
99.44%

- (2) Same as above but stood 20 - 25 mins this time.

Results. - 99.40%

- (3) Same as above but used 30 ccs. N/10 alkali at 25°C. for 20 - 25 mins.

Result. - 99.40%

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Expt. Archangelsky's method of boiling with NaOH.

- (1) 0.4125 gms chloral hydrate in 1 litre of water, treated with 30 ccs N -soda, and evaporated down. solution neutralised and pptd.

Result. - 113.8%

- (2) 0.3500 gms in 1 litre of water treated as before, but evaporation done in separate portions in a Pt.dish. (100 cc.)

Result. - 117.3%

- (3) 0.2000 gms. in litre of water treated as before, but evaporated in air bath (100°C)

Result. - 142 %.

- (4) Chloroform boiled with alkali, and tested for formic acid. Result. 97 mlgms HgCl (one case)
30 mlgms (in another)

Archangelsky's method.contd.

- (5) 2,000 gms chloral hydrate in 500 ccs water treated with 40 ccs N -soda, and placed on the water bath. Bath was shut off for the night shortly afterwards, and the beaker remained uncovered. On evaporating down the next day, a good result was obtained

Result. - 99.73%

- (6) Above experiment repeated exactly, except that after treatment with alkali, it was brought rather rapidly to the boil, and then evaporated as usual on the water bath.

Result. - 107.01%

(7) Comparative series

25 ccs. special solution used. Diluted to 725 ccs. with water. Added 30 ccs. N -soda. Evap'd to 30- 50 ccs. Neutralized with acetic acid (free from formic acid.) Added equal volume of sat'd $HgCl_2$ solution, covered with clock glass and placed on water bath for several hours.

Results. - 148.5%
151.0%
150.5%

-----00000-----

Modification of Archangelsky's Method.

Expt. Comparative series

- (1) 25 ccs solution used as before. Diluted to 725 ccs. Added 30 ccs. N -soda for 10 mins. Neutralised with HCl and added 3 drops N -HCl in excess. Added 0.1 gm. $CaCO_3$ stirred, filtered, evap'd to 30 - 50 ccs. Added 1 cc. 10% HCl. and ppt. with $HgCl_2$.

Results. - 98.72%
99.04%

Modification of Archangelsky's method. (contd).

- (2) Last expt. repeated but tried omitting the filtration after adding CaCO_3 .

Result. - 98.36%

Further examination of method.

- (3) To meet objection that the 10 min. treatment with alkali used in this method, might increase the formic acid value, an artificial mixture of formic acid and chloroform in the correct proportions, was treated exactly as in Expt. (1) of this series. (See last expt. on page 56.)

Preliminary tests were made without chloroform, to obtain the amount of formic acid actually present.

Result. No increase was observed.

- (4) Distillate from animal tissues (sausage meat) was treated with HgCl_2 .

Result. 400 ccs distillate gave about 3 mgms. flocculent ppt.

Steam Distillation Experiments.

Preliminary To get a general idea of the course of the distillation.

Expt. (1) 2 gms. chloral hydrate in small amt. water. Added 4-5 drops 10% tartaric acid. Steam distilled and titrated every 50 ccs. dist. with N-soda, standing with alk. for few mins.

1st 50 ccs.....	0.98	gms	chloral	hyd.
2nd " "	0.48	"	"	"
3rd " "	0.24	"	"	"
4th " "	0.12	"	"	"
	<u>1.82</u>	"	"	"

Steam distillation (contd).Expt.

- (2) 0.2 gms in about 100 ccs. water and made slightly acid, steam distilled and titrated using tenth normal alkali.

1st 50 ccs.....	0.0758	gms chl.hyd.
2nd " "	0.0501	" " "
3rd " "	0.0336	" " "
4th " "	0.0199	" " "
	<u>0.1794</u>	" " "

- (3) 4.0 gms chl.hyd. in 100 ccs. water. Added equal wt. of 20% phosphoric acid (Arch's instructions) and steam distilled slowly. Titrated first portions with N -soda and later ones with N/10 soda.

1st 50 ccs.....	2.20	gms.
2nd " "	0.89	
3rd " "	0.46	
4th " "	0.23	
5th " "	0.130	
6th " "	0.063	
7th " "	0.036	
8th " "	0.020	
	<u>4.029</u>	gms.

- (4) 4.0 gms as above, but total vol. of liquid was 1 litre this time. 9 portions of 100 ccs. taken off, and titrated as above.

1st 100 ccs.....	0.952	gms.
2nd " "	0.712	
3rd " "	0.554	
4th " "	0.430	
5th " "	0.347	
6th " "	0.256	
7th " "	0.198	
8th " "	0.153	
9th " "	0.122	
	<u>3.723</u>	gms.

Steam distillation (contd.)

Expt.
(5) Complete series using pptn method.

2 gms chloral hyd. in 500 ccs water with equal weight phosphoric as before, making 1 litre of liquid. Steam distilled and used the modification of Arch's method to determine the recovered chloral.

1st 100cc...	0.4764 gms.		
2nd "	0.3558		
3rd "	0.2713		
4th "	0.2074		
5th "	0.1569		
6th "	0.1210		<u>500 cc. portions.</u>
7th "	0.0885		
8th "	0.0717		
9th "	0.0551	1st 500cc...	1.4685
10th "	0.0433	2nd "	.3776
11th "	0.0288	3rd "	.0871
12th "	0.0224	4th "	.0230
13th "	0.0163	5th "	.0048
14th "	0.0115	6th "	.0009
15th "	0.0081	7th "	.0003
<u>1500 ccs.</u>	<u>1.9350 gms.</u>	<u>3500 ccs.</u>	<u>1.9620 gms.</u>

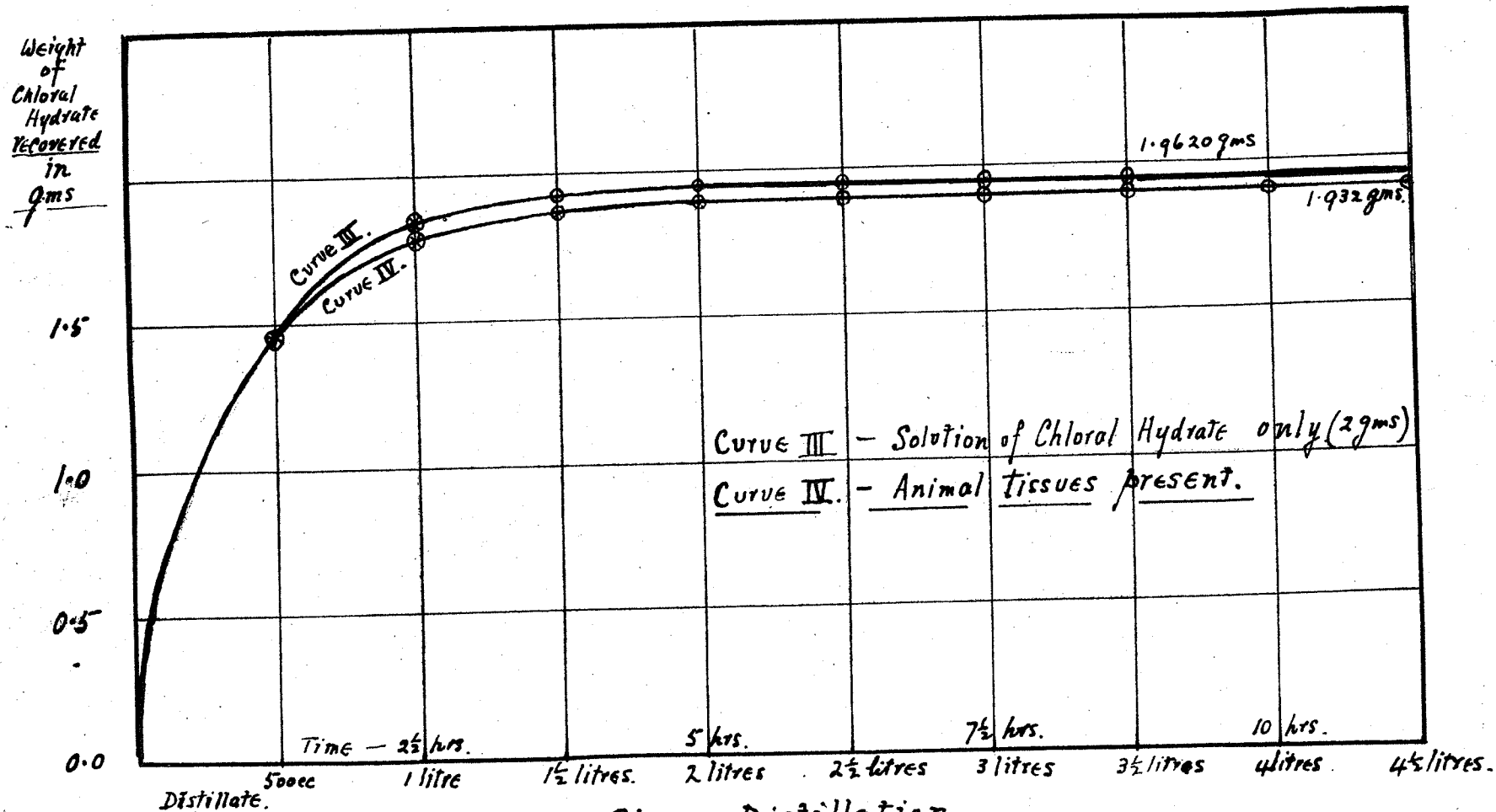
Result - 98.1% total recovery.

Expt
(6) Above distillation was repeated, but sausage meat was allowed to remain with the chloral overnight, after thoroughly grinding together, 500 cc. fractions separated.

1st 500cc...	1.4610 gms.	
2nd "	.3160	
3rd "	.0943	
4th "	.0313	
5th "	.0108	
6th "	.0062	
7th "	.0052	
8th "	.0038	
9th "	.0033	

$4\frac{1}{2}$ litres 1.9319 gms.

Result - 96.59% total recovery.



Steam Distillation
by Modification of Archangevsky's Method.

Steam distillation (contd.)

Expt.
(7)

The second last experiment (No.5) was repeated, but no separate determinations made. The various fractions were each treated by the modified procedure, evaporated down and washed into a graduated flask from which an aliquot part was taken.

Result. - 3.918 gms. - 98.0% recovered.

cf. Expt (5) which gave 98.1%

-----ooOoo-----

Method of
Refluxing with MgO.

Expt.

- (1) 0.25 gms chl.hyd. in 175 ccs water refluxed for $\frac{1}{2}$ hr. with $1\frac{1}{2}$ gms MgO. Filtered off MgO, and ppt'd. $HgCl_2$ solution.

Result. - 71.0%

- (2) 0.25 gms chl.hyd. in 150 ccs water refluxed for some time with 2 gms MgO. Dissolved excess MgO and ppt'd.

Result. - 91.8%

- (3) Repeated above using rubber stopper between reflux flask and condenser. Used a spiral condenser. Refluxed for 1 hr. 2 gms MgO.

Result. - 84.9%

- (4) Repeated using a trap containing alkali, to see if any loss at top of condenser. Slight loss observed but total recovery was still low.

Result. - 85.0%

Refluxing with MgO. (contd.)

Expt.

- (5) Increased length of time under reflux to
2½ hrs. 1 gm MgO.

Result. - 89.6%

- (6) Refluxed as before, but used a water bath,
and increased time to 5 hours. 2 gms MgO.

Result. - 88.8%

- (7) Refluxed as in (6) but filtered off an
aliquot part, and tested for the complete
conversion of the chloral. This was done
by treating with NaOH for short time. The
results on the part treated with NaOH, were
the same as the untreated part.

Conclusion. The chloral is completely
converted into chloroform and formate,
but the formate may be further decom-
posed. (See Kunz-Krause³².)

- (8) To confirm the decomposition of Mg Formate
on boiling, a definite and known quantity of
formic acid was refluxed with MgO. The amount
of formate remaining was then determined.

Result. 93.9% only, was recovered.

- (9) Comparative series. 25 ccs solution (1%) made
to 100ccs. Refluxed for 2 hrs with 1 gm MgO.

Results. 92.88% - 92.40%.

Reaction with MgO in the Cold.

Expt. 0.25 gms in 25 ccs. water, stood overnight
in a stoppered bottle with 1 gm MgO.

Result. - 97.80%
96.84%

Expt. to see what action between chloroform
and MgO under the same conditions as the last.

Took 0.12 ccs chloroform and 1 gm MgO
with 25 ccs water. Stood overnight in same
bottle.

Result. No formic acid produced.

Decomposition by MgO (Contd.)Effect of refluxing chloroform and MgO.

Expt. Chloroform was refluxed for some time with 0.6 gms MgO, and the solution tested for chloride.

Result 9 migs of AgCl were pptd.

Only a slight decomposition occurs therefore, when pure chloroform is used.

Expt. 0.12 ccs chloroform refluxed with 1 gm
(1) MgO, and tested for formic acid.

Result. None found.

(2) This was repeated using 5 ccs chloroform.

Result. No formic acid found.

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Decomposition by Alcoholic alkali.By refluxing.

Expt.
(1) 10 ccs. 1% solution chl. hyd. were refluxed for 3- 4 hrs with 1 gm KOH and 70 ccs alcohol (95%). The alcohol was evaporated while alkaline, on the water bath. Nitric acid was added and excess of AgNO₃.

Result. - 96.85%

(2) Comparative Series. Repeated above exactly, using the special solution, but only for 1 hr.

Result. - 96.23%
95.31%

Decomposition by alcoholic alkali. (contd.)By using a closed bottle.

Several expts valueless - corks pushed out.

Expt.

- (1) 10 ccs of 1% solution chl.hyd. treated with 0.4 gms NaOH and 10 ccs alcohol in a rather wide necked bottle fitted with a rubber stopper. The stoppers were believed to have leaked slightly as the results were lower than those obtained by Wallis himself, and those in the next expt.

Results were all about 98.5%

Expt.

- (2) The above was repeated using round-bottomed Jena glass flasks with necks about 3 inches long, and taking a small stopper. These were not so apt to be forced out as the larger stoppers used in the last expt.

Results. 99.54%

99.84%

99.77%

99.38% after allowing for a blank of about 7 mlgms of AgCl.

-----ooOoo-----

Reduction to chloride by Zinc dust

Preliminary. Zn of different fineness, and different periods of refluxing tried.

Expt.

- (1) 2½ gms of 20 mesh Zn, 15 ccs acetic acid, added at the beginning, and half an hour's refluxing with 0.25 gms chl.hyd. in 65 ccs. water.

Result. - 80.96%

Reduction to chloride by Zinc Dust. (contd.)

Expt.
(2)

Repeated using Zn dust and acid on a water bath for $1\frac{1}{2}$ hrs.

Result. - 99.68%

Expt.
(3)

A similar test using 30 mesh Zn, and standing in the cold for 2 days, after refluxing.

Result. - 99.16%

Expt.
(4)

Comparative Series. Following Self's⁵ directions implicitly, special solution.

Results. - 84.60%
85.91%

Expt.
(5)

Special solution. modified above by adding the acid at the beginning, and refluxing for $1\frac{1}{2}$ hrs.

Results. - 99.90%
99.75%
99.95%

-----00000-----

Iodometric Methods.

Rupp's method following the later instructions to add the alkali last.

Expt.
(1)

10 ccs 1% solution chl. hyd. Added 25 ccs. N/10 Iodine. Added $2\frac{1}{2}$ ccs. N- soda. Stood 5 mins. Diluted added HCl and titrated with N/10 Thio-sulphate solution.

Results. - 94.76% 96.43% 97.01%
94.76% 97.09% 97.67%
94.76% 95.02% 97.67%

Iodometric methods (contd.)

Expt.

- (2) Tried to improved method. Added more Iodine, and more alkali. Conducted test as before but used 40 ccs Iodine, and 4 ccs N- soda. The soda was added very slowly with constant shaking.

Result. - 99.9%

- (3) Thought Rupp's amounts might give better results if the alkali was added slowly.

Result. - 99.41%

- (4) Comparative series. The above was repeated using the special solution.

Results. - 98.25%
98.66%

- (5) Alkali of N/10 strength was substituted, and 25 ccs was poured in very slowly, with constant stirring.

Results. - 99.49%
99.49%

- (6) Application to large volume of liquid.

The last experiment was repeated, but the chl. hyd. was diluted to 750 ccs. with water. Since freshly boiled water was not used, a blank expt. was run.

Result. - 99.6%

Kolthoff's Method.

Expt.

- (1) 25 ccs Iodine (N/10) was mixed with 10 ccs. 2N- soda. 10 ccs of 1% chl. hyd. soln. was added in a stoppered bottle and stood for 1 hr. It was then acidified and titrated with Thiosulphate (N/10)

Result. - 56.4%

- (2) By adding the alkali last a higher result was obtained.

Results. - 59.10%
62.44%

TABLE OF

Titration with Alkali.		Treatment with alkali and reduction of mercuric chloride				
N/10 at room temp.	N/10 at 15°.	NaOH diff. times & strengths.	NaOH then CaCO ₃ & evap- orati.	NaOH boil- ed. A's meth- od.	MgO refl- ux'd.	MgO in cold.
(1)	(2)	(3)	(4)	(5)	(6)	(7)
99.3	98.7	99.36	99.04	99.73#	92.88	96.84
99.3	98.7	99.44	98.72	107.0 #	92.40	97.80
		99.40		150.5		
		99.40		148.5		
				131.0		
Mean values.						
99.3	98.7	99.40	98.88	-	92.64	97.32

- Conditions of heating were different.

Average of Columns (1) 99.3
 (2) 99.4
 (3) 99.63
 (11) 99.87
 (13) 99.49
 Mean. 99.54

COMPARATIVE VALUES.

Estimation as Chloride				Iodometric methods.		
Alcohol- ic KOH reflux- ed.	alcohol- ic KOH closed bottle.	Zn dust.	Zn dust & acid.	N- soda added slowly	N/10 soda added slowly	2N- soda Kolthoff's method.
(8)	(9)	(10)	(11)	(12)	(13)	(14)
96.23	99.54	84.60	99.90	98.25	99.49	36.4
95.31	99.84	85.91	99.75	98.66	99.49	59.1
	99.77		99.95	99.41		62.4
	99.38					
95.77	99.63	85.25	99.87	98.77	99.49	-

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**GENERAL
CONCLUSIONS.**

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GENERAL CONCLUSIONS.

Certain rather interesting conclusions may be drawn from a consideration of the results obtained in this little study.

With regard to the volumetric method, treating with caustic alkali and then titrating the excess of alkali, it seems pretty well established that in fairly high concentrations, such as obtains when a sample is being assayed, for instance, by Hinrich's B.P. method, the required decomposition is completed quite rapidly, and great care must be exercised to follow the stated time quite closely.

It seems also a fact, however, that in quite dilute solution, say an initial concentration of alkali equal to only 0.02 normal, $\frac{1}{2}$ gm. of chloral hydrate is completely converted in about ten minutes. A short time longer at this dilution, has not the same harmful effect in decomposing the chloroform, and a very much reduced error is therefore introduced, should the time be varied slightly.

It should be remembered that, by this vol. method, an error of about 0.4% is introduced, if the solution is not first neutralized.

Self's method of correcting the high results obtained on long standing with alkali, by determining the chloride produced, appears to be quite satisfactory, when the solution has not been heated with alkali. Otherwise a high result is obtained even with the use of the correction.

The volumetric method is, in general, quite rapid, and applicable to ordinary routine analysis. Where interfering substances are present, however, or in working with unknown concentrations, the method is hardly to be recommended.

The method depending on the reduction of mercuric chloride, by the formic acid produced from a preliminary treatment with caustic alkali, is a much more accurate method. Being gravimetric, it is capable of giving better results, but this is not the sole reason. The chief objection of the volumetric method, the production of hydrochloric acid, does not affect the results obtained by this method, and therefore, so long as the solution is treated with alkali in the cold, for a length of time within reason, no measurable error is introduced.

This leads up to a statement of the conclusions in regard to the method of Archangelsky⁷.

It seems well established from the evidence submitted by the writer and by other investigators, that the method of boiling down distillates with free caustic alkali must be abandoned if accurate results are desired. The simple modification of converting with caustic soda in the cold, for a reasonable time, then making exactly neutral by addition of acid and CaCO_3 , overcomes the former objection, and at the same time allows large volumes of liquid to be evaporated without change or loss of the formate. The objections to a reduction method, when dealing with animal tissues which may contain other reducing substances, were discussed in an earlier section. The evaporation in neutral solution, as indicated above, effectually removes, in the opinion of the writer, practically all bodies which would be likely to interfere with the subsequent reduction of mercuric chloride.

The course of the distillation under several different conditions has been indicated, and, in general, it seems that a shorter length of time might be used, than that stated in Archangelsky's method. The safest way is, no doubt, to test the last runnings from the condenser with mercuric chloride, until satisfied that no weighable amount is left behind.

The method is, in the writer's opinion, open to less serious criticism than the method of Kippenberger⁶ although the latter is designed to be

used for forensic purposes.

Kippenberger's method has the merit of weighing and identifying the chloroform as such, but in the courts, it is doubtful whether this would prove the presence of chloral hydrate any more effectually than the first method. The order of accuracy seems to be higher in Archangelsky's method, as modified, than in that of Kippenberger.

It is of interest to note that Bettink and Mareeuw⁴⁴ found divergent values by the two methods. This difficulty would probably disappear, almost completely by the use of the modified treatment of the distillate, as indicated.

The method of refluxing chloral hydrate with MgO with a view to estimating the formic acid produced, seems to have been definitely shown to be unreliable.

The difficulty experienced by Kippenberger when using MgO, in regard to the partial decomposition of the chloroform produced, indicates that the estimation of chloroform after using MgO is also a doubtful method.

Results on decomposing the chloral hydrate in the cold were also insufficiently accurate to be used.

The poor results by Nieloux¹¹ method of refluxing with alcoholic alkali, seems to indicate that this method is not so suitable as some others, yet it appears to be widely used, and with a correction may be considered to give sufficiently accurate results for the purposes required.

The bottle method of Wallis¹³ gave quite good results. It must be remembered that a considerable blank value was obtained, and according to Wallis, this amount decreases as the bottle is used. This detracts from the usefulness of the method considerably. In addition, the evaporation of the alcohol and the filtration of the insoluble material which comes from the glass, are operations which take some time. It has, besides the objection that it is quite useless for any considerable quantity of liquid.

Self's⁵ method of treatment with Zinc dust, gave quite good results, but only when modified by the addition of the acid before refluxing. However, I see no reason why this should not be done. The method is accurate, rapid, free from effect on the glass vessels, and has much to recommend it. It could probably be used with success on fairly large amounts of liquid also.

The iodometric method, after modification, gave satisfactory results, and was found to work quite well with a large volume of liquid. A distillate from animal organs might quite conceivably contain bodies which would react with Iodine, however, so that this method would be open to some criticism, for that purpose. For ordinary assay work, ^{however} it would be preferable, I should think, to the much discussed volumetric method

The comparative series of tests, using one common solution is interesting, and serves to show which methods are the most reliable. It was a matter of interest to see that, at least four or five of the methods listed in the table (under Experimental Work) which methods are all mentioned in the literature, were not reliable as they stood. The iodine method was modified before good results could be obtained, and the Zinc dust method had to be changed slightly. The refluxing method using alcoholic alkali was not capable of improvement, except by making the conversion under pressure. The method of determining the formic acid, after refluxing with MgO could not be improved except by treating in the cold, and even then was not considered satisfactory. The volumetric method of Garnier²⁴ gave rather doubtful results, and better results were obtained at room temperature and using

a little larger excess of N/10 soda.

The methods considered fairly satisfactory, then, were those given in the table under Columns (1), (3), (9), (11), and (13).

The writer's modification of Archangelsky's method, given in Column (4) is a little low, and it has occurred to me that perhaps the Calcium formate present, after addition of the CaCO_3 , may be decomposed to a slight extent, in an analogous way to that of Mg formate, but to a much less extent.

The average value of the five satisfactory results is found to be 99.50% which may be taken as the degree of purity of the sample used.

SUMMARY.

SUMMARY.

- (1) Archangelsky's method of determining chloral hydrate in organs and tissues, has been modified to give more reliable results. Results by the old method ranged from 100 to 150%; by the new method, uniform results, about $\frac{1}{2}$ to 1% low are obtained.

It is suggested that the distillation need not be quite so long, 7- 8 hours, instead of 12- 20.

Kippenberger's method is discussed and compared with Archangelsky's method.

- (2)2) The determination of formic acid after refluxing with MgO is unreliable, as Mg formate is unstable at the boiling temperature. Low results are always obtained.
- (3) The low results attributed to the method of refluxing with alcoholic alkali and subsequent determination of chloride, were confirmed.
- (4) The decomposition with Zinc dust was found to be incomplete in twenty minutes, but good results were obtained when slightly modified.
- (5) The method of decomposition with alcoholic alkali under pressure gave good results.
- (6) The iodometric method of Rupp¹⁵ was found to give low results, but was modified to overcome the interfering secondary reaction.

Summary (Continued.)

- (7) The volumetric method for the determination of the formic acid produced by the action of N/10 caustic soda, was found to give good results, if a fairly large excess of alkali was added, and if done at room temperature.

Garnier's method indicated that the change was hardly complete below 15°C, and when using only a small excess of alkali.

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