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A CRITIQUE OF CERTAIN METHODS OF GASTRIC ANALYSIS.

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If there is a wide diversity of opinion concerning the best method of accomplishing a purpose, it is generally fair to conclude that no one method is satisfactory. Such a conclusion seems wholly justified in the case of the quantitative determination of the HCl of the stomach contents. It would be difficult to find any small field of work in which there has recently been more active endeavor to provide measures suitable for clinical purposes and in which there are at the same time so many records of fruitless labor. There is no serious difference of opinion concerning the simple qualitative tests for free acids in general, for free HCl or for lactic acid. The presence of free acids in general can be readily and satisfactorily determined by using Congo paper or solution. Free HCl can be quickly shown with phloroglucin-vanillin or resorcin solutions, and lactic acid can be demonstrated with Uffelmann's reagent, using extraction with ether if necessary. The total acidity also is easily calculated by mere titration with decinormal sodium hydrate solution. When, however, it is desired to determine the total quantity of HCl or the total free HCl present, one finds a number of widely different procedures recommended. It is customary in the more extensive treatises upon the subject of gastric disease to give descriptions of numerous methods, but of the more

elaborate of these it is generally admitted that the only one that has any strong claim to accuracy is that devised by Sjoeqvist and subsequently modified by Sjoeqvist himself and by a number of other authors, particularly by Salkowski and Fawinszy and v. Jaksch. If everything claimed for this method be admitted, it must still be granted that the necessity for somewhat elaborate apparatus and for numerous solutions, together with the fact that it requires a good deal of time, make it practically useless to the clinician and rob it of much of its value to the research student. Further, several investigators consider the method unreliable, and Leo in particular has demonstrated satisfactorily that the phosphates are a constant source of error, and that if any ammonium chloride chances to be present in the gastric contents it adds to the uncertainty. The method is now used almost solely for testing the accuracy of results obtained in research, and its use even for this purpose is of doubtful value. The difficulty in obtaining satisfactory results in determining the total HCl is indicated by Sjoeqvist himself, who states that "any attempt to provide an exact method of determining the HCl quantitatively is certain to meet with obstacles that cannot be overcome."

Much of the eagerness to discover such a method was the result of the somewhat unreasoning enthusiasm that ensued upon the introduction of chemical methods for the diagnosis of gastric diseases and the rather general tendency to believe that such methods could be made to a large degree to supersede bedside diagnosis. I think that one is now justified in stating that the value of chemical examination of gastric contents has been largely overrated and is still overrated by many special writers upon this subject. It is in most instances much less valuable than proper physical examination of the patient, particularly when the

latter procedure includes inflation and the investigation of the motor power of the stomach. In carcinoma the chemical alterations are more suggestive than in any other condition, and are very important as indications of the actual nature of the disease; but even in carcinoma no change is constant and no chemical condition can be said to be absolutely indicative of malignant disease. All forms of changes in the chemical condition of the gastric contents, from hyperacidity down to anacidity, may be due to numerous primary causes which cannot be directly discovered by chemical methods, and the exact difficulty in each case can be learned only by eliciting a proper history and by proper physical examination. In most instances an elaborate chemical examination of the gastric contents does not lend any real assistance in definitely determining this difficulty. Recognition of this fact is seen in the recent writings of a number of authorities, and in the more or less general custom now in use of investigating in most cases only the presence or absence of free HCl and of lactic acid, the total acidity and in some instances the free HCl. The fact that the results from more elaborate methods have been shown to be of uncertain value, together with the general recognition that usually more elaborate results, even if trustworthy, are not of much importance, has led to simplicity in clinical examinations instead of the elaborate measures of chemical diagnosis that were previously adopted. The most simple and yet the most rational description of the chemical examination of the gastric contents and of the measures necessary may be found in Fleiner's book.

But there are a few conditions in which exact chemical methods, including the determination of the amount of combined HCl, are either necessary or of much interest. They are often of importance in

research, since in order to establish a point the total HCl should be definitely known. In ordinary clinical work only two conditions are frequently met with in which exact estimations are of value. One of these is hyperacidity, in which it is often important to determine the amount of acidity which is due to combined HCl, for it is now recognized that both the free and combined HCl are active in producing symptoms, not the free HCl alone. The other common cases in which such a determination is of importance are those which show absence of free HCl but still show some acidity. In such instances one frequently desires to know whether any HCl has been secreted and is present in combination with protein, and this fact can be learned only by a quantitative estimation of the HCl. It has been my custom in cases of the latter class to make a test for the presence of the milk-curdling ferment, which can be easily and rapidly done. If this ferment is absent the case may at once be considered to be one of achylia, as the milk-curdling ferment is apparently almost always the last to disappear of the substances secreted by the stomach. This is of course a rough method of diagnosis, and even though the test were positive, it does not always indicate the presence of HCl, but it is practically as accurate as most of the more direct clinical methods, and leads one into no more error. This rough method was adopted because no method in use until very recently seemed to furnish more trustworthy results.

There are four methods, however, of determining the total HCl of the gastric contents which may be easily used by clinicians, and which are worthy of consideration. These are Leo's and Töpfer's, one recently described by Hewes, of Boston, and a still more recent one described by Cohnheim and Krieger. Leo's method is perfectly satisfactory in cases in which organic acids are absent, and if simple qualita-

tive tests for lactic acid and for volatile organic acids are negative, it may be used clinically with entire confidence, remembering always that calcium chloride must be added to the stomach contents before the primary titration for total acidity is carried out. But in cases in which organic acids are absent, mere titration for total acidity is a close index of the amount of HCl present, and is entirely satisfactory for clinical purposes. Except, therefore, for research purposes, there is no frequent good in using Leo's method; and since it cannot be used in all cases, it is unsatisfactory in research, for results in research are not satisfactory unless a constant method of procedure is adopted. As to Töpfer's method, I have previously stated that I consider it of little value even for clinical purposes, because the color-changes in the titrations are so difficult to recognize that they can be satisfactorily appreciated only by one who is well acquainted with them and who has sharp appreciation of color-changes. The results also in the titration for free HCl are often erroneous, as organic acids are included if present in any considerable amount. I have had no reason to change my opinion concerning this method. I have rather, as will be explained later, had reason to hold this opinion more emphatically, and I believe that I am in agreement with the majority in this position. The method is certainly not widely used, as reports published from various parts of the world rarely indicate its use, and a number of those who previously recommended the method somewhat enthusiastically have discarded it. Hewes, for instance, who spoke favorably of it some years ago, has recently stated that he considers it insufficient even for clinical purposes. Of the two remaining methods, that of Hewes and that recommended by Cohnheim and Krieger, I think the latter, so far as it has yet been studied, seems to be

by all means the most simple and at the same time the most accurate method that has yet been devised. As to Dr. Hewes' method, for reasons which I will state later, I believe that it is not sufficiently accurate to be of any value. Cohnheim and Krieger's method depends upon the fact that phosphotungstic acid and the salts of this acid precipitate native albumins and the products of their digestion in combination with the phosphotungstic acid. The method, in essence, consists in a determination of the total acidity of the gastric contents, then adding to another portion of gastric contents a solution of phosphotungstate of calcium, which precipitates the albumins and albumoses and sets free the HCl in combination with them; the HCl combines with the calcium of the phosphotungstate and forms neutral calcium chloride. There occurs, therefore, a reduction of acidity corresponding to the amount of combined HCl present, and the combined HCl is at once indicated by titrating a second time after the precipitation and by determining the difference between the second and the first titration. The authors report a series of results which they obtained with stomach contents, controlling these results by Sjoeqvist's method, and also results obtained when working with known quantities of HCl in solutions containing Witte's peptone. They also tried the method with solutions containing peptone, HCl and lactic acid. Their results were strikingly accurate.

The method seems extremely rational and simple, but it is of importance to confirm the results of the originators of the method and to determine whether the phosphates produce any error through a possible combination with albumins or through changes in the phosphates themselves, such as that which occurs in Leo's method. The latter is not to be anticipated because free HCl is always present in this method,

while it is not in Leo's method. Nevertheless conclusions can be reached only by actual examination of the method. In using the procedure with solutions of Witte's peptone in HCl of known strength, I obtained results which were practically absolutely accurate in every instance. The free HCl in the mixture was determined by using phloroglucin-vanillin as an indicator and the combined HCl by this method. The added results gave the total HCl; in almost every instance a quantity of HCl practically equal to the amount known to be in the solution was shown by the method. The results, when lactic acid was present, were similarly accurate. Solutions of HCl, Witte's peptone, and of acid sodium and calcium phosphates were then tested, the phosphates being added in a strength of 0.2 per cent. Larger quantities of phosphates are practically never found in the gastric contents. The results in all these instances indicated only the HCl present and indicated that in full amount. The method is therefore apparently entirely accurate, certainly much more accurate than any other method which has been suggested with the possible exception of Sjoeqvist's; and it is so simple and so rapidly carried out that it is always at the service of the clinician.

The method is as follows: The calcium phosphotungstate is prepared by making a 4 per cent solution of commercial phosphotungstic acid, heating, and adding calcium carbonate until, after gentle boiling, the reaction becomes neutral; then filter. This solution may be kept indefinitely. In carrying out the test determine the total acidity; then to ten cubic centimetres of gastric contents add thirty cubic centimetres of the calcium phosphotungstate solution, filter off the precipitate, wash the filter, collecting the washings with the filtrate, and titrate the filtrate and washings. Subtract the second result from the first, and the figures obtained represent the acidity due to

combined HCl. Rosolic acid is used as an indicator in each case. The free HCl is estimated by titration with phloroglucin-vanillin, and the total amount of HCl is obtained by adding the results for the free and the combined HCl.

If free HCl is absent decinormal solution is added until a marked reaction for free HCl appears, the amount added being known, and the method is then carried out. Any excess over the amount added that may be found is then due to combined HCl.

As a modification of the tset that is so simple as to be undeserving of such a name, but one that hastens the result decidedly, I would suggest filtering into a cylinder graduate after the precipitation, taking twenty cubic centimetres of the filtrate, titrating this and doubling the result. This avoids loss of time in filtering and washing. The results in the first titration are better if the stomach contents are diluted about five times, as the color-change with rosolic acid is then sharper.

To refer once more to Töpfer's method. I stated reasons for considering this method inaccurate, and my conviction on this point has become more fixed since I have tested Cohnheim and Krieger's method. In the latter method, as I stated, I have, with the originators of the procedure, found practically regularly in solutions of HCl of known strength, containing peptones (and therefore containing both free and combined HCl), that titration with phloroglucin-vanillin and the use of Cohnheim and Krieger's method, gave results which, when added together, represented accurately the total amount of HCl present. I think that this demonstrates that both the phosphotungstic precipitation and the phloroglucin-vanillin titration give results which are, practically speaking, entirely accurate so far as such

mixtures are concerned. Now as to Töpfer's method, Strauss found that the results for free HCl obtained by this method (dimethylamidoazobenzol) were nearly always decidedly higher than those obtained with phloroglucin-vanillin. I had the same results. Phloroglucin-vanillin being generally considered to give accurate results, the proper conclusion seemed to be that the results with the dimethylamidoazobenzol were variable and usually too high. Those who adhered to Töpfer's method claimed that this was not true. I think the results with phosphotungstate and phloroglucin-vanillin, used on the same specimen, while demonstrating the accuracy of both these tests, show the inaccuracy of dimethylamidoazobenzol as an indicator for free HCl.

When one inaccurate indicator is used in estimating the free HCl, when the combined HCl is estimated by using as an indicator alizarin, which gives an end reaction which is extremely difficult to recognize and very likely to lead to error (if indeed it can be considered to be accurate at best) and when the method includes the use of three different indicators, it is practically impossible to obtain accurate results, for it is well known that an error attends upon the use of practically every indicator, and that this error varies for each indicator. The errors of three indicators are therefore introduced in this method, and this is in itself sufficient to make the method probably unreliable.

The method suggested by Hewes is in brief as follows: The total acidity is estimated as usual by using phenophthalein as an indicator, but Hewes recommends that it be continued until a deep-red color is obtained rather than stop at the first appearance of pink. The free acidity is then estimated by titrating with dimethylamidoazobenzol until the end reaction is nearly obtained, when tropælin is used

to determine the end reaction definitely, as Hewes does not now consider the other indicator sufficiently accurate. The total acidity due to free acids and acid salts (chiefly phosphates) is then estimated by titrating with Congo red as an indicator, as Hewes states that Congo red is an indicator of the amount of phosphates as well as of free acids. By this means the total acidity and amount of free HCl are of course directly indicated, and if free HCl be present, the combined HCl is indicated by the difference between the total acidity and the result obtained by titration with Congo red. If no free HCl is present and one wishes to know whether any combined HCl is present he may use Ewald's modification of Sjoeqvist's method.

The first error in this method is in the recommendation that the titration for total acidity (with phenophthalein) be carried to the point where a deep-red color is obtained and not stopped, as is usually recommended, when a pink color first appears. Töpfer pointed out that phenophthalein always gives too high results when used as an indicator in albuminous solutions. I have tested this statement and confirmed its accuracy, and any one can similarly convince himself by titrating solutions of peptones in HCl of known strength with phenophthalein. To continue the titration until a deep color is obtained would therefore increase the error always attendant upon the use of phenolphthalein.

The most important error lies, however, in the use of Congo red as an indicator for the free acids and acid salts. The statements that I have previously made concerning dimethylamidoazobenzol apply with equal force to Congo as an indicator of free acids. Phloroglucin-vanillin is generally recognized to be an accurate indicator, and its accuracy is well demonstrated by Cohnheim and Krieger's method—it always

gives much lower results than Congo red even in solutions containing no acid salts but containing albumins. It seems wholly proper therefore to decide that Congo red is not an accurate indicator of free acids. I have personally recommended Congo red as an indicator for free acids, but am quite willing to acknowledge my error in so doing, since I have used the phosphotungstate method and compared results. The more serious portion of the error in using Congo red lies, however, in considering it to be an indicator of the amount of acid salts. There is a general statement in many works which discuss gastric analysis that Congo red reacts to acid salts, but I am not aware that there is any statement that it is a good indicator for these salts. If such a statement is made I think it is wholly erroneous. I have made solutions of monosodic and monocalcium phosphates in about 0.2 per cent strength (about the maximum strength in which acid phosphates are found in the stomach contents) and have titrated these solutions with phenolphthalein, rosolic acid and Congo red as indicators. With the monosodic phosphate solution Congo gave no recognizable reaction. With monocalcium phosphate solution a slight though distinct reaction was obtained, but the reaction vanished after the addition of 0.3 cubic centimetres of decinormal sodium hydrate, while it took 2.3 cubic centimetres and 2.5 cubic centimetres of the soda solution to give a reaction with the other indicators. It is evident, therefore, that while Congo red reacts to monocalcium phosphate, it does so only in solutions of considerable strength, and is by no means an accurate indicator of the amount present, as it shows only about one-eighth of an acidity which is due to this salt.

This error is sufficient to make the method unreliable. But one of the most serious errors in this method, as well as in Töpfer's, is the use of three

indicators, thus multiplying the errors due to indicators.

No method is satisfactory that demands the use of several indicators. In Cohnheim and Kreiger's method the two titrations which are used in estimating the combined HCl are done with the same indicator. Another indicator is brought into use in estimating the free HCl and therefore in determining the total HCl, but both rosolic acid and phloroglucinvanillin are very accurate—much more accurate than the other indicators, as is testified by results obtained in solutions of known strength.

The use of Ewald's modification of Sjoeqvist's method for determining the combined HCl when free HCl is absent entails the most serious errors attending upon the use of Sjoeqvist's method, and the objections raised by Leo and others are sufficient to demonstrate that the results, even though they are used only to indicate qualitatively the presence or absence of combined HCl, are not at all trustworthy.

It may be said that the objections I have made to some of the methods discussed are of no clinical consequence. As was stated at the outset, however, the necessity for knowing the amount of combined HCl arises in only a relatively small number of cases; but when it does arise, close accuracy is necessary. If the methods used are not accurate the results have merely a specious appearance of value, and much time would be saved and quite as useful a purpose served by simply determining the total acidity and observing the intensity of the qualitative reaction for HCl, judging roughly of the total amount present by these factors, or, if free HCl is absent, by determining whether the milk-curdling ferment is present.

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