XXXVIII. STUDIES IN THE ACETONE CONCENTRATION IN BLOOD, URINE, AND ALVEOLAR AIR: I. A MICROMETHOD FOR THE ESTIMATION OF ACETONE IN BLOOD, BASED ON THE IODOFORM METHOD.

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SCANDINAVIAN readers will find in my book [Widmark, 1917] a summary of the most important methods hitherto used for the estimation of the acetone concentration in blood and tissues. It will be found that none of the methods there referred to is entirely satisfactory or applicable to more extended measurements. Series of estimations upon living material cannot be obtained.

Of a method that is to be used both in the physiological laboratory and in clinical work the following demands, among others, should be made:

(a) It should not require more than about 100 mg. of blood. (b) The carrying out of the estimation should be simple, and the apparatus not too complicated or expensive. (c) The error should not exceed 10 %.

In the blood of diabetics with fairly pronounced acidosis the concentration of the total acetone fluctuates among values lying at about 0.20 per mille, *i.e.* in 100 mg. of blood there are about $20\gamma^1$ total acetone. A micromethod should therefore have a mean error in the single estimation not greatly exceeding about $\pm 2\gamma$, which would therefore constitute an error of 10 % with a concentration of 0.20 per mille.

A detailed investigation of the iodoform method has shown that a modification of this renders possible an estimation of these small quantities of acetone with a mean error which remains approximately that just named. The carrying out of the estimation is simple and requires little practice. No more than 100 cmm. of blood is required, and the apparatus is not complicated.

We shall now describe the process, giving first the main outlines of the method and the carrying out of the estimation, and in a further section entering into a detailed examination of the method.

¹ We use the symbol γ for the quantity 0.001 mg., according to the proposal of the "Ausschuss für Einheiten und Formelgrößen" [1913].

A. DESCRIPTION OF THE MICROMETHOD.

1. *The method of obtaining the blood sample.*

The requisite amount of blood is obtained in the usual way by pricking with a stilette the ball of the finger or the ear-lobe. In disinfecting the skin care should be taken to remove all traces of alcohol and ether before the puncture is made. It is best merely to wash with soap and water. With a capillary pipette, graduated up to 100 cmm., the blood is sucked up as it issues from the wound. It is of great importance that the blood should not be subjected to evaporation, since in that case too low values may be obtained. The sucking up and measuring of the blood may be carried out most easily and most quickly by the aid of a precision regulator as constructed by Weichardt [1909]¹. The blood is brought into the flask of the distillation apparatus, in which there has previously been placed 10 cc. of 1 % phosphoric acid. The pipette is rinsed out four or five times with the phosphoric acid, so that every trace of blood passes down into the flask, which is immediately corked up.

The pipette should not be cleaned with alcohol and ether. The best method is to place it in the tube of a vacuum pump. After distilled water has been sucked through the pipette for a few moments, wipe the point. After a few minutes' further suction the pipette will be dry.

2. *The distillation.*

The distillation should be undertaken not more than two or three hours after the sample of blood has been secured. The shape of the distillation apparatus is shown by Fig. 1. From the distillation flask, the capacity of which is 100 cc., passes a detachable, twice-bent distillation tube. The tube is provided with a small drop-receiver and is blown out, immediately under the second angle, to a bulb of a few cc. capacity. It is drawn out at the end into a point the opening of which is about $\frac{1}{4}$ mm. across. A test-tube 150 mm. long and 15 mm. wide serves as receiver. The test-tube is enclosed by a cooler, the form of which is shown by the figure. The distillation tube reaches down into the test-tube and should end a couple of mm. above the bottom. The flask, distillation tube, and test-tube with accompanying cooler can be fixed in a wooden stand with elastic metal clamps in such a way that the test-tube can be loosened and withdrawn from the stand by a single movement.

In the test-tube, immediately before distillation, are placed 3 cc. of $N/2$ sodium hydroxide solution [natr. hydricum puriss. e natrio], and 2 cc. of $N/200$ iodine solution are added from a well-calibrated pipette (or in the estimation of greater quantities of acetone than 50 γ , 2 cc. of $N/100$ iodine solution).

¹ A modification of this is sold by the A. B. Vetenskapliga Instrument (Scientific Instrument Company), Lund, Sweden.

Before distillation, the flask and the distillation tube should be boiled out for a few minutes with 1 % phosphoric acid. This is especially important if a cork connection is used between the tube and the flask.

In the distillation of pure water or phosphoric acid solution the strength of the titer of the alkaline iodine solution is diminished to some extent. In order to determine the titer two blind tests are carried out before the distilling

Fig. 1

of the blood sample, and one blind test afterwards. In these 10 cc. of 1 % phosphoric acid are placed in the flask, and the distillation is carried out as described below for the blood sample. The first blind test usually shows too great a consumption of iodine and should not be counted in the determination of the titer. The last will serve as a test by which it may be ascertained if any error has slipped in during the distillation of the blood sample.

The distillation of the blood sample is carried out as follows. The cork is removed from the flask and the distillation tube is attached. The apparatus is placed in the stand, and the condensing water turned on. The fluid is then boiled, the burner being held in the hand and the flame kept small. As soon as the steam has reached the contents of the test-tube a loud rattling will be heard. From this moment the boiling is continued for 100 seconds, the heat being moderated by raising and lowering the burner, so that the boiling remains even and the blood does not froth up into the distillation tube. A test of the evenness of the boiling exists in the rattling noise, which ought not to cease. When the 100 seconds are up, the heat is increased a little and at the same time the test-tube is loosened from its clamp, so that the point of the distillation tube comes above the surface of the fluid. No fluid must be allowed to remain clinging to the point; any that does so remain can be blown down into the test-tube by heating the flask for a moment. When the test-tube has been further cooled down for a few minutes, the condensing water is shut off and the test-tube is taken out of the cooler. We may now proceed to the next distillation; 10 distillations can be carried out in an hour. A precipitate appears only on the distillation of quantities of acetone exceeding 50 γ , the iodoform being to a certain extent soluble in water.

3. *The titration.*

When the tube has stood for at least three minutes, 3.5 cc. of $N/2$ sulphuric acid are added from a burette. In every tube is placed a thin glass rod, the lower end of which is flattened out into the form of a disc, in order to facilitate the stirring of the fluid. The solution is stirred by moving this glass rod a few times up and down. A couple of drops of a 1 % starch solution are now added with a pipette, and titration is carried out with $N/200$ sodium thiosulphate solution (or $N/100$ if $N/100$ iodine solution be used). In the titration we use a burette (Bang's burette for the estimation of blood sugar) graduated in 0.02 cc. Thiosulphate is added drop by drop as the fluid is stirred with the glass rod, until the blue colour almost vanishes. Towards the end of the titration only fractions of a drop are added. The titration is continued until every trace of colour has vanished; the easiest way to find out when this is the case is to look at the fluid through the mouth of the test-tube. With a little practice the change can be determined to somewhat less than half a drop. Care must be taken that no drops of thiosulphate remain clinging to the upper parts of the test-tube. They can be brought down with the glass rod.

The blind test enables us to determine the titer of the iodine solution. The amount of acetone is calculated from the difference between the quantities of thiosulphate used in the blind test and in the blood test. 0.02 cc. of $N/200$ thiosulphate corresponds to 0.968 γ acetone.

B. DETAILED EXAMINATION OF THE METHOD.

For the testing of the method pure acetone was used, prepared in the following way from the bisulphite compound [see Plimmer, 1915]. To 125 cc. of crude acetone were added 70 g. of sodium bisulphite in saturated solution. The mixture was well shaken in a closed flask and, after being left to stand for some time, the liquid was drawn off by means of a suction filter and the precipitate dried between filter-paper. It was then placed in a distillation flask and decomposed with 40 g. of soda. The mixture was subjected to fractional distillation, and the distillate was dried with calcium chloride and subjected to a fresh distillation. The fraction that passed over at a temperature of between 56 and 57° was used in the experiments.

The pure acetone boiled at a temperature of + 56·8°, and had a sp. g. of 0·798 at 15°. Its reaction was neutral and it showed no aldehyde reaction (equal volumes of 10 % silver nitrate and sodium hydroxide were mixed together, ammonia was added drop by drop until the precipitate was dissolved, and a couple of drops of acetone were then added. No mirror was formed).

A solution prepared from 1·462 g. acetone in 1 litre of water showed the following concentration, when titrated by Messinger's method:

1·465 ± 0·0023 per mille (6 estimations).

The acetone may therefore be regarded as sufficiently pure for the testing of the micromethod.

In order to render possible the preparation of an acetone solution of accurately known concentration and to avoid any loss through evaporation in the measuring of the pure acetone, the following method of procedure was adopted.

A number of small bulbs of 1–2 cc. capacity were blown out from narrow glass tubes and the point of each bulb was drawn out into a capillary tube. The bulb, after being weighed, was filled with the pure acetone by gently heating it and then dipping the capillary into the acetone. As cooling took place the bulb was filled with the fluid. The capillary was then melted off, and the filled bulb weighed again.

In the preparation of dilute acetone solutions a 1 litre volumetric flask was half filled with water, and a bulb was dropped into the water and broken with a glass rod. The flask was then filled up to the mark. Finally there was further added a small quantity of water corresponding to the volume of the glass of the bulb, the sp. g. of which was assumed to be 2·5.

1. *Measurement of the blood sample.*

The greatest risk of error in the measurement of the blood sample lies in the fact that the acetone easily volatilises. Opportunity for this is found in the time which elapses from the moment when the blood comes out of the puncture until it is sucked up into the pipette. In the pipette the danger of evaporation is extremely small.

Generally I have proceeded in the following way: 4-5 drops of blood from the puncture were allowed to drop on to a clean watch-glass, from which the blood was quickly sucked up into the pipette. The time required from the falling of the first drop of blood upon the glass until the requisite quantity had been sucked up into the pipette usually ran to 15-20 seconds. During this period the evaporation is so small that it is not noticeable with the micromethod.

This method has however been sharply criticised, and it must be granted that the method is associated with a certain risk for persons without practice. I recommend therefore that the blood be not allowed to drop upon a watch-glass. Instead it may be sucked up directly by placing the point of the pipette over the puncture and sucking up the blood as it drips out. This can be done very easily and conveniently by means of the Weichardt regulator mentioned above. With a blood sample from the ear of the rabbit it is best to let an assistant hold the animal's ear stretched out upon a square of glass. This will give the necessary support and firmness for the sucking up of the blood as it drips out.

That the taking of the sample in this way gives a perfectly satisfactory result is shown by the following experiments.

Experiment 1. A quantity of acetone was subcutaneously injected into a rabbit. Three hours later seven blood samples were taken; nos. 1, 3, 5 and 7 by means of the pipette, nos. 2, 4 and 6 by direct dropping of the blood from the ear vein into weighed flasks. Room temp. 17° .

The samples taken with the pipette gave the following values:

1	0.392 ‰
3	0.392
5	0.390
7	0.387
Average	0.390

The weighed samples gave the following results:

2	123.2 mg. blood contained 46.5γ acetone	=0.377 ‰
4	66.6 " " 24.4γ "	=0.366
6	124.0 " " 44.0γ "	=0.355
Average		0.366

Experiment 2. Similar experiment with a greater quantity of acetone. Room temp. 16.5° .

With the pipette:

2	0.629 ‰
4	0.624
6	0.615
8	0.620
Average	0.622

By dropping directly into weighed flasks:

1	68.8 mg. blood contained 43.3γ acetone	=0.629 ‰
3	84.4 " " 50.3γ "	=0.596
5	113.4 " " 67.8γ "	=0.598
7	106.0 " " 67.3γ "	=0.635
9	80.2 " " 46.9γ "	=0.585
Average		0.609

By weighing, somewhat lower values are found than when the pipette is used, this being evidently due to the fact that in the former case the blood is measured in mg., in the latter in cmm. If we assume the sp. g. of the blood to be 1.05 and calculate the weighed samples according to this, we find the average in Exp. 1 to be 0.384 ‰ and in Exp. 2 0.639 ‰, the former value a little lower, the latter a little higher, than the average of the samples measured with the pipette.

The measurement with the pipette is simpler and easier than weighing and is therefore to be preferred.

The risk of evaporation however is sufficiently great to put out of the question a sucking up of the blood and weighing with a torsion balance in the same way as in the blood-sugar test according to Bang. On the other hand it is possible that this method may be very well suited to an estimation of aceto-acetic acid. For this purpose the blood may be sucked up on to thin filter-paper and weighed, and the free acetone may be allowed to evaporate either by letting the filter-paper hang for some time under ordinary atmospheric pressure, or by enclosing it for a few minutes in a flask which is then evacuated by means of a water suction pump. This process is at present being worked out, but the details are not ready for publication. If Shaffer's [1911] method for converting β -hydroxybutyric acid into acetone and then estimating the acetone should prove to be practicable, this micromethod could probably also be used with advantage for a micro-estimation of β -hydroxybutyric acid in the blood.

2. *The distillation.*

The distillation of the blood sample presents no noteworthy difficulties. The construction of the distillation apparatus may vary in different ways. An indispensable condition however is that the steam must not be allowed to come into contact with either rubber stoppers or tubes. It seems, in fact, as if the steam possessed the power of carrying along with it substances which unite with iodine. The consequence of this is that the estimations become too high: the error may often amount to several tenths per cent of the real value. Cork may be used, but it must be well boiled previously. It is safest to use an apparatus entirely of glass, with distillation tube ground in. The disadvantage of such an apparatus however is that it is impossible without great expense to get a considerable number of distillation flasks of the right size to fit into one and the same distillation tube. This fact makes it difficult to undertake series of estimations.

The advantage of the apparatus previously described is that it is particularly simple both in construction and management. I have generally used cork connections between distillation tube and flask. This was the case in all the estimations in this part of the work. Care should be taken that the open end of the distillation tube is not too large, and that the tube does not end more than 4 mm. above the bottom of the test-tube.

The fact that I allow the acetone distilled over to be absorbed without previous cooling by the alkaline iodine solution, which in the course of the distillation has been heated to a considerable extent, might possibly lead to the supposition that great loss would be unavoidable, or at least would very easily arise. Practical experience however shows—as appears quite clearly in the chapter on the precision of the method—that the risk of a loss of acetone is very small. Moreover

there is also some theoretical support for the view that the absorption of the acetone ought not to be impaired by a gentle heating of the alkaline iodine solution. The decisive tests for the retention of the acetone in the iodine solution are in the first place its vapour-pressure in the solution, and secondly the velocity of the reaction in the formation of iodoform. With a rise of temperature both these factors are intensified, but they act in opposite directions. The increased vapour-pressure facilitates the evaporation of the acetone, the increased speed of the reaction renders it possible for greater quantities of acetone to be bound as iodoform within the unit of time. The loss of acetone will depend on the question which of these two factors is increased most with a given rise of temperature. According to Regnault [1862] the tension of the acetone is increased from 179.6 mm. Hg at 20° to 420.2 mm. at 40°, a rise therefore in the proportion of approximately 1 : 2. The partial pressure in an aqueous solution of 1 % is increased, according to my own measurements, in approximately the same proportion with a rise in temperature from 20 to 40°. If we assume that the velocity of the reaction in the formation of iodoform follows van't Hoff's law, the velocity for a rise in temperature of 20° should be increased in the proportion of 1 : 4. Hence it follows that the retention of the acetone in the alkaline iodine solution is facilitated to an appreciable extent by heating. In this connection however another circumstance of great importance is to be mentioned. It is an established fact that when iodine is added to alkali the resulting solution does not remain unchanged if left to stand. The hypoiodite formed at the beginning passes over by degrees into iodate:

The iodate does not form iodoform with acetone. The formation of iodate proceeds slowly in cold solutions, but almost instantaneously in warm. This fact would therefore seem to argue that the binding of the acetone is rendered more difficult by the heating of the solution, and that an error in the estimation might arise owing to the fact that the quantity of hypoiodite necessary for the formation of iodoform is not forthcoming. In my own experiments I have never been able to find that the circumstance just mentioned plays any part in the estimations, although I have often made use of the supply of iodine in the alkaline iodine solution up to 70–80 % (see *e.g.* experiment 9). Possibly the case is somewhat otherwise with these extremely dilute solutions than with the more concentrated.

Determination of the most suitable time of distillation.

Experiment 3. In the distillation flask are placed 10 cc. of 1 % phosphoric acid and 0.1 cc. of 0.732 % acetone solution. The flask therefore contains 73.2γ acetone. The alkaline iodine solution is introduced into the test-tube in the usual way, 2 cc. of *N*/200 iodine solution being used. The experiment is repeated eight times, with variations in the time of distillation, the latter being reckoned from the point at which the rattling sound begins.

Table I.

Time of distillation in min.	Acetone in γ
$\frac{1}{4}$	55.1
$\frac{1}{2}$	64.5
$\frac{3}{4}$	68.0
1	72.8
$1\frac{1}{4}$	74.3
$1\frac{1}{2}$	72.8
$1\frac{3}{4}$	74.8
2	73.8

The experiment shows that after 1 minute practically all the acetone has already been distilled over.

Experiment 4. A similar experiment, with the use of 168.6 γ acetone in the flask and 2 cc. of *N*/100 iodine solution.

Table II.

Time of distillation in min.	Acetone in γ
$\frac{1}{2}$	143.9
$\frac{3}{4}$	161.4
1	169.1
$1\frac{1}{2}$	170.8
2	168.1
$2\frac{1}{4}$	168.6
$2\frac{1}{2}$	173.0

Here too the acetone has passed over quantitatively after 1 minute.

The experiment shows that a time of 90 seconds is sufficient for the distillation of pure solutions. In distilling blood samples it is necessary, on account of the frothing, to boil somewhat more carefully. I recommend therefore a somewhat longer time—100 seconds—for the boiling.

The dilution of the blood sample with 10 cc. of 1 % phosphoric acid is designed partly to check the frothing in the distillation, and partly to decompose the aceto-acetic acid. The dilution is carried out in the proportion of 1 : 100. In the distillation about 4 cc. pass over, so that afterwards the dilution is in the proportion of about 1 : 60. This dilution is more than ten times as great as that recommended by Embden for the urine distillation. There can therefore be no reason to suppose that any disturbing iodine-binding substances will, through the concentration due to the distilling process, be made to pass over into the distillate. A dilution of the sample with water while distillation is in progress is superfluous.

3. *The titration.*

After the distillation the tube should stand for 1 minute in the cooler. After a further period of 3 minutes the formation of iodoform is finished. This is shown by the two following experiments.

Experiment 5. To each of six tubes containing 3 cc. of sodium hydroxide and 2 cc. of *N*/200 iodine solution is added 1 cc. of an acetone solution containing 47.4 γ per cc. The first tube is acidified immediately, the second after 1 minute, the third after 2 minutes, and so on. The results of the titration are shown by the following table.

Table III.

Time in min.	Acetone in γ
0. -	22.5
1	36.3
2	45.5
3	47.4
5	47.6
20	47.4

The first column shows the time that elapsed between the adding of acetone to the alkaline iodine solution and the addition of acid.

In this experiment therefore the iodine is already bound after 3 minutes.

Experiment 6. To each of four flasks, containing 10 cc. of 1 % phosphoric acid, 0.1 cc. of acetone solution containing 53.2 γ acetone was added. Distillation was then carried out in the usual way. The test-tubes were allowed to stand in the cooler for 1 minute. The first tube was acidified immediately after this, the second after 2 minutes, and so on.

Table IV.

Time in min.	Acetone in γ
0	52.6
2	53.3
15	52.2
20	52.2

The experiment shows that the mixture may be acidified 3 minutes after the tube is taken out of the cooler.

After the acidification and when the solution has been sufficiently stirred with the glass rod, starch may be added and titration carried out.

Experiment 7. To each of five tubes containing alkaline iodine solution was added 1 cc. of an acetone solution containing 68.8 γ acetone per cc. When the tubes had stood for 5 minutes they were acidified with sulphuric acid. The contents of the first tube were titrated immediately, those of the second after half a minute, and so on.

Table V.

Time in min.	Acetone in γ
0	67.4
$\frac{1}{2}$	67.4
1	67.4
10	67.9
30	66.9

The first column shows the time that elapsed between the addition of sulphuric acid and the titration.

It is of a certain importance that the titration should be carried out in the test-tubes, partly because the loss of colour can be more clearly observed there than in flasks and beakers, and also because the risk of evaporation of the iodine is then quite inconsiderable. Titrations in flasks and beakers always require a somewhat smaller consumption of thiosulphate than titrations in test-tubes. An iodine solution which, when titrated in a test-tube, used up 1.98 cc. of $N/200$ thiosulphate, used in a 50 cc. flask 1.95 cc., and in a beaker, after 5 minutes' gentle stirring, only 1.40 cc.

The diminution which appears on distillation in the titer of the iodine generally reaches a degree corresponding to 0.03–0.05 cc. of $N/200$ iodine solution. Probably this diminution is due to several causes.

In the text-books of quantitative analysis stress is laid upon the fact that the quantity of iodine demanded for the production of the blue colour will vary considerably, according to the concentration of the iodide present. Care must therefore be taken that the analyses are carried out as far as possible in equal volumes of fluid. With the use of *N*/10 iodine solution however the error becomes so small that it may as a rule be neglected. If *N*/100 iodine solution be used the error may become very great. Treadwell [1913] therefore carries out the following experiment.

To different quantities of water were added 1.5 cc. of starch solution, and *N*/100 iodine solution until the first perceptible blue coloration appeared.

Water in cc.	<i>N</i> /100 iodine solution in cc.
50	0.15
100	0.30
150	0.47
200	0.64

When, on the other hand, there was added to the solutions 1 g. of potassium iodide, the consumption of iodine was reduced to about 0.04 cc. up to a volume of 200 cc., which required 0.14 cc. of iodine solution.

In the micromethod the volume of fluid is about 12 cc. and varies within very narrow limits. I have not been able to find that the result of the titration is changed by adding potassium iodide immediately before titrating. Probably sufficient quantities are already present.

The reason for the change in the titer of the iodine solution is probably to be sought in part in another direction. The consumption of iodine becomes greater if, in distilling, the boiling is prolonged beyond the time prescribed. It is greater when a rubber stopper is used as connection between flask and distillation tube than with the use of cork, and is least of all with only glass connections. Finally we find a diminution in the titer of the iodine as soon as we pass 100 cc. of air free of dust through the alkaline iodine solution.

The determination of the titer can however be carried out with perfectly satisfactory accuracy by means of the two blind tests, provided only that the titer of the thiosulphate is quite correct. The result shows that the titer remains sufficiently constant even during very long series of experiments. It is however of great importance for the obtaining of good results that the purest possible chemicals should be used. Only the sodium hydroxide prepared from the metal is of perfectly satisfactory purity. The other preparations often give, even with the test-sample without distillation, too great a consumption of iodine. Ordinary distilled water (according to recent investigations in connection with the salvarsan treatment) often contains important quantities of organic substances which may of course bind iodine. It has been proved also that newly-distilled water gives considerably more even results than ordinary distilled water. I have therefore, in the preparation of all the solutions required by the method, myself distilled the water in a glass distillation apparatus¹.

4. *The standard solutions.*

The preparation and conservation of these is effected by means of the methods that will be found in most of the text-books of analytical chemistry².

¹ A very convenient apparatus of this kind has been constructed by Hamner and may be obtained from Grave, Stockholm.

² Naturally there can here be no question of the preparation of *N*/200 iodine solution according to Bang by the addition of titrated acid to an excess of iodate and potassium iodide. For when the solution after the distillation is mixed with excess of acid, there is liberated a further quantity of iodine corresponding to the excess of acid.

I have found it safest and most convenient to prepare every day from well-determined $N/10$ iodine and thiosulphate solutions new solutions of $N/100$ and $N/200$ respectively. We have a control of these in the blind tests. The standard solutions should be tested at least once a week. This is most easily done by first testing the thiosulphate solution with bichromate and potassium iodide in a solution acidified with sulphuric acid.

5. Accuracy of the method.

The two following experiments are designed to illustrate the accuracy of the method in the estimation of the amount of acetone in pure solutions before and after the distillation.

Experiment 8. In 12 test-tubes were measured out 3 cc. of $N/2$ sodium hydroxide + 2 cc. of $N/200$ iodine solution + 3 cc. of freshly distilled water. From an acetone solution containing 73.1γ acetone per cc. 0.1 cc. was measured off (with a 1 cc. measuring pipette) in the first tube, 0.2 cc. in the second, and so on; 1 cc. in the tenth tube therefore. The eleventh and twelfth tubes served as blind tests for the determination of the titer of the iodine. After 5 minutes 3.5 cc. of $N/2$ sulphuric acid was added to each of the tubes. After stirring with a glass rod titration was immediately carried out. The following table and Fig. 2 show the results of the titration.

Table VI.

	Calculated	Found (γ)
1	7.3	10.0
2	14.6	15.4
3	21.9	20.8
4	29.2	30.6
5	36.6	37.8
6	43.9	44.9
7	51.2	51.3
8	58.5	61.0
9	65.8	66.8
10	73.1	73.9

The table shows the titration results in the estimation of pure acetone solutions without previous distillation. The second column gives the calculated quantity of acetone, the third column the quantity found by the estimation.

Fig. 2 illustrates this experiment.

Experiment 9. In each of 13 flasks 10 cc. of 1 % phosphoric acid was measured off. Into ten of these flasks rising quantities (from 0.1–1.0 cc.) of an acetone solution containing 73.1γ acetone per cc. were measured off with a 1 cc. measuring pipette. Distillation was then carried out in the usual way, with two blind tests before, and one blind test after, the acetone tests. In the test-tubes 3 cc. of $N/2$ sodium hydroxide + 2 cc. of $N/200$ iodine solution. The acetone values when plotted as in Fig. 2 fall on a straight line.

Fig. 2. The calculated values lie upon the oblique line, the values found are marked by circles. Abscissa: the volume of acetone solution added. Ordinate: acetone γ . Estimations without distillation according to experiment (8).

Table VII shows the titration results in an estimation of pure acetone solutions after distillation. Arrangement as in the previous table.

Table VII.

	Calculated	Found (γ)
1	7.3	11.1
2	14.6	16.0
3	21.9	21.8
4	29.2	29.5
5	36.6	38.0
6	43.9	44.0
7	51.2	50.8
8	58.5	57.1
9	65.8	66.2
10	73.1	73.0

The mean error of the single estimation in an estimation of pure acetone solutions appears from the following series.

Experiment 10. Ten estimations were carried out on a pure acetone solution containing 7.32 γ per 0.1 cc. The acetone was measured out with a 0.1 cc. pipette. Distillation as usual. In the test-tubes 3 cc. of hydroxide + 2 cc. of *N*/200 iodine solution. The following values were obtained¹:

Acetone in γ : 9.7, 7.7, 7.3, 7.1, 7.3, 7.5, 7.3, 7.3, 8.0, 6.8.

Average: 7.6, $E = \pm 0.805$, $e = \pm 0.255$.

Experiment 11. Ten estimations on an acetone solution containing 57.1 γ per 0.1 cc. Estimations carried out as before.

Acetone in γ : 56.7, 56.2, 56.6, 56.7, 56.7, 56.2, 56.0, 57.9, 53.7, 55.7.

Average: 56.24, $E = \pm 1.071$, $e = \pm 0.339$.

From these two experiments it follows that the mean error of the separate estimation hardly exceeds 1 γ . The absolute value of the error is about the same, independently of the amount of the acetone. In the first series the error expressed as a percentage of the mean is 10.5 %, and in the second series 1.9 %.

In the first series the mean is somewhat higher than the calculated value, in the second series somewhat lower. I have not been able to find—provided that sufficient attention is paid to the estimation of the consumption of iodine in the blind experiment—that the method has any appreciable constant error. In general, however, the values are a little too low (about 1 γ). This is especially the case if a distillation tube is used having a point with a wider opening than that mentioned in the description of the apparatus. Under such circumstances it may happen that in the distillation the air which passes out before boiling is set up is not completely freed from its acetone in passing through the alkaline solution. Further it is necessary to provide carefully that the glass connection shall close quite tightly. It is advisable to damp the ground glass with a drop of distilled water when the glass tube is fitted into the flask. If a cork connection is used the same cork should not be used too long, but should frequently be replaced by a new one.

It is evident that the method is not suitable for estimations of the acetone percentage in the normal blood. For this 0.1 cc. of blood is too small a quantity. The estimation of the concentration in normal blood falls entirely within the limits of error of the method. In an estimation of normal blood we therefore regularly get titration results which are identical with those of the blind test, or at most show 0.01–0.02 cc. less *N*/200 thiosulphate consumption than these.

The following experiment shows the mean error of the separate estimation in a determination of the amount of acetone in calves' blood, to which acetone was added:

Calculated γ	79.6	39.8	31.8	19.9	15.9	8.0
Found γ	77.1	39.9	32.7	18.9	14.4	8.0
	77.1	39.4	30.3	19.4	15.4	8.0
	77.0	41.3	30.7	19.6	15.9	7.5
	76.7	39.4	30.3	19.1	16.8	7.5
	73.8	40.4	32.2	19.8	16.1	7.7
Average	76.3	40.1	31.2	19.4	15.7	7.7

¹ By E , here and in the following experiment, is meant the mean error of the single estimation; by e the mean error of the mean.

It is my impression that as a rule we are rather more likely to get too low values than too high. In order to illustrate the agreement between the two-fold estimations in the determination of the total acetone concentration in the blood of *diabetics* with acidosis, I give the following report of all the estimations in a case. This series is intended to demonstrate the clinical practicability of the micromethod. The samples of blood were taken by the Hospital laboratory nurse, the distillation was performed by my own laboratory assistant, and finally I myself carried out the titrations.

Table VIII.

Date	Acetone ‰	
	I.	II.
24. i.	0.302	0.321
25. i.	0.334	0.334
26. i.	0.310	0.314
27. i.	0.189	0.184
28. i.	0.114	0.111
29. i.	0.080	0.082
30. i.	0.116	0.097
31. i.	0.264	0.276
1. ii.	0.165	0.160
2. ii.	0.172	0.174
3. ii.	0.126	0.102
4. ii.	0.109	0.114
5. ii.	0.097	0.097
6. ii.	0.106	0.087
7. ii.	0.106	0.087
8. ii.	0.068	0.073
9. ii.	0.204	0.216
10. ii.	0.174	0.174
11. ii.	0.135	0.135
12. ii.	0.094	0.082

In order to show that in the short period of distillation there is time for all the aceto-acetic acid present to be decomposed, a few analyses of the total acetone concentration in urine have been made. In these the total acetone has been estimated by the method of Embden and Schliep and the micro-method simultaneously. The results are shown by the following table.

Table IX.

Subject	Macromethod	Micromethod
A. S.	0.198	0.208 } 0.206
		0.203 }
A. S.	0.397	0.392 } 0.385
		0.375 }
Mrs L.	1.083	1.060
H. S.	0.329	0.314 } 0.315
		0.315 }
H. S.	0.295	0.305 } 0.303
		0.300 }

The experiments show that with distillation by the micromethod aceto-acetic acid is decomposed to the same extent as when the macromethod is used.

As we saw in regard to the blood estimations, so also it is impossible to estimate the normal acetone percentage in the urine with any claim to accuracy by the micromethod. As an average figure for the quantity of total acetone present in normal urine may be given approximately 20 mg. per daily quantity of urine. Assuming a daily quantity of 1500 cc. the quantity of acetone present in 0.1 cc. is therefore only a little more than 1%. The estimation of this quantity falls entirely within the limits of error of the method, which fact was also shown by series of estimations.

Finally it should be emphasised that we do not yet know with perfect certainty whether the values obtained by estimating by means of the iodoform process correspond in every respect to the acetone concentration in question. The possibility is still open that besides acetone other volatile iodoform-producing substances pass over into the distillate in an estimation of the blood and urine of diabetics. Such substances may in general be characterised as having the group CH_3C united with oxygen.

The presence of *ethyl alcohol* in the blood of diabetics demands special attention for the reason that these patients are often given a certain amount of wine in their diet.

However the presence of ethyl alcohol in the blood has no effect upon the results of titration.

Experiment 12. Subject: the author. At 2.15 p.m. on April 15, 1917, 50 cc. of whisky were taken $2\frac{1}{2}$ hours after a meal. This quantity contains about 25 cc. of absolute alcohol (and about 9 mg. of aldehyde, reckoned as acetaldehyde). Before the spirit was drunk two blood samples were taken, and a double sample was also taken 25 and 45 minutes respectively after drinking.

Iodine solution consumed in the blind tests: 1.930 and 1.935 cc. $N/200$ thiosulphate. The iodine solution consumed in the blood tests corresponded to the following quantities of $N/200$ thiosulphate:

Table X.

Blood test.		
Before the taking of the alcohol	1.940	1.940
At 2.40 p.m. (25 min. after taking)	1.940	1.940
At 3.0 p.m. (45 min. after taking)	1.940	1.930

In this experiment the alcohol concentration in the blood during the time taken for the last two tests certainly exceeded 0.5 ‰ notwithstanding the fact that no change in the titer of the iodine can be observed in the tests.

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