XXXII. STUDIES IN THE ACETONE CONCENTRATION IN BLOOD, URINE, AND ALVEOLAR AIR. III: THE ELIMINATION OF ACETONE THROUGH THE LUNGS.

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In the following an attempt is made at an exact analysis of the passage of acetone into the expiratory air, and in so doing I have tried in the main to follow the method of analysis adopted in modern experiments in regard to the exchange of respiratory gases[1].

If the passage of acetone into the alveolar air is a pure diffusion process, the partial pressure of the acetone vapour in the latter should never be higher than the partial pressure in the blood. But if, on the contrary, an energetic, secretory activity is contributed by the lungs, the partial pressure of the acetone in the alveolar air may be higher than in the blood. The decision of these points becomes much more simple in the case of acetone than in the case of the respiratory gases. The latter are partly dissolved and partly in chemical combination in the blood, and their concentrations are subjected to great variations in the venous and the arterial blood. The acetone, on the other hand, is exclusively dissolved in the blood, and the concentration remains pretty nearly constant for hours [Widmark, 1919]. Certainly after resorption no demonstrable difference in concentration occurs in the arterial and venous blood.

In the physiology of respiration the absorption coefficient, as defined by Bunsen [1877], has been used in the estimation of the pressure of the gases in blood and air. The custom of thus expressing by the absorption coefficient the partition of a gas between a fluid and a gas above the fluid is associated with the fact that the gas analyses have chiefly been carried out by the eudiometric method.

The absorption coefficient may evidently be regarded as a partition coefficient for the gas in question with reference to a fluid and a gaseous phase, respect being taken to the particular pressure and temperature of the gases. If we estimate in the direct chemical way *the concentration* of a substance which is divided between a gaseous and a fluid medium it is to a certain

[1] No historical review is given in this paper The reader is referred to my book [1917].

extent a roundabout method to express the division by the absorption coefficient. It is simpler to express it in the same way as when we are dealing with the distribution of a substance between two fluid media. Here the concentration of the substance in the gaseous phase must naturally be calculated upon the volume which the gas has when diffusion equilibrium is attained.

As far as acetone is concerned, therefore, we need not express the concentration in partial pressure; it is simplest to give the quantity by weight of acetone per cc. of air and per cc. of fluid. The coefficient of partition (λ) therefore takes the following expression:

$$\lambda = \frac{[\text{acetone in air}]}{[\text{acetone in fluid}]}.$$

This method of expression is made use of in the following pages. Naturally the values can easily be calculated again in mm. of mercury-pressure. In that case we should have to do with fractions of a mm., although the exposition would not gain in clearness thereby.

The above method of expression can naturally only be used when the partial pressure of the binary fluid complex is directly proportional to the dilution. It is not deducible *a priori*—nor indeed is it probable—that such a simple state of affairs exists, in regard to acetone, throughout the whole dilution from 100–0 %.

For our purpose, however, it is only of interest to know the coefficient of partition between air and fluid within the domain represented by the acetone concentration in the fluid from 0–1 $^{0}/_{00}$. This is consequently only a thousandth part of the whole curve of partition, and in regard to this part we may assume that it will be approximately straight, *i.e.* that a constant relationship will exist between the acetone concentration in fluid and in air. This is also shown by the experiments.

The partial pressure in the case of the system water-acetone has previously been studied by Carveth [1899]. He has determined the composition of the vapour from this binary fluid and has calculated the partial pressure of the acetone. His measurements however cannot be used for the particular relationships in question. It is therefore necessary to elucidate these experimentally.

Our first object then will be to determine the partition of acetone between air and fluid for solutions under 1 $^{0}/_{00}$. Further, in order that it may be possible to apply the measurements to physiological conditions, the coefficient of partition should be determined for a temperature of 38°.

The determination of the coefficient of partition has been carried out in two different ways, first by determining the quantity of acetone which an acetone solution loses when shaken with a certain volume of air at 38° (method *A*), and secondly by an analysis of air shaken at the same temperature with acetone solution of known strength (method *B*).

After determining λ for water, blood, and serum I have proceeded to examine whether the acetone concentration in alveolar air shows a value that is in agreement with this coefficient.

A. DETERMINATION OF λ BY ESTIMATIONS OF THE CONCENTRATION OF THE ACETONE SOLUTION BEFORE AND AFTER SHAKING WITH A DEFINITE QUANTITY OF AIR.

For these determinations a litre flask was used, the neck of which was drawn out into a glass tube about 13 cm. long and 5 mm. in diameter. Over the tube was passed a glass cup which was fixed to the lower end of the tube by a piece of rubber tubing. The cup was filled with mercury up to a certain mark. An inverted test-tube was fitted over the glass tube, its opening reaching down into the mercury. The test-tube was supported by the bottom of the cup and was further fixed by means of a perforated rubber stopper passing over the test-tube and fitting into the orifice of the cup, so that this became perfectly air-tight. The space thus enclosed was accurately measured with water and found to contain 1230 cc.

The flask was shaken in a machine driven by a motor. It was enclosed in a case which could be kept at a constant temperature of 38°, at which the shaking was performed. The temperature was regulated by a spirally twisted bimetallic spring which changed its curvature with variations in temperature, and according to the movements of this an electric current was shut off or opened, this current in its turn affecting an electro-magnetic key which regulated the flow of gas to a burner placed inside the case. The temperature was regulated to about 0·1°. The apparatus was constructed at the Lund Physiological Institute.

The experiments were carried out as follows. In the flask was placed an accurately measured quantity of acetone solution, usually 12 cc., in some cases 3 cc. The flask was then closed, placed in the shaking-machine, and shaken for one hour at 38°. A test experiment showed that this period was long enough to admit of the establishment of a balance between the acetone concentration in the air and in the fluid. After the shaking the flask was taken out of the machine, the mercury in the cup poured off, and the flask turned upside down so that the acetone solution might run down quickly into the test-tube. Samples for analysis were then taken from the latter. In experiments with pure aqueous solutions the analysis was performed according to Messinger; in experiments with blood and serum the estimation was carried out after distillation in the usual way, either by the macro- or the micro-method.

Full details are here given of one experiment. The other estimations were carried out in exactly the same way.

Experiment 1. February 27, 1917. In the flask was placed 12 cc. of 0·809 $^{o}/_{oo}$ acetone solution. After one hour's shaking at 38° the solution was

found to be 0·648 °/₀₀. By heating from 20–37° the volume of the solution was diminished through evaporation of water from 12 cc. to 11·965 cc. (calculated according to *Chemiker-Kalender*, II, 1917, Table 88).

Amount of acetone introduced	$12 \times 809 = 9708\,\gamma$
Found in the solution after shaking	$11{\cdot}965 \times 648 = 7753\,\gamma$
Amount of acetone evaporated	$1955\,\gamma$

The volume of the flask was 1230 cc. After the introduction of 12 cc. of fluid, therefore, the volume of air over the fluid became $1230 - 12 = 1218$ cc. The quantity of acetone per cc. of air is therefore $\frac{1955}{1218} = 1{\cdot}605\,\gamma$.

From this we calculate

$$\lambda = \frac{1{\cdot}605}{648} = 0{\cdot}00248.$$

In addition to this estimation two further estimations were carried out on pure acetone solutions. These were performed exactly as has just been described. In the table given below all the estimations will be found set out together.

Table I. Summary of estimations of the partition of acetone between air and water. The second column contains the results of the analysis of the acetone solution after the shaking, the third column gives the calculated quantity of acetone per cc. of air, and the fourth the values of λ.

	Acetone found in solution after shaking γ/cc.	Acetone in air (by difference) γ/cc.	λ
1	648	1·61	0·00248
2	646	1·62	0·00251
3	342	0·82	0·00240
			Average: 0·00246

The coefficient of partition for acetone between water and air at 38° is therefore, according to these experiments, 0·00246.

The coefficient of partition *between serum and air* ought presumably to be somewhat higher than for pure aqueous solutions, on account of the saltness of the serum. An estimation however showed no certain difference.

Experiment 2. For this estimation a quantity of defibrinated calves' blood was centrifuged, the serum pipetted off, and a small quantity of pure acetone added. Into the flask was introduced 3 cc. of serum, which was shaken for one hour at 38°. The acetone concentration was determined in 0·2 cc. by the micro-method, and was

Before shaking °/₀₀	After shaking °/₀₀
0·392	0·199
0·387	0·184
0·378	0·187
Average: 0·386	0·190

This gives $\lambda = 0{\cdot}00255$.

In the determination of the coefficient of partition between *air and total blood* higher values were obtained for λ.

Experiment 3. 15 cc. of defibrinated cow's blood was shaken for one hour at 38°. The determination of the acetone concentrations was carried out by the macro-method. The following values were obtained:

Acetone concentration before shaking, 0·826 and 0·823, average 0·825 $^{o}/_{oo}$.

Acetone concentration after shaking, 0·661 $^{o}/_{oo}$.

Hence we get for the acetone concentration in the air, 2·04 γ/cc., and for λ 0·00309. Another measurement gave 0·00329.

The measurement of the coefficient of partition between total blood and air therefore gives a value considerably higher than those previously found. This is easily understood if we assume that the acetone concentration in the blood corpuscles is lower than in serum—an assumption the probability of which is strengthened by other experiments. The total blood should, theoretically speaking, yield the same amount of acetone per cc. of air as the plasma obtained after centrifugalisation. But on the other hand the partition coefficient comes to be somewhat higher for the total blood, since the determination of the acetone concentration in the total blood gives a lower value than in serum.

B. Determination of λ by an analysis of air shaken with acetone solution.

From the experiments just described it follows that the proportion between the amount of acetone per cc. of air and per cc. of solution is about 3:1000. In 100 cc. of air we ought therefore to find about three times as much acetone as in 0·1 cc. of solution. Consequently there should be quite sufficient quantities of acetone in the air for an estimation by the micro-method to be possible, and the error can therefore be actually three times as great as in the blood estimations without making the error per cent. any greater than in these.

The analysis of air shaken with acetone solution was carried out as follows. In a tube of about 120 cc. capacity, provided with glass taps at both ends, were placed about 50 cc. of acetone solution of known concentration. The tube was then shaken in the shaking-machine for 1½ hours at 38°. A test experiment showed that after one hour's shaking equilibrium had already been set up between the acetone concentration in the air and in the fluid. A couple of times during the shaking process one of the taps of the tube was opened in order to equalise the pressure, which had been increased by the heating from room temperature to 38°. After the shaking the tube was taken out and placed in a small chamber heated by a gas flame to 38°, the temperature of which was kept constant by means of a regulating arrangement constructed like that of the shaking-machine. The air in the tube was here passed over into a Hempel gas burette, provided with correction-tube and manometer. Acetone solution of the same strength as that used in the

shaking served as fluid in the pipette. When the amount of air had been measured at the temperature and pressure which prevailed during the shaking, the air was passed over into three test-tubes, arranged like a series of wash-bottles. The glass tubes, which reached down to the bottom of the test-tubes, were drawn out into fine capillary points. Each of the test-tubes contained 3 cc. of $N/2$ sodium hydroxide + 2 cc. of $N/200$ iodine solution (or 2 cc. of $N/100$ iodine solution in the estimation of greater quantities of acetone). The air was passed very slowly through the solutions in the three test-tubes bubble by bubble.

The titre proved to be diminished when 100 cc. of air from the laboratory had been passed through. In the first tube this diminution amounted to about 0·04 cc. of $N/200$ iodine solution; in the two others it was somewhat less, but still quite perceptible. The change of titre was determined before every series of experiments, and the results of titration were corrected by the values thus obtained from the blind tests.

The acetone was absorbed almost completely by the iodine solution of the first test-tube. Into the second 5–10 % usually passed over, in the third the formation of iodoform could not as a rule be demonstrated with certainty.

The acetone solution used—50 cc.—had in proportion to the volume of air so great a volume that no change in the concentration of the solution after shaking could be observed by titrating. In the calculation I have therefore used the original concentration of the acetone solution.

As an example of the method the following report is adduced.

Experiment 4. January 26, 1917. 50 cc. of 0·164 °/₀₀ acetone were introduced into the shaking tube and shaken for 1½ hours at 38°. The volume of the air analysed was 57·2 cc. and it contained 24·5 γ acetone, *i.e.* per cc.

$$\frac{24{\cdot}5}{57{\cdot}2} = 0{\cdot}43\ \gamma \text{ acetone.}$$

Hence $$\lambda = \frac{0{\cdot}43}{164} = 0{\cdot}00262.$$

In this way 27 estimations were carried out, in which the strength of the acetone solution varied from 0·164 to 0·822 °/₀₀. Details of these estimations are given in the following table.

Table II. Determinations of the partition coefficient of acetone between air and water at 38°.

Acetone per cc. solution	Acetone per cc. air						Average acetone per cc. air	
γ	γ						γ	λ
164	0·43	0·46	0·44	0·40	0·43	0·39	0·425	0·0026
328	0·94	1·01	0·93	0·99	0·80	0·68	0·891	0·0027
369	0·98	1·11	0·92	0·87	1·03	0·87	0·963	0·0026
576	1·31	1·49	1·30	1·61	1·34	1·21	1·377	0·0024
822	2·15	1·87	2·14	—	—	—	2·053	0·0025

The weighted mean of these values for λ is $0{\cdot}00257 \pm 0{\cdot}000055$. The values show no rise or fall in the case of these solutions, therefore a constant relationship may be considered to exist between the concentration in the air

and in the solution. Fig. 1 (p. 387) gives a graphic representation of the position of the different values in regard to the mean. The abscissa represents the concentration in the aqueous solution expressed in γ/cc., the ordinate the concentration in the air in γ/cc. The values obtained are represented by crosses. The circles represent the values obtained according to method *A*. The straight line is drawn in accordance with the weighted mean value for λ in method *B*. The error is somewhat considerable: $\pm$ 0·00028. This is probably due to the fact that it is very difficult to determine satisfactorily the titre of the blind tests. It has been found also that both rubber tubes and greased glass taps have an extremely injurious effect upon the determinations and cause the values to become irregular; possibly rubber tubes and grease give off small quantities of iodine-binding substances or absorb the acetone. The determination of the mean value is however of sufficient accuracy to admit of the use of this value in the investigation which follows. The values obtained by this process are somewhat higher than those given by the method previously described; the difference however is unimportant.

C. Estimation of acetone in the alveolar air.

If the elimination of acetone through the lungs is a pure diffusion-process we should find—provided that a complete balance of diffusion can be set up—in determinations of the acetone concentration in alveolar air and blood a coefficient of partition which is in agreement with that obtained by the shaking experiments with blood. This will naturally be the case only if free acetone exclusively is found in the blood. If aceto-acetic acid is also present we ought to obtain a coefficient lower than that just mentioned, on account of the fact that in the estimation in the blood a total acetone value is obtained. If, on the other hand, an active secretion of the acetone occurs, we should obtain a coefficient higher than that obtained in the shaking experiments. In the following a simple method for determining the acetone concentration in the alveolar air is described. The results of the determinations obtained by this method show that the coefficient in the case of blood/alveolar air has a value which agrees so closely with those obtained in the shaking experiments that *the elimination of the acetone may be entirely explained as a diffusion-process*. There is not the slightest reason to suppose that any secretion takes place.

The method seems to promise sufficient accuracy to permit of the use of the alveolar air analyses in determining the percentage of free acetone in the blood. Thus in a diabetic, by a combined blood estimation and analysis of alveolar air, we may arrive at an understanding of the relationship between the free acetone and the total acetone in the blood. The method has this great advantage, that the relationship between the acetone and the aceto-acetic acid can in no way be disturbed by the analysis: the separation of the free acetone from the aceto-acetic acid is effected so to speak with the organism itself as distillation apparatus.

Method.

A necessary presupposition, if these measurements are to give exact and reliable results, is that the acetone must not come into contact with rubber tubes and greased glass taps. This condition is fulfilled by the following method, which, with all its simplicity, seems to give quite satisfactory results.

The apparatus is composed of the following parts:

(*a*) A Haldane tube, made entirely of glass, length about 1·3 metres, diameter 23 mm. The mouth of the tube is produced by squeezing together one end of it, so that the tube may conveniently be stopped up by the tip of the tongue. 1 dm. inside the mouth of the tube another tube 5 cm. long is melted on at right angles to it. The diameter of this tube is 7 mm.

(*b*) A well calibrated collecting pipette for the air, about 100 cc. in capacity. This can be made out of an ordinary 100 cc. pipette by cutting off the lower end 4 cm. under the bulb and drawing it out into a 5½ cm. long capillary tube. On the tube is placed a small cup intended to serve as a mercury seal in the manner shown by Fig. 2. The other end of the pipette is attached to a rubber tube and mercury receiver. Into the rubber tube a glass tap is fitted. The rubber tube can further be compressed with a clamp.

(*c*) A test-tube prepared by fusing together the upper part of a wider and the lower part of a narrower test-tube. The upper part has a length of 4½ cm. and a diameter of 18 mm., the lower part a length of 10 cm. and a diameter of about 6 mm. (cp. Fig. 3).

(*d*) A doubly bent glass tube, one end of which is somewhat widened and the other drawn out into a 10 cm. long capillary tube of even thickness. This tube can be fixed in the test-tube by means of a perforated cork so that the point of the capillary reaches to within a couple of mm. of the bottom of the test-tube. The cork has also another perforation into which a short rectangularly bent tube is fitted (Fig. 3).

(*e*) A small V-shaped capillary tube.

When the sample of alveolar air is to be taken, the pipette is placed in such a position in regard to the Haldane tube (horizontally supported by two stands) that its capillary reaches up through the T-tube to about 1 cm. above the inner orifice of the latter (see Fig. 2). The lower orifice of the T-tube will then be closed by the mercury seal of the pipette. In this position the pipette is now filled with mercury by raising the receiver up to a certain position ascertained by experiment beforehand.

The subject of the experiment is now allowed to take the mouth of the tube between his lips. After one inspiration the nose is closed and he must now hold his breath for 20 seconds, after which he should breathe out as much air as possible into the tube. Immediately after this expiration he closes the opening of the tube with the tip of his tongue. He must now in this position breathe through the nose while the mercury is made to run out quickly from

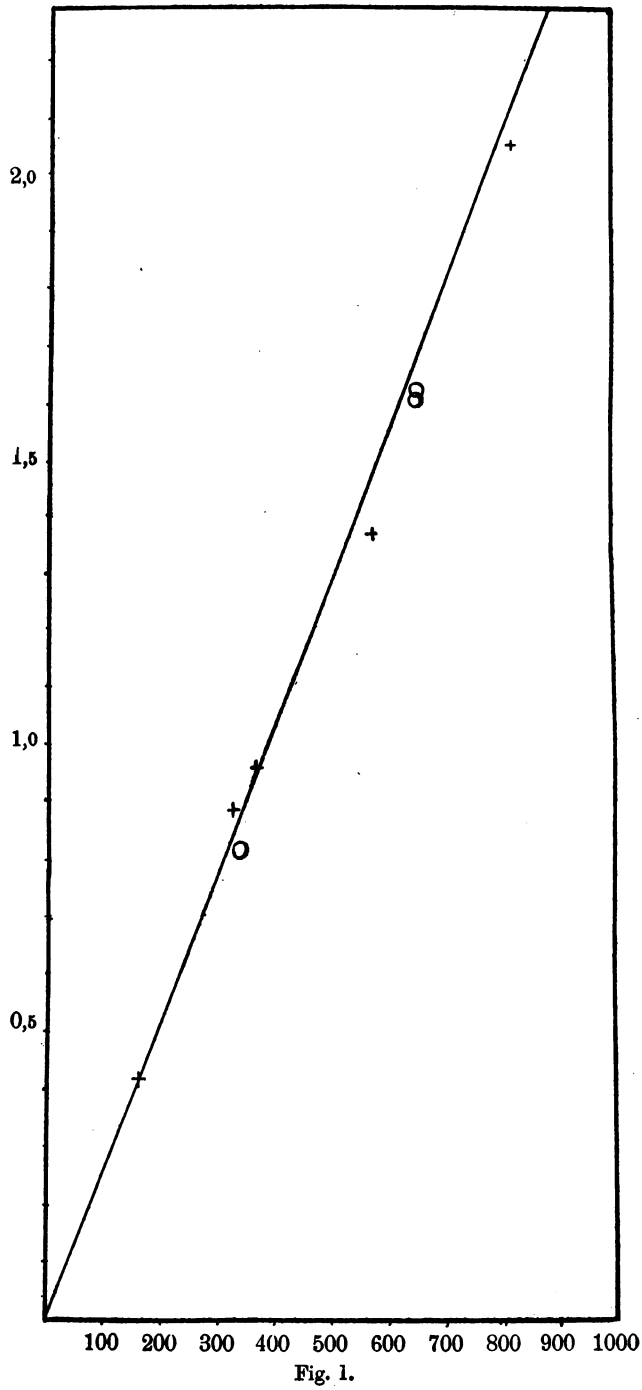

Fig. 1.

the pipette by lowering the vessel. The mercury must sink down to a certain mark on the lower tube of the pipette.

When this is done the glass tap is closed and the pipette promptly lowered so that the bent tube (*d*) can be pushed over the capillary tube of the pipette and closed by the mercury. The test-tube, containing 2 cc. of *N*/200 iodine solution and 3 cc. of *N*/1 NaOH, can be placed in position beforehand. During the whole operation the pipette should not be touched with the hands but should be held by a stand or clamps in the lowering and raising processes.

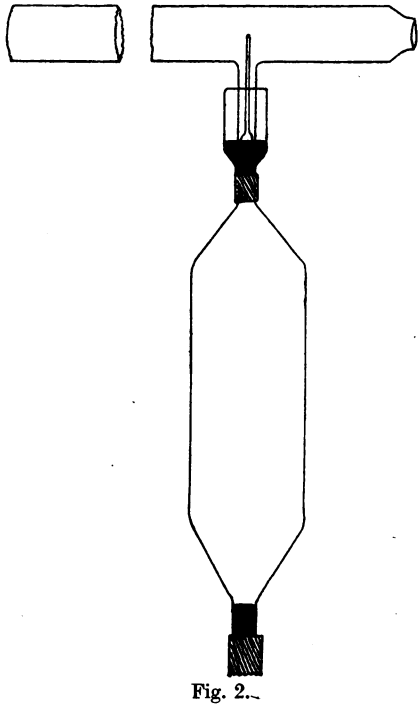

Fig. 2.

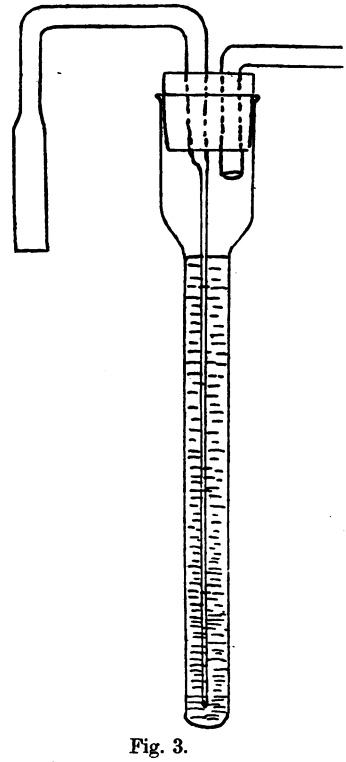

Fig. 3.

The mercury vessel is now raised, the clamp screwed on, and the glass tap opened, after which the speed of flow of the mercury is regulated by a screw-clamp so that the air is driven bubble by bubble through the alkaline iodine solution. The passage of 100 cc. ought to take about an hour. After all the air has been driven out of the pipette it is necessary to pass through the iodine solution the volume of air—amounting to about 1·5 cc.—present in the connecting tube. For this purpose the small bent glass tube in the cork of the test-tube is attached to a short piece of rubber tubing provided with a mouth-piece consisting of a capillary tube 4 cm. long. By gently sucking through this the mercury is first made to rise half a centimetre from the seal up into the connecting tube. Then the small V-shaped capillary tube is dipped into the mercury and manipulated so that one end of it emerges above the

surface into the connecting tube. A few more cc. of air are now sucked through the iodine solution, and in this way the acetone gas present in the connecting tube is washed out.

The absorption of the acetone in the alkaline iodine solution becomes in this fashion practically complete; I have not been able to find, by coupling several test-tubes together, that any acetone passes the first test-tube. The conditions for the absorption are more favourable with this apparatus than with that previously described, partly because the narrower test-tube makes the layer of fluid through which the air bubbles have to pass deeper, and partly because the stronger hydroxide increases the speed of reaction in the iodoform formation.

It was found, however, that the passage of 100 cc. of room air is sufficient to change the titre of the iodine. This change is very constant and amounts to a diminution of 0·05 cc. of $N/200$ iodine. It should always be determined by means of a blind test. It is further of great importance to provide carefully that both test-tube and connecting tube shall be extremely well cleaned and rinsed out with freshly distilled water immediately before the beginning of the experiment.

Before the titration, which in other respects proceeds in the manner previously described, the fluid is acidified with 3·5 cc. of N/sulphuric acid. The mixture is at once stirred and titration carried out immediately.

In the case of healthy persons, in whom an estimation by the micro-method reveals no trace of acetone in the blood, we do not obtain by the alveolar air test any greater binding of iodine than we find in the blind tests with room air. The percentage of carbonic acid in the alveolar air therefore plays no part in the estimations, and the normally occurring amount of acetone is too inconsiderable to be demonstrated.

The measurement of the acetone contained in the alveolar air is performed at room temperature. In the calculation of the acetone concentration in the air we must therefore reckon the volume of the air from room temperature to 38°, at which temperature the air is assumed to be saturated with water. This calculation is most easily performed by the aid of the tables in "Landolt and Börnstein." The air is assumed to have stood the whole time under atmospheric pressure; the slight increase of pressure arising in the lungs before the expiration into the Haldane tube may be neglected in the calculation. We add complete details for one case.

The acetone in the alveolar air after the administration of acetone.

Experiment 5. March 16, 1917. Bar. 768 mm. Room temperature 16°. Volume of the analysing pipette 95·86 cc. Subject: the author. At 10.45 a.m. about 9·5 g. of acetone were taken in 500 cc. of water. Alveolar tests at 2.22 p.m. with 20 seconds' breathing pause. The volume of the air measured, saturated with water at a pressure of 768 mm. and a temperature of 38°, was 108·3 cc.

Total quantity of acetone found: 46·9 γ.

Acetone concentration in the alveolar air: $\frac{46{\cdot}9}{108{\cdot}3} = 0{\cdot}433$ γ/cc.

Acetone concentration in the blood: at 2.20 p.m. 0·127
" " " at 2.25 p.m. 0·124 } 0·126 ‰.

$$\lambda = \frac{0{\cdot}433}{126} = 0{\cdot}00344.$$

In the same way a number of other determinations of the partition of the acetone between blood and alveolar air in experimental acetonaemia were carried out. These measurements are collected in the following table.

Table III.

Blood acetone γ/cc.	Air acetone γ/cc.	λ
222	0·59	0·00266
220	0·72	0·00327
210	0·63	0·00300
208	0·62	0·00298
199	0·59	0·00296
143	0·45	0·00315
126	0·43	0·00341
94	0·28	0·00298
63	0·16	0·00254
60	0·18	0·00300
		Average: 0·002995
		$E = \pm 0{\cdot}000257$
		$e = \pm 0{\cdot}000081$

All the determinations were carried out with the author as subject. Most of the values for the blood acetone concentration were obtained by twofold estimations. The error in these blood estimations may be estimated at about $\pm$ 15 γ per cc. of blood. The mean error for γ in the separate estimation is 0·000257. The average for λ is 0·002995 with $e = \pm$ 0·000081. The acetone percentage of the alveolar air therefore agrees very well with the concentration obtained by shaking air with blood containing acetone. In the experiments referred to (p. 383) the two values 0·00309 and 0·00329 were obtained.

The error in the estimation of λ arises partly from the error in the determination of the concentration in the blood and partly from the error in the determination of the concentration in the air. If the mean error in the blood estimation is assumed to be $\pm$ 15 γ, the error in the blood analysis alone will amount to about $\pm$ 0·00015. The air analysis cannot therefore give any appreciable error.

Hence it follows that the estimation of the free acetone in the blood can be performed with about the same accuracy by an analysis of the alveolar air as the total acetone estimation in a blood sample by means of the micro-method.

It is probable that the establishment of diffusion equilibrium between blood and alveolar air takes place almost instantaneously. In an experiment upon myself with 20 seconds' breathing pause I obtained $\lambda = 0{\cdot}00296$, and upon expiration into the Haldane tube in immediate succession to a normal expiration I found the values 0·00284 and 0·00273. With another person, B. J., as subject, the value 0·0034 was obtained after 20 seconds' breathing pause, 0·0032 after 10 seconds' and 0·0032 after a normal expiration. Since the mean error of the separate estimation is $\pm$ 0·00026 we cannot therefore demonstrate that the diffusion equilibrium has not been able to establish itself in normal respiration. Probably the difference between the acetone partial pressure of the blood and the acetone partial pressure of the alveolar air is quite inconsiderable. The experiments show at all events that a breathing pause of 20 seconds is quite sufficient to allow diffusion equilibrium to be established. This time is further taken with so wide a margin that it must be presumed that even in persons whose lungs may offer less favourable conditions for diffusion than the normal, equilibrium must be established. For further information upon these points we refer to Marie Krogh [1914].

If the acetone within the organism is found in diffusion equilibrium in the different fluids of the tissues, *the acetone in these fluids should have a partial pressure agreeing with that of the blood.* These fluids should therefore give off to the air an amount of acetone per cc. equal to that found in the alveolar air. This may be shown indirectly by the following experiment:

Experiment 6. March 16, 1917. Subject: the author. After taking a certain quantity of acetone the alveolar air contained at 2.22 p.m. 0·433 γ/cc.

Of the amount of urine collected in the bladder from 2.15–2.30 p.m. 3 cc. were shaken in a flask according to method *A*.

Concentration in the urine before shaking was 0·168 $^{0}/_{00}$ (three estimations).

Concentration in the urine after shaking was 0·085 $^{0}/_{00}$ (three estimations).

From these values λ for the urine is found to be 0·00241. The 0·168 $^{0}/_{00}$ urine can therefore give off: $168 \times 0{\cdot}00241 = 0{\cdot}405\,\gamma$ per cc. of air, which is only 7 % lower than the value found in the alveolar air. The small difference may possibly be due to the circumstance that method *A* regularly gives somewhat lower values than method *B*. The urine sample was obtained during strong diuresis (7·33), and if the value 0·00262—which was the mean value of the determinations with aqueous solutions according to method *B*—be used for λ, we obtain the value 0·437 for the acetone concentration in the air.

The acetone in the alveolar air of diabetics.

These air analyses, as has already been pointed out, render possible *the determination of the concentration of the free acetone in the blood of diabetics.*

This determination is carried out precisely in the manner previously described for the alveolar air analyses. The percentage of free acetone in the

blood is calculated by dividing the air acetone concentration by the value for λ, *i.e.* approximately 0·003[1].

The estimation of the percentage of aceto-acetic acid in the blood can be carried out in the usual way by a simultaneous blood estimation; the value of the free acetone is subtracted from the total acetone value.

In the following I give a couple of examples of these estimations of the percentage of free acetone and aceto-acetic acid in the blood of diabetics[2]. The estimations are only intended to demonstrate the possibility of the process here described. A systematic investigation of the relationship between the acetone and the aceto-acetic acid in the blood is reserved for a later work.

Experiment 7. March 14, 1917. Subject: J. N., diabetic with moderate acidosis. Breathing pause 20 seconds. Three estimations were made:

(1) At 1.30 p.m. the alveolar air contained 0·108 γ/cc.
(2) „ 2.15 „ „ „ 0·117 „
(3) „ 3.17 „ „ „ 0·135 „

From these values the free acetone in the blood was calculated, using the factor 0·003:

(1) 0·032 ‰.
(2) 0·035 ‰.
(3) 0·041 ‰.

In the same case estimations of the total acetone concentration in the blood were carried out.

2.25 p.m. $\left.\begin{matrix}0{\cdot}102\\0{\cdot}109\end{matrix}\right\}$ 0·106 ‰.

3.20 p.m. $\left.\begin{matrix}0{\cdot}121\\0{\cdot}126\end{matrix}\right\}$ 0·124 ‰.

The complete analysis of the relationship between the acetone and the aceto-acetic acid in the blood is given in the following table:

Table IV. Relationship between acetone and aceto-acetic acid in J. N. according to Exp. 6.

Time	Total acetone in blood ‰	Acetone in air γ/cc.	Free acetone in blood ‰	Combined acetone in blood ‰	Free acetone in per cent. of total acetone
1.30 p.m.	—	0·108	0·032	—	—
2.15 p.m.	0·106	0·117	0·035	0·071	33
3.17 p.m.	0·124	0·135	0·041	0·083	33

[1] A certain irregularity in the values may of course arise if the proportion between the volume of the blood corpuscles and that of the plasma departs excessively from the normal. This is however not usually the case with diabetics.

[2] In both examples the total acetone concentration in the blood is rather low, so that the estimations lie very near the limits of error of the method. Unfortunately while these experiments were being carried out I had not access to any case with a higher degree of acidosis.

Experiment 8. March 21, 1917. Subject: J. N., diabetic with moderate acidosis. Breathing pause 20 seconds. Two estimations were carried out:

Time: 2.40 p.m. 0·119 γ/cc.
3.35 p.m. 0·119 γ/cc.

The blood estimations gave the following results:

Time: 3.00 $\left.\begin{matrix}0{\cdot}073\\0{\cdot}068\end{matrix}\right\}$ 0·071 °/$_{oo}$.

3.40 $\left.\begin{matrix}0{\cdot}077\\0{\cdot}080\end{matrix}\right\}$ 0·079 °/$_{oo}$.

All the estimations are shown in Table V.

Table V. Relationship between acetone and aceto-acetic acid in J. N. according to Exp. 8.

Time	Total acetone in blood °/$_{oo}$	Acetone in air γ/cc.	Free acetone in blood °/$_{oo}$	Combined acetone in blood °/$_{oo}$	Free acetone in per cent. of total acetone
3.00 p.m.	0·071	0·119	0·036	0·035	51
3.40 p.m.	0·079	0·119	0·036	0·043	46

These estimations give values which may be quite well compared with those obtained by Marriott [1913]. In the four estimations published by him the pre-formed acetone constituted 63, 30, 61 and 28 % of the total acetone.

Formula for the elimination of acetone through the lungs.

A simple expression similar to that derived in Part II for the elimination of the acetone through the kidneys may also be obtained for its elimination through the lungs.

Assuming that the acetone in the alveolar air is in diffusion equilibrium with the acetone in the blood, the quantity of acetone, M, eliminated through the lungs per unit of time is evidently directly proportional to the concentration of the free acetone in the plasma and to the alveolar ventilation, *i.e.* the alveolar air exchanged during the unit of time:

$$M_l = a.V.\lambda,$$

where a = the concentration of the free acetone in the plasma;
V = the alveolar ventilation;
λ = the coefficient of partition between air and plasma.

An increase in the ventilation, *e.g.* in coma, consequently increases the amount of acetone eliminated through the lungs. Naturally this law only applies so long as the ventilation does not exceed a degree which would make it impossible for diffusion equilibrium to be set up. In the latter case the amount eliminated through the lungs is estimated not only from the factors here mentioned but also from the lung diffusion constant for acetone, and from other factors which we shall not go into here (see Marie Krogh [1914]).

The formula given above naturally applies to the free acetone only. The aceto-acetic acid does not take part in the elimination through the lungs, since its salts are not volatile. Nor is there any reason to suppose that a greater decomposition of the aceto-acetic acid takes place in the lungs than in other organs of the body[1].

As far as the mutual *relationship between the amount of acetone given off through the lungs and through the kidneys* is concerned, it is obvious that this can be estimated from the factors here mentioned.

If in the blood free acetone alone is found, the relationship will be as follows:

$$\frac{M_l}{M_n} = \frac{aV\lambda}{av\kappa} = \frac{V\lambda}{v\kappa}.$$

where λ and κ may be regarded as constants. Besides these we therefore determine the relationship between two factors that are independent of one another: the ventilation and the quantity of urine. With increased ventilation the ratio is altered to the advantage of the elimination through the lungs; with an increased quantity of urine it is changed in the opposite direction. The quotient is always greater than 1.

If aceto-acetic acid occurs in the blood we obtain the following expression:

$$\frac{M_l}{M_t} = \frac{a.V.\lambda}{a.v.\kappa + c},$$

where a still represents the concentration of the free acetone in the blood.

Since the aceto-acetic acid (c) is given off exclusively through the kidneys, the quotient will of course become smaller the more aceto-acetic acid there is in the blood. This explains why the quotient in diabetes is less than in the experimental acetonaemia induced by the taking of acetone. In severe cases of diabetes it is small for the same reason.

Summary.

By determining the acetone concentration in the alveolar air it is established:

(1) that the elimination through the lungs is a pure diffusion process;

(2) that from a simple determination of the acetone concentration in the alveolar air it is possible to calculate the free acetone concentration in the blood.

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[1] For further details see Widmark [1919].