

## THE DIFFUSION OF CARBON DIOXIDE IN TISSUES

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The diffusion of CO<sub>2</sub> in animal membranes has received the attention of but few investigators and the data presented have been inadequate and apparently contradictory. Krogh (1919) published a permeability constant for CO<sub>2</sub> in connective tissue based on a single determination. He considered his value too high although it is lower than a similar constant given by Hagenbach (1898) for CO<sub>2</sub> diffusing in 20 per cent gelatin. Fenn (1928 *b*) published diffusion coefficients for CO<sub>2</sub> in nerve and muscle and found values considerably lower than the diffusion coefficient that he calculated from Krogh's permeability constant. Considering the contradictory results obtained by the above authors and the fundamental importance of the knowledge of the rate of diffusion of CO<sub>2</sub> in physiological problems it seemed desirable to make a study of the diffusion of this gas in different types of membranes. Two methods of determining the rate of diffusion of CO<sub>2</sub> in tissues and the results of measurements made on connective tissue, muscle, and frog skin are given below.

### *Apparatus and Methods*

*Measurement of Permeability.*—The method of measuring permeability of tissues to CO<sub>2</sub> depends on the fact that small amounts of CO<sub>2</sub> can be detected by conductivity changes in a barium hydroxide solution (Fenn, 1928 *a*). Essentially the method is to pass CO<sub>2</sub> of a known tension over one face of a tissue and catch the CO<sub>2</sub> that leaves the opposite face in barium hydroxide. Measurements of the conductivity of the barium solution are made by means of a suitable Wheatstone bridge and the changes are expressed in cc. of CO<sub>2</sub> at standard pressure and temperature.

The apparatus used is shown in Fig. 1. It consists of a conductivity cell with two platinum electrodes sealed into the side arm of a bottle, fitted with a ground glass stopper. The brass tube (*A*) is sealed into the glass stopper with de Khotinsky cement. Two brass rings (*B*) are used to hold the tissue and these fit snugly

over the lip at the base of the brass tube (C). When the tissue is in place, the tube (C) is inserted into tube (A) and the tissue is trapped under the lip of tube (C), leaving an area of 0.317 cm.<sup>2</sup> exposed for diffusion. The gas is saturated with water at the experimental temperature (22°C.) and then enters the diffusion chamber through the glass tube (D). The side arm with the stop-cock is open until the apparatus has come to temperature equilibrium with the water bath in which the whole apparatus is immersed.

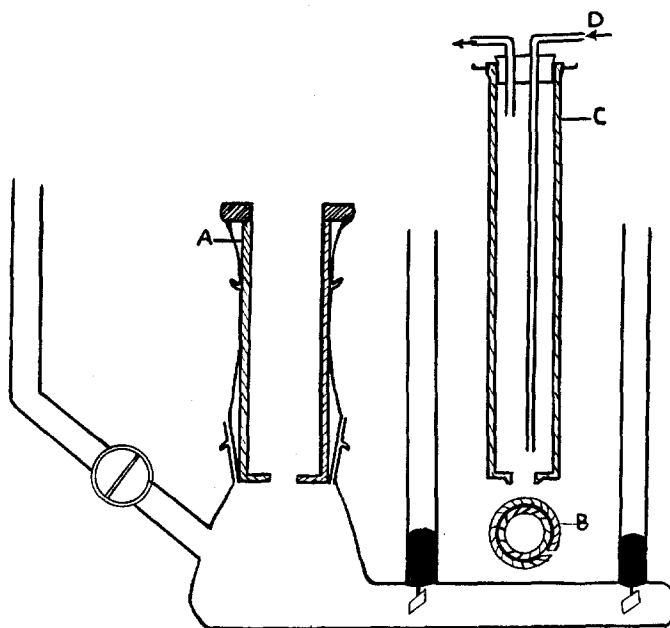


FIG. 1. Apparatus for measuring the permeability of tissues to carbon dioxide.

The values given by Fenn for the change in conductivity per c. mm. of CO<sub>2</sub> at different specific conductivities of barium hydroxide have been (1) multiplied by 0.95 to reduce them from 25°C. to 22°C.; (2) multiplied by  $\frac{9.28}{9.75}$  to correct for the difference in cell constants; (3) the ordinates have been multiplied by  $\frac{3.97}{6.00}$  to correct for the volume of barium hydroxide used.

The routine procedure for an experiment is to prepare the tissue in the rings, wash well with Ringer's solution, make several determinations of its thickness, and then immediately mount it in the conductivity cell containing a known quantity (6.0 cc.) of barium hydroxide solution. The small space between the two brass tubes is well sealed with wax and the cell is immersed in a constant temper-

ature bath. The gas, previously mixed in two 20 liter bottles, is then passed slowly through a saturator (also in bath) before entering the diffusion chamber and the whole apparatus is allowed to stand until an equilibrium is established. 20 minutes were found to be sufficient to flush out all parts with the CO<sub>2</sub> mixture and to arrive at the bath temperature. The conductivity of the barium hydrate is now measured and subsequent readings taken at intervals until the rate of passage of CO<sub>2</sub> has remained constant for a period of 2 hours. The results are expressed in cc. of gas (N.T.P.) passing through 1 cm.<sup>2</sup> under a pressure gradient of one atmosphere per cm.

*Measurement of Absorption Coefficients.*—According to Fick's law of diffusion, the quantity of gas ( $ds$ ) which passes in a time ( $dt$ ) across an area ( $A$ ) under a concentration gradient  $\frac{dc}{dx}$  is given by the formula

$$ds = KA \frac{dc}{dx} dt \quad (1)$$

where ( $K$ ) is the true diffusion coefficient for the substance. With appropriate units  $K$  has the dimensions  $\frac{\text{cm.}^2}{\text{minute}}$ . In order to derive a diffusion coefficient from the permeability constant  $\left(\frac{\text{cm.}^2}{\text{minute atmosphere}}\right)$  as determined by the barium hydroxide method it is necessary to know the absorption coefficient  $\left(\frac{\text{cm.}^3}{\text{cm.}^3 \text{ atmosphere}}\right)$  for CO<sub>2</sub> in the tissues. Dividing the permeability constant by the absorption coefficient the true diffusion coefficient  $\left(\frac{\text{cm.}^2}{\text{minute}}\right)$  is obtained.

A differential volumeter of the type used by Fenn (1927) was modified as shown in Fig. 2 for the determination of the absorption coefficient. The experimental bottle ( $A$ ) has a side pocket ( $B$ ) large enough to contain 2.5 cc. of mercury and a side arm with a stop-cock ( $C$ ) for flushing the bottle with gas. A ground glass stopper fits into the base of the experimental bottle and during an experiment contains a flat piece of acidified tissue stretched over a brass disc and held in place by a split ring. The volume of the tissue is determined from the weight (specific gravity 1.04) and the thickness determined by the method described below. The whole apparatus is immersed in a water bath at  $22^{\circ} \pm 0.01^{\circ}\text{C}$ .

The tissue is weighed and placed in the stopper. The apparatus is then placed in the water bath and the experimental bottle flushed out with hydrogen, saturated with water at 22°C. The stop-cocks are turned to the position connecting both bottles with the capillary and an equilibrium established. At this stage the tissue is saturated with hydrogen and mercury is tipped from the side pocket, trapping the hydrogen in the tissue. The stop-cocks are now turned to permit flushing of the bottle with CO<sub>2</sub>. 5 minutes are allowed for thorough washing out of all

hydrogen and then the cocks are turned again to the capillary. When equilibrium is again reached (2 to 3 minutes) the mercury is quickly dumped into the side pocket and CO<sub>2</sub> is exchanged for the hydrogen in the tissue, resulting in a rapid movement of the kerosene drop toward the experimental bottle. When the drop in the capillary again comes to equilibrium the tissue has become saturated with

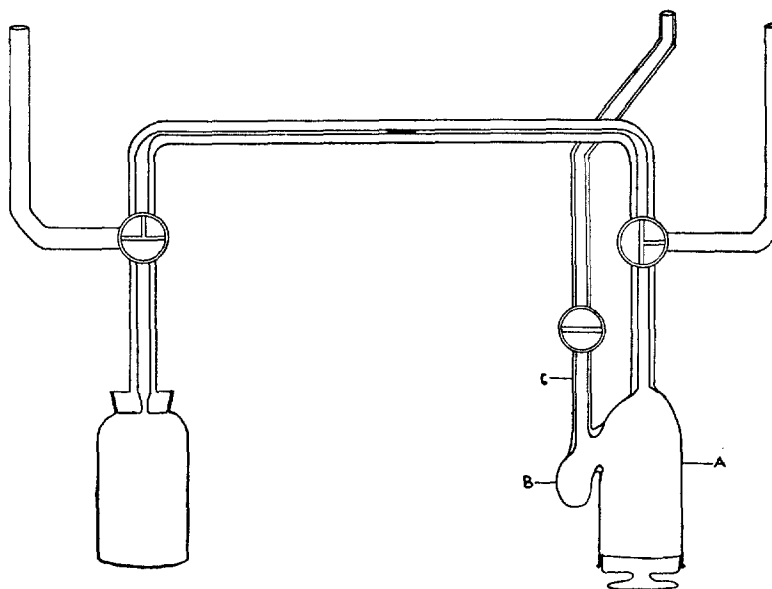


FIG. 2. Apparatus for the determination of the carbon dioxide absorption and diffusion coefficients for tissues.

CO<sub>2</sub> and the amount required for saturation can be calculated by means of the following formula from Fenn (1928 a):

$$x = d \frac{V_c + V_r (P - y)}{V_c + d} \frac{1}{P} \quad (2)$$

where

$x$  = volume of CO<sub>2</sub> (temperature and pressure of experiment) required for saturation;

$d$  = volume of capillary ( $8.6 \times 10^{-3}$  cc. per cm.) per unit length;

$V_c$  = volume of control bottle (19.4 cc.);

$V_r$  = volume of experimental bottle (30.3 cc.);

$P$  = barometric pressure;

$y$  = vapor pressure in the bottles.

The value  $x$  is corrected to standard temperature and pressure in these experiments.

Some determinations of the amount of CO<sub>2</sub> leaving the tissue have been made by covering the CO<sub>2</sub> saturated tissue with mercury and flushing the bottle with hydrogen. When the mercury is dumped into the side pocket the CO<sub>2</sub> escapes and the kerosene drop moves away from the bottle in proportion to the amount of CO<sub>2</sub> dissolved.

A correction must be made for the hydrogen leaving the tissue when calculating the amount of CO<sub>2</sub> necessary for saturation. This correction is only small due to the low solubility coefficient for hydrogen in H<sub>2</sub>O (0.018 cc./cc.). A further correction is made for any constant drift of the kerosene drop previous to dumping the mercury and after saturation. Such a correction, while not large, becomes significant over a period of 20 or more minutes. The maximum rate of movement noted was 0.04 cm./minute which in 20 minutes amounts to about 5 per cent of the total movement. The average drift was 0.02 cm./minute.

*Measurement of the Diffusion Coefficient by the Volumetric Method.*—In the experiments for determination of the absorption coefficient, we have a condition where a flat sheet (of known thickness) is suddenly exposed to a gas and the percentage saturation at any time thereafter is indicated by the position of the kerosene drop. Under such conditions a diffusion coefficient for the gas in the material of the sheet can be calculated by the formula

$$\frac{Q}{Q_1} = 1 - \frac{8}{\pi^2} \left\{ e^{-\frac{\pi^2 Kt}{4a^2}} + \frac{1}{9} e^{-\frac{9\pi^2 Kt}{4a^2}} + \frac{1}{25} e^{-\frac{25\pi^2 Kt}{4a^2}} + \dots \right\} \quad (3)$$

taken from the work of Andrews and Johnston (1924).

$Q_1$  is the total amount of the gas present in the sheet at saturation.  $Q$  is the amount present at the time  $t$ .  $a$  is the thickness of the tissue and  $K$  the diffusion coefficient of the gas.

This equation can be reduced to the simpler form

$$\frac{Kt}{a^2} = -0.0851 - 0.933 \log \left( 1 - \frac{Q}{Q_1} \right) \quad (4)$$

if all terms but the first are neglected, a condition sufficiently accurate for present purposes. When  $\frac{Kt}{a^2}$  is greater than 0.1, corresponding to 36 per cent saturation, the error is less than 1 per cent.

When the mercury is tipped off the tissue, during a determination of the absorption coefficient, a stop-watch is started and the position of the kerosene drop read at intervals of 30 seconds for the first 2 minutes, and appropriate intervals thereafter, until equilibrium; *i.e.*, saturation is reached. From the position of the drop, at any time after tipping, the percentage saturation can be determined and  $K$  can be readily calculated from Equation 4 when  $a$  is known. The position of the drop must be corrected for the H<sub>2</sub> diffusing from the tissue as well as any constant drift as mentioned above. The rate at which H<sub>2</sub> leaves the tissue may be calculated by means of Equation 4, assuming a diffusion coefficient of 28.0 ×

$10^{-4}$ . This figure is obtained by assuming that the rate of diffusion is inversely proportional to the square root of the gas density and the coefficient of diffusion for CO<sub>2</sub> is  $6.0 \times 10^{-4}$ . A number of determinations of the CO<sub>2</sub> diffusion coefficient in acidified tissues have been made by this method.

The CO<sub>2</sub> used in all experiments was taken from cylinders as supplied by the Ohio Chemical Company for medicinal purposes. This CO<sub>2</sub> is pure within a small fraction of 1 per cent by analysis. All CO<sub>2</sub> mixtures above 30 per cent were analyzed by flushing out a capillary tube (with stop-cock) and allowing the open end to stand in NaOH. The ratio of the height to which the NaOH rises in the tube to the total length of the tube gives the percentage CO<sub>2</sub> in the gas mixture within 1 per cent. All CO<sub>2</sub> mixtures below 30 per cent were analyzed on the Haldane-Henderson gas analyzer.

*Measurement of Membrane Thickness.*—Probably the greatest source of error in all permeability and diffusion measurements lies in the determination of the

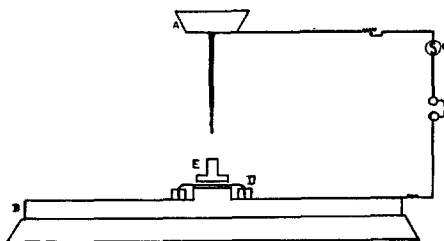


FIG. 3. Apparatus for measuring the thickness of tissues.

thickness of the membrane used. Many preliminary experiments in this work have been rejected due to variability, traceable directly to errors in the methods of measuring thickness. The apparatus used in all the experiments below is shown in Fig. 3. (A) represents the objective of a microscope fitted with a tapered metal pointer and (B) a brass plate with an elevated disc resting on the stage of a microscope. An electric circuit is completed from the pointer to the plate through a rheostat and a pair of head-phones. An oscillating current is furnished by the vacuum tube oscillator (C). The tissue, already in the rings (D), is placed over the elevation on the brass plate and the objective is lowered by means of the fine adjustment screw (calibrated) until the pointer just makes contact as signified by the hum in the phones. By taking alternate readings with the 3 gm. weight (E) on the tissue and directly on the platform, a series of figures is obtained the average of which gives an accurate determination of the thickness. For example, three thickness determinations on the same membrane gave the following values: 0.235 mm., 0.238 mm., and 0.230 mm., each an average of six to ten measurements. The deviation of any single reading from the mean of ten readings seldom exceeds 5 per cent.

## RESULTS

*Permeability Constant.*—Fig. 4 shows the graph of a typical experiment using the barium hydroxide method for the determination of the permeability of tissues for  $\text{CO}_2$ . This graph shows the constancy of the rate at which  $\text{CO}_2$  passes through a tissue after an initial period required to reach equilibrium.

Table I contains a summary of all the determinations of membrane permeability obtained by the barium hydroxide method. The average

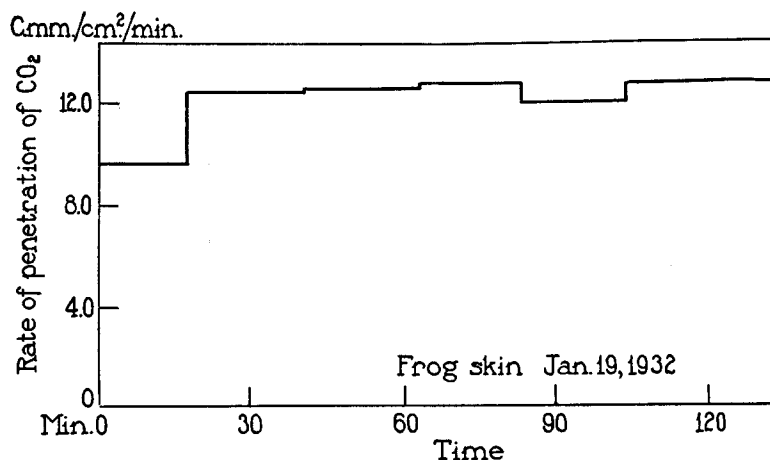


FIG. 4. Graph showing constancy of the rate at which carbon dioxide penetrates frog skin. Thickness 0.237 mm.;  $\text{CO}_2$  tension 734 mm. Hg; temperature  $22^\circ\text{C}$ .

thickness of the tissue is given, together with the extreme range. The tissues decreased in thickness during the period of the determination. This decrease was, on the average, 10 per cent of the total thickness. The fourth column shows the extreme range of  $\text{CO}_2$  tensions used and the last column the permeability constant together with the probable error.

The three determinations on the permeability of rubber were made on membranes cut from the same sheet. Rubber was chosen as a means of checking the reliability of the method by comparison with the permeability as determined by other workers. The individual determinations gave the following values: 0.47, 0.50, and  $0.47 \times 10^{-4}$

which are in good agreement with the permeability constant  $0.44 \times 10^{-4}$  as found by Daynes (1920). The determinations on rubber also show the variation to be expected using a membrane of constant properties.

Twenty-five determinations on frog skin, cut from the belly of the frog, gave an average permeability constant of  $3.05 \times 10^{-4}$  with a probable error as shown. Acidification (4 or more hours in N/10 HCl)

TABLE I  
*Permeability Constants for CO<sub>2</sub> Diffusing in Various Media*

	No. of determinations	Average membrane thickness	Range of CO <sub>2</sub> tension	Permeability (P) × 10 <sup>4</sup>
		<i>micra</i>	<i>mm. Hg</i>	
Rubber . . . . .	3	50	84-86	0.48
Frog skin . . . . .	25	248 (175-325)	57-744	3.05±0.23
*Frog skin (acidified N/10 HCl) . . . . .	4	283 (270-300)	722-734	4.47±0.01
Connective tissue (dog diaphragm) . . . . .	2	215 (175-255)	127-148	2.65
Frog muscle . . . . .	26	354 (255-500)	74-731	5.29±0.54
Mammalian muscle (dog diaphragm) . . . . .	1	700	152	†4.7
Smooth muscle (cat bladder) . . . . .	1	360	109	†5.0
Parchment paper . . . . .	2	233	728-732	1.35
‡Parchment paper (NaHCO <sub>3</sub> ) . . . . .	2	235	727-730	1.54

Permeability is expressed as cc. per cm.<sup>2</sup> per min. with a gradient of one atmosphere per cm. × 10<sup>4</sup>.

Figures in parentheses in Column 3 indicate the extreme ranges of thickness of tissues.

\* Frog skin soaked for 4 or more hours in N/10 HCl.

† Corrected for connective tissue.

‡ Parchment soaked for 12 hours in 0.15 M NaHCO<sub>3</sub>.

All experiments at 22°±0.01°C.

of frog skin markedly reduces the resistance offered to the passage of carbon dioxide as can be seen from the average value of  $4.47 \times 10^{-4}$ . This change may be attributed to several factors such as sloughing off of the outer epithelial layer, a 5 to 10 per cent increase in water content, and general structural changes accompanying precipitation of proteins.

Mammalian connective tissue offers slightly more resistance to the passage of CO<sub>2</sub> than does frog skin. These membranes were taken



from the central tendon of the diaphragms of two dogs. The permeability was  $2.65 \times 10^{-4}$ .

Muscles, both of frogs and mammals, are more freely permeable to  $\text{CO}_2$  than connective tissue or frog skin. Twenty-six determinations, on the combined externus and internus obliquus muscles from the abdominal wall of the frog, gave an average value of  $5.29 \times 10^{-4}$ , uncorrected for the thin layers of connective tissue. A correction for connective tissue, estimated at 5 per cent of the total thickness, gives a value of  $5.4 \times 10^{-4}$ . The permeability seems to vary somewhat with the season, being somewhat lower in the winter months. This difference, however, may be due only to the difference in batches of frogs used. The single determinations on the diffusion through mammalian striated and smooth muscle are not greatly different from the average value for frog muscle. The values for mammalian muscles have both been corrected for the layers of connective tissue. The thickness of these layers (measured) was equal to 15 per cent of the total thickness and the correction made on the basis of the permeability measurements given for connective tissue in Table I. Allowance for connective tissue raises the permeability constant from  $4.4 \times 10^{-4}$  to  $4.7 \times 10^{-4}$  as given.

The frog muscles were still slightly irritable at the end of a determination and the frog skin appeared to be in good condition. The mammalian tissues were used directly after removal from the animal and were definitely not "normal" after 2 to 4 hours at  $22^\circ\text{C}$ . However, there was no marked change in permeability over this period.

The amount of  $\text{CO}_2$  produced by metabolism in the tissue has been neglected in the calculation of the above constants, since it forms as a maximum only 1 part in 100 of the total gas diffusing.

*Absorption Coefficient.*—Diffusion rates expressed as a permeability constant ( $P$ ) are useful when considering the diffusion of gases in a steady state; *i.e.*, when equilibrium has been established and the concentration gradient remains constant. When dealing with the kinetics of diffusion, however, Fick's diffusion coefficient ( $K = P/\alpha$ ), as given above, must be known. The determination of the absorption coefficient ( $\alpha$ ) presents some difficulty since  $\text{CO}_2$  is present both in the physically dissolved and in the bicarbonate form. If the bicarbonate ion assists in the diffusion of  $\text{CO}_2$  in the steady state then the diffusion coefficient for total  $\text{CO}_2$  will be  $K = \frac{P}{\alpha + B}$  where  $B$  is a modifying fac-

tor due to the presence of CO<sub>2</sub> in the form of bicarbonate. If the bicarbonate ion does not diffuse appreciably we can proceed as though it were not present except for the slight salt effect which it may have on the solubility of CO<sub>2</sub>. The following evidence indicates that the diffusion of bicarbonate is negligible when determining permeability.

In order that the permeability ( $P$ ) should remain constant at different experimental gas tensions, the amount of diffusible CO<sub>2</sub> dissolved must be a linear function of the gas tension. That the total CO<sub>2</sub> absorbed by tissues is not proportional to the tension is evident from the CO<sub>2</sub> dissociation curves of muscle and nerve as determined by Fenn (1928 *b*). Above 50 mm. Hg tension of CO<sub>2</sub>, the amount of combined CO<sub>2</sub> (bicarbonate) is relatively constant. Assuming that bicarbonate diffused alone, the gradient or diffusion pressure would be practically the same for tensions of CO<sub>2</sub> above 50 mm. of Hg. This would mean that approximately equal amounts of CO<sub>2</sub> would be transported over a wide range of experimental gas tensions and the permeability constant would vary with the tension used. Inasmuch as the permeability is found to be constant and independent of tension, it might be inferred that bicarbonate contributes but little to the total diffusion.

The experiments on the permeability of parchment paper (Table I) give a further indication of the small part played by bicarbonate in the total carbon dioxide transport. The first two determinations on parchment soaked in Ringer's solution (bicarbonate free) gave permeability constants 1.34 and  $1.36 \times 10^{-4}$ . The two determinations on the same membrane soaked in 0.15 M bicarbonate solution gave the permeability constants 1.52 and  $1.55 \times 10^{-4}$ , an increase of about 12 per cent. If such a concentration of bicarbonate increases the rate of diffusion only by 12 per cent, it seems reasonable to assume that in tissues where the bicarbonate concentration is but one-tenth as great (approximately 0.015 M), bicarbonate would contribute a negligible amount to the total diffusion.

It was thought at first that acidification of the tissue would solve this difficulty by removing the bicarbonate. However, such a procedure (Table I) so lowers the resistance offered by the tissue that diffusion is even faster with only dissolved CO<sub>2</sub> present.

Since bicarbonate contributes so little to the total diffusion, only the physically dissolved CO<sub>2</sub> has been considered in the determination

of the absorption coefficient. This amount may be estimated, assuming that  $\text{CO}_2$  dissolves in the tissue as though it were 80 to 83 per cent water and the solids were inert. The carbon dioxide absorption coefficient for water at  $22^\circ\text{C}$ . is 0.829 cc. (Landolt-Börnstein tables). On this assumption the tissues would take up 0.67 cc. per cc. at  $22^\circ\text{C}$ . under a pressure of one atmosphere.

The work of Van Slyke (1928) on the solubility of  $\text{CO}_2$  in acidified blood corpuscles shows that the organic constituents of tissue are not inert but dissolve  $\text{CO}_2$ . On the basis of Van Slyke's figure of 0.45 cc. for the absorption coefficient of  $\text{CO}_2$  in acidified ox red blood corpuscles (71.7 per cent  $\text{H}_2\text{O}$ ) at  $37^\circ\text{C}$ . an estimate can be made of the solubility in acidified muscle or other tissue at  $22^\circ\text{C}$ . Assuming that the temperature coefficient for  $\text{CO}_2$  solubility in tissues is the same as for water ( $-0.017$  cc. per degree) and correcting for the difference in water content, one arrives at the figure 0.77 for the solubility coefficient of  $\text{CO}_2$  in acidified tissues. This value is higher than the solubility (0.67 cc. per cc.) as calculated from the water content.

Van Slyke was unable to analyze for  $\text{CO}_2$  on acid solutions containing more than 25 per cent cells due to the viscosity of the solution. His value of 0.45 cc. for the solubility in 1 cc. of cells was obtained by extrapolation from 25 per cent to 100 per cent cells. A determination of the solubility of  $\text{CO}_2$  in tissues based on his extrapolated figure and requiring correction for temperature and water content is rather indirect. It was, therefore, thought advisable to make some actual determinations of the absorption coefficients for  $\text{CO}_2$  in the tissues used. The apparatus and procedure used for this purpose are given above.

Table II, Column 2, shows the results of the solubility determinations made on frog skin and muscle acidified with  $\text{N}/10$   $\text{HCl}$ . The average value 0.78 cc. per cc. for the absorption coefficient for  $\text{CO}_2$  in frog skin agrees almost exactly with the value calculated from Van Slyke's data on acidified red blood corpuscles and is somewhat lower than the coefficient of solubility for  $\text{CO}_2$  in water (0.829 cc. at  $22^\circ\text{C}$ .) as given in the Landolt-Börnstein tables. The average value for the solubility of  $\text{CO}_2$  in acidified muscle is somewhat higher (0.84 cc. per cc.). The water content of the frog skins increased on an average 6 per cent, due to acidification, so that the value for the solubility of  $\text{CO}_2$

in normal tissue is probably somewhat less, 0.73 cc. per cc. for frog skin. A similar correction for increased water content of muscle (average increase 8 per cent) gives a value 0.78 cc. per cc. for the solubility of CO<sub>2</sub> in normal muscle. The corrected values have been used in all calculations below.

TABLE II  
*The Absorption Coefficients and Diffusion Coefficients for CO<sub>2</sub> in Acidified Tissues, as Determined by the Volumetric Method*

Tissue	No. of determinations	Absorption coefficient	Determinations	Diffusion coefficient
Acidified frog skin . . . . .	11	0.78±0.022	9	6.7±0.23
Acidified muscle . . . . .	10	0.84±0.024	7	6.4±0.33

All determinations at 22°C.  
Probable error is given.

TABLE III  
*Averages of Carbon Dioxide Diffusion Coefficients Determined by the Barium Hydroxide Method*

Membrane	No. of determinations	Permeability constant	Absorption coefficient	Diffusion coefficient
		× 10 <sup>4</sup>	cc./cc.	cm. <sup>2</sup> /min. × 10 <sup>4</sup>
*Rubber . . . . .	3	0.48	0.93	0.51
Frog skin . . . . .	25	3.05	0.73	4.18
Frog skin (acidified) . . . . .	4	4.47	0.78	5.7
Frog muscle . . . . .	26	5.29	0.78	6.8
Muscle (dog) . . . . .	1	4.7	0.78	6.0
Smooth muscle (cat) . . . . .	1	5.0	0.78	6.4
Connective tissue (dog) . . . . .	2	2.65	0.73	3.6

Permeability expressed as cc. per cm.<sup>2</sup> per min. under a gradient of one atmosphere per cm. × 10<sup>4</sup>.

\* Absorption coefficient based on Wroblewski's equation for the solubility of CO<sub>2</sub> in rubber, as given by Glazebrook.

*Diffusion Coefficient.*—Table III contains the averages of the CO<sub>2</sub> permeability constants as determined by the barium hydroxide method, together with the CO<sub>2</sub> diffusion coefficients as calculated by means of the solubility coefficients of Table II. The absorption coefficient for

rubber has been calculated from Wroblewski's equation (see Glazebrook) for the solubility in water-saturated rubber ( $\alpha = 1.2779 - 0.01576t$ ). The absorption coefficient for connective tissue has been arbitrarily taken as equal to that of frog skin.

The figures given for the diffusion coefficient indicate that all types of muscle offer approximately the same resistance to the diffusion of  $\text{CO}_2$ . On the other hand this gas is very much retarded when passing

TABLE IV  
*The Diffusion of Carbon Dioxide in Various Media*

Medium	Temperature	Permeability constant	Absorption coefficient	Diffusion coefficient	
	°C.	$\times 10^4$	cc./cc.	$\text{cm.}^2/\text{min.} \times 10^4$	
Water.....	16	9.4	0.99	9.5	Hüfner, 1897
Rubber.....	17	0.44	0.86	0.51	Daynes, 1920
Rubber.....	22	0.48	0.93	0.51	Wright
Gelatin 20 per cent.....	15	5.9	1.0	5.9	Hagenbach, 1898
Connective tissue (frog).....	20	4.0	0.73	5.5	Krogh, 1919
Connective tissue (dog).....	22	2.7	0.73	3.7	Wright
Muscle (frog).....	22	0.85	0.78	1.17	Fenn, 1928
Muscle (frog).....	22	5.3	0.78	6.8	Wright
Muscle (dog).....	22	4.7	0.78	6.0	Wright
Smooth muscle (cat).....	22	5.0	0.78	6.4	Wright
Nerve (frog).....	22	0.55	0.78	0.71	Fenn, 1928
Frog skin.....	22	3.1	0.73	4.2	Wright

Permeability is expressed in cc. per  $\text{cm.}^2$  per min. under a pressure gradient of one atmosphere per cm.  $\times 10^4$ .

The coefficient of Daynes has been multiplied by 60 and the coefficients of Hüfner and Hagenbach multiplied by  $\frac{1}{1440}$  to change the units to minutes.

Krogh's value has been divided by and Fenn's figure multiplied by the proper absorption coefficients to complete the table.

through connective tissue or frog skin, the diffusion coefficient for these tissues being only 60 per cent of that for muscle. The explanation for this difference must lie in a difference in structure of the tissues. For instance the muscle probably has a greater percentage of lymph interspaces than skin or connective tissue, and diffusion in such spaces should be as fast as in water (Table IV).

In this connection it should be mentioned that the term diffusion coefficient is here applied to a membrane of tissue composed of many layers, involving different absorption coefficients and diffusion coefficients (Osterhout, 1933). However, the fact that the rate of saturation (Fig. 6) so nearly follows the theoretical based on an equation developed for an homogeneous medium, warrants the use of the term until more is known concerning diffusion in each layer.

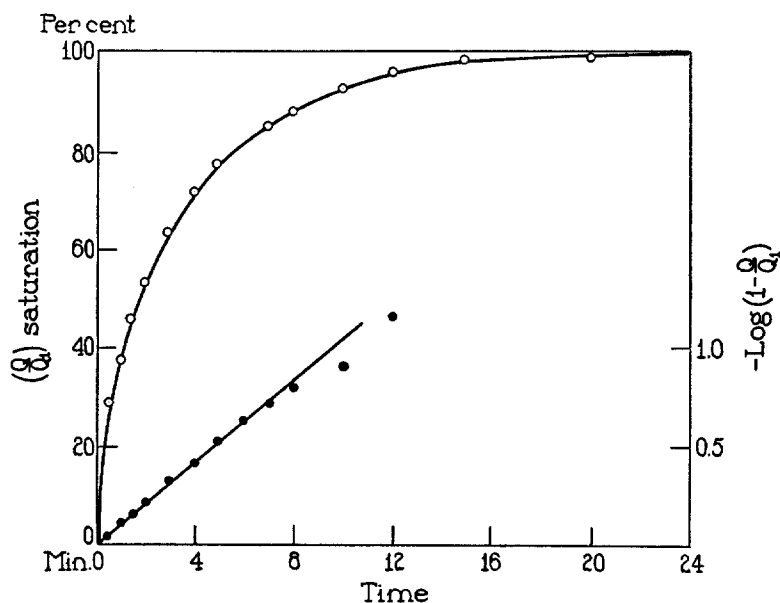


FIG. 5. Circles show the rate of saturation of a sheet of acidified frog skin with CO<sub>2</sub>. Curve shows the theoretical rate of saturation when the diffusion coefficient is  $6.8 \times 10^{-4}$ . Lower line shows the relation between  $-\log(1 - Q/Q_1)$  and  $t$  in Equation 4.

*Diffusion Coefficients Determined by Volumetric Method.*—Fig. 5 shows the graph of a typical experiment in which a piece of acidified frog skin saturated with hydrogen, was suddenly exposed to an atmosphere of CO<sub>2</sub> in a volumeter. The circles show the rate of saturation as indicated by the movement of the kerosene drop. The theoretical rate of saturation at different times as determined by substitution of  $6.8 \times 10^{-4}$  for  $K$  in Formula 4 above is shown by the curve. The experimental values follow closely the theoretical. The points on the

lower line represent  $-\log(1 - Q/Q_1)$  plotted against time and they fall regularly about a straight line up to 8 minutes, corresponding to 90 per cent saturation. The slope of this line is an average value for  $\frac{-\log(1 - Q/Q_1)}{t}$  which when substituted in Equation 4 gives the value  $6.8 \times 10^{-4}$  for  $K$ , the tissue having a thickness of 0.083 cm.

From Formula 4 it is seen that all values of  $Q/Q_1$  (per cent saturation) when plotted against  $t/a^2$  should fall on a single curve. Fig. 6

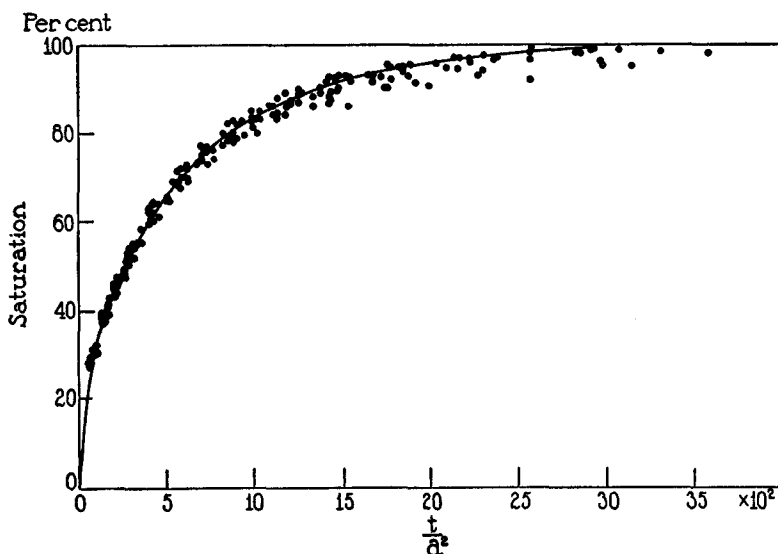


FIG. 6. Graph shows all the experimental points (per cent saturation) obtained by the volumetric method on acidified frog skin and muscle, plotted against  $t/a^2 \times 10^2$ . The curve shows the theoretical saturation when the diffusion coefficient is  $6.5 \times 10^{-4}$  at all values of  $t/a^2 \times 10^2$ .

shows a graph of all the experimental points obtained on acidified frog skin and muscle together with the theoretical curve for  $K = 6.5 \times 10^{-4}$ . The points fall regularly about the theoretical curve up to 90 per cent saturation. The last 10 per cent of saturation lags behind the theoretical rate. The reasons for this probably are (1) that the tissues were not entirely uniform in thickness over the total area and (2) that a small fraction of the tissue was held between a brass disc and a split ring approximately doubling the effective thickness at the edges.

A summary of the diffusion coefficients obtained with acidified tissues by the volumetric method is given in Table II. The average coefficient for frog skin is  $6.7 \times 10^{-4}$  and for muscle  $6.4 \times 10^{-4}$  with the probable error as shown. The value for frog skin can be directly compared with the diffusion coefficient ( $5.7 \times 10^{-4}$ ) as obtained on acidified skin by the barium hydrate method (Table IV). The agree-

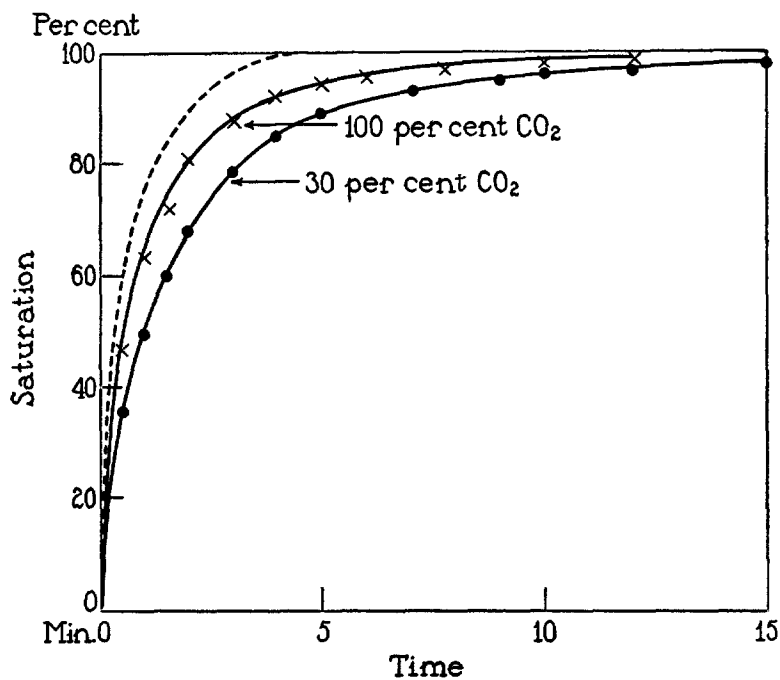


FIG. 7. Graph showing the different rates of saturation of non-acidified muscle (0.380 mm. thick) when exposed to different tensions of CO<sub>2</sub>. The lower curves show the rate of saturation in atmospheres of 30 per cent and 100 per cent CO<sub>2</sub> as indicated. The broken curve shows the theoretical rate of saturation ( $K = 6.0 \times 10^{-4}$ ).

ment must be considered good. Larger variations in the determination of the thickness of a relatively large area of tissue when stretched over a brass plate together with the fact that fewer volumetric determinations were made lead the author to believe that the value  $5.7 \times 10^{-4}$  is the more exact. Acidified muscle tears and splits very easily and a satisfactory determination of its permeability has not been possible.



*The Rate of Saturation of Non-Acidified Tissues*

When base is available for the formation of bicarbonate the rate of saturation of a tissue with  $\text{CO}_2$  is definitely changed. This is shown in Fig. 7 where the rate of saturation of a disc of frog muscle (0.380 mm. thick) is plotted against time in minutes. The two lower curves show the experimentally determined rates of saturation of the same tissue at the  $\text{CO}_2$  tensions indicated. The upper curve

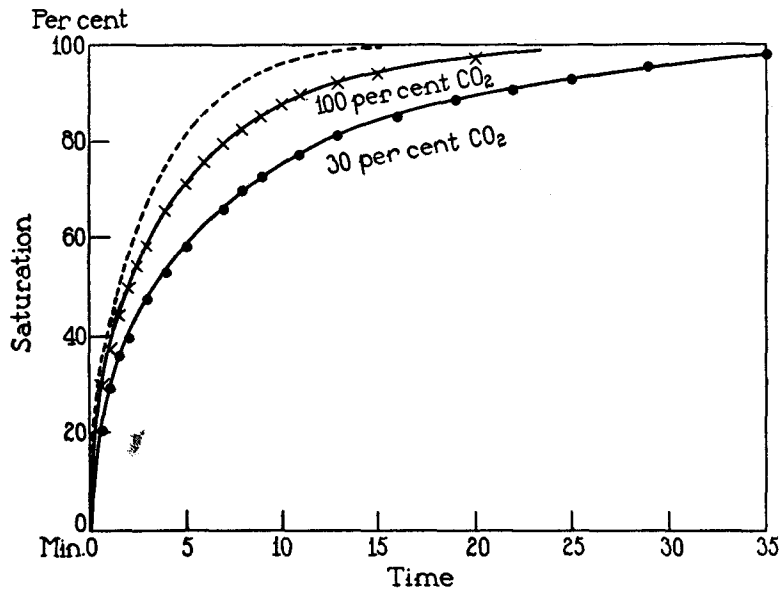


FIG. 8. Graph showing the different rates of saturation of a double layer of non-acidified frog skin (0.610 mm. thick). The lower curves show the rates of saturation at the  $\text{CO}_2$  tensions indicated and the broken curve the theoretical rate of saturation ( $K = 4.1 \times 10^{-4}$ ) if bicarbonate were not present.

shows the theoretical rate of saturation if bicarbonate were not present. Fig. 8 is a similar graph for a double layer frog skin (0.610 mm. thick). The curves given are typical of twenty-two determinations on muscle and eleven determinations on frog skin. The rate of saturation is slower in the presence of bicarbonate and furthermore is dependent on the  $\text{CO}_2$  tension to which the tissue is exposed, being slower at 220 mm. Hg than at 730 mm. Hg  $\text{CO}_2$  tension.

It has been assumed that saturation is complete when the kerosene drop of the volumeter has reached a constant rate of movement. After saturation (20 to 30 minutes) the drop moves uniformly toward the experimental bottle at an average of 1.3 mm. per minute, representing a CO<sub>2</sub> absorption of 12 c.mm. per gm. per minute. This is more than five times the maximum rate of absorption due to phospho-creatine hydrolysis when sartorius muscles are exposed to an atmosphere of CO<sub>2</sub> (Lipmann and Meyerhof, 1930).

It should also be mentioned that after allowing for the large drift the amount of bound CO<sub>2</sub> (total minus H<sub>2</sub>CO<sub>3</sub>) at saturation is approximately twice as great as that found by Fenn (1928*b*) and Root (1933) at tensions of 70 mm. and 220 mm. Hg. The only apparent reason for this discrepancy is that the tissues were stretched tightly over a brass disc and in the work quoted they were at rest.

#### DISCUSSION

Table IV contains a collection of diffusion coefficients for CO<sub>2</sub> in various media. CO<sub>2</sub> diffuses in muscle approximately 65 per cent as rapidly as in water. In connective tissue and frog skin the diffusivity is even slower, about 40 per cent of that in water. The diffusion coefficient for gelatin is very nearly that for muscle. Krogh's value for the permeability of connective tissue is somewhat high as he judged. Fenn's "approximate" values for the diffusion coefficients for CO<sub>2</sub> in muscle and nerve are too low. They were calculated from the experimentally determined rate of saturation by means of Formula 4 given above. This formula does not apply when a chemical reaction takes place to change the rate of diffusion, in this case CO<sub>2</sub> to bicarbonate.

From Graham's law, that diffusion is inversely proportional to the square root of the molecular density, O<sub>2</sub> should diffuse 1.18 times as fast as CO<sub>2</sub>. This law holds for these gases diffusing in water and is approximately true for diffusion in connective tissue. However, Krogh's value (1919) for the diffusion coefficient for O<sub>2</sub> ( $K = 4.5 \times 10^{-4}$ ) is less than the diffusion coefficient for CO<sub>2</sub> ( $6.8 \times 10^{-4}$ ) in frog muscle. This is not a unique exception to Graham's law since Daynes (1920) found that H<sub>2</sub> diffused in rubber much more rapidly than would be expected in comparison with other gases.

It is a pleasure to acknowledge my indebtedness to Professor W. O. Fenn who suggested this problem and assisted me with advice and encouragement throughout the progress of the work.

## SUMMARY

1. Two methods are given for measuring the rate of diffusion of CO<sub>2</sub> in tissue membranes. Methods are also given for the determination of tissue thickness and the absorption coefficient for CO<sub>2</sub> in tissues.

2. The values obtained for the permeability constant ( $P \times 10^4$ ) at 22°C. for CO<sub>2</sub> in the following tissues are:—frog skin, 3.05; connective tissue (dog), 2.65; smooth muscle (cat), 5.00; frog muscle, 5.29; striated muscle (dog), 4.70.  $P$  is expressed as cc. per cm.<sup>2</sup> per minute under a pressure gradient of one atmosphere per cm.

3. Evidence is presented to show that in a "steady state" bicarbonate contributes a negligible amount to the diffusion of CO<sub>2</sub>.

4. The absorption coefficient for CO<sub>2</sub> in frog skin is 0.73 cc. per cc. and for frog muscle 0.78 cc. per cc.

5. In all of the tissues studied the diffusion of CO<sub>2</sub> is slower than in water. The diffusion coefficients ( $K \times 10^4$  in cm.<sup>2</sup>/minute) at 22°C. for tissues as compared with water are:—water (16°C.), 9.5 (Hüfner, 1897); frog skin, 4.1; connective tissue, 3.7; frog muscle, 6.8; striated muscle (dog), 6.0; smooth muscle (cat), 6.4.

6. The time course of saturation of a tissue with CO<sub>2</sub> is altered in the presence of available base. Non-acidified tissues saturate more slowly than acidified tissues and the rate of saturation is dependent on the CO<sub>2</sub> tension.

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