THE RATE OF DIFFUSION OF GASES THROUGH ANIMAL TISSUES, WITH SOME REMARKS ON THE COEFFICIENT OF INVASION. By AUGUST KROGH.

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The rate at which gases and especially oxygen will diffuse through tissues has, so far as I am aware, never been systematically investigated and practically never been investigated at all, though a knowledge of the gas diffusion is obviously essential for the solution of one of the problems of the physiology of to-day: the supply of oxygen to the cells. The brilliant work of Barcroft and his collaborators has made it comparatively easy to obtain a quantitative idea of the average oxygen tension in the capillaries and has furnished many of the necessary data concerning the call for oxygen of the tissues, but in order to make out how this call can be met it is necessary (1) to measure and calculate the average distances which the oxygen molecules have to travel from the capillaries until they enter into chemical combination, and (2) to know the rates at which they travel, that is the diffusion coefficients for oxygen in the different tissues. The present paper is intended to supply the second of these desiderata for certain tissues.

The diffusion of gases through animal tissues must take place in essentially the same way as their diffusion through fluids or colloidal membranes. The gases are dissolved in the tissue fluids and diffuse in a liquid state. The laws governing the diffusion of gases through water and watery solutions have been worked out by Exner(1), who found that the rates of diffusion for different gases in the same fluid are proportional to the absorption coefficients of the gases in the fluid and inversely proportional to the square roots of their molecular weights. Exner could only measure relative diffusion rates for different gases. Stefan(2) measured directly the rate of diffusion of carbon dioxide. Hüfner(3) devised a method for measuring directly the diffusion rates

of different gases through water. He defined the diffusion coefficient of a gas as the quantity diffusing through 1 square cm. and 1 cm. thickness in 24 hours, when the pressure difference is 1 atmosphere, divided by the absorption coefficient for the gas in question. This unit is very unpractical for physiological work, the more so as the absorption coefficients for gases in tissues are generally unknown and their accurate determination very difficult. I prefer therefore to define the diffusion constant simply as the quantity diffusing through 1 sq. cm. and 1μ (0·001 mm.) thickness in 1 minute at a pressure difference of 1 atmosphere. For comparison purposes Hüfner's measurements of diffusing quantities must be multiplied by $\frac{10000}{1440}$ and his coefficients further by the absorption coefficients of the gases in water. Hüfner found for oxygen in water a diffusion coefficient of 1·62 or, expressed in my terms, a diffusion constant of 0·34. Later Hagenbach(4) measured the diffusion of gases through gelatine of about 20 p.c. concentration. He found that most gases diffuse somewhat more slowly through gelatine than through pure water, the diffusion coefficients being on an average only 67 p.c. of those found by Hüfner for water. For oxygen he obtained a value which was very much higher than anticipated namely 1·6 expressed in my terms instead of 0·23. As animal tissues must *a priori* be assumed to show properties similar to those of gelatine a renewed investigation is obviously necessary.

Methods. In my researches on the diffusion rates of gases through tissues I have employed two fundamentally different methods. The first involves the diffusion of a gas (O_2, CO or CO_2) either from one fluid through a tissue membrane to another fluid or from air through the membrane to a fluid. The second involves simply the diffusion of two pure gases from either side of a tissue membrane to the other. The use of the first method appeared necessary in order to settle certain questions about invasion which will have to be dealt with in their place. The second method is by far the simpler and easier of the two and, as it turned out, the one giving the most reliable results. I shall describe the methods and the results obtained by each of them separately.

1. The apparatus[1] which is made entirely of brass and gilded inside consists of two vessels *A* and *B* which can be put firmly together by means of three binding screws. *A* has a capacity of 1·5 c.c. It can be filled through the tubes 1 and 2 with the fluid into which the diffusion is to take place. *A* is separated from *B* by the diffusion membrane which

[1] I am indebted for several important details in this apparatus to the constructive skill of the laboratory mechanician, Mr H. Pedersen.

is prepared as follows. A piece of suitable membranous tissue is stretched out on a cork plate over a brass ring 3 which is of such a size that it fits loosely on the outside of the slightly projecting circular orifice of *A*. A second ring 4 which is slit open on one side is pushed down on 3 and secures the piece of membrane to be employed. When *A* and *B* are screwed together a circular area of the membrane becomes available for diffusion. The size of this area is in my apparatus 0·794 sq. cm. *B* has been made in two pieces which are screwed apart for cleaning purposes. It will hold about 50 c.c. A gas can be led in through the tube 5 and when a fluid is employed in *B* a froth chamber is usually placed on the tube 6. In both vessels adequate mixing arrangements are provided and special precautions have been taken to secure the completest possible renewal of the fluid along both surfaces of the membrane. The mixing

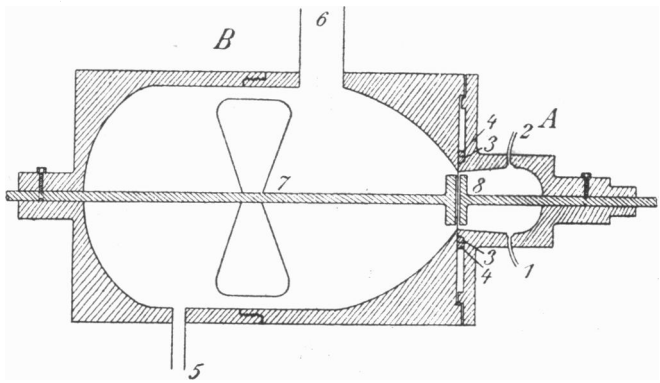

Fig. 1.

screws are driven by a small motor at the rate of 1000 to 2000 revolutions per minute. During experiments the apparatus is placed in a large air thermostat and a constant temperature maintained.

Diffusion experiments have been made chiefly with oxygen and a constant oxygen pressure of 1 atmosphere was maintained in *B* by allowing a current of the gas (98·0 p.c.) to pass through at a uniform but generally slow rate. *B* was in some experiments filled with 40 c.c. blood (or hæmoglobin solution) while in others it contained only gas, saturated with moisture. *A* was at the beginning of each experiment filled with reduced blood (or hæmoglobin solution). The reduction was easily carried out by bubbling pure nitrogen through the solution in the vacuum obtained by a good filter pump. During the filling of *A* two 0·5 c.c. samples of the reduced solution were taken and the amount of gas which

they would take up from air at a temperature of 17° was determined by means of Barcroft's method[1].

During the experiment oxygen will diffuse from B to A through the membrane. In A the oxygen will enter into combination with the hæmoglobin. At the low temperature employed the tension of dissociation of the oxyhæmoglobin formed is so low that it can be left out of account, at least so long as the hgbl. is not more than half saturated. We have on one side therefore a constant O_2 pressure of one atmosphere —due allowance being made of course for the vapour tension and the small impurity of the oxygen—while the pressure on the other side remains at 0. After a suitable time two 0·5 c.c. samples are taken from A and the amounts of oxygen which they will take up from air determined as before. The differences between these determinations and those made at the beginning of the experiment give, when multiplied by the volume

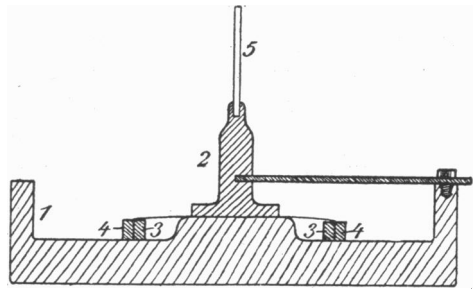

Fig. 2.

of A, the quantity of oxygen which has traversed the membrane during the experiment.

In order to obtain the rate of diffusion per unit length of way it was necessary further to measure the thickness of the membrane. This was in most cases carried out by means of the simple device shown in Fig. 2. The membrane with the rings 3 and 4 was placed in saline in the small circular trough 1 which was mounted on a horizontal microscope. The small weight 2 was placed upon it. This weight carried a glass plate 5 on which a horizontal and a vertical fine line were engraved. The microscope was provided with a screw micrometer eyepiece. The vertical line on the glass plate was made to coincide with the vertical thread in the micrometer and the position of the horizontal line was measured alter-

[1] A description of certain small and not very important modifications of Barcroft's differential blood gas analysis, which we have introduced in this laboratory, has been given by Andresen (5).

nately with the weight standing directly on its small platform in the trough and with the membrane interposed. Usually a series of at least ten alternate readings were taken and in most cases a very good agreement was obtained.

Most of the diffusion experiments were made with oxygen but some also with carbon monoxide and a few with carbon dioxide. In the experiments with CO_2 a solution of sugar isotonic with normal saline and containing 0·005 p.c. NaOH was used instead of blood, and air with an analysed content of 6—10 p.c. CO_2 was employed instead of the pure gas because the diffusion would otherwise become too rapid for accurate or convenient measurement. The carbon dioxide taken up by the alkaline solution was determined in the Barcroft apparatus in the usual way.

As an example to show the experimental procedure and the manner in which the results are calculated I reproduce the protocol of one of the determinations.

Bar. 762·5 Tp. of diffusion apparatus 16·0°.

Membrane: abdominal wall of frog (muscles and connective tissue), thickness 221 μ.

Fluid: corpuscles of ox blood, washed with NaF, dissolved in water. Added 0·9 p.c. NaCl. In *B* hgbl. solution treated with a current of 98·0 p.c. O_2. The solution for *A* reduced (incompletely). The reduced solution run into *A* through 1 at 11.17. Two samples of 0·5 c.c. of the solution passing out through 2 put into Barcroft bottles (*a* and *b*). Mixing started, diffusion exp. begun 11.18. Stopped at 3.40. Duration of exp. 262 m. Two samples from *A* through 1 into Barcroft bottles (*a* and *b*). The determinations gave

Taken up from air c.mm.	a	62·9	b	60·0	
	a_1	28·8	b_1	27·5	
Difference		34·1		32·5	or for 1 c.c. 66·6.
Liberated with FeCy	a	77·7	b	77·5	
	a_1	75·0	b_1	75·9	

In the final samples the volumes of O_2 liberated with FeCy are on an average 2·1 c.mm. less than in the initial. This must mean that a corresponding amount of methæmoglobin has been formed in *A* during the experiment and as the oxygen for this has probably passed through the membrane the corrected difference per c.c. is 70·8 c.mm. In most experiments this correction is insignificant or absent. The oxygen pressure in *B* has been $\frac{98}{100}(762{\cdot}5 - 13) = 734$ mm. The volume of O_2 which has diffused into *A* is $0{\cdot}0708 \times 1{\cdot}47 = 0{\cdot}104$ c.c. that is per minute and sq. cm. through a thickness of 1μ and with a pressure difference of 760 mm. $\frac{0{\cdot}104}{262} \cdot \frac{221}{0{\cdot}794} \cdot \frac{760}{734} = 0{\cdot}114$ which is called the diffusion constant for the membrane in question.

In this case and in a few others with very thick muscular membranes

a slight correction has to be applied, because the membrane has used up oxygen during the experiment. The respiratory exchange of such membranes has been determined in special experiments and it has been assumed that the amount of oxygen used has on an average diffused through half the thickness of the membrane. The correction will raise the diffusion constant to 0·117. A second experiment of 234 minutes duration on the same membrane gave also the value 0·117 for the diffusion constant for O_2 while an experiment with CO lasting 260 minutes gave 0·082 for this gas.

The membranes investigated by this method were the following:

(*a*) A rubber membrane of 37μ thickness.

(*b*) The muscular wall from the sides of the abdomen of a frog. A very uniform piece of tissue can be obtained consisting of two layers of connective tissue each between 15 and 30μ thick and a much thicker layer of striped muscle.

(*c*) In two experiments the muscular tissue was removed from the abdominal wall by gentle scraping and the diffusion through the connective tissue alone measured. One of these experiments was made with the double layer of connective tissue from a large frog, measuring 63μ, the other with a single layer from a small frog measuring only 17·5μ.

(*d*) Several experiments were made finally with a membrane consisting chiefly of smooth muscle from the "uterus" of a pregnant frog.

(*a*) On rubber three separate determinations were made with oxygen giving for the diffusion constant 0·066, 0·062 and 0·064 respectively, average 0·064; three determinations with CO gave 0·038, 0·034 and 0·036, average 0·036, while a single experiment with CO_2 gave 0·32. All the experiments were made at temperatures between 16° and 17°.

A calculation of some of Graham's(6) experiments, in which atmospheric air diffused through rubber into a vacuum and the resulting gas mixture was measured and analysed, gives for oxygen:

Rubber tube of 2 mm. thickness (p. 567) Tp. 20°–23°	Diff. constant	0·050
Sheet rubber of 1 mm. thickness (p. 568), Tp. 20°	,,	0·059
Rubber balloon said to be 0·02 mm. thick* (p. 569)	,,	0·062

* The weight and diameter of this balloon have been given. Assuming a specific gravity of 0·92 the thickness works out as 0·029 mm. This would increase the diffusion constant to 0·090.

Graham has found the following relation between the diffusion constants of different gases. A comparison with my values shows an agreement which must be considered as satisfactory. My figures have been

obtained on rubber saturated with moisture, Graham's on the dry substance:

	Graham	Krogh
Nitrogen	0·39	—
Carbon monoxide	0·44	0·56
Methane	0·84	—
Oxygen	1·0	1·0
Hydrogen	2·15	—
Carbon dioxide	5·3	5·0

(*b*) The experiments on muscle have been given in detail above.

(*c*) The connective tissue membranes. Two determinations were made on each with oxygen. In one of these the diffusion took place from gas to blood and in the other from blood to blood, but this difference apparently had no influence upon the rate of diffusion, though in the experiments gas-blood an invasion of O_2 into the surface of the membrane had to take place in addition to the diffusion. The significance of this fact will be discussed below. The results were:

	Tp.	Diffusion constant	O_2	CO	CO_2
Connective tissue 17·5μ thick	16°	Gas-blood	0·095	0·069	—
		Blood-blood	0·087	—	—
Connective tissue 63μ thick	18°	Gas-blood	0·106	—	—
	20°	Blood-blood	0·118	0·083	4·0

(*d*) On the frog's uterus I found it impossible to obtain constant results, and the diffusion constants found were in all cases much lower than in striped muscle or connective tissue. The reason for this was revealed by the histological examination of the membrane which showed that its surface was very uneven. A reliable determination of its effective thickness was therefore impossible. The following experiments were made:

		Diffusion constant	
Thickness	Tp.	O_2	CO
24μ	15°	0·079	0·043
21μ	36°	0·069	—
15·5μ	16°	0·038	—
	36°	0·050	—
	36°	0·037	—
	17°	0·035	—

2. The results obtained on all the tissues examined by the first method are much lower than Hüfner's on water, viz. 0·34 for oxygen, and I suspected therefore that there might be some unknown source of systematic error in my experiments. To investigate this point and also

in order to study the diffusion at different temperatures I worked out the second method briefly mentioned above. Two glass vessels of approximately equal volume (35 c.c.) and the form shown in Fig. 3 are at 1 provided with metal flanges exactly similar to those used for mounting the diffusion membrane in the first series of experiments. The lower end of both vessels is connected with a single mercury reservoir (not shown in the figure). By raising this mercury can be run into both vessels simultaneously and at the same rate. Through the tube 2 each vessel can be washed out with a current of pure gas—oxygen or nitrogen—saturated with moisture. The upper ends of both vessels are connected with the end of glass tubes 3 of 1 mm. bore carrying two three-way taps. By means of these taps the vessels can be arranged to communicate through the tube 4 containing a long drop of kerosene acting as a manometer. The free branches of the three-way taps are utilised for connecting with gas sampling vessels. These are of 20 c.c. capacity and of the type usually employed in this laboratory, but the pair of them are connected below through a wide Y-piece with a single mercury reservoir in order to secure a uniform pressure in both during the taking of samples. 5 are needles of steel enclosed in glass tubes provided with plates of mica. The needles can be moved up and down from outside by means of a magnet, and the mica plates serve for the thorough mixing of the gases in each vessel before the taking of samples.

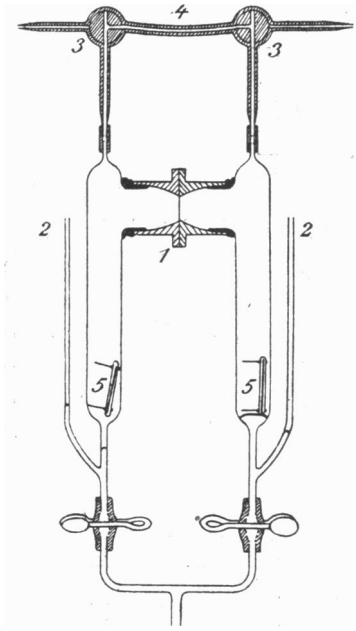

Fig. 3.

An experiment is made as follows. A suitable membrane is mounted in the rings 3 and 4 (Figs. 1 and 2). The thickness is measured and it is put in place between the two vessels (1, Fig. 3). The apparatus is placed in a constant temperature water-bath. A current of pure nitrogen is led through the left-hand vessel and a current of nearly pure oxygen through that to the right. Care is taken that the spaces on each side of the manometric drop are also filled with nitrogen and oxygen respectively. When the vessels are completely washed out with the pure gases—about 1 l. gas has generally been used for this purpose—samples are taken

from the current on both sides. The vessels are closed at the bottom by raising the mercury above the junctions, and at the top they are connected with the manometer. The diffusion experiment is taken to begin at the moment when the gas currents are interrupted. The diffusion of oxygen through a membrane being more rapid than that of nitrogen the drop of kerosene will move from left to right, and the rate at which it moves gives some indication of the diffusion rate. The levels of mercury in the two vessels are adjusted from time to time so as to maintain the same pressure throughout in both vessels.

When the experiment has to be finished (several times also during its course) the gases in each vessel are thoroughly mixed. The taps 3 are closed. Mercury is run into both vessels and when a positive pressure has been obtained the taps are opened to wash out the connections (lead tubes of very narrow bore) down to the sampling vessels and a sample of 20 c.c. is drawn from each side of the membrane. The experiment is considered as finished when the first half of the samples has been collected. In most cases the experimentation time has been so long that the unavoidable uncertainties about the time of beginning and finishing the experiment have been unappreciable. The samples drawn are analysed for oxygen and nitrogen respectively. In a few cases with very thick muscular membranes the CO_2 liberated by the respiratory exchange of the membrane has also been determined and a corresponding absorption of O_2 (R.Q. assumed = 0·8) allowed for in the calculation of the diffusion experiment. In many cases the nitrogen samples only have been analysed for oxygen. The determination of a small percentage of nitrogen in oxygen is not nearly so accurate as the determination of a small percentage of oxygen in nitrogen, and as the diffusion of nitrogen is, moreover, of very little biological interest the determination was often omitted.

As an example I reproduce the protocol of one of the determinations.

Temp. during exp. 20·0°. Membrane: chitine from last dorsal segment of Oryctes larva preserved in spirit, washed with water. Thickness measured by means of screw micrometer divided in 0·01 mm. Measurements: 5·5, 6·0, 5·2, 5·1, 5·4, 5·7, 6·0. Average 5·6

Index correction 0·2

Thickness 54 μ

		O_2 in nitrogen %	N_2 in oxygen %
Exp. begun 19th	4.47 p.m.	0·01	1·62
Finished 20th	10.52 a.m.	0·59	1·99
Difference	1085 m.	0·58	0·37

The volumes of the vessels are left 37·3 c.c., right 35·5 c.c. The volume of oxygen which has diffused into the nitrogen is therefore $\frac{0{\cdot}58}{100}$ 37·7. It is reduced to 0° by multiplication with $\frac{273}{293}$. It could be reduced to normal dry pressure by multiplication with $\frac{B-f}{760}$, but in order to find the diffusion at a full atmosphere's pressure difference the result would have to be divided by $\frac{B-f}{760}$. All corrections for total pressure can therefore be omitted. The O_2 pressure difference between the two vessels has been at the beginning

$$\frac{100-(0{\cdot}01+1{\cdot}62)}{100}, \text{ at the end } \frac{100-(0{\cdot}59+1{\cdot}99)}{100},$$

or on an average $\frac{97{\cdot}9}{100}$. The result must be multiplied by $\frac{100}{97{\cdot}9}$ to get the diffusion at a full atmosphere's pressure difference, and we obtain the diffusion constant by further division by the time (1085 m.), multiplication by the thickness (54μ) and division by the area of the membrane (0·794 sq. cm.) as follows:

$$\frac{0{\cdot}58}{100} \cdot 37{\cdot}7 \cdot \frac{273}{293} \cdot \frac{100}{97{\cdot}9} \cdot \frac{1}{1085} \cdot \frac{54}{0{\cdot}794} = 0{\cdot}013.$$

The reliability of this method was tested by means of determinations made on gelatine and on a rubber membrane. A membrane of gelatine which would stand the unavoidable pressure differences at the beginning and end of each experiment could not be prepared, but the difficulty was overcome by making metal plates fitting into the diffusion apparatus and provided with a number of small holes. Such a plate was immersed in a hot gelatine solution of about 15 p.c., which was allowed to set by cooling whereupon the plate was cut out and the gelatine removed on both sides by means of a razor. Two such plates were tested. One was 319μ thick and had 36 holes of 0·930 mm. diameter. The total effective area was 0·245 sq. cm. The other of 380μ thickness had 69 holes with a total area of 0·143 sq. cm. A series of determinations of the diffusion through these plates at 20° gave

Plate	Duration m.	Diffusion constant O_2	Diffusion constant N_2
I	1009	0·301	—
I	1580	0·235	0·114
II	1622	0·294	0·214
I	731	0·286	—
I	362	0·296	—
	Average	0·28	(0·16)

The figure for oxygen is 82 p.c. of Hüfner's value for water. It agrees well with Hagenbach's general result that the diffusion through 20 p.c. gelatine is about 33 p.c. slower than through water, but is incompatible with Hagenbach's single determination of the diffusion of oxygen through gelatine which gave 1·6 as the value of the constant.

A series of determinations on a dry rubber membrane of 37μ thickness made at 20·8° gave

Duration m.	Diffusion constant O_2	Diffusion constant N_2
40	0·075	—
92	0·073	—
79	0·082	0·04
Average	0·077	

The average is somewhat higher than that obtained in the former series of experiments, viz. 0·064, but the temperature is 5° higher and Graham found that the diffusion of gases through rubber increases rapidly with the temperature.

After these tests which show that the method is on the whole reliable, though rather deviating results are occasionally met with, several series of experiments were made on membranes composed of animal tissues.

(*a*) *The influence of temperature upon the rate of diffusion.* Hüfner assumes in his calculation of the diffusion through the lungs of warm-blooded animals that the influence of an increased temperature is to diminish the rate of diffusion, because the diffusion is proportional to the coefficient of absorption, which decreases rapidly with increasing temperature, and is taken by him to be proportional further to the square root of the absolute temperature. It must be borne in mind, however, that the internal friction in water is greatly diminished by increasing temperature, and it is quite conceivable that this may cause an increase in the rate of diffusion of gas molecules. It is absolutely necessary therefore to study the problem experimentally.

The following series of determinations were made on peritoneal membrane from small dogs. The thickness of these membranes is sufficiently small (8–12μ) to allow a determination to be made in about half-an-hour.

I		II		III	
Tp.	Diff. const. O_2	Tp.	Diff. const. O_2	Tp.	Relative diff. rate* O_2
20·1°	0·117	20·0°	0·123	20°	75
0·4°	0·088	0·2°	0·093	36°	86·5
20·0°	0·102	0·2°	0·100	20°	72
35·4°	0·133	20·2°	0·121	36°	92
20·0°	0·120	36·2°	0·124	—	—

* The thickness of this membrane was not measured.

If these results are averaged and the diffusion at 20° taken as unity we find in the three series

Tp.	I	II	III	Averages
0·2°–0·5°	0·78	0·76, 0·82	—	0·79±0·02
20°	1·04, 0·91, 1·06	1·01, 0·99	0·98, 1·02	1·00±0·02
36°	1·18	1·02	1·18, 1·26	1·16±0·05

The experiments show conclusively that the rate of diffusion of O_2 is increased with increasing temperature and an approximation to the rate of increase is obtained. According to Hüfner the diffusion rates for O_2 in water at the different temperatures should have been proportional to

	Abs. coeff.	$\sqrt{T}$	Hüfner's relative diffusion rates
0°	0·0485	16·51	1·51
20°	0·0310	17·11	1·00
36°	0·0242	17·59	0·80

When these figures are divided by the relative figures for the internal friction of water at the same temperatures we get

	Calculated diffusion rates for O_2 in water	Observed diffusion rates for O_2 in conn. tissue
0°	0·83	0·79±0·02
20°	1·00	1·00±0·02
36°	1·12	1·16±0·05

The calculated figures agree with the observed within the possible errors of the latter and the results therefore lend some support to the assumption that the rate of diffusion is inversely proportional to the internal friction of water. On this assumption the diffusion constant could be calculated for any desired temperature. For practical purposes it is, however, safer and more convenient to utilise the actual determinations and to allow 1 p.c. increase or decrease in the diffusion constant for each degree the temperature is higher or lower than 20°. In the following all the determinations mentioned have been reduced to 20° in this way.

(*b*) *The diffusion of oxygen through connective tissue membranes of different thickness.* The following determinations have been made:

	Connective tissue from abdomen of frog			Peritonæum of dog	
Thickness	63 μ	35 μ	17·5 μ	11·5 μ	7·65 μ
	Gas-blood 0·106	g.-g. 0·101	g.-bl. 0·099	g.-g. 0·120	g.-g. 0·123
	Blood-blood 0·118	„ 0·120	bl.-bl. 0·091	„ 0·117	„ 0·121
	—	„ 0·137	—	„ 0·102	„ 0·122
	—	—	—	„ 0·112	„ 0·107
	—	—	—	„ 0·115	—
Averages	0·112	0·119	0·095	0·113	0·118

The general average is 0·113. It is obvious that there is no systematic difference between the results obtained with blood and those obtained with air and further that membranes of very different thickness give practically the same diffusion constant. If the invasion coefficient for oxygen as defined by Bohr(7) and "determined" by myself(8) were even approximately correct this ought not to be so.

The invasion coefficient for oxygen is defined by Bohr as the amount of oxygen which will penetrate in 1 minute into 1 sq. cm. of water surface when the O_2 pressure difference between the surface itself and the gas is 1 atmosphere. When determinations have to be made the gas pressure in the surface must be assumed to be equal to that in the interior of the fluid—that is the mixing must be absolutely complete and no diffusion whatever must take place. This condition cannot be fulfilled and it is moreover always very uncertain how near it has been approximated. I showed in 1910 that Bohr's determinations were at least six times too low and I pointed out that mine might also be too low and even much too low. My value was 0·076 c.c. or considerably lower than the diffusion constant. In the diffusion experiments according to the second method both invasion and evasion have to take place. When this is not taken into account the diffusion constant should be found too low, because the pressure head available for diffusion must be the difference between the total pressure and the amount necessary to bring about the invasion and evasion, which should, according to my determination of the invasion, correspond very nearly to the pressure necessary for the diffusion through a thickness of 3μ. If such a correction were applied it is obvious that the diffusion determinations would become absurd, the thin membranes giving much higher values for the diffusion constant than the thick ones. In order to be at all compatible with the present determinations of the diffusion constant the invasion coefficient would have to be at least about five times higher than my determination in 1910.

I have made a renewed attempt to determine the invasion coefficient which it may be useful to record briefly, because it demonstrates clearly why such attempts are bound to fail. I employed the apparatus shown in Fig. 4. 1 is a smooth cylinder (diameter 13 mm., length 18 mm.) dipping into the trough 2 filled with a fluid into which the diffusion takes place. The cylinder can be revolved by means of the shaft 3 at a maximum rate of 4000 revolutions per minute. When revolving it will be covered with a film of fluid. The mixing arrangements in the trough and the rubber band 4 sliding against the cylinder secure a complete renewal

of the film for each $\frac{2}{3}$ revolution. Now let us suppose the fluid to be water at 37° and take it to form a film of only 1μ thickness. The film of water being renewed 6000 times per minute 6 c.c. of water will pass over each sq. cm. of the cylinder surface per minute. If the water is free from dissolved gas and the apparatus revolves in an atmosphere of pure oxygen it should take up (according to my 1910 experiments) 0·076 c.c. O_2 per sq. cm. per minute, supposing the oxygen pressure in the film could be maintained approximately at 0. The absorption coefficient of O_2 in water at 37° being 0·024 this quantity would require 3 c.c. water for its solution at 1 atmosphere pressure. If 0·076 c.c. are actually taken up the O_2 tension difference between the gas and the whole of the water passing over the cylinder must be about $\frac{1}{2}$ atmosphere and of course much smaller still between the actual surface of the water and the gas.

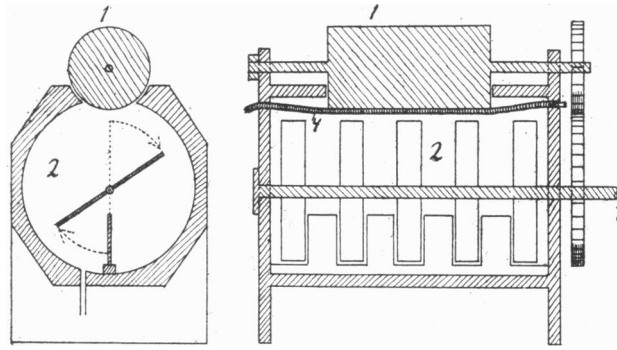

Fig. 4.

This means that the real invasion coefficient must be many times larger than any figure which could be found in experiments with water.

I have made a few experiments using completely reduced blood as the fluid. The invasion took place at room temperature and with a comparatively low oxygen pressure in the gas above the apparatus. After a suitable time the total quantity of oxygen taken up was determined by means of Barcroft's method. Assuming that the oxygen tension of the blood surface itself were not higher than that calculated for the total quantity of blood at the end of the experiment the "invasion coefficient" worked out as 0·3 or four times the earlier value. It is easy to see that this value must be a great deal too low. The oxygen molecules must diffuse a certain distance through plasma and through the walls of the red corpuscles before they can combine with the hæmoglobin. Supposing this distance to be on an average 1μ the "invasion coefficient"

found, which is about equal to the diffusion constant for water, would just represent the pressure difference necessary for the diffusion and the true invasion coefficient must be infinite. The average distance through which diffusion must in this case take place is perhaps less than 1μ, but it is obvious that as a determination of the invasion coefficient γ the result ought to be written $0{\cdot}3 < \gamma < \infty$, the more so as the combination of O_2 with hgbl. is not instantaneous, but involves the existence of a certain O_2 pressure within the corpuscles during their passage across the cylinder.

Bohr maintained that in fluids in which the gas molecules were combined chemically in the very surface the process of absorption was different in character from a true invasion and much more rapid. I do not see why the process should be really different, but when chemical combination takes place the gas pressure in the surface can be maintained at a very low figure, though always above 0, considering the reaction time of the combination. I have made some experiments with potassium pyrogallate in the above apparatus. In this case the rate of "invasion" rose rapidly with increasing temperature, indicating in my opinion the increased rate of combination between oxygen and the solution. The maximum "invasion coefficient" obtained for this fluid at a temperature of 20° was about 12 which is, I suppose, still much below the real figure.

The processes of invasion and evasion of a gas must, as far as I can see, be similar to condensation and evaporation of a pure fluid. When such a fluid is in contact with an atmosphere saturated with its vapour evaporation and condensation balance each other, just as evasion and invasion must balance each other when tension equilibrium is maintained between a gas and a fluid. Martin Knudsen(9) has shown in the case of mercury that the rate of evaporation and consequently of condensation is so great that every molecule striking the surface of the fluid phase will be taken up. It seems to me very probable that this would hold also in the case of gas molecules striking a fluid in which they can become absorbed.

In any case invasion is a process which is so rapid that it can be left out of account altogether in dealing with the rate of absorption of gases in animal tissues.

(c) *The average diffusion constant for oxygen through connective tissue.* This at 20° is 0·113 and there does not seem to be any difference between the connective tissue obtained from frogs and that from mammals. The reason why the diffusion through connective tissue should be so much

slower than through gelatine or water is unknown, but the fact that it is cannot, I think, be called in doubt. A single experiment has been made on goldbeater's skin of 23μ thickness which is connective tissue that has undergone some preparation. It shows a much lower diffusion constant, viz. 0·04. Still lower is the diffusion constant for chitin 0·013.

(*d*) *Determinations on the abdominal muscular wall of the frog.* The chief difficulty in this case is to obtain a reliable determination of the thickness of the tissue. In three cases, including the experiment according to method 1, I have measured the thickness by means of the arrangement described above. In one case I have made the measurement by means of a screw micrometer acting directly on the membrane, and in a fifth case I have cut out the membrane, determined the weight and calculated the thickness, assuming a specific gravity of 1·04. The results of the determinations, reduced to 20° and with two exceptions made at that temperature are:

Thickness of membrane μ	Measured by	Diff. const. at 20	
140	Screw micrometer	0·116	
141	Microscope	0·145	
148	—	0·161	
221	—	0·122 0·122	Experiment according to method 1 at 16°
290	Weighing	0·131 0·136	
	Average	0·133	

The diffusion constant is unmistakably higher than for connective tissue though the difference is not considerable. When the fact is allowed for that the abdominal wall employed includes two layers of connective tissue of a total thickness averaging about 50μ the diffusion constant for oxygen through muscle must be higher still and works out as 0·139 or approximately 0·14. By means of this figure combined with measurements of the capillaries and their distribution in muscles it becomes possible to study a little more closely than hitherto the mechanism of the oxygen supply to muscles during rest and during work and it is chiefly with this purpose in view that the present investigation has been undertaken.

(*e*) *The relative diffusion constants of different gases.* Determinations were made with CO and CO_2 by the first method and with N_2 by the second, but these latter do not agree very well. Taking the diffusion constant of oxygen as a unity I have obtained the following results:

Membrane	Thickness μ	N_2	CO	CO_2
Conn. tissue	63	—	0·74	35·7
,,	17·5	—	0·76	—
Muscle	221	—	0·70	—
,,	290	0·70	—	—
,,	148	0·64	—	—
,,	140	0·45	—	—
Conn. tissue	35	0·52 0·49	—	—
Peritonæum	11·5	0·42 0·42	—	—
Chitin	54	0·6	—	—

When these results are averaged and compared with those obtained by other investigators I find:

	Relative diffusion constants			
	calculated for water	found by Hüfner for water	Exner soap solution	Krogh animal tissues
N_2	0·53	0·53	0·44	0·4–0·7
CO	0·89	—	—	0·73
O_2	1	1	1	1
CO_2	23·1	27·5	24·2	35·7

My figure for CO_2 is based on a single determination and may possibly be too high. The figure for CO which is, I believe, reliable is distinctly lower than the theoretical deduced for water. In the investigation of the rate of diffusion of gases through the alveolar wall by Marie Krogh(10) it is assumed that the different gases diffuse at the relative rates deduced theoretically for water and the results are based on determinations of the diffusion of carbonic oxide—this being the only gas available for the purpose. If the alveolar wall consists of ordinary connective tissue the diffusion constants calculated for oxygen through the lungs of man will have to be increased about 20 p.c. A direct determination of the relative rates of diffusion of O_2 and CO through the alveolar wall has been planned.

Summary.

Methods are described for measuring the rates of diffusion of gases through membranes of animal tissue and other substances.

The diffusion constant for a gas through a substance is defined as the number of c.c. (0°, 760 mm.) penetrating through 0·001 mm. (1μ) thickness and 1 sq. cm. surface per minute when the pressure difference is 1 atmosphere.

The diffusion constant for oxygen through animal tissues increases

with increasing temperature—about 1 p.c. per degree, taking the rate at 20° as unity.

Diffusion of gases through animal tissues is much slower than through water or gelatine. The absolute diffusion constants for oxygen at 20° are:

Water	0·34	Hüfner
Gelatine	0·28	Krogh
Muscle	0·14	—
Connective tissue	0·115	—
Chitin	0·013	—
Indiarubber ...	0·077	—

The "invasion coefficient" for oxygen into water is many times higher than the "determinations" hitherto made would indicate.

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