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DIFFUSION RELATIONS OF UREA, INULIN AND CHLORIDE IN SOME MAMMALIAN TISSUES[1]

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For a substance contained in the fluid external to a tissue, the equilibrium amount per 100 g. tissue divided by the amount per 1 ml. of fluid gives a measure of the tissue 'space' in which the substance is dissolved, provided that at the same time it is neither appreciably adsorbed nor concentrated within. If instead of the amount per 100 g. tissue we consider that in 100 g. of tissue water, divided as before by the amount per ml. external fluid, we have the value already defined as a 'permeation' [Conway & Cruess-Callaghan, 1937]. Such a quantity, which can be treated quite empirically and at the outset as only numerically descriptive, is useful, for example, in comparing the equilibrium values of urea dissolved in tissues. Usually a 'permeation' of about 100 may be expected. If definitely higher than 100 it indicates that urea is being formed in appreciable amounts as in the liver, or is being concentrated for excretion, as shown with suitable controls for the isolated kidney of the frog [Conway & Kane, 1935], or again is being adsorbed on surfaces. If lower than 100 it shows that some tissue water is not free for solution or that there is a region impermeable to urea. Urea has already been used to investigate the 'free water' for solution in muscle [e.g. Eggleton, 1930], a question dealt with at some length in a previous paper from this laboratory [Boyle & Conway, 1941]. Here we are concerned rather with comparisons between tissues and with respect to inulin, chloride and urea, and use the 'permeation' values throughout. These can be readily converted to 'spaces' in the usual sense by multiplying by the tissue water fraction.

[1] A preliminary account of some of the findings in the present paper (including the use of inulin to measure the intercellular spaces) has been already presented by one of us in thesis form [FitzGerald, 1937].

The 'permeation' of inulin or alternatively the inulin 'space' may be expected to give a measure of the free intercellular spaces. It has been used here to determine the spaces in the isolated sartorius muscle of the frog [Boyle, Conway, Kane & O'Reilly, 1941] and by McCance [1938] to determine the total intercellular spaces in the human subject after intravenous injection.

Where the inulin 'permeation' considerably exceeds any probable estimate of the intercellular spaces, and provided we eliminate the possibility of an adsorption explanation, as by comparison with other tissues, we may conclude that the cells are in part at least permeable to inulin. Again, if the inulin 'permeation' is definitely less than the chloride value it may be assumed that chloride is either adsorbed on the surface of the tissue elements or is present within them, or probably distributed in both these ways. Such considerations are theoretically important for muscle, renal and nerve tissue.

Diffusion coefficients for urea and inulin have also been determined for renal cortical sections, and also for urea in muscle, liver and brain tissues. Chloride data have been used only for 'permeation' comparisons with urea and inulin in muscle and nerve tissue.

Throughout we have used concentrations in plasma as if the volume of this could be regarded as entirely free water for solution. This, of course, is not strictly correct, and would involve small reductions in the 'permeations' as given. For such plasma values, however, we have considered it advisable to keep to the conventional usage.

METHODS

Experimental. Urea 'permeations' were measured simply from analyses of tissue samples and of blood plasma taken at the same time. This was varied by raising the blood urea with intravenous injections of 5% urea in 0·6% NaCl (10–40 ml./kg.), carried out usually under ether anaesthesia. For renal tissue this procedure is not applicable, and here, in order to remove the lumen concentration, a cannula was very quickly introduced into the renal artery or the aorta, and the freshly excised kidney was perfused with a solution recommended by Krebs & Henseleit [1932] but containing 0·01–0·02 *M* cyanide, the gas mixture being either 5% CO_2 with 95% O_2 or 95% N. The perfusion was conducted at 37° C. under a pressure from the above gas mixture of about 100–160 mm. Hg, the external surface of the kidney being also maintained at about 37° C. The perfusion was usually carried out for about 15 min., about 200 ml. fluid being sent through. It was shown that under these

conditions the cyanide removed any appreciable concentrating effect in the flowing urine, and that with a urea-free solution all the urea in the kidney was washed out after the first few minutes. When the perfusing fluid contained 0·2 % urea the 'permeation' was obtained by determining the renal cortical concentration after perfusion. Alternatively, after perfusing with a urea-free solution the cortex was sectioned, the sections being allowed to diffuse in a urea-Krebs fluid, 30–60 min. being quite sufficient time. The diffusion chambers used are described below.

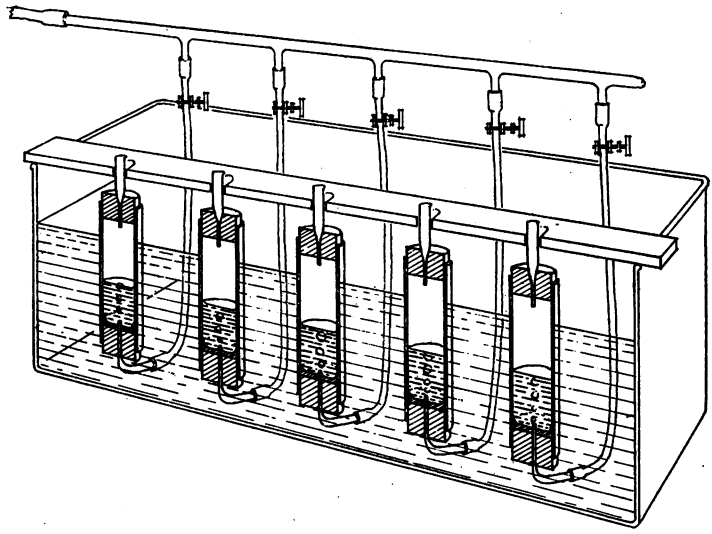

Fig. 1. Sectional view of diffusion chambers in thermostat. See text.

Inulin permeations were determined similarly after intravenous injections of 0·6 % NaCl with 1 % inulin (50 ml./kg.). For the renal cortex the procedure was similar to that for urea, but cortical sections were also diffused without preliminary perfusion of the kidney. Tissue sections were prepared by the method of Warburg [1930] from animals killed by a sharp blow on the cervical spine, no anaesthetic being used. The sections were not trimmed until after the diffusion, the area being then determined by using trans-illuminated squared paper. From this and the weight of the trimmed tissue a mean value for the thickness could be obtained. The mean thickness of a large series was found to be 0·7 mm., but cortical brain sections could not be obtained finer than 1·0 mm., and for muscle tissue it was found preferable to use the whole diaphragm of the rat (mean thickness of 0·9 mm.).

Diffusion chambers. Diffusion was conducted in chambers made out of simple pyrex tubes 8 × 2 cm. The bottom of each chamber was formed by a rubber cork sealed with an internal layer of a high-melting paraffin, through which passed a finely pointed tube. Through this latter the appropriate gas mixture was introduced serving also to mix the solution. The arrangement of a series of chambers is shown in Fig. 1.

Chemical methods

Urea was determined in the various fluids and tissues by the improved micro-diffusion technique [Conway, 1939].

Inulin. In determining the inulin content of tissues, the principle used was to determine the reducing power (as glucose) of a protein-free extract of the tissue before and after hydrolysis with 0·1 *N* HCl. The results expressed as glucose were then converted to inulin by multiplying by 0·9.

The cadmium sulphate solutions as described by Van Slyke, Hiller & Miller [1935] were used as protein precipitants.

Tissue extracts were made by grinding the tissue (50–150 mg.) with 3·0 ml. water with the aid of a little pure quartz sand (Merck's) transferring with some washing to a small conical flask (25 ml.) and heating momentarily to boiling, cooling, and adding sufficient excess of the strong $CdSO_4$ solution to precipitate all proteins and then sufficient *N* NaOH to neutralize the excess. The mixture was then made up to 5 or 10 ml. and centrifuged. Aliquot volumes made up to 5 ml. were taken for subsequent analysis by a modification of the Hagedorn-Jensen method, after hydrolysing at 100° C. with 0·5 ml. *N* HCl added. A similar determination was carried out without hydrolysing for the free glucose or reducing substances present. Subtracting this value from the hydrolysed sample gave gross values for the inulin. Such values are too high, since without any inulin present a slight increase in reducing substance occurs on heating with acid. An allowance for this, as measured from many determinations on different tissues, is sufficiently small and constant to obviate the need of special determinations for each animal. Expressed as mg. glucose/100 g. tissue it amounts to 21 for the kidney (28 determinations), 25 for muscles (4 observations) and 24 for the cerebral cortex (4 observations).

The above method was checked by recoveries of added inulin (Pfanstiehl's used throughout) to the macerated tissue mixtures. These gave a mean value of 99% of the added quantity.

The inulin in the diffusing and perfusing fluids was determined in a similar manner, the protein being directly precipitated with the weak $CdSO_4$ solution.

Chloride. For tissues, this was determined both by the micro-diffusion method [Conway, 1935] and the open Carius method after Eisenman's modification of the Van Slyke procedure [1929]. Blood chloride (plasma) was determined by the micro-diffusion method. This latter method, when applied to rabbit muscle determinations and under the given conditions, requires longer times for the liberation of the free chlorine than that already considered. (This point is being further investigated.)

Water content of tissues. As the 'permeation' values are given in terms of the water content, it was necessary to determine this value for the special conditions used. In the procedure used the loss of weight per 100 g. of tissue dried for 12 hr. at 105° C. in a platinum crucible was reckoned as the water content. In this way the water content of the rabbit's kidney was found as 76·4; after perfusing with cyanide-Krebs's solution it was 85·6. For renal sections diffused in Krebs's fluid it was 80·0, and in cyanide-Krebs's fluid 81·8. For

muscle Hill & Kupalow's [1930] value of 80·0 for the total water content (similar to Katz's figure [1896] of 76·8) was taken, and for the brain, Matsumoto's [1933] figures for the rabbit's brain—69·4 and 81·8 for white and grey tissue—were used.

Haemoglobin. This was determined in arterial blood samples by the Bürker method as modified for spectrophotometric use by Heilmeyer [1933].

Inulin 'permeations'

Skeletal muscle. The 'permeations' were determined for rabbit muscle after injecting intravenously 50 ml./kg. of 10% inulin in 0·6% NaCl into the animal anaesthetized with ether and allowing at least 30 min. for equilibrium between plasma and muscle. The blood was then removed through a carotid cannula and immediately centrifuged, the plasma being taken for analysis. Simultaneously with the removal of blood, samples of skeletal muscle were excised, weighed and analysed for inulin as described. The results for four rabbits are given in Table 1. The mean

Table 1

Rabbit	Conc. in plasma %	Tissue	No. of observations	Range of 'permeations'	Mean 'permeation'
		Inulin permeations			
1	1·50	Skeletal muscle	7	9·7– 6·0	7·9
2	1·56	,,	4	16·2– 8·1	11·8
3	1·01	,,	4	7·7– 4·6	6·1
4	(1·36)	,,	7	2·0–15·7	7·9
2	1·56	Cerebral cortex	4	4·4– 2·0	3·0
3	1·01	,,	3	0·2– 1·7	0·8
		Chloride permeations (similar conditions)			
5	0·601	Skeletal muscle	2	—	12·8
6	0·582	,,	2	—	16·5
7	0·576	,,	2	—	16·1
8	0·590	,,	2	—	16·7
9	0·573	,,	2	—	12·8
10	0·574	,,	2	—	16·3
8	0·590	Cerebral cortex	1	—	53·8
9	0·573	,,	1	—	45·2
10	0·574	,,	1	—	49·6
9	0·573	Cord	1	—	59·2
10	0·574	,,	1	—	56·3

inulin 'permeation' for the three animals for which plasma figures were available is 8·6 (or an inulin 'space' of 7%), or $8·5 \pm 0·6$ for the whole fifteen observations (giving standard error of mean). Thirty minutes would appear to be sufficient for inulin equilibration across the capillaries, if we are to judge by the fact that no appreciable change occurs in the plasma inulin after 30 min. (see Fig. 2). It is true that the volume of the circulation may be decreasing after this time and a loss of inulin be obscured. Determinations of blood haemoglobin carried out on two rabbits similarly treated with inulin injections showed no appreciable

increase from 30 to 120 min., but rather a small decrease, as shown in Fig. 2. From this it would appear reasonable to suppose that no appreciable inulin was *leaving* the capillaries 30 min. after the injection.

Over 30–120 min. after injection the mean plasma inulin concentration was 2·7 g./100 ml. Since 5 g. inulin per kg. were injected, then in 1 kg. rabbit there were $5/2{\cdot}7 \times 100$ ml. intercellular space, hence 18·6 % of the weight of the animal. Since the fluid in which the inulin was injected was 0·6 % NaCl it may be presumed that the water in about

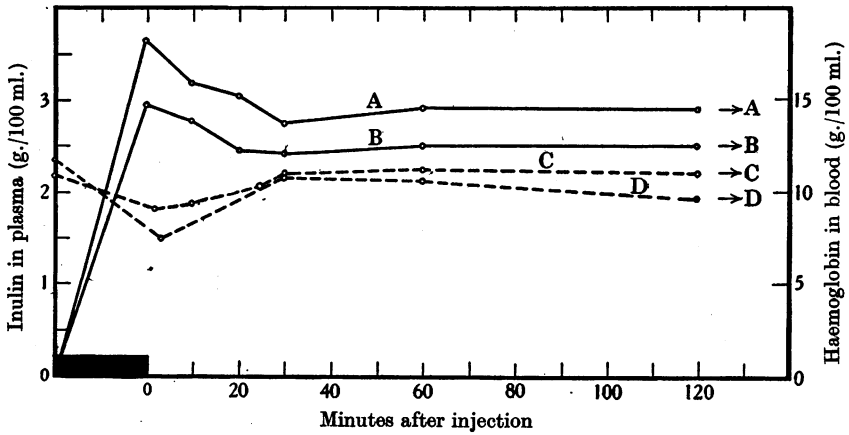

Fig. 2. A and B curves represent inulin concentrations in plasma of two rabbits after intravenous injection of 5 g. inulin/kg. as a 10 % solution in 0·6 % NaCl. The times of sampling begin from the end of the injection, which took about 20 min. C and D give haemoglobin concentrations in the blood of two rabbits similarly treated. ▬ represents continuous injection of 10 % inulin solution in saline.

one-third its volume passed into the tissue cells and two-thirds (3·3 % body weight) remained to swell the intercellular fluid. Consequently the original intercellular fluid would have been 15·3 % of the body weight, which may be compared with McCance's figure of 15·7 % for the human subject [1938].

Comparison with chloride 'permeation'. This value of 8·5 % for the inulin 'permeation' we may take as giving a maximum value for the interspace water (as a percentage of the total water) in the excised skeletal muscle of the rabbit, and it is very appreciably less than the generally accepted value derived from chloride analyses (e.g. Fenn [1936] for references). The mean chloride 'permeation' was determined for a further six rabbits, using the leg muscles with duplicate determinations and was found, under the same conditions, to be $15{\cdot}2 \pm 0{\cdot}7$ (being some-

what lower than for similar observations on frog muscle [Conway & Kane, 1934; Boyle *et al.* 1941]. The difference between the mean inulin and chloride 'permeations' as measured for the excised rabbit muscle is therefore $6{\cdot}7 \pm 0{\cdot}9$.

Renal cortex. Cyanide perfused kidney (rabbit). The freshly excised kidney of a rabbit was perfused for 15 min. with a cyanide-inulin-Krebs's solution as described in the section on methods. The cyanide content was $N/200$, and the inulin 1·0 %, no appreciable concentration of inulin occurring in the urine. The results are given in Table 2, six duplicate determinations being carried out with two rabbits. The mean value of the inulin permeation was 56·5 % with a range of 42·6–63·8.

TABLE 2

Tissue	Inulin conc. in perfusate	Inulin 'permeations'	Mean inulin 'permeations'
Renal cortex	1·0	56·6; 42·6; 47·7	49·0
Renal cortex	1·0	63·8; 63·0; 62·5	63·1

Renal cortex. Sections diffused in inulin solution. Sections from the kidneys of rabbits which had received no injections were introduced as quickly as possible into the diffusion chambers containing Krebs's solution with 1 % inulin stirred with the gas mixture as described. They were removed at varying times, dried between filter paper, rapidly but carefully, so that all adherent fluid was well removed. Table 3 gives a

TABLE 3

External inulin conc. g./100 ml.	No. of sections analysed	Mean time of immersion of renal sections min.	Mean thickness of tissues mm.	Mean 'permeation'
0·900	2	45	0·9	29·8
9·670	2	45	0·7	56·3
0·900	2	52	0·7	25·0
0·800	3	61	0·6	47·5
0·800	3	65	0·7	30·2
0·800	3	70	0·7	38·0
0·900	2	120	0·8	51·7
0·900	2	180	0·8	53·2

summary of the results obtained from experiments on seven animals. The total average 'permeation' (nineteen sections) was 41·5 % or five times as great as that found for muscle tissue. After 2–3 hr. it was 52·4 %. The majority of these sections were analysed for inulin without using the momentary boiling of the ground tissue extract, and may have given values somewhat under the true figures.

Cerebral cortex. Inulin 'permeations' determined out on two of the rabbits examined for inulin 'permeations' in muscle, the results being extremely low (Table 1), ranging from 0·2 to 4·4 % with a mean of 1·9 %.

Chloride analyses carried out under similar conditions give 'permeations' ranging from 45·2 to 53·8 for the cerebral cortex and 59·2 and 66·3 were found for the cord.

Urea permeations

Skeletal muscle. Determinations were carried out on two normal rabbits and two rabbits and two rats after intravenous injections of urea, 40 ml./kg. of 5 % urea in 0·6 % NaCl. The mean value of the 'permeation' in eleven determinations was 93 % when 80 % of the tissue weight was taken as that of the total water present [Hill & Kupalow, 1930].

Renal cortex. Cyanide perfused kidneys. The kidney was perfused as for the inulin determinations, the perfusing fluid containing 0·2 % urea. The mean of nineteen determinations on six cats and two dogs was 94·4 % with a standard deviation of the mean of 2·1 %.

Table 4

Tissue	No. of animals (no. of estimations in brackets)	Time after perfusion or immersion min.	Conditions	Mean conc. of urea in blood plasma or ext. fluid %	Mean 'permeation' (with S.D. of mean)
Renal cortex	6 cats (19) 2 dogs	20	Perf. with Krebs's fluid containing 0·01 % NaCN	0·2	94 ± 2·1
Renal cortex	6 cats (25) 1 dog	30–60	Sections diffused in Krebs's solution containing urea and cyanide	0·03–0·2	107 ± 3·2
Renal cortex	6 cats (23) 1 dog	30–60	Sections diffused in Krebs's solution with urea and no cyanide	0·2	109 ± 2·8
Skeletal muscle	3 rats 2 rabbits	5–60	Intrav. inj. of 10–40 ml. 5 % urea (in 0·6 % NaCl) per kg.	0·479	92·9 ± 4·0
Cerebral cortex	2 rabbits		No injections	0·061	86·5
	2 rabbits	30	Inj. of 40 ml. 5 % urea (in 0·6 % NaCl) per kg.	0·363	34·6
Cerebral cortex	2 rabbits	60	Inj. as above	0·368	35·1
Cerebral cortex	1 rabbit	270	Inj. as above	0·344	62·0
Cerebral cortex	1 cat	25	Inj. as above	0·535	44·0
Cord	2 rabbits		No. inj.	0·061	63·0
Cord	2 rabbits	60	Inj. of 40 ml. 5 % urea (in 0·6 % NaCl	0·385	32·8
Cord	1 cat	25	Inj. as above	0·535	15·0

Renal cortex. Diffused sections (30–60 *min. in chambers*). For six cats and one dog the following procedure was used. The kidneys were perfused for 15–20 min. immediately after excision and as described under Methods with a cyanide-urea solution (0·2 % urea and *N*/200 NaCN). Sections were made of the renal cortex, some of which were

taken for immediate analysis, some transferred to a diffusion chamber containing 0·2 % urea without cyanide and others into a diffusion chamber containing a similar fluid to that used in perfusing. The sections in the chambers were allowed to diffuse for 30–60 min. being then dried and analysed for urea. The results are shown in Table 4 and in the histograms of Fig. 3.

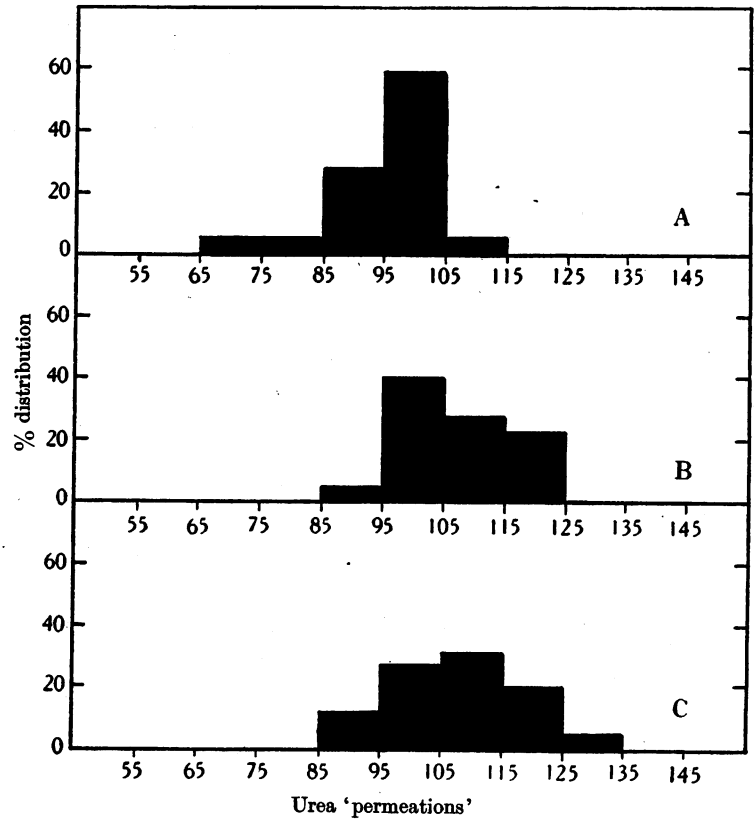

Fig. 3. Urea 'permeation' for renal cortical sections from six cats and one dog, and for which there are corresponding figures for sections (A) immediately after the cyanide-urea perfusion of whole kidney, and (B) after subsequent diffusion of sections in the same fluid as used for perfusion and (C) after diffusion as in (B), but without cyanide.

The mean value after perfusion (nineteen sections) was $94{\cdot}4 \pm 2{\cdot}1$. After a subsequent diffusion of twenty-five sections in cyanide urea it was $107 \pm 3{\cdot}2$ and in urea solutions without cyanide it was $109 \pm 2{\cdot}8$. The significance of these results is considered in the discussion.

Cerebral cortex, medulla and spinal cord. Without injections the mean urea 'permeations' of the cerebral cortex and cord of two rabbits were 86·5 and 67 % respectively. Half an hour after the intravenous injection of 40 ml./kg. of 5 % urea in 0·6 % NaCl the 'permeation' for the cerebral cortex was 34·6 %, the plasma being 0·363 g./100 ml.; 1 hr. after injection the value was 36·6 % for three rabbits; and 270 min. afterwards it was 62 % (one rabbit). The 'permeations' for the cord as observed 1 hr. after the urea injection were similar to the cerebral cortex. As shown in Table 4 the results for the cat (one animal) resemble those for the rabbit.

Diffusion coefficients

Diffusion coefficients through an agar gel at 38° C.

An agar gel (2·3 %) was prepared containing 2·0 % inulin and 0·5 % urea. This gel was allowed to settle upright in Hagedorn-Jensen tubes at 38° C. When solid the tubes were placed in a water-bath for some

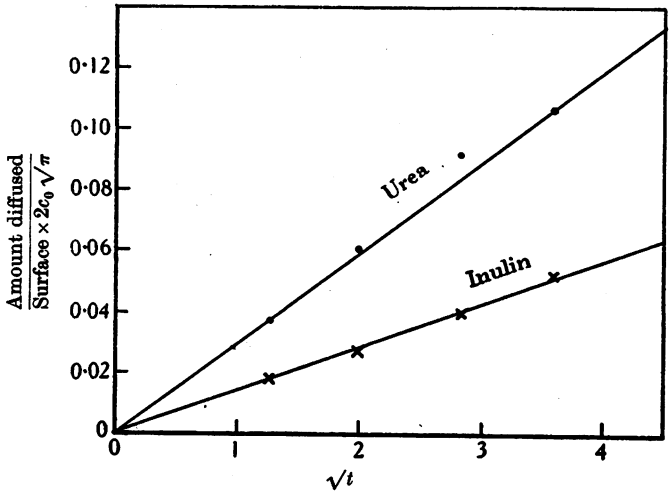

Fig. 4. Diffusion of urea and of inulin through an agar gel. The slopes of the curves give the square roots of the diffusion coefficients.

time to allow for temperature equilibration. Then at a noted time 10 ml. of Krebs's fluid at 38° C. was run over the surface and stirred by bubbling with the 5 % CO_2-oxygen mixture. Samples were removed for analysis of inulin and urea at different times, and a graph plotted of amounts diffused against $\sqrt{t}$. The diffusion coefficients (cm.²/min.) were determined from the slopes of the lines in Fig. 4, in accordance with the equation

$$\frac{\text{Amount diffused}}{\text{Surface}} = 2c_0 \sqrt{\frac{kt}{\pi}}.$$

The value for inulin was found as $19{\cdot}6 \times 10^{-5}$ and for urea as $88{\cdot}0 \times 10^{-5}$ (cm.^{2}/min.). These figures are of a similar order to those found by Bunim, Smith & Smith [1937], using a somewhat different method.

Diffusion coefficients in mammalian tissues

Urea. The general procedure consisted in diffusing sections of tissues, prepared immediately after killing the animal, either in a Krebs's solution containing urea or in one free from urea, and determining the urea concentration in the tissues after a definite time. The diffusion coefficient is then calculated in accordance with the following formula [Conway & Kane, 1934]:

$$\frac{\text{Amount diffused}}{\text{Surface}} = 2\ (ec_0 - c_1)\ \sqrt{\frac{kt}{\pi}},$$

where c_0 is the concentration in the tissue, c_1 in the external fluid and e an equilibrium factor. Since the urea in the external solution will be in simple equilibrium with the urea in the tissue water and since the tissue water is 76 g./100 g. of tissue or 0·81 ml./1 ml. tissue (sp. gr. being taken as 1·05), e will therefore be 0·81. (If this equilibrium factor be neglected the observed diffusion coefficient for urea diffusion *inwards* will be only 0·66 of the value *outwards*, and for other substances the ratio may be as low as 0·05 or less.)

Sections of the renal cortex were diffused under a variety of conditions, i.e. either directly in cyanide-Krebs's fluid or after an initial perfusion of the kidney with or without cyanide. For skeletal muscle the diaphragm of the rat was used (mean thickness of 0·9 mm.), it being realized at the same time that mammalian skeletal muscle swells in the usual 'isotonic' fluid.

The results are summarized in Table 5. The mean values of the diffusion coefficients for different animals and tissues range from 5·3 to $12{\cdot}3 \times 10^{-5}$ with a general mean of $8{\cdot}65 \times 10^{-5}$. The observations on single sections show considerable variation as shown in the table. The main source of this variation is apparent when the diffusion coefficients are graphed against the section thickness (omitting observations on cerebral cortex and cord which appear different in kind). From Fig. 5 it is obvious that increasing thickness in section causes an increase in the diffusion coefficient, the correlation between d and k being 0·78. From the line of best fit the mean coefficient of $2{\cdot}5 \times 10^{-5}$ was found at a section depth of 0·5 mm., an extrapolation of the curve beyond this point being scarcely permissible from the data. These data, it may also be noted, are mainly from the renal cortical sections. From Fig. 5 it

TABLE 5

Tissue	Animal	No. of animals	No. of sections	Mean depth of sections	Range of k ($\times 10^5$)	Mean k ($\times 10^5$)
		Urea diffusion coefficients				
Kidney	Rabbit	4	14	0·76	0·5 –10·4	5·3
	Cat	1	2	0·77	6·7 – 7·8	7·3
	Dog	2	10	1·14	2·7 –21·2	12·3
Muscle	Rat	2	3	0·85	7·5 –10·3	8·2
Liver	Rabbit	1	1	1·11	—	7·3
	Rat	2	3	1·13	7·6 –13·2	11·2
Cerebral cortex	Rabbit	2	2	1·56	4·22– 5·9	5·1
	Cat	1	1	1·29	—	11·8
Spinal cord	Rabbit	2	2	2·42	6·5 –80	7·2
		Inulin diffusion coefficients				
Kidney	Rabbit	1	3	1·11	3·6 – 3·8	3·8
Cortex	Rabbit	1	4	0·88	2·3 – 4·3	3·1

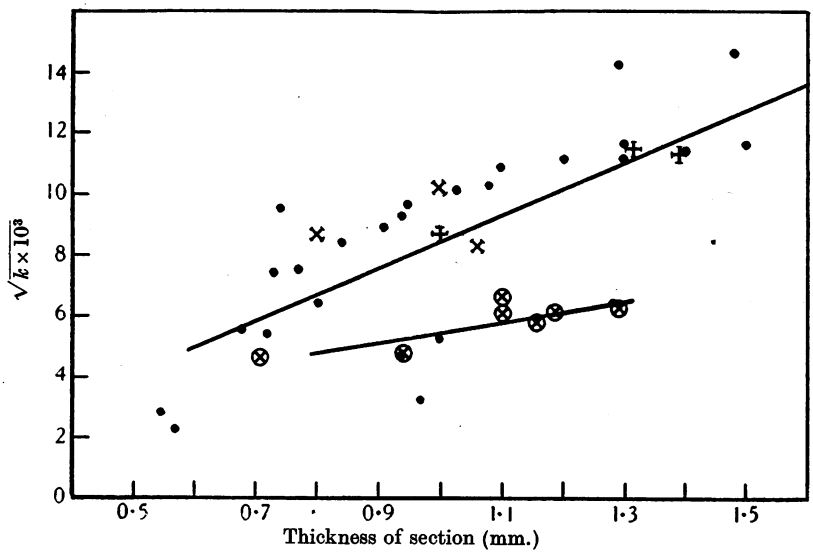

Fig. 5. Influence of section thickness on diffusion coefficients of urea through renal cortex (dots), diaphragm muscle (×) and liver (+); also on the diffusion coefficient of inulin through renal cortical sections (circles with crosses).

appears also that the values for muscle and liver fall into the distribution of renal values and the mean coefficients for these tissues with large numbers of data will probably be very similar to that for the renal cortex. Why the thickness of the section should affect the diffusion coefficient is not immediately obvious since when the bulk of the sections were diffused in a cyanide-Krebs fluid, oxygen lack was scarcely operative; yet the

effect is probably associated with a higher gradient of metabolites in the thicker sections.

Inulin. Inulin diffusion coefficients were determined only with renal sections, which gave a relatively high 'permeation' value. The mean for seven sections from two rabbits was $3{\cdot}34 \times 10^{-5}$ (range 2·2–4·6). These are indicated in Fig. 5 (circles with crosses) and show also—though few in number—a similar effect of section thickness.

Discussion

If, for a given tissue, we consider the 'permeations' of different substances injected intravenously, the lowest sets a maximum value for the intercellular fluid continuous with the plasma, provided it can pass the capillary walls and that a sufficient time is allowed for equilibration with the blood concentration.

For most tissues we may expect inulin to be a very suitable substance for determining the intercellular spaces. From the data considered in the results after inulin injection, it is clear that it passes freely across the capillary walls (Fig. 2) and would appear to have reached equilibration in about half an hour. Thirty minutes after injection the inulin 'permeation' for excised leg muscle is $8{\cdot}5 \pm 0{\cdot}6$ and for chloride $15{\cdot}2 \pm 0{\cdot}7$, the difference being $6{\cdot}7 \pm 0{\cdot}9$, so that the mean difference for a very large number of observations may be taken as lying somewhere between 4 and 9 (taking ± 3 times the standard error of the mean). These figures may be compared with the inulin 'permeation' for the isolated sartorius muscle of the frog immersed in an inulin-Ringer fluid [Boyle *et al.* 1941] which is 11–12% (or 9–10 as a 'space'), the chloride figure being 17·6 (this latter figure referring to the freshly excised muscle and the corresponding plasma value).

Considering how the difference between the inulin and chloride 'permeations' is made up in the case of rabbit muscle, we have first the question of the circulatory space and the red corpuscles therein, which though containing chloride may be presumed to contain no inulin. For the isolated sartorius of the frog it was shown [Boyle *et al.* 1941] that the circulatory space is only about 2% and a similar determination for freshly excised rabbit muscle has given a figure of 2% as a maximum value (i.e. neglecting the error arising from the muscle haemoglobin). Only about 1% at most can therefore be assigned as arising from the chloride and inulin difference in the red corpuscles.

The major part of the difference between the inulin and chloride 'permeations' arises from the presence of some chloride within the fibres

and related to the potassium concentration by an equality of the products of these ion concentrations on each side of the membrane [Boyle & Conway, 1941]. For the estimation of this chloride fraction we have the following data:

K in muscle and in plasma	97·4 and 5·5 m. equiv./kg.
Cl in plasma	97·6 m. equiv./kg.
Water in muscle and in plasma	0·8 and 0·926 l./kg.

(The figure for potassium in rabbit muscle is from Costantino's data [1911], and the plasma potassium and plasma water from Abderhalden's figures [1909].) For the calculation the interfibre volume may be taken as 0·1 l./kg. (or approximately 10 % of the weight, but a few per cent more or less will here have no appreciable effect). In such intercellular space there will be 0·5 m. equiv. potassium/kg. muscle so that 96·9 m. equiv. potassium will exist in the fibres and in 0·7 l. of water. The concentration of potassium in the fibre water is therefore 138 m. equiv./l. The product of the potassium and chloride concentrations in plasma water is 626, so that the concentration of chloride in the fibre water must be 626/138 (=4·6) if the Donnan relation applies. This would give $4{\cdot}6 \times 0{\cdot}7$ or 3·2 m. equiv./kg. muscle. This divided by the external plasma concentration and by 0·8 l. for the total muscle water gives a 'permeation' allowance of 4·2. Adding to this a red corpuscle allowance of 1·0 we obtain finally 5 as the approximate expected difference between the inulin and chloride 'permeations'. The figure found is $6{\cdot}7 \pm 0{\cdot}9$, which differs from 5 by no more than may be expected from the sampling error. It would seem unnecessary therefore to consider with such data further causes for the increase in the chloride 'permeations' over the inulin value for rabbit muscle.

Inulin 'permeation' of the renal cortex. The best values for the renal cortical 'permeation' gave figures of over 50 % or about seven times more than for muscle and 25 times more than for the cerebral cortex. The total average for the diffused sections was 41·5.

It is unlikely that so high a figure represents merely free space for diffusion or intercellular space. It is true that some increase in water content occurred as judged by the increase in water proportion of the tissue sections from 76·4 to 79·4 % and that the conditions were abnormal, but a practically identical figure is given for excised frog kidneys (as shown in experiments it is hoped to describe later). These kidneys lose weight instead of gaining it in Ringer fluid isosmotic with the blood, and are demonstrably active. Further, the haemoglobin 'permeation'

determined at the same time in such experiments is much lower than the inulin 'permeation'.

Urea 'permeations' in the renal cortex and evidence for active excretion. It was shown [Conway & Kane, 1935] for the isolated kidney of the frog that all the urea was freely diffusible therefrom into an external Ringer fluid, and that it re-entered the kidney immersed in cyanide-Ringer fluid to a full 'permeation', or, in other words, the water in the tissue had the same urea concentration as the external solution. Without cyanide, it entered to a much higher level. With 20 mg./100 ml. urea outside the mean increase in 37 min. over the inactive kidney was 17 mg./100 g. kidney, but the range was as high as a 47 mg./100 g. increase. In 37 min. therefore there was accumulated by the special activity of the kidney as much urea as was contained in a volume of external fluid 0·85 of the renal volume and the accumulation could reach more than double this figure. The accumulated urea diffuses as freely from the kidney as urea entering the cells passively as shown by a study of the diffusion curve and coefficients of diffusion. Could the urea accumulation be explained by the entrance of external fluid—produced by some pressure difference—at one region of the kidney, and then the urea be concentrated by water absorption at another region? Such an explanation has been advanced for similar accumulation of phenol red in the isolated kidneys of *Rana catesbiana* by Richards & Barnwell [1928]. These authors, however, did not consider either the total accumulation with respect to the water absorption this would entail or the pressure requirements. It may be noted, first, that from Krause's data as given by Pütter [1926] and calculating for a mean frog weight of 25 g. (as used in the experiments described above) an average of 55 mg. urine will be excreted by one kidney in 37 min., or 1·4 times the mean volume of the kidney. As considered above, the accumulation of urea would require 0·85 of the renal volume absorbed within 37 min., but since from the diffusion rate of accumulated urea from the kidney immersed in Ringer fluid (free from urea), 73 % will have been lost within this period, then upwards of $4 \times 0{\cdot}85$ the renal volume (or about twice the amount of urine normally passed within the period), will need to be absorbed into the isolated organ. If this amount of fluid were interpreted as coming along the blood vessels and through the glomeruli under the suction pressures produced somewhere down the tubule, then it may be held that the capsule of Bowman and glomerular capillaries would close like a valve, since from their histological structure they can offer no appreciable resistance to the pressure differences necessary. It would be similar to expecting a flow of fluid through an

immersed organ by suction on its vein, and the same would apply to fluid entering from the ureter. If again we were to suppose the fluid to have been sucked across the upper tubule walls, then even with large differences in osmotic pressure, produced by a considerable dilution of the external fluid, water itself does not enter isolated tissues with anything like the rapidity required. Thus, as shown by Buglia [1909] for the isolated gastrocnemius of the frog, an increase of only 9% of its weight occurs after 37 min. immersion in $N/25$ saline. (The special excretion of urea appears to be confirmed by a study of inulin accumulation under conditions similar to the urea experiments and from a Ringer fluid containing 2% or more of inulin. Such experiments are in progress here, and so far they show that, compared with urea, inulin is not appreciably accumulated by the active kidney over that entering the inactive organ. Such inulin experiments do not in themselves outrule active excretion of inulin since a much shorter section of tubule may be involved.)

The difficulties against showing the same effect of urea accumulation in the mammalian kidney are considerable. First, only sections of the cortex can be used in order to secure an adequate oxygen supply, and in these the tubules will open freely into the external solution. Seeing that the diffusion of urea will take place from these openings at anything from 12 to 50 times as fast as through the cells, it is obvious that this will greatly lower the lumen concentration and the chances of demonstrating secretion. Also, even with thin sections, the tissue will scarcely be functioning at its normal level. We have, however (as indicated above), repeated such experiments with the kidneys of the cat and dog, and the results are rather curious. After perfusing the kidney with urea 0·2% and cyanide (securing good urine flow and thereby largely diminishing the urea in tubules if there remained any secretory activity) the distribution of the urea permeation gave 100 as the mode and a mean value of $94\% \pm 2{\cdot}1$. When the sections were transferred after perfusion to a diffusion chamber containing the same fluid as that used in perfusing, the 'permeation' rose to 107%; with cyanide omitted from the diffusing fluid, it reached 109% (see Fig. 3). Here then it may be said that a definite increase over 100 occurred in the diffused sections, but that the effect is almost as great in the presence of cyanide which in the mammalian kidney, at least, leaves a very appreciable residual oxidation. The results, therefore, do not give the same clear-cut decision as with the frog's kidney.

Urea 'permeations' in the central nervous system. Whereas the urea

permeation of muscle or of renal cortex is at or close to the expected value of 100, that of the central nervous system of the rabbit and cat is definitely less, indicating that some region is quite impermeable to urea or that some proportion of the water is bound.

A curious result follows the injection of urea. A certain region of the nerve tissue is rapidly permeated, there being very little or no increase in the permeation value from 30 to 60 min., and the value of 30–40 % is probably reached very quickly. From this region of the brain the urea diffuses with the same rate as from kidney or muscle sections, as evidenced by the diffusion coefficient. After this rapid entrance into about 30 % of the tissue water there is a very slow diffusion into another region which is not complete even after 4½ hr.

It is clear, therefore, that the total volume of the tissue may be divided into four volumes or 'spaces' when we include the free intercellular space (inulin 'permeation') and the water into which the urea does not appear to enter even after an indefinite contact with the tissue.

The following explanation may be advanced as to what these tissue 'spaces' signify. The considerable difference in rate of entrance of urea may be explained by a rapid entry into nerve cells and a comparatively slow passage into the conducting fibres. This should be evident also for the spinal cord, but a smaller 'permeation' should be observed in the initial short period. The few observations made tend to support this view, since only a 15 % 'permeation' was found with the cat's spinal cord after 25 min., whereas the cerebral cortex of the cat gave 44 % 'permeation' in the same time, and the fullest 'permeations' for both tissues (corresponding to the normal values with respect to the blood urea) are 63·0 and 86·5 % as judged from rabbit experiments. The question arises as to what is the significance of the 'space' indicated by the failure of urea to reach a 100 % 'permeation' in nerve tissue. Since this 'space' is much greater for the cord than for the cerebral cortex, we may suppose it is connected with the conducting tissue and not with the nerve cells. Alternatively, it may not be a 'space' in any real sense but merely the effect of a solubility difference between urea in the myelin sheaths and in the blood.

Here account may be taken of the results of Riser, Valdiguie & Guiraud [1938], for the urea concentration of brain, muscle, blood, etc. after intravenous injection of urea into dogs (2–3 g./kg.). About 30 min. after the injection of urea 'permeation' in the brain would appear from their figures to be approximately 70 % and that of muscle 90 %. The data for the brain agree with ours (for the urethanized rabbit and cat) only

in so far as they show a considerably smaller value than for muscle, but differ much in the magnitude of the relative brain concentration. This is about twice what we have found. Our experiments were performed before those of Riser *et al.*, and since reading an account of their paper we have repeated and confirmed our results. Wherein lies the cause of the different effects described, whether it be due to different animal species or experimental conditions, does not at present appear.

Diffusion coefficients. It has been already shown that both for the frog's muscle and renal tissue the diffusion coefficient of urea is $1{\cdot}1 \times 10^{-5}$ (cm.^{2}/min.) [Conway & Kane, 1934]. These values were obtained at a mean temperature of 18° C. From the usual effect of temperature on diffusion coefficients in vitro we could expect a value of about $1{\cdot}6 \times 10^{-5}$ at 38° C., but the mean value for the mammalian tissues at 38° C. is found to be $8{\cdot}0 \times 10^{-5}$. As already indicated, however, the value depends largely on the thickness of the section (though for this most of the observations were made on renal tissue). Allowing for this effect the mean coefficient at a thickness of 0·5 mm. is $2{\cdot}5 \times 10^{-5}$ which is not far from the anticipated value. The coefficients for muscle and liver and cerebral cortex (first permeable region) appear to be of the same order as for the kidney, but for the cerebral cortex the effect of thickness of section does not seem to be of nearly the same consequence.

The diffusion coefficients of urea and of inulin through an agar gel at 38° C. are $88{\cdot}8 \times 10^{-5}$ and $19{\cdot}6 \times 10^{-5}$ or a ratio of 1·0 : 0·22. Through renal cortical tissue at 1 mm. thickness of section, the coefficients are $8{\cdot}30 \times 10^{-5}$ and $3{\cdot}36 \times 10^{-5}$ or a ratio of 1·0 : 0·38, the inulin coefficient being relatively higher. This is probably due to the greater relative effect for the inulin of the amount dissolved in extracellular water, since if we were to suppose only 8 % of the external inulin diffusing freely as through water, it would account for such a difference.

Summary

1. The inulin 'permeation' in rabbit muscle has a mean value of 8–9 % indicating that this fraction of the total water (or 7 % of the total muscle volume) is freely permeable to external inulin. This figure sets a maximum value for the intercellular spaces in the excised tissue. The value is much lower than the chloride 'permeation' which was found to be 15 % under similar conditions.

2. The inulin 'permeation' of the cerebral cortex has a mean value of only 2 %.

3. The inulin 'permeation' of the renal cortex contrasts markedly with that for muscle and brain tissue, being 41 % (mean value) for renal cortical sections immersed after sectioning in Krebs's fluid containing inulin, and of 56·6 % for freshly excised kidneys perfused with an inulin-Krebs fluid.

4. The mean urea 'permeation' in muscle was found to be 93 % (eleven observations).

5. The urea 'permeation' of renal cortical sections after perfusing with modified Krebs's fluid containing urea and cyanide was $94 \pm 2{\cdot}1$ (standard error of mean). When cortical sections of such perfused kidneys were immersed in modified Krebs's fluid containing urea and cyanide the value rose to $107 \pm 3{\cdot}2$, and to $109 \pm 2{\cdot}8$, when the cyanide was omitted.

6. The urea 'permeation' of the brain and cord of the rabbit was found to be 86·5 and 63·0 (two animals) respectively. On raising the blood urea to about six times its normal level by an intravenous injection, a urea permeation of 30–40 % is quickly reached with a subsequent slow increase, so that after 270 min. it stands at 62 %.

7. The mean diffusion coefficient of $8{\cdot}30 \times 10^{-5}$ (cm.^{2}/min.) for urea has been found for all the tissues examined (thirty-eight observations) and of $3{\cdot}36 \times 10^{-5}$ for inulin in the kidney (seven observations).

The scatter of the figures is wide, but this is mainly due to varying thickness of section; for the diffusion coefficient without any very obvious reason increases with the section thickness, and when this is 0·5 mm. k is $2{\cdot}5 \times 10^{-5}$.

The coefficient appears to be the same or similar for the different tissues; but the number of figures do not warrant any exact comparison.

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