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On the permeability of urea and some non-electrolytic substances through cell membrane.

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Abstract

(1) We studied the permeability of erythrocytes (human, an, chicken and frog), collodion membrane_ and frog's urinary bladder, to urea and its associated substances, alcohols and glucose. (2) Hemolysis of human erythrocytes to urea, its derivates and alcohol, is due to the penetration of these substances through the erythrocytes membrane. (3) Among urea and its associated substances, it was observed that the hemolysis time of human erythrocytes was inversely proportinal to the molecular weight of these substances. (4) Chicken erythrocytes were far less permeable to urea than human erythrocytes. (5) Hemolysis time to guanidine showed no difference between human and chicken erythrocytes. (6) Frog's urinary bladder in a living condition has a high degree of semipermeability to urea. When it is injured or dead, it behaves like collodion membrane.

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On the permeability of urea and some non-electrolytic substances through cell membrane.

Von

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Introduction.

The living cell membrane is characterized by semipermeability. Although there is little difference in morphological structure among different cells, it is noteworthy that very great differences in their permeability are observed under a high grade of morphological similarity.

Investigations hitherto performed in respect of permeability, have been concerned mainly with the permeability of electrolytic substances, non-electrolytic ones having been relatively ignored. Erythrocytes of various animals are the most suitable material for the study of permeability, just as is muscle for the study of contraction, and nerve for the study of the conduction of excitation, because their principal function consists in giving out and taking in various materials. Among the methods of studying the permeability of erythrocytes, the hemolysis and hematocrite methods for the estimation of volume change are the most usefull.

According to *Gryns* (1896) and *Hedin* (1897), the non-electrolytes which can readily penetrate the erythrocytes are the following: monohydric alcohols, esters, ketones, ethers, amids, as well as a number of other substances such as urea, urethane, chloral hydrate, pyridine, etc.. The results of *Overton* (1895) and of many later workers, obtained from the study of other cells of both plant and animal origin, confirm the above results. But one striking difference between the erythrocytes and other cells is in the behavior of urea. *Overton* classed it as a substance of relatively slow permeability, whereas it penetrates in the mammalian erythrocytes far more rapidly than other cells.

Interested in this point, the author studied the permeability of human, chicken and frog's erythrocytes, collodion membrane, and urinary bladder of frog by urea and some non-electrolytic substances.

Permeability of erythrocytes.

Methods:

The experiments were performed chiefly on human erythrocytes, partly on chicken and frog erythrocytes. The blood was defibrinated, and erythrocytes used in this natural condition or washed with isotonic dextrose solution. The blood samples 0,5 cc. were mixed with 5 cc. of isotonic solution of various substances. After the mixtures had been prepared we observed the volume changes of erythrocytes by the hematocrite method and the osmotic activity of the substances to the erythrocytes by hemolysis method. The substances afforded for the experiments were as follows: Urea and its allied substances (urethane, guanidine, methylguanidine, creatine), theophylline (1. 3. dimethylxanthine) and caffeine (1. 3. 7. trimethylxanthine) as purine base; ethylalcohol and glycerol as mono- and trihydric alcohol respectively; glucose as carbohydrate,

Results:

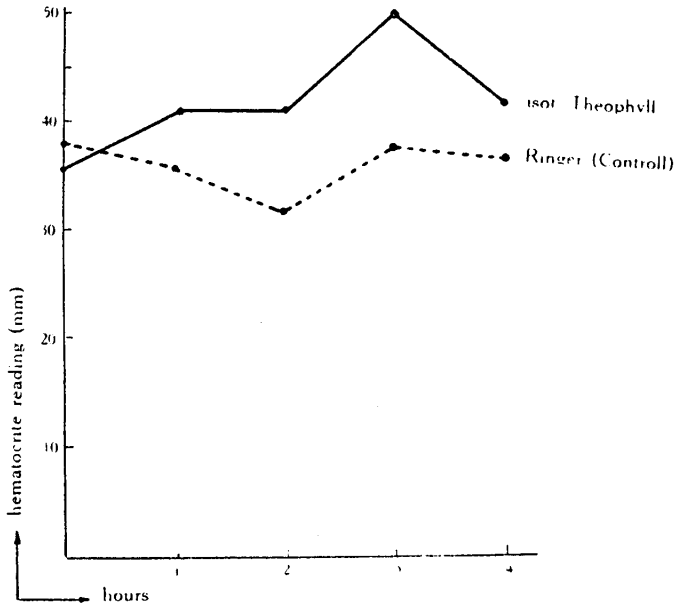
All the substances above described, except glucose, caused hemolysis of both human and chicken erythrocytes more or less quickly. The rate of hemolysis of human erythrocytes may be classified as follows: Urea, ethylalcohol > glycerol, urethane > guanidine, methylguanidine, creatine > theophylline and caffeine. The hemolysis caused by the 1 class substances, for example isotonic urea solution, is so rapid (as if the blood were added to pure water) that we cannot determine the volume change of erythrocytes by usual methods. But it is clear from the following observations that the hemolysis occurred osmotically by the rupture of erythrocytes membranes as a result of the penetration of water into the erythrocytes: (1) now a suspension of erythrocytes was observed under the microscope, and the isotonic common salt solution replaced by isotonic urea solution. We could then observe that erythrocytes increased their volume rapidly and were destroyed by the rupture of the erythrocytes membrane, (2) the time for hemolysis was lengthened by an increase of the concentration of urea. Chicken erythrocytes behaved differently from urea. The time required for hemolysis of human erythrocytes to isotonic urea solution is usually about 5 sec., while that of chicken erythrocytes requires about 10 minutes. The time taken for hemolysis of both human and chicken erythrocytes for guanidine was 3–4 hours after the mixture, there being no difference between the two. The same relations hold for the isotonic solution of theophylline and

caffeine and their hemolysis time was similarly 6-7 hours. On the assumption that all these hemolyses are caused by the rupture of erythrocytes membranes in consequence of volume increase of water penetration, the volume change of human and chicken erythrocytes to relatively slow penetrating theophylline was studied by the hematocrite method. The results with chicken erythrocytes are summarized in Table 1, and an example of those of human erythrocytes is represented graphically in Fig. 1, showing that the above assumption is true.

Table 1. Volume change of chicken erythrocytes after the mixture with isotonic theophylline solution.

No.	Hours after the moment of mixture	Volume change of chicken erythrocytes			
		In Ringer's solution		In isoton. theophyll solut.	
		Hematocrite reading (mm)	Vol. change in p. c.	Hematocrite reading (mm)	Vol. change in p. c.
1	0	30,0		29,0	
	1	28,8	−4	39,5	+36
2	0	11,5		13,5	
	1	11,5	0	15,0	+11
	2	11,5	0	17,0	+26
3	0	34,5		34,0	
	1	35,5	+2,8	42,0	+23,5
	2	34,0	−1,5	40,0	+17,5
	3	34,5	0	40,0	+17,5
4	0	21,0		27,0	
	1	21,5	+2,4	30,5	+13
	2	20,5	−2,4	30,5	+13

Fig. 1. Volume change of human erythrocytes after the mixture with isotonic theophylline solution



Permeability of collodion membrane.

Methods:

A sack of collodion membrane of 50 cc. capacity was prepared after *Michaelis'* method. The permeability of urea and ethylalcohol through the membrane was studied in the following manner: A known concentration of urea or alcohol was introduced in the sack, immersed in a vessel, in which water was renewed incessantly from the tap. I took out samples of the solutions from time to time, determined the decrease of concentration. It was measured on urea after *Knop-Häfner's* hypobromite method, using *van Slyke's* apparatus of manometric type, and on alcohol by the gravimetric method.

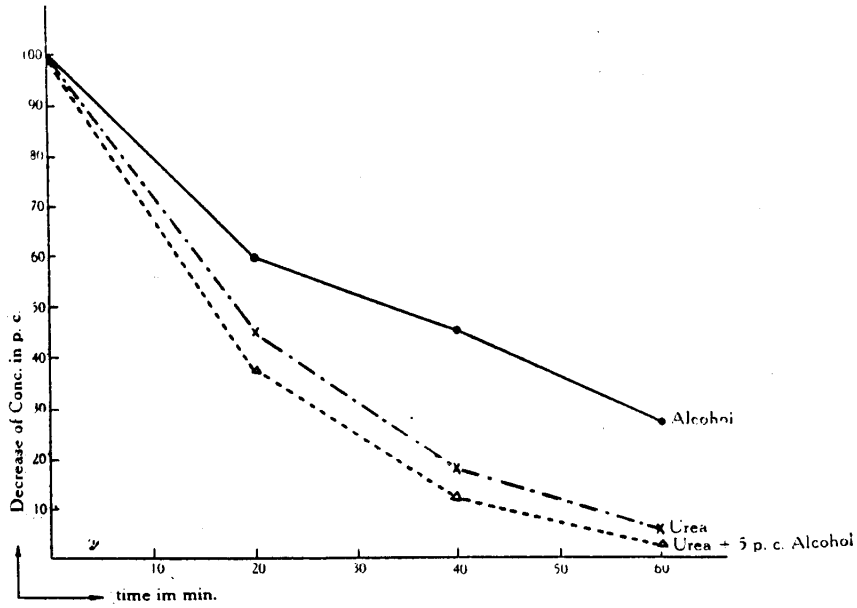
Results (*see Fig. 2*):

Typical examples of these results are shown graphically in Fig. 2. The decrease in concentration is calculated as a percentage, taking the original concentration as 100.

These curves show clearly that, (1) the permeability of urea and alcohol through the collodion membrane is in its nature similar, (2) the rate of penetration of urea is a little higher than that of alcohol, even if their molecular weight is in the reverse relation. Also it was studied on the influence of alcohol on the permeability of

collodion membrane to urea. One of the results is shown in Fig. 2. As will be described below, alcohol (narcotica) has no influence on the permeability of collodion membrane, while it has a marked influence on the permeability of frog's urinary bladder by urea.

Fig. 2. Permeability of collodion membrane by urea and alcohol.



Permeability of frog's urinary bladder.

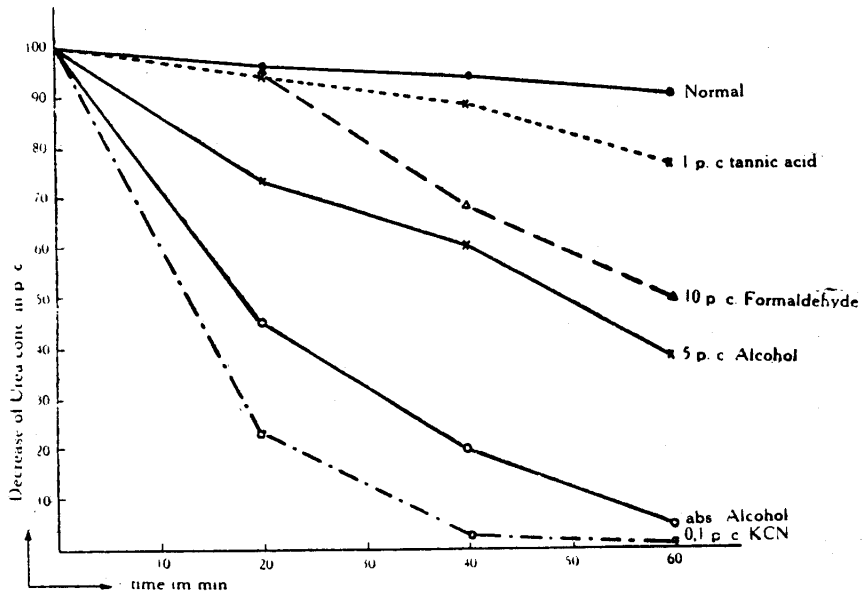
Methods :

So far as I am aware, no one has used the frogs urinary bladder for permeability experiments. The practical advantages of frog's urinary bladder as a living membrane over other kinds of material used in permeability studies may be pointed out. It may be obtained in a fresh living condition at all times. Its thickness is perhaps 10μ or so and is considered to be composed of one or very few cell layers. Details for the preparation are as follows : The frog was decapitated and pitched, then laid on its back and his abdominal wall was opened. A pipette suitable for the opening of cloaca was inserted deep into it and about 5 cc. of *Ringer*'s solution inserted. Then the urinary bladder extended, enabling us to isolate it easily without injury from other tissues. A suitable canula was introduced into the urinary bladder via the cloaca, tied up at its neck with a cotton thread, and cut out. The permeability of this membrane to urea, as well as influence of alcohol, tannic acid, formaldehyde and potassium cyanide upon it, were investigated in the same manner as in the case of the collodion membrane.

Results (see Fig. 3):

Examples of these results are represented in Fig. 3. In spite of the extreme thinness of the urinary bladder membrane, it is remarkable that the permeability to urea is almost negligible and entirely different in its nature from that of the collodion membrane, so long as the membrane is kept in a living condition. While if the integrity of the membrane is once impaired, for example through KCN or alcohol of high concentration, the permeability becomes similar to that of the collodion membrane (see Fig. 3). Between these two extremities we can observe intermediate types of permeability by the application of alcohols of relatively lower concentrations, tannic acid and formaldehyde. When alcohol of high concentration was applied, the permeability to urea as well as to alcohol was increased till it equalled that of the collodion membrane.

Fig. 3. Permeability of frog's urinary bladder to urea, and the influence of some drugs upon it.



Discussion

As representative of the substances studied, I shall discuss below mainly effect of urea in relation to cell permeability. Urea is a metabolic product of protein, and thus one of common constituents of body fluid. Therefore, it is naturally conceivable that it penetrates freely through walls of cell membrane. Those substances which are

able to pass freely through membrane, (i. e. the membrane is no more semi-permeable to those substances, as *Höber* described, although they have the same osmotic pressure as blood plasma,) cause hemolysis with more or less rapidity, according to the difference of the rate of penetration, as solvent pure water or hypotonic solutions of various concentrations were used. Among the series of urea derivates, the rate of penetration of these substances through human erythrocytes is inversely proportional to their molecular weight. As *Gryns*, *Hedin*, *Overton* and *Höber* confirmed, urea is freely passable to erythrocytes, equally distributed in erythrocytes and plasma soon after the addition, while it does not penetrate muscle cell membrane. It is proved experimentally that the same reason holds also for those substances which are in their chemical constitution akin to urea (urethane, guanidine, methylguanidine, creatine, etc.). But we cannot understand for what reasons erythrocytes membranes are permeable urea and not muscle cell membrane. Urea is less permeable to chicken erythrocytes than human erythrocytes, and it is said that fish erythrocytes are practically inpermeable to it (*Jacobs*, 1931). Guanidine, which is a normal constituent of bird blood, as is urea in mammalian blood, shows no difference in the permeability between human and chicken erythrocytes.

Frog's urinary bladder in a living condition shows a relative higher semi-permeability to urea. According to the experiments made by *Cazeneuve* and *Livon* (1878), and *Reid* (1890) with dissected bladders of dogs, and cats respectively, they found that no urea had passed out from a fresh bladder, also that scraping away epithelium from within at once rendered the wall permeable to the contained solution of urea. My own experiments on frog's urinary bladder also show that the same relation exists. When alcohol, potassium cyanide, etc., are applied and the integrity of the living state is impaired, the permeability of the urinary bladder behaves as that of collodion membrane. Concerning the problem as to through what mechanism the living membrane represents such a high degree of selective permeability, the two principal theories — the molecular sieve and lipoid theories — afford no satisfactory explanation, we must therefore wait until new experimental evidence has been accumulated.

Summary

(1) We studied the permeability of erythrocytes (human, chicken and frog), collodion membrane and frog's urinary bladder, to urea and its associated substances, alcohols and glucose.

(2) Hemolysis of human erythrocytes to urea, its derivates and

alcohol, is due to the penetration of these substances through the erythrocytes membrane.

(3) Among urea and its associated substances, it was observed that the hemolysis time of human erythrocytes was inversely proportinal to the molecular weight of these substances.

(4) Chicken erythrocytes were far less permeable to urea than human erythrocytes.

(5) Hemolysis time to guanidine showed no difference between human and chicken erythrocytes.

(6) Frog's urinary bladder in a living condition has a high degree of semipermeability to urea. When it is injured or dead, it behaves like collodion membrane.

In conclusion, I wish to express my sincere thanks to Prof. *Oinuma* for his kind suggestions and advice during the investigation.

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