# Inhalation Anesthetics and Permeability of Human Erythrocytes to Monosaccharides

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Studies of the effects of inhalation anesthetics on membrane carrier systems were carried out using, as a prototype, entry of monosaccharides into human erythrocytes. The data obtained lead to the proposal that, by acting as penetrating competitive inhibitors in a system which involves uphill counter-transport mechanisms, anesthetics can result in either acceleration or inhibition of the rate of transport of monosaccharides across cell membranes, depending upon the relative concentration of the anesthetic.

MEMBRANE carrier systems represent one of the mechanisms by which certain substances, notably monosaccharides, are transported across cell membranes. The present investigation is concerned with the effect of therapeutic concentrations of inhalation anesthetics on such carrier systems, using as a prototype the transport mechanisms by which monosaccharides enter human erythrocytes. The advantage of the use of erythrocytes include the large amount of information already available on the subject [1-5] plus the ready availability of such human membranes in large numbers. In addition, since the movement of monosaccharides into erythrocytes is not hormonally influenced, the studies can be carried out independently of any possible effects of anesthetics on the activity of substances such as insulin. The results of this study permit formulation of the hypothesis that inhalation anesthetics may, depending upon their relative concentration, act as penetrating competitive inhibitors either to accelerate or to in-

hibit the rate of transport of monosaccharides by virtue of the role they play in the counter-transport mechanisms involved in membrane carrier transport.

## Methods

The methods employed were those previously reported.[6] They are based upon the fact that when human erythrocytes are exposed to appropriate low concentrations of monosaccharides, the monosaccharides gradually enter the red cells and as they do so the volume of red cell increases. The osmotic movement of water across the human red cell membrane being so much more rapid than the movement of monosaccharides and, in fact, being so rapid as to be essentially instantaneous, the osmotic pressure within the cells under these conditions may be assumed to be identical with that of the surrounding medium at all times. The relatively slow volume changes which occur in erythrocytes upon their exposure to a medium containing a monosaccharide reflect, therefore, not differences in tonicity between the red cells and the surrounding medium but instead passage of the monosaccharide from the surrounding medium into the erythrocytes.[7-9]

In the past, methods for measuring changes in red cell volume following exposure to monosaccharides have ranged from simple hematocrit determinations to more complex photoelectric techniques.[8] In the present study changes in red cell volume were measured by determining serial microhematocrit values [10-12] following admixture of equal volumes of whole blood and 0.286 $M$ (i.e., slightly hypotonic) solutions of various monosaccharides. An advantage of this method is that it is devoid of explosive hazards during investigation of inhalation anesthetics at high flow rates for periods of hours. The method is also simple and

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TABLE 1. Hematocrit Readings During
L-Sorbose Exposure

(Mean ± Standard Error of the Mean)

| Agent | N | 5 Min. | 60 Min. | 120 Min. |
|---|---|---|---|---|
| 0% O₂ | 6 | 100.2 ±0.3 | 124.8 ±1.2 | 138.7 ±1.1 |
| 100% O₂ | 12 | 101.9 ±0.8 | 131.1 ±1.4 | 143.0 ±1.2 |
| 80% N₂O | 6 | 103.6 ±1.4 | 132.5 ±1.5 | 147.2 ±0 6 |
| 0.7% halothane | 6 | 101.0 ±1.1 | 117.9 ±1.4 | 128.9 ±1.0 |
| 0.7% methoxy-flurane | 6 | 98.6 ±1.1 | 111.4 ±0.9 | 123.6 ±0.8 |
| 25% cyclopropane | 6 | 103.6 ±0.4 | 129.7 ±0.9 | 142.6 ±0.4 |
| 2.3% ether | 8 | 97.4 ±0.4 | 116.3 ±1.7 | 127.0 ±1.3 |

accurate. Its disadvantage is that it cannot measure rapid changes in cell volume. In the present investigation, the monosaccharides studied were D-galactose, D-fructose, D-arabinose, L-arabinose, D-xylose, L-sorbose and D-ribose. Each monosaccharide was studied separately. Control studies were performed using an alcoholic sugar, inositol (1,2,3,4,5,6-cyclohexanehexol), which has physical characteristics (molecular weight, molecular configuration, degree of dissociation) comparable to monosaccharides but which does not penetrate human erythrocytes.[2]

Heparinized venous blood samples were obtained from normal volunteers. Within five minutes 2.0 ml. of blood were added to 2.0 ml. of monosaccharide solution which had already been equilibrated by bubbling with the anesthetic gas (or oxygen) under study. The anesthetic gas (or oxygen) continued to bubble through the blood-monosaccharide mixture for the next two hours. The microhematocrit of this mixture of blood and monosaccharide solution was determined 5, 60, and 120 minutes after admixture. The hematocrit of the undiluted blood sample was also determined. The hematocrit readings in the experimental series were reported as a percentage differ-

ence from the hematocrit reading which would have been expected if no volume changes had occurred after exposure of the red cells to the monosaccharide solution, i.e., as a percentage difference from one half of the control (undiluted) hematocrit. For example, if the control undiluted hematocrit were 40, it was then assumed that if no changes in red cell volume occurred after mixing 2.0 ml. blood and 2.0 ml. sugar solution, the hematocrit would be 20. If upon measurement at 5, 60 and 120 minutes the microhematocrit values were found not to be 20 but to be instead 22, 25 and 30, then the three experimental hematocrits would be reported as 110, 125 and 150, respectively. Each hematocrit reading reported was the average of five simultaneous determinations. This, combined with the fact that all hematocrit readings were done by one of 3 experienced persons, resulted in a methodological error inherent in the technique of hematocrit determination of less than 2 per cent as determined by serial hematocrit readings in the same blood sample over a period of hours, by serial determinations on samples drawn from the same individual throughout the period of a day, and by up to 80 microhematocrit determinations in blood obtained at the same time of day over a period of a year from the same individual. No seasonal variation in microhematocrit readings was found, but diurnal variations were observed. Accordingly, all blood samples were matutinal and all determinations made at the same time of day.

Fresh 0.286 M solutions of the individual monosaccharides were made weekly or more often if necessary by mixing the sugar (analytic grade) with distilled, demineralized water in glassware specially prepared to prevent chemical contamination. The sugar solution was

TABLE 2. Hematocrit Readings During
D-Ribose Exposure

(Mean ± Standard Error of the Mean)

| Agent | N | 5 Min. | 60 Min. | 120 Min. |
|---|---|---|---|---|
| 0% O₂ | 16 | 102.8 ±0.4 | 128.8 ±0.9 | 142.4 ±1.0 |
| 100% O₂ | 16 | 102.8 ±0.5 | 132.2 ±1.4 | 145.9 ±1.3 |
| 80% N₂O | 6 | 102.4 ±1.5 | 125.4 ±2.3 | 141.9 ±0.4 |
| 0.7% halothane | 6 | 97.6 ±0.5 | 118.7 ±0.7 | 132.0 ±0.5 |
| 0.7% methoxy-flurane | 6 | 99.0 ±0.2 | 117.9 ±0.3 | 133.4 ±0.4 |
| 25% cyclopropane | 8 | 103.1 ±0.4 | 130.9 ±1.0 | 144.9 ±1.0 |
| 2.3% ether | 6 | 101.3 ±0.5 | 127.5 ±0.4 | 141.9 ±1.0 |

TABLE 3. Hematocrit Readings During
D-Fructose Exposure

(Mean ± Standard Error of the Mean)

| Agent | N | 5 Min. | 60 Min. | 120 Min. |
|---|---|---|---|---|
| 0% O₂ | 6 | 96.2 ±0.5 | 103.6 ±0.8 | 111.8 ±0.8 |
| 100% O₂ | 6 | 93.6 ±0.5 | 103.9 ±0.8 | 113.3 ±0.9 |
| 80% N₂O | 6 | 96.5 ±0.6 | 108.6 ±0.8 | 118.2 ±0.4 |
| 0.7% halothane | 6 | 94.6 ±0.5 | 105.0 ±0.6 | 115.3 ±0.2 |
| 0.7% methoxy-flurane | 7 | 93.1 ±0.3 | 101.0 ±0.6 | 111.8 ±0.9 |
| 25% cyclopropane | 8 | 94.5 ±0.8 | 105.3 ±0.9 | 118.8 ±1.3 |
| 2.3% ether | 6 | 96 2 ±0.7 | 107.8 ±1.4 | 116.6 ±1.6 |

equilibrated with the gas (oxygen or anesthetic) to be studied prior to addition of the blood by bubbling gas through the 2.0 ml. sample for at least 10 minutes prior to admixture with blood. The gases studied were: 100 per cent oxygen; zero per cent oxygen (100 per cent helium); 80 per cent nitrous oxide; 25 per cent cyclopropane; 2.3 per cent ether; 0.7 per cent halothane and 0.7 per cent methoxyflurane. All anesthetic gases were mixed with oxygen. The concentrations of anesthetics were selected as being those approximately equivalent to those employed for maintenance of surgical anesthesia under clinical conditions. The methods by which these concentrations were achieved and the methods by which constant concentrations were delivered for two hours were as previously reported.[6] Helium was employed instead of nitrogen to achieve completely anoxic conditions since it, unlike nitrogen, has no anesthetic nor analgesic effect at one atmosphere. The effect of carbon dioxide was also evaluated and will be separately reported.

The temperature of the blood: monosaccharide mixture was maintained at 21–23° C. The effect of temperature on sugar transport[13] is such that the temperature variability allowed in the present studies did not influence the results, especially since temperature variations were narrow in degree and randomly introduced.

Control runs wherein anesthetic gases were bubbled through undiluted blood for periods of two hours without change in hematocrit demonstrated that neither the bubbling nor the anesthetics in themselves caused the observed changes in red cell volume.

That changes in cell volume were associated with the entry of sugar into erythrocytes was

TABLE 5.   Hematocrit Readings During
L-Arabinose Exposure
(Mean ± Standard Error of the Mean)

| Agent | N | 5 Min. | 60 Min. | 120 Min. |
|---|---|---|---|---|
| 0% O₂ | 8 | 133.1±2.0 | 163.1±1.1 | 161.6±1.6 |
| 100% O₂ | 13 | 141.4±2.5 | 158.2±1.4 | 157.4±1.8 |
| 80% N₂O | 8 | 137.5±3 2 | 155.3±1.8 | 153.0±1.9 |
| 0.7% halothane | 6 | 140.9±3.4 | 161.5±1.8 | 162.8±1.6 |
| 0.7% methoxy-flurane | 6 | 130.5±1.7 | 161.3±1.1 | 163.1±0.9 |
| 25% cyclopropane | 6 | 136.8±1.3 | 153.6±1.3 | 150.5±1.4 |
| 2.3% ether | 6 | 131.4±2.7 | 160.2±0.7 | 163.7±1.2 |

confirmed by measuring sugar concentrations in red cells and in plasma after admixture of whole blood and 0.286 M sugar solution. The results of such studies with glucose have been reported.[6] When D-fructose solution (table 3) was added to whole blood, for example, the concentration of fructose in erythrocytes as a percentage of the total amount of fructose in whole blood increased from an average of 1.6 per cent 5 min. after admixture to 14.3 per cent 2 hours after admixture. Comparable values for D-ribose (table 2) were 6.0 and 16.8 per cent, respectively.

Statistical significance of observed hematocrit readings was evaluated by determining the significance of the difference between the means of two experimental runs. The minimum number of observations for each sugar exposed to each gas was six. As mentioned, each observation represented the average of five simultaneously determined microhematocrits. Statistical significance was assumed present if the difference between two means corresponded to $P$ values of 0.01 or less.

## Results

In tables 1–7 are tabulated the hematocrit readings for each of the 7 sugars after 5, 60,

TABLE 4.   Hematocrit Readings During
D-Arabinose Exposure
(Mean ± Standard Error of the Mean)

| Agent | N | 5 Min. | 60 Min. | 120 Min. |
|---|---|---|---|---|
| 0% O₂ | 8 | 97.1±1.3 | 114.8±1.3 | 127.0±2.1 |
| 100% O₂ | 6 | 96.9±1.8 | 111.8±0.8 | 122.2±1.0 |
| 80% N₂O | 7 | 94.8±0.5 | 113.1±0.7 | 124.5±0.8 |
| 0.7% halothane | 6 | 90.9±2.8 | 103.3±1.3 | 115.4±1.5 |
| 0.7% methoxy-flurane | 10 | 93.7±0.8 | 102.3±0.8 | 110.4±1.2 |
| 25% cyclopropane | 7 | 93.4±0.6 | 112.5±0.5 | 126.7±0.6 |
| 2.3% ether | 6 | 95.4±0.5 | 106.9±1.9 | 121.5±1.3 |

TABLE 6.   Hematocrit Readings During
D-Galactose Exposure
(Mean ± Standard Error of the Mean)

| Agent | N | 5 Min. | 60 Min. | 120 Min. |
|---|---|---|---|---|
| 0% O₂ | 8 | 133.0±1.8 | 158.2±1.1 | 158.2±1.2 |
| 100% O₂ | 6 | 126.6±2.2 | 155.7±0.6 | 157.8±0.3 |
| 80% N₂O | 6 | 121.7±0.3 | 154.4±0.9 | 155.9±0.5 |
| 0.7% halothane | 6 | 121.6±1.0 | 147.5±1.4 | 153.6±0.6 |
| 0.7% methoxy-flurane | 6 | 119.2±1.2 | 148.0±2.3 | 155.0±0.7 |
| 25% cyclopropane | 6 | 126.8±1.2 | 152.2±0.7 | 153.6±0.6 |
| 2.3% ether | 6 | 120.3±0.7 | 149.1±1.1 | 156 7±0.4 |

TABLE 7. Hematocrit Readings During
D-Xylose Exposure

(Mean ± Standard Error of the Mean)

| Agent | N | 5 Min. | 60 Min. | 120 Min. |
|---|---|---|---|---|
| 0% O₂ | 10 | 127.2±2.4 | 150.6±1.9 | 153.6±1.9 |
| 100% O₂ | 9 | 129.4±0.8 | 153.7±1.8 | 155.1±2.2 |
| 80% N₂O | 6 | 135.9±3.4 | 157.5±2.5 | 158.2±2.0 |
| 0.7% halothane | 6 | 125.8±2.5 | 150.0±1.3 | 153.6±2.3 |
| 0.7% methoxy-flurane | 6 | 124.6±2.3 | 148.8±1.9 | 153.2±1.8 |
| 25% cyclopropane | 6 | 133.4±2.4 | 152.8±1.2 | 156.3±1.2 |
| 2.3% ether | 6 | 131.2±2.3 | 158.9±2.9 | 160.8±2.5 |

and 120 minutes of exposure to each of the gaseous mixtures studied, N representing the number of observations. All sugars are seen to penetrate the erythrocyte as indicated by hematocrit values which increase with time. When exposed to the same gas, the rate at which penetration occurred differed for each sugar. Similarly, the rate of entry of the same sugar varied according to the gas to which it was exposed. In no instance did hemolysis occur.

Statistical comparisons were made using only the data obtained at 120 minutes. Other equilibria, including reaction velocities at other times and, perhaps most especially, the initial reaction velocities occurring within the first five minutes, were not statistically analyzed. The experimental procedure and methods were designed to obtain qualitative data that could evaluate relative changes rather than to obtain quantitative data adequate to derive thermodynamic equations. The data obtained, for example, at 5 minutes were not as accurate, as shown by their relatively larger standard errors of the mean, as were the data at 120 minutes because of the inability to stop a rapidly occurring dynamic reaction at exactly the same time (5.00 minutes) in every instance. The data at 5 minutes and 60 minutes did, however, give evidence of the trend of events prior to two hours. Thus, in every instance in which an anesthetic had a significant effect ($P < 0.01$) upon a 2-hour equilibrium, this effect was invariably present at one hour, though usually with a significance corresponding only to a $P$ value of 0.05.

Within this framework, there was no significant difference for any of the sugars between the hematocrit values after exposure for

120 minutes to 100 per cent oxygen and the values obtained after exposure of the same sugar for 120 minutes to 100 per cent helium. Since there was no difference which could be ascribed to the presence or absence of oxygen, evaluations of the effect of anesthetics were made by comparing the hematocrit values observed after 120 minutes of exposure to 100 per cent oxygen with the hematocrit values obtained for the same sugar after 120 minutes exposure to each of the 5 anesthetic agents. It was elected to employ the results obtained with 100 per cent oxygen as the sole basis for all comparisons instead of using 100 per cent in some comparisons and zero per cent oxygen in others. This was done to simplify comparisons and to reduce chances of spurious results due solely to changing the basis of comparisons. Since the results with 100 per cent oxygen and with zero per cent oxygen were not always identical, though not significantly different, statistical errors were undoubtedly introduced, but these were less than would result from capricious changing of controls for each observation. The significance of analyses using 100 per cent oxygen as the basis for comparison is summarized in table 8. Each arror indicates the presence of a significant difference ($P < 0.01$) between the results obtained when the sugar was tested in the presence of an anesthetic and the results obtained when the sugar was tested in the presence of 100 per cent oxygen. An upward arror indicates that the rate of entry in the presence of the anesthetic was significantly greater than in the absence of the anesthetic, a downward arrow indicating the reverse. The absence of an arrow indicates that sta-

TABLE 8. Summary of Results (see text)

| | Nitrous Oxide | Ether | Cyclopropane | Halothane | Methoxy-flurane |
|---|---|---|---|---|---|
| L-Sorbose | ↑ | ↓ | | ↓ | ↓ |
| D-Ribose | ↓ | | | ↓ | ↓ |
| D-Fructose | ↑ | | ↑ | | |
| D-Arabinose | | | ↑ | ↓ | ↓ |
| L-Arabinose | | | ↓ | | |
| D-Galactose | ↓ | | ↓ | ↓ | ↓ |
| D-Xylose | | | | | |
| D-Glucose | | | | | |

tistically there was no difference between the hematocrit values in the presence of and in the absence of the anesthetic. In table 8 the data from previously reported glucose studies [6] are included to facilitate comparisons.

When 2.0 ml. 0.286 $M$ inositol was added to 2.0 ml. of blood and exposed to 100 per cent oxygen for 2 hours, the hematocrit values at 5, 60, and 120 minutes were 90.7 ± 0.5, 84.1 ± 0.6, and 82.4 ± 0.6, respectively ($N$ = 8).

## Discussion

The data obtained with inositol emphasize the difference between physical permeability of erythrocytes and physiological permeability. Although inositol closely resembles monosaccharides from a molecular and structural point of view, it does not penetrate red cell membranes and so is not associated with an increase in red cell volume when erythrocytes are exposed to a 0.286 $M$ solution. On the other hand, erythrocytes increase in volume when exposed to monosaccharides which permeate red cell membranes. The control studies with inositol also emphasize that observed changes in cell volume were not due to differences in tonicity. In fact, red cell volume decreased following exposure to inositol, a direction of change perhaps unexpected since hypotonic solutions usually cause red cells to increase in size. One explanation for the decrease in erythrocyte size during exposure to inositol is that intracellular glucose, normally at equilibrium with extracellular glucose, left the erythrocytes after exposure to a glucose-free inositol medium. The validity of this was confirmed by observing an increase in extracellular glucose following admixture of whole blood and inositol. Undoubtedly shifts in other osmotically active substances also contributed to the changes.

The present data show that the entry of monosaccharides into human erythrocytes is not related to oxygen concentration. The rate of entry in the absence of oxygen is no different than in the presence of 100 per cent oxygen for all the monosaccharides tested. Entry of glucose has similarly been shown to be independent of oxygen concentration.[6]

The present data also demonstrate that certain anesthetics inhibit the rate of entry of some monosaccharides into erythrocytes, while other anesthetics accelerate the rate of entry; still others have no effect (table 8). There is no immediate relationship apparent among any of the physical properties of the anesthetics studied and their ability to alter permeability. The effects are not, for example, related to the molecular weights. The one generalization that can be stated is that the two halogenated compounds, halothane and methoxyflurane, have similar effects. Both inhibit entry. Furthermore, neither halogenated compound accelerated entry, though nitrous oxide and cyclopropane did so with certain sugars. But even halothane and methoxyflurane did not inhibit the entry of all monosaccharides. They had no effect on D-fructose, L-arabinose, D-xylose, or D-glucose while at the same time inhibiting the entry of D-galactose, D-arabinose, L-sorbose and D-ribose. The extremely high blood:fat solubility coefficients of these two anesthetics (over 300) might be expected to bear some relation to the observed effects, but if fat solubility were a determinant of the effect of anesthetics on permeability to sugars, then methoxyflurane would be expected to have more inhibitory effect than halothane, because its lipoid solubility is at least twice as great and furthermore, cyclopropane, with a blood-fat solubility coefficient greater than that of ether, would be expected to have effects between those of ether and the halogenated compounds. Instead, cyclopropane has effects quite different from those of ether on the one hand or halothane or methoxyflurane on the other. Attempts to relate air:blood solubility or vapor pressures of the various anesthetics to the effects of the anesthetics are equally unproductive. There is also no relation between hydrogen bonding capacities of the anesthetics considered or their ability to form clathrate micro-crystals.[14]

There is also no relationship between the physical characteristics of the various sugars and the effects of anesthetics. Whether the sugar is a hexose or a pentose makes no difference, nor does it make a difference whether the sugar is an aldose or a ketose. There is no relation between the D- and L-forms and the effect of anesthetics, nor does it make any

difference whether the sugars have a positive or a negative specific rotation.

Although there is no apparent rhyme or reason in the data, a hypothesis can be offered to suggest how anesthetics exert such diverse effects. But first, it is necessary to consider certain aspects of the transfer of monosaccharides across human erythrocyte membranes in the absence of anesthetics. This mechanism is not simple physical diffusion.[5, 15-20] Neither, however, is the passage accomplished by a "pump" process involving active transfer wherein metabolic energy is expended to move molecules against a concentration gradient. Instead, the movement of monosaccharides across red cell membranes is achieved by altered or facilitated diffusion.[1-3, 7, 21-24] By this is meant that, although the configuration and physical properties of monosaccharides are such that they normally are excluded from the cell membrane, monosaccharides are capable of forming complexes at reactive sites on the outer surface of the membrane to become more soluble in the membrane. The complexes so formed move toward the inner part of the membrane by a process of thermal agitation which requires the expenditure of no metabolic energy and which demonstrates the kinetics of diffusion. Transfer of monosacchardies continues until a steady state is achieved in which, as with simple diffusion, the monosaccharides is equally distributed within the aqueous phases on both sides of the membrane. The transfer process differs from simple diffusion in several aspects, among which are the rate of transfer, the fact that the kinetics of saturation may be demonstrated, and the competition observed when two or more monosaccharides are simultaneously presented to the exterior of the membrane. The difference between physical permeability, which is negligible in the case of monosaccharides, and physiological permeability due to facilitated transfer is emphasized by the fact that erythrocytes are impermeable to other molecules similar in size, structure, and physical properties, e.g., inositol.

Several model systems have been proposed to describe the details of the transfer of monosaccharides across red cell membranes by a process which is not diffusion yet not an energy-consuming one. Although other inter-pretations of the observable facts are tenable,[1, 15-17, 25] the most appropriate model is that proposed by LeFevre,[2, 3, 7-9, 22-24, 26] Widdas,[19] and Rosenberg and Wilbrandt.[27] This theory, though unproven, is more consistent with observed facts than any other. For further details, including derivation of thermodynamic equations, the reader is referred to the references. The essentials of theory may, however, be summarized for the present purposes according to the principles outlined by Le-Fevre [8] and Wilbrandt and Rosenberg.[27] The penetrating sugar molecule, S, enters into a reversible association with a mobile carrier, C, which is present at the external as well as at the internal interface of the membrane. The result of this association is the formation of a freely mobile complex, SC:

$$S + C \underset{k_2}{\overset{k_1}{\rightleftharpoons}} SC \qquad (1)$$

In this system, C remains entirely within the membrane which represents a barrier separating the aqueous compartment on the outside from the aqueous compartment on the inside. Within this membrane, C may either exist in the free state or bound with S as the complex, SC. Free S exists only in the aqueous phase. The association-dissociation reaction described by equation 1 occurs at either the internal or outside interface. The reaction is, however, always essentially at the equilibrium point because, compared to other steps in the transfer of the sugar across the membrane, this reaction occurs so rapidly. Therefore, $k_1[S][C] = k_2[SC]$ or, in terms of a dissociation constant, $K$: $K = [S][C]/[SC] = k_2/k_1$. Since the total amount of C which is available in the membrane remains constant, the percentage of C at each interface in the form of SC is [8]

$$\frac{[S]}{[S] + K}$$

Because of the thinness of the human erythrocyte membrane, a nearly linear, if not a completely linear gradient for SC may be assumed to be present within the membrane, or, in other words, a steady state is approached. The rate of transfer (V) of monosaccharide, S, from one aqueous compartment (the outside

of the cell) to a second aqueous compartment (the inside of the cell) is therefore [4, 5, 19] proportional to the difference in the concentration of $SC$ at the two interfaces, or:

$$V = D'C_t \left( \frac{[S]_1}{[S]_1 + K} - \frac{[S]_2}{[S]_2 + K} \right) \quad (2)$$

where $D'$ is the permeability constant determined by the density of the reactive sites and the physical characteristics of the membrane and $D'C_t$ is a term for the capacity of the system.[27] This equation is valid only if the diffusion constant of the free carrier, $C$, is equal to the diffusion constant of the monosaccharide-carrier complex,[18] $CS$, i.e., if $D' = D'_o = D'_{os}$.

Since the rate of transfer of a monosaccharide depends upon $[S]_1$ and $[S]_2$ relative to $K$, if $K$ is large (i.e., if a low affinity exists between sugar and carrier), then the kinetics of transport of the monosaccharide across the membrane will resemble those of free diffusion. On the other hand, if $K$ is low (i.e., a high degree of affinity exists between $S$ and $C$), then the kinetics of transport resemble more those of facilitated transfer than of free diffusion.[8] $K$ values have been calculated for many aldoses.[26] Representative values, in mols/liter, are (approximately): D-glucose, 0.007, D-mannose 0.025, D-galactose 0.04, D-xylose 0.06, L-arabinose 0.14, D-ribose 0.19, D-arabinose 1.6, with L-xylose, L-galactose, and L-glucose having values greater than 3. Calculation of $K$ values does much to explain the complex competition kinetics apparent when two different aldoses are simultaneously presented to erythrocyte membranes. The different $K$ values for aldoses furthermore explain why when individual monosaccharides are separately exposed to erythrocytes at a constant concentration (i.e., if $[S]_1$ is kept constant) the rate of entry of the monosaccharides varies.[10, 28] The relationship between $K$ values for aldoses and their rate of entry as well as competition kinetics are not equally applicable for ketoses. It has been suggested that the reason for this is that in an aqueous medium ketoses form furanose components at mutarotational equilibrium whereas aldoses are in the form of $\alpha$ and $\beta$ pyranoses.[8]

The present data demonstrate that different sugars penetrate erythrocytes at different rates. For example, after exposure of D-arabinose to 100 per cent oxygen, the hematocrit values at one and two hours were, respectively, 111.8 and 122.2 per cent of control, whereas comparable values at similar times for L-sorbose were 131.1 and 143.0 per cent. These relative rates of penetration are in general agreement with those previously reported by others.[1, 10, 28] That they are not identical is due to the fact that rates of entry depend upon details of experimental conditions in different studies, including for example, differences in concentration of sugar.[2] The relative rates of sugar movement were also influenced by the fact that although erythrocytes were exposed to only one test sugar at a time, glucose was present within the erythrocytes at the time of exposure. This intracellular glucose tied up a portion of the membrane monosaccharide transport system, the extent depending upon the ability of the test sugar to compete. Finally, listing the sugars presently studied in the order of their rates of entry is impossible on the basis of the present data (nor was it the purpose of the study) because some sugars had achieved equilibrium at one hour when exposed to 100 per cent oxygen as shown by the fact that the hematocrit values were the same at 2 hours as they were at one hour. D-Galactose, L-arabinose, and D-xylose showed essentially no change in hematocrit during the final hour of observation. In the case of these monosaccharides, all the molecules of sugar which were to enter erythrocytes during the course of two hours had done so by the end of the first hour. It, therefore, becomes impossible to say on the basis of the present data which of these three had the fastest rate of entry. Indeed, the experimental design of the present investigation was such that although an inhibitory effect of an anesthetic on the rate of entry of these 3 sugars would be and was detected (e.g., the inhibition of galactose entry by 4 of the 5 anesthetics tested), it would be impossible to determine whether any of the anesthetics accelerated their rates of entry. The present data do not preclude the possibility of the existence of such a stimulatory effect. The present data do, however, allow the conclusion that there

is no relationship between $K$ values and the effect of anesthetics. Anesthetics had their effect on rate of entry of individual sugars regardless of whether corresponding $K$ values were high or low. If anesthetics produced their effect by altering $K$ values, a more consistent relationship between the anesthetics and the $K$ values might be expected than is discernible from the present data. Furthermore, the effect of varying the anesthetic concentration on the rate of entry of sugars (*vide infra*) is difficult to explain on the ground that $K$ is affected. On these bases it is unlikely that the effects of anesthetics on monosaccharide permeability is mediated through alterations produced in the dissociation constants of the sugars.

Befor passing on to further consideration of transport mechanism, it should parenthetically be noted that the 3 sugars which had achieved equilibrium after one hour in oxygen, namely, D-galactose, L-arabinose, and D-xylose, had essentially comparable hematocrit values at the time of equilibration. There was no significant difference between the hematocrit values for the 3 sugars after one hour, all being in the range from 153 to 158. This homogenity of hematocrit values persisted at two hours for these 3 sugars. This indicates that when equilibrium is achieved the level at which equilibrium is achieved is comparable for all sugars.

In equation 2 above the transport rate is determined by two factors, the capacity $(D'C_t)$ and a saturation factor (the difference between two degrees of saturation). Because the largest value possible for the saturation factor is 1 (for the saturation degrees 1 and 0), $D'C_t$ represents the maximum transfer rate possible. Increasing $S_1$ (or $S_2$) cannot raise $D'C_t$ and so equation 2 represents a form of saturation kinetics.[31] $D'C_t$ may therefore be replaced by $V_{max}$ and the equation written [27]:

$$V = V_{max} \left( \frac{[S]_1}{[S]_1 + K} - \frac{[S]_2}{[S]_2 + K} \right) \quad (3)$$

It is apparent, therefore, that transfer of monosaccharides across red cell membranes is dependent upon $K$ (the affinity factor) and $V_{max}$ (the capacity factor). When the *relative* substrate concentration $S' \equiv S/K$ is introduced into equation 3, the equation may be rewritten

$$V = V_{max} \left( \frac{[S']_1}{[S']_1 + 1} - \frac{[S']_2}{[S']_2 + 1} \right) \quad (4)$$

In addition to the above consideration of transport of monosaccharides, the phenomenon of counter-transport within membranes may also occur under certain conditions. "Counter-transport" refers to uphill transport of a substrate against a concentration gradient but without the expenditure of metabolic energy, the energy instead being obtained from the simultaneous downhill movement of a second substrate using the same carrier. Thermodynamic and kinetic analyses of counter-transport in red cells have been derived.[18, 19, 29, 30] The details of such a process need not be reviewed, but it can be summarized by assuming a situation in which a substrate $A$ is equilibrated on both sides of a red cell membrane, *i.e.*, a membrane with a carrier system in it. If a second substrate, $S$, is added to the external aqueous medium an inwardly directed gradient for the carrier complex $CS$ is created. This in turn creates an outwardly directed gradient for the free carrier $C$. Under these conditions substrate $A$ which was originally equilibrated on either side of the membrane will then be exposed to concentrations of the carrier $C$ which are unequal on the 2 sides of the membrane. Because of this inequality of the concentration of $C$ on the two sides of the membrane, a gradient of $CA$ will arise in the membrane with more of the complex, $CA$, at the inner interface of the membrane than at the outer. Because a gradient of $CA$ is established, there will be a movement of $CA$ outwards with a release of $A$ at the external surface thereby accomplishing an uphill transport of $A$.

In considering the effect of inhalation anesthetics on the above transport mechanisms, the not unlikely assumption will be made that inhalation anesthetics are real or potential inhibitors. This assumption is valid since anesthetics are certainly not biologically inert (they produce anesthesia). Neither are inhalation anesthetics characterized by excitatory effects, though under certain conditions they may appear to be stimulatory (table 8). Indeed, once the assumption is made that the inhalation anesthetics tested are primarily inhibitors it becomes impossible to formulate a

hypothesis to explain how they could either increase or decrease the rate of membrane transport of monosaccharides. It is convenient to regard inhalation anesthetics within the context of a general classification of inhibitors of transfer systems into 5 main types.[27] Two of these 5 types would be noncompetitive inhibitors ($I_N$) which react either with enzymes ($I_{EN}$) or with carriers ($I_{CN}$). Two additional types of inhibitors would be substrate-competitive or carrier-competitive inhibitors reacting with enzyme (s) ($I_{ES}$ and $I_{EC}$, respectively). The fifth type of inhibitor of transfer systems would be substrate-competitive inhibitors reacting with a carrier ($I_{CS}$). Inhibitors of the $I_{CS}$ type can be further classified into those inhibitors which act by fixing the carrier in the interface (nonpenetrating inhibitors, $I_{CS(NP)}$) and those which act by resembling the substrate and penetrate the membrane in the form of a carrier complex (penetrating inhibitors, $I_{CS(P)}$). Within such a classification inhalation anesthetics as real or potential inhibitors of transfer processes would be classified amongst those of the $I_{CN}$ or $I_{CS}$ group rather than of the $I_{EN}$, the $I_{ES}$, or the $I_{EC}$ types since enzymes do not play a significant role in the red cell membrane transport of monosaccharides.[8, 18, 27] As indicated above, the kinetics of monosaccharide transfer in human erythrocytes can be and are best defined without invoking the presence of enzymes. Even those theories which do evoke enzyme-related mechanisms depend upon the presence of freely mobile dimers (instead of carriers).[25] Furthermore, clinical and experimental experience to date with inhalation anesthetics indicates the existence of dose-response relationships which are characteristic of competitive rather than of noncompetitive inhibitors. Therefore, inhalation anesthetics as inhibitors are better further classified as $I_{CS}$ rather than as $I_{CN}$ inhibitors. Finally, although inhalation anesthetics may have as their primary site of anesthetic action the cell membrane, inhalation anesthetics also penetrate cells and are present on both sides of cell membranes. This would therefore make them $I_{CS(P)}$ or penetrating types of inhibitors rather than surface-active, nonpenetrating $I_{CS(NP)}$ inhibitors.

The importance of the classification of inhalation anesthetics as inhibitors lies in the fact that inhalation anesthetics must be differentiated from other types of inhibitors of sugar penetration into red cells [16, 21, 23, 24, 31] because the kinetics of $I_{CS(P)}$ inhibitors are associated with distinct and characteristic reactions.[27] These reactions are those associated with the phenomenon of counter-transport. In the presence of an inhibitor of the $I_{CS(P)}$ type, after equilibration has occurred the concentration of the inhibitor, $I$, in the aqueous phase at the outside of the membrane will equal the concentration of the inhibitor in the aqueous phase at the inside of the membrane, i.e., $[I]_1 = [I]_2$. When such occurs in the presence of a carrier system, the kinetics of the transport of a substrate monosaccharide, S, added to the external medium will be:

$$V = V_{max}$$

$$\times \left( \frac{[S']_1}{[S']_1 + [I'] + 1} - \frac{[S']_2}{[S']_2 + [I'] + 1} \right)$$

$$(5)$$

where $[I']$ is the relative concentration of the inhibitor. In such a situation, an inhibitor of the $I_{CS(P)}$ type may alter transport of monosaccharides in one of two ways: either by decreasing the rate of transfer by acting as a substrate-competitive inhibitor reacting with the carrier; or, on the other hand, by accelerating the rate of transfer if the concentration of inhibitor (anesthetic) is low enough so that counter-transport becomes a significant factor. This latter circumstance can be demonstrated by consideration of the fact that the difference in the rates of transport, $\Delta V$, of substrate, S, in the absence of inhibitor $I$ and in the presence of inhibitor $I$ is obtained by substracting equation 5 from equation 4. When this is done, the difference contains the factor $(I' + 1 - [S']_1 [S']_2)$. If, then, $[I'] + 1 > [S']_1 [S']_2$ then $\Delta V$ has a positive sign and then, and only then, will there be inhibition of transport. On the other hand, if $[S']_1 [S']_2 > [I'] + 1$, then $\Delta V$ has a negative sign, in which case transport will be accelerated. (Because $[I']$ is also an independent factor in the difference between equations 4 and 5, greatest acceleration does not occur at lowest possible $[I']$ because if $[I']$ goes to zero, the difference goes to zero too). With other words, low concentrations of $I_{CS(P)}$ inhibitors can accelerate

transport [27, 31] because the force associated with counter-transport mechanisms which are activated outweigh the increase in resistance produced by the competitive inhibition. In the present investigation, the anesthetics which rapidly (within moments) equilibrate on either side of the membrane, could have served either to accelerate or to decrease the rate of entry of the more slowly penetrating sugars by competing with the same carrier system. The effect, whether one of acceleration or decreased rate of entry, would depend upon the relationship between the relative concentration of the anesthetic and the relative concentration of the sugar. It follows that an $I_{CS(P)}$ inhibitor in a concentration low enough to accelerate transport (*i.e.*, when $[S']_1[S']_2 > [I'] + 1$) can be made to inhibit transport if its concentration is increased to the point where $[I'] + 1 > [S']_1[S']_2$. In the present experiments, therefore, an anesthetic which showed a stimulatory effect on transfer at a therapeutic concentration should either have no effect (elimination of stimulatory effect) or an inhibitory effect (reversal of stimulatory effect), if administered in a higher concentration, depending on how high the concentration was increased. Furthermore, an anesthetic which exhibited no inhibition at a therapeutic concentration would be expected to show inhibition at a higher concentration. This was tested by performing experiments in which 100 per cent cyclopropane was administered in the same manner as 25 per cent cyclopropane. When D-fructose was the substrate monosaccharide and when 25 per cent cyclopropane was administered (a concentration accelerating the rate of transfer compared to oxygen-fructose) the hematocrit values were $105.3 \pm 0.9$ and $118.8 \pm 1.3$ after 1 and 2 hours, respectively. When 100 per cent cyclopropane was administered, comparable hematocrit readings for D-fructose were $96.8 \pm 0.8$ and $104.4 \pm 0.9$ ($N = 4$). Similar experiments using D-arabinose as the substrate sugar were $112.5 \pm 0.5$ and $126.7 \pm 0.6$ for 25 per cent cyclopropane (representing a stimulatory effect of cyclopropane compared to the readings in the absence of cyclopropane), and $107.4 \pm 0.3$ and $119.6 \pm 0.6$ after 100 per cent cyclopropane. Ether, on the other hand, had no effect

on the rate of transfer of either D-fructose or D-arabinose when administered in a 2.3 per cent concentration. When administered as a 9.2 per cent concentration, however, there was inhibition of transfer, the hematocrit readings for D-fructose at 1 and 2 hours being $92.8 \pm 1.6$ and $98.3 \pm 1.6$, those for D-arabinose being $103.2 \pm 1.7$ and $111.5 \pm 1.3$ Nitrous oxide, an agent with stimulatory effects on D-fructose and L-sorbose transport, was not tested at higher concentrations to see if the stimulatory effect could be reversed since it was felt unlikely that $[I']$ of nitrous oxide could be increased sufficiently at one atmosphere to result in $[I'] + 1$ being greater than $[S']_1[S']_2$.

On the basis of the above, it is proposed that inhalation anesthetics act as inhibitors of the transport mechanisms by which monosaccharides are transported across human red cell membranes. As inhibitors they are competing for the substrate sugar after reacting with the mobile membrane carrier involved in sugar transport. By virtue of the penetrating qualities of inhalation anesthetics whereby they act on both sides of the membrane, and because of counter-transport mechanisms occurring within the membrane the rate of transfer of a monosaccharide may be either increased or decreased by an anesthetic, depending upon the relationship between the concentration of anesthetic and the concentration of monosaccharide. Such a hypothesis would do much to explain how inhibitors such as anesthetics can under certain conditions result in excitation of biologic processes. Final proof of this hypothesis will depend upon kinetic analyses.

## Summary

The effects of oxygen concentration (zero per cent and 100 per cent) and the effects of therapeutic concentrations of anesthetics (nitrous oxide, diethyl ether, cyclopropane, halothane, and methoxyflurane) on the rate of entry of monosaccharides into human erythrocytes was measured *in vitro* by observing changes in red cell volume. The monosaccharides studied were D-galactose, D-fructose, D-arabinose, L-arabinose, D-xylose, L-sorbose, and D-ribose. Certain anesthetics had no effect on the rate of entry of some sugars, certain

anesthetics inhibited the rate of some sugars, and certain anesthetics accelerated the rate of entry of some sugars. On the basis of the results it is proposed that inhalation anesthetics act as inhibitors of the transport mechanisms by which monosaccharides are transported across human red cell membranes. The inhibition is a competitive one for the substrate sugar after the anesthetic has reacted with the mobile membrane carrier involved in sugar transport. Because inhalation anesthetics penetrate cells and so act on both sides of a membrane, and because of countertransport mechanisms within red cell membranes, the rate of transfer of a monosaccharide may be either increased or decreased depending upon the relationship between the concentration of anesthetic and the concentration of monosaccharide.

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**LOCAL ANESTHETIC TOXICITY**   The $LD_{50}$ of drugs continues to be used
without reference to rate of administration.   To determine the effect of intravenous
administration rate on rabbits, the critical disposal rate was determined as the
maximum rate at which each local anesthetic could be administered intravenously and
just fail to elicit signs of central nervous system stimulation.   For procaine this
rate was 1.5 mg./kg./minute as compared to 1.25 for liodocaine.   According to this
method the relative toxicity of lidocaine to procaine is less than reported by other
authors who used a rapid rate of administration.   Besides rate of hydrolysis, rate of
excretion and inherent toxicity influence overall toxicity.   (*Cahill, J. F., Aldos, J. G.,
and Wenning, A. S.: Relation Between Acute Toxicity and Critical Rate of Disposal
of Several Local Anesthetics, Canad. J. Physiol. 43: 343 (May) 1965.*)

**INTRAVENOUS REGIONAL ANESTHESIA**   In 77 patients having opera-
tions on the hand, the average dose of 0.5 per cent lidocaine was 182 mg. and the
average time before tourniquet release was 26 minutes.   Analgesia, operating condi-
tions and patent acceptance were good.   Neurologic side effects occurred in 7 patients
after tourniquet release, and of these two became unconscious.   Fall in blood pres-
sure and bradycardia also was seen, while various electrocardiographic changes oc-
curred in 7 patients.   One patient developed asystole which was successfully treated
with external massage.   (*Kennedy, B. R., and others: Intravenous Regional Analgesia:
An Appraisal, Brit. Med. J. 1: 954 (Apr. 10) 1965.*)

**INTRAVENOUS LOCAL ANESTHESIA**   Intravenous regional anesthesia was
used with good results in 90 per cent of 128 patients with doses of not over 200 mg.
of lidocaine for arms and 400 mg. for legs.   In arms it was noted that hand injection
gave much better results than anticubital injection since in the latter valves prevented
back flow of agents.   Anesthesia appears to be produced by agent gaining access to
relatively large nerve trunk through good sized veins since nerves which are small
distal to the tourniquet are poorly anesthetized while large nerves are affected distal
to two tourniquets when agent is injected between the two.   Ischmia may contribute
to the late analgesia produced by this method but not to the immediate analgesia.
(*Sorbie, C., and Chacha, P.: Regional Anaesthesia by the Intravenous Route, Brit.
Med. J. 1: 957 (Apr. 10) 1965.*)