REVIEW ARTICLES

THE PHARMACOLOGY OF MEMBRANES

THE PASSAGE OF SUBSTANCES ACROSS BIOLOGICAL MEMBRANES*

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THE meaning of the term "membrane" in biology is not well defined. In the widest sense of the word any structure or complex of structures through which substances or ions pass at rates significantly different from those at which they would pass through a similar layer of water may be considered to be a membrane.

The Variety of Membrane Structures

The cell membrane should be considered first, or perhaps we should rather say the plasma membrane. Secondly the term is applied to sheets of cells separating two different phases, as for instance in the capillary wall, the intestinal epithelium or the acini and ducts of glands. Finally the term is applied to various intracellular structures, such as the nuclear membrane and the mitochondrial membrane. Probably we should also include the walls of certain vacuoles and the cytoplasmatic reticulum. I wish to restrict my field to the processes which may be involved in the passage of substances across biological membranes and to select a few examples for illustration. For this latter purpose I have chosen to confine myself to a single group of non-electrolytes, namely the monosaccharides.

The Structure of the Cell Membrane

As far as the structure of the cell membrane is concerned it is only in recent years that methods for rational investigation have become available, and we have to admit that our knowledge is still superficial.

The prevalent view to-day is that the cell membrane consists of a bimolecular leaflet of lipids covered on the outside and on the inside by a layer of unfolded protein or mucoprotein.

The main evidence for a structure of this kind has come from British studies on the X-ray diffraction patterns of the myelin sheath of nerves. From electron micrographs this sheath is known to be built up by concentric layers and from the embryological studies of Geren (1954) it appears that each of these layers must be formed from two layers of the cell membrane of the Schwann cell, which become wrapped around the axon after its invagination into the Schwann cell. From studies on the radial repeat periods of fresh as well as dried and lipid extracted myelin sheaths, Finean came to the conclusion that two such Schwann cell membranes in apposition had a thickness of about 170 Å, and that each of these membranes consisted of a double layer of lipids with protein monolayers

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attached on either side. Fig. 1 from Engström and Finean's book (1958) gives a schematic representation of these interpretations. The radial repeat period representing two membranes is indicated, and in each of these are seen the "hairpins" of the phospholipid and cholesterol molecules with their non-polar ends facing each other and with protein layers attached to the polar ends.

One major function of the cell membrane is evidently to prevent or at least greatly to impede the loss of essential components of the cytoplasm, but at the same time the metabolism demands that some substances can pass or be passed at fairly high speeds across the membrane. A highly discriminative handling of different substances is therefore a main feature of the function of the cell membrane. A membrane like the one suggested by Finean may well account for some of this discriminative handling but

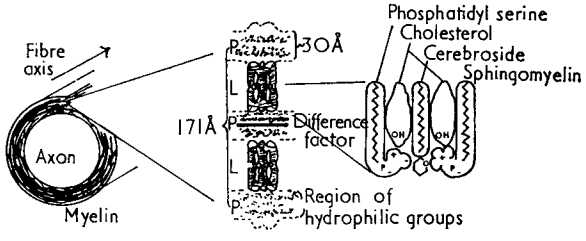

FIG 1. Drawing showing possible arrangement of molecules (including phospholipid-cholesterol complex) in the structural unit of the myelin sheath.

there are a number of observations which call for considerable modification of this crude model.

At the present stage of our knowledge it seems reasonable to distinguish between five main groups of mechanisms by which substances or ions may pass the cell membrane. These are set out in Table I.

If the cell were completely surrounded by a lipid double-layer with no crevices in it simple diffusion of substances into and out of the cell would be impossible. To diffuse through such a membrane a substance would first have to make a jump from a water phase into the lipid layer and later another jump from the lipid layer into a water phase. To make the first jump a strongly polar substance would require a considerable activation energy in order to break the hydrogen bonds to the water molecules, and since this energy would greatly exceed the average thermal agitation energy of the molecules such jumps would occur only on rare occasions. A non-polar substance—not being held by such strong ties to the water phase, could much more frequently obtain sufficient energy to make the jump into the lipid layer, but it would, on the other hand, require some energy to pass from the lipid layer into the cytoplasm.

Activated Diffusion

A theory for such activated diffusion, as it has been called, has been developed by Danielli (Davson and Danielli, 1952). Very briefly stated it predicts that the permeability of a cell membrane for various substances

is a function of their lipid-water partition coefficients and their molecular sizes. These predictions are, as we all know, in good agreement with the findings in a number of studies on the rates of penetration of a variety of substances into different cells, and there is therefore hardly any doubt that the penetration of a considerable number of substances through the cell membranes is a matter of activated diffusion.

Water-filled Pores

There are, however, a number of exceptions.

One of these is the penetration of some lipid-insoluble substances of very small molecular size, as for example water and methanol which pass many cell membranes much faster than one might expect from their oil-water partition coefficients. It is therefore generally accepted that they penetrate by simple diffusion through minute water-filled "pores".

The major evidence for the existence of water-filled channels through the cell membranes has come from studies of the passage of water across the membranes. Experiments on a variety of cells have shown that the

TABLE I

MECHANISMS OF PASSAGE THROUGH CELL MEMBRANES*

"Passive"	*Simple diffusion*	—through continuous water phase	Rate of passage proportional to concentration difference across membrane
	Activated diffusion	—through a non-water phase	
	Facilitated diffusion	—reversible binding to membrane carrier moving by thermal agitation	
"Active" (transportation immediately dependent upon cellular energy)	*Facilitated (propelled) penetration*	—reversible binding to membrane carrier, the movement of which is accelerated by cellular energy	Rate of passage showing upper limit ("saturable system")
	Unidirectional uphill transportation	—transportation mechanism undefined, carrier system possibly involved	

* Formation of vacuoles by pinocytosis may be important in some cases of cellular uptake but does not per se involve passage through the cell membrane.

permeability constants for diffusion of water across the membranes, as determined by isotopically labelled water, are generally considerably smaller than those determined from the net flux of water, produced by differences in the osmotic pressures on the two sides of the membrane. As emphasized by Ussing (Koefoed-Johnsen and Ussing, 1953), this fact that the membrane offers a smaller resistance to bulk flow of water than to diffusion of water molecules must mean that there are continuous water filled channels through the membranes. Our present means for evaluation of the equivalent diameter of these pores are beset with many uncertainties, but for what they are worth, they have led to figures of the magnitude of 7 Å (Paganelli and Solomon, 1957; Mullins, 1959). Such pores would obviously allow free diffusion of nothing but the smallest molecules. Even the diffusion of very small ions through the pores would be greatly restricted if the pore walls were occupied by fixed electric charges of the same sign.

There are a number of substances which should be unable to pass a lipid membrane with pores of this size at significant rates by either simple diffusion or activated diffusion because their molecules are too big to pass through the pores and too polar to become detached from the water molecules with any frequency worth mentioning. Among these we should expect to find the monosaccharides, and indeed a number of monosaccharides have been found to be unable to cross many cell membranes. It is, however, a fact that certain others, for example in some instances their stereoisomers may pass the same membranes at fairly high rates.

Mechanisms other than Simple Diffusion and Activated Diffusion

Evidently, some monosaccharides, and only some, are capable of passing the cell membranes by mechanisms other than simple diffusion and activated diffusion. To illustrate this point I shall discuss in a little more

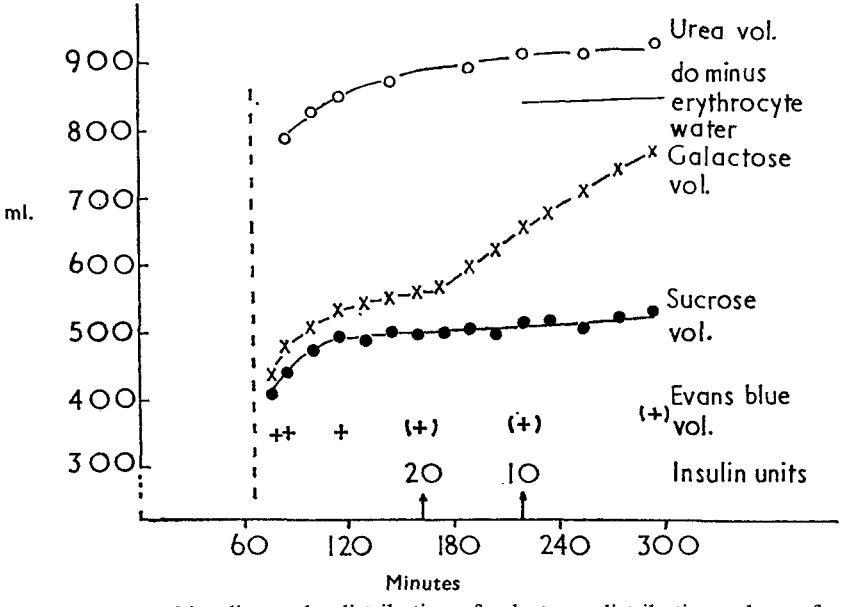

FIG. 2. Effect of insulin on the distribution of galactose; distribution volumes for sucrose, urea and Evans blue determined for comparison. Weight of hind limb preparation 1,050 g. Start of perfusion at zero time. 2·3 g. of galactose + 0·8 g. of sucrose + 1·8 g. of urea + 20 mg. of Evans blue dissolved in 10 ml. added at 65 min.; addition indicated by vertical broken line.

detail the passage of monosaccharides across the cell membrane of skeletal muscle and heart muscle and the effect of insulin on these passages.

My own limited experience in this field started in the early forties when, together with Professor Lundsgaard and Dr. Gammeltoft, I was engaged in some studies on isolated, perfused hind-limb preparations of the cat (Gammeltoft, Kruhøffer and Lundsgaard, 1944). As an indication of the marked steric specificity of the processes involved in passage of monosaccharides into muscle cells it was found in these studies that insulin is

without effect upon the cellular uptake of fructose, in contrast to its well-known uptake-promoting effect on glucose.

Some years later Levine and his colleagues (Levine, Goldstein, Huddlestun and Klein, 1950; Goldstein, Henry, Huddlestun and Levine, 1953) made the important discovery that the uptake-promoting effect of insulin is not confined to glucose. In eviscerated, nephrectomized dogs these investigators demonstrated that the rate of distribution in the body of certain sugars, such as D-galactose, D-xylose and L-arabinose, was greatly accelerated by insulin, whereas no such effect was exerted on the distribution of others, such as D-fructose.

Levine's publications gave rise to intensive studies on the passage of various monosaccharides across the muscle cell membrane. Personally I

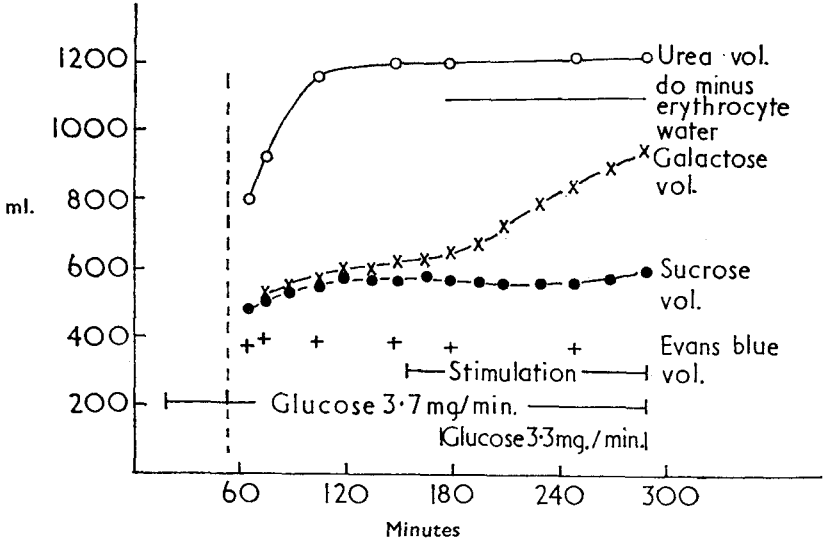

FIG. 3. Effect of muscular exercise on the distribution of galactose; distribution volume for sucrose, urea and Evans blue determined for comparison.

was able to confirm his findings in the somewhat simpler preparation of the isolated, perfused hindlimbs of the cat. Figs 2 and 3 show some results from these studies (Huycke and Kruhøffer, 1955).

Some time after the recirculation of blood through the hindlimb preparation had been established in these experiments a mixture of known amounts of D-galactose, Evans blue, sucrose and urea was added to the perfusion blood. The figures show the apparent distribution volumes of these substances as a function of the subsequent time. It will be noted that initially the galactose volume increases only slightly faster than the sucrose volume.

However, as seen in Fig. 2, shortly after the addition of insulin the galactose volume starts to increase much more rapidly and after some 2 hr. it has approached the urea distribution volume.

Fig. 3 shows the results from a similar experiment in which, however, a period of muscular exercise (induced by electric stimulation) was substituted for the addition of insulin. It is apparent that muscular exercise has the same effect on the rate of distribution of galactose as has insulin. It may be added that other experiments showed that muscular exercise, just like insulin, is without effect upon the rate of cellular uptake of fructose.

Later experiments, in particular those of Park, Reinwein, Henderson, Cadenas and Morgan (1959) on isolated perfused rat hearts, have added a great deal of further information which strongly supports the view that the passage of glucose and various other monosaccharides across the muscle cell membrane occurs by one of the processes commonly called facilitated diffusion and facilitated penetration.

Facilitated Diffusion and Facilitated Penetration

Before discussing these more recent results it seems appropriate to say a few words on these types of transport mechanisms.

Both of them are characterised by the fact that the substance under transport does not pass the membrane in the free state, but is attached to a carrier molecule or a carrier group. In facilitated diffusion the carrier is assumed to shuttle back and forth through the membrane merely by thermal agitation, whereas in facilitated penetration the back and forth movement of the carrier is speeded up at the expense of metabolic energy.

In both types of transportation the reaction between carrier and substance is assumed to be a simple reversible chemisorption, requiring no cellular energy and taking place in the same way at both sides of the membrane.

The blood haemoglobin in the body constitutes a good macro-scale model for the mechanism of facilitated penetration, with the propelled movement of the carrier between alveolar air and tissues being taken care of by the circulation.

A non-circulating haemoglobin solution separating two other phases serves as a good model for the mechanism of facilitated diffusion.

From our knowledge of such systems it is readily seen what the fundamental properties of the systems of facilitated diffusion and penetration must be. I shall propose then to enumerate these basic properties and for each of them to mention briefly the related findings from experiments on the passage of monosaccharides across the muscle cell membrane.

Increased transportation capacity. (1) As the first point it is obvious that the presence of the carrier will greatly increase the transportation capacity for those substances which can be attached to it. This tallies well with the fact that some monosaccharides may pass the cell membrane at fairly high rates, whereas others hardly pass at all.

Saturation of the transport system. (2) The transport system is saturable, that is, it is capable only of transportation up to a certain rate, but this and the saturation concentration may vary from one substance to another. Thus the haemoglobin system will be saturated with CO at a lower tension than with O_2, but the more ready release of O_2 from haemoglobin may

endow the Hb-system with a higher transporting capacity for O_2 than for CO, if only O_2 is offered at a sufficiently high tension.

The system of glucose uptake in muscle cells is also saturable, that is, the rate of glucose uptake increases only up to a certain limiting value with increasing extracellular glucose concentrations. It appears, furthermore, that this limiting rate is the same whether insulin is present or not. Much higher external glucose concentrations are, obviously, required to reach the limiting rate when insulin is absent (Lundsgaard, Nielsen and Ørskov, 1939; Park, Reinwein, Henderson, Cadenas and Morgan, 1959).

The release of the transported substance unchanged. (3) The substance transported should be released as such on the exit side, and if not subsequently converted it should be demonstrable there, and it should finally reach a similar concentration to that on the entrance side.

For non-metabolisable, penetrating sugars like D-galactose and 3-methyl-glucose it has been found that the intracellular concentration in muscle cells, after adequate time, approaches, but does not exceed a maintained extracellular concentration. Glucose is, however, converted chemically inside the muscle cells and it is therefore not surprising that it has only been possible to demonstrate free glucose there under special conditions, namely, with high extracellular concentrations and in the presence of insulin.

Ambi-directional operation. (4) The transport systems will work equally well in both directions.

It has been found that once heart cells have been loaded with a non-metabolisable, penetrating sugar it can be washed out of them at a comparable rate by switching to a perfusion fluid not containing the sugar.

Competition between transport substances. (5) When two substances are present which both combine with the carrier one will tend to depress the transportation of the other, and the strongest depressive effect will be exerted by the substance which has the higher affinity for the carrier.

Such mutual effects are well known for CO and O_2 and similar competitive effects have been demonstrated for the uptake of different sugars in muscle cells. It seems also likely that the depressive effect of phloridzin on such uptakes is due to a competition for a membrane carrier.

These examples will suffice to show, that there is substantial evidence for the view that the passage of various monosaccharides across the muscle cell membrane occurs by facilitated diffusion or facilitated penetration, and that the carrier involved has a definite specificity, but not at all a specificity which is entirely confined to glucose.

Monosaccharides in Other Cells

Similar systems are unquestionably engaged in the uptake of monosaccharides in other cells. Intensive studies, in particular by Le Fevre (1954) have strongly suggested that this may be so in the case of erythrocytes. There is, however, one major difference, namely that insulin has no effect upon the entrance of monosaccharides into erythrocytes.

At the moment we are without knowledge of the intimate nature of the membrane carriers involved in these transports. It seems possible,

however, that some clue to the chemical structure of the sorptive site of the carrier may be derived from a recent suggestion by Le Fevre and Marshall (1958). They pointed out that those monosaccharides which penetrate the erythrocyte membrane are apt to assume a particular bending of the ring, the so-called C–1 resting chair conformation, whereas those which do not penetrate are more apt to assume other conformations.

Another problem which remains unsolved is how insulin is capable of accelerating the influx and outflux of various sugars from muscle cells. From the promptness of this effect and from the size of the insulin molecule it seems likely that it is caused by an action on the outside of the membrane. In trying to visualise the type of action involved it would seem to be a fact of importance that the maximum rate of glucose uptake appears to be the same whether insulin is present or not. This, in my opinion suggests that insulin does not affect the carrier system directly, but that it rather acts by removing some diffusion hindrance located between the free extracellular fluid and that site in the membrane outside, where glucose becomes attached to the carrier.

The Passage of Monosaccharides across Sheets of Cells

Let us now consider a somewhat different subject namely the passage of monosaccharides across membranes made up by sheets of cells, and let us among these first consider the capillary wall.

It is a common view to-day that only two processes are involved in the passage of substances across the capillary wall. These are diffusion through pores for lipid-insoluble substances like monosaccharides, and activated diffusion through the entity of the endothelial cells for highly lipid-soluble substances.

That pores do exist in some capillaries has been demonstrated by electron-microscopy, but this method has failed to visualise any pores in muscle and connective tissue capillaries and in the capillaries of the central nervous system (Bennett, Luft and Hampton, 1959).

It appears that in the case of muscle and connective tissue capillaries we still have to believe in the existence of pores considerably larger than those traversing the common cell membrane since various small-molecular, lipid-insoluble substances appear to pass through these capillary walls at rates which are fairly proportional to their free diffusion rates.

In the case of the capillaries of the CNS the situation may be quite different. Some recent observations by Dr. Crone (Crone, 1960) in our department may serve to illustrate this point.

The technique in these experiments was as follows. A single brief injection was made into an artery supplying the organ under investigation. The solution injected contained known proportions of Evans blue and the substance, the transcapillary passage of which was to be studied. If the substance was already present in the blood, the substance was given in a labelled form. Over a subsequent period of some 10–15 sec. fractional collections were made of the venous blood leaving the organ—in the case of the brain these collections were made from the sagittal sinus. On the assumption that no Evans blue was lost during a single passage

through the capillaries the extraction percentage for the substance under study could be determined. For the first venous samples these extraction percentages should very nearly represent the true outflux of the substance through the capillary wall, since, with the rapidly rising arterial concentration, the back-flux would be negligible in these early periods.

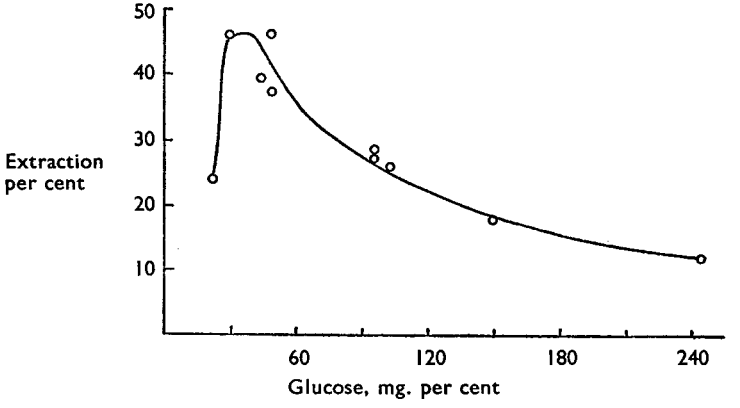

FIG. 4. Initial percentage loss of radioglucose from blood during a single passage through the brain vessels of dogs as determined at various blood glucose concentrations.

Now, in the case of the brain it was found that the initial extraction percentage for fructose was only a few per cent, and that up to plasma concentrations of some 200 mg. per cent there was no indication of a variation in its magnitude. These findings were interpreted to mean that

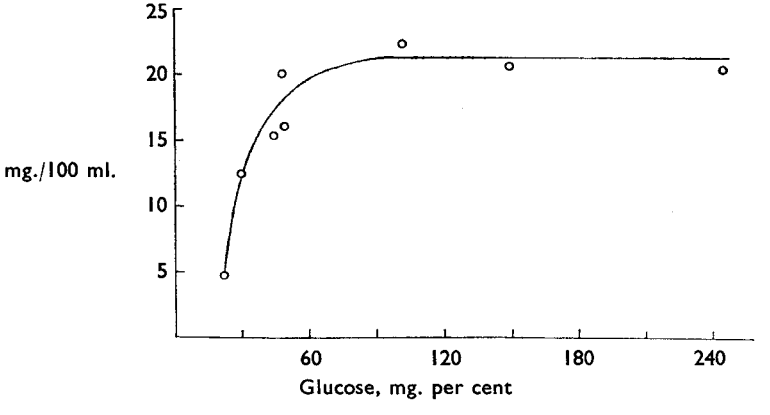

FIG. 5. Relationship between the blood glucose concentration (abscissa) and the outflux of glucose per 100 ml. of blood passing through the brain vessels (ordinate). Calculated from the data of Fig. 4 by the application of a small correction for simple diffusion outflux.

small amounts of fructose could be lost by simple diffusion through the capillary walls.

In the case of labelled glucose the findings were quite different. From Fig. 4 it will be seen that the initial extraction percentage for this substance was found to be smaller and smaller at increasing levels of the blood glucose concentration. These findings obviously indicate that a saturable process is involved in the blood to brain transport of glucose. A more striking illustration of this is seen in Fig. 5. Here the amounts of glucose lost per 100 ml. of blood have been calculated, and from these have been subtracted those amounts which, according to the fructose experiments, should have been lost by simple diffusion. It is seen that this excess amount of glucose lost per 100 ml. of blood (ordinate) rises with increasing blood glucose concentrations (as determined by a non-specific "reduction" method) until at a value of some 60 mg. per cent it reaches a limiting value.

Two important facts have thus been borne out by Crone's studies. Firstly, the very low extraction percentage for fructose as compared with figures of 40–60 per cent obtained in other capillaries, adds further evidence to the view that the blood-brain barrier is much more tight than other blood-organ barriers. Secondly, they show that there is a preferential penetration of glucose by a saturable process through the blood-brain barrier.

The nature of this important process, which provides for adequate supplies of glucose to the brain at even quite low blood glucose concentrations is not yet known. It seems quite reasonable that it could be a matter of facilitated diffusion or penetration across each of the cell membranes of the endothelial cells.

On the other hand, it cannot be excluded that it may be a matter of an uphill transportation. As we all know transportation of this type is involved in the passage of monohexoses across other cell sheets such as the renal proximal tubules and the intestinal epithelium.

The intimate nature of these directional mechanisms by which glucose can be taken in at a lower concentration at one end of the cell and delivered at the other end at a higher concentration remains obscure.

The phosphorylation-dephosphorylation hypothesis has been dismissed because it has turned out that sugars, which are not phosphorylated, may be transported uphill almost as fast as glucose. All present views are therefore based upon mere speculations.

If I should make a guess as to the nature of these mechanisms I would make it in accordance with the economy principle which seems to have a wide application in biology. In other words, I would suggest that the uphill transportations of monosaccharides make use of the same carriers as those involved in facilitated diffusion and penetration, but that at the exit side of the membrane the carrier is converted, somehow, into a form with reduced sorptive affinity for monosaccharides, and that upon return to the entrance side, it is reconverted into the original form.

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