GENERAL DISCUSSION.

Professor A. Krogh (*Copenhagen*) (*communicated*) added : Lundegårdh has recently demonstrated a secretory uptake of anions from very dilute solutions by active cells in plant roots and measures the metabolism necessary to bring about this transport; he further suggests a possible mechanism for this process. The analogy to the active absorption observed in my laboratory in a number of fresh water animals is very striking and I have been able to show for the frogs skin that in addition to Cl^- and Br^- NO^- is also actively absorbed although at a slower rate.

I desire to emphasise again that these active processes, which require energy, should not be classed with membrane properties.

Dr. T. Teorell (*Uppsala*) said :

(1) When discussing " active transport " of a substance by living cell systems as suggested by Professor Krogh, it is advisable to distinguish between *permeability* (ability of penetration) and *driving forces*. The *flow* of the substance across a cell or tissue boundary is proportional to these two independent factors. So far, previous investigators seem to have concentrated on the nature of the permeability (pore-, solubility-theory, etc.). It is generally not at all clear what is meant by driving forces. Hitherto, only the " osmotic " force has been emphasised by biologists, and this has been spoken of as pressure or concentration gradients. If transport of electrically charged particles is considered, another driving force must be taken into account, namely that of an *electrical potential gradient*. This may be located within the boundary or membrane across

which the transport occurs. As can be shown both theoretically and experimentally, the combined influence of osmotic and electrical gradients may lead to transport of material *against* a concentration gradient.[1] Many such " up-hill migrations," in biology described as " active transport," may be related to the existence of a P.D. in the secreting or resorbing area. It should be emphasised that such an effect of a P.D. does not require any actual flow of current (no local current, therefore no identity with electro-phoresis !). Some fundamental problems to be considered in relation to permeability forces are :—(*a*) The magnitude of P.D. within the cell or tissue boundaries (membranes) performing active transport, (*b*) The nature of such P.D.'s and how they are maintained.[2]

(2) The stomach mucosa provides a very suitable object for investigations of permeability problems *in vivo*. The following results were obtained in experiments on narcotised cats.[3] The mucosa seemed to show the same behaviour as a collodion sack. Interdiffusion of ions could take place between the stomach contents and the mucosa cells or the blood. Surprisingly, H ions seem to be freely permeable through the mucosa surface. The concentration-time curves were generally pronouncedly exponential, thus allowing an approximate determination of a diffusion constant D . ($C_t = C_o e^{\frac{-D.A.t}{v}}$, where C_t and C_o are the conc. at the time t and o, A is area and v is volume).

The following somewhat averaged figures were found (all solutions were approximately isotonic with the blood) :

A. *Diffusion from the Stomach Cavity into the Mucosa—*

(D figures are given per cm² per minute when the concentration gradient is unity. The figures have to be multiplied by 10^{-3}.)

Glucose	0·3	$(Na)HCO_3$	0·6
H(Cl)	1·4	(K)Br	0·7
$H(ClO)_4$	2·4	(Na)Ac	0·7
$H_3(PO_4)$	0·5	(H)Ac	2·5
H(La)	4·3	(" free " diffusion of HCl against water : $D = 1{\cdot}6 . 10^{-3}$)	
H(Ac)	7·2		

B. *Diffusion of Cl Ions from the Mucosainto the Stomach Cavity—*

Solution in the cavity.	$D \times 10^{+3}$.
H_2O	0·2
KNO_3	0·4
KBr	0·5
HCl	0·7
HLa, HAc	>0·9

The acidity of the weak acids, H-lactic and HAc, is seen to decrease " abnormally " fast, probably because of a solubility effect on the undissociated molecules.

The possibility of a " back diffusion " to the mucosa of hydrochloric acid initially secreted into the stomach cavity and a simultaneous outward diffusion of alkali chloride from the stomach cells has been suggested as an explanation for the variations of gastric juice acidity (and chloride content).[4]

Professor H. Handovsky (*Ghent*) said : Professor Krogh, in discussing the morphological variations in animal cell membranes, stated that " the membrane is an integral and perhaps variable part of the cell." From a physiological point of view this variability seems to me to be of highest significance and as it does not appear to have been sufficiently emphasised at this meeting, I should like to put forward some suggestions.

Animal cell membranes are, upon one side at least, in contact with colloidal suspensions. If this suspension is blood, the composition is that of sols of different proteins, hydrophilic lipoids, hydrophobic sterols,

[1] *Proc. Nat. Acad. Sci.*, Wash., 1935, **21**, 152 ; *J. Gen. Physiol.*, 1937, *in press.*
[2] In this connection, see remarks on pp. 1054 and 1086.
[3] For details as to the technique see *Skand. Arch. f. Physiol.*, 1933, **66**, 225.
[4] *Loc. cit.*[1] and *Acta Med. Scand.*, 1935, **85**, 518.

etc. The numerous studies of single cell (erythrocyte) permeability have shown that many physical properties are altered after the cells have adsorbed colloids upon their surfaces. Other cell membranes, such as those of the blood capillaries, also adsorb colloids. Since the colloid composition of the blood changes under varying physiological and pathological conditions, the cell membrane will then have different exterior layers. These changes may (and occasionally must) considerably alter the behaviour of the cell membrane. Thus, permeability constants, measured by analyses of substances on either side of the membrane, are probably related directed only to the physiological state of the membrane at the moment of measurement, and change with change in the physiological state.

For instance, consider a porous membrane. The laws of diffusion and dialysis, the Donnan equilibrium, determine the permeability of the membrane. If, however, the pores are filled with albumen or with a sterol, the membrane is no longer porous, and other laws, the laws of solubility in the membrane, govern its permeability. As an illustration of the great variability of a membrane under different conditions, I should like to cite one of our experiments: Through an artificial membrane of nitrocellulose (Zsigmondy's *Ultrafeinfilter*), at about one atmosphere pressure, there passed 41 c.cm. of water in 100 minutes. The addition of caffeine (0·3 per cent.) did not affect the velocity of the passage of the water. After a 2½ per cent. seralbumen solution had been passed, however, only 13·2, 13·7, 13·5 c.cm. of water was transmitted in 100 minutes, and none of the albumen went. With a caffeine-seralbumen solution having an albumen concentration of 2½ per cent. and of caffeine 0·3 per cent., the membrane passes 20·7, 21·8, 18·3 c.cm. of water in 100 minutes, and again none of the albumen passes. In this we seem to have a good model for the study of the varying physiological behaviour of a membrane.[5]

Dr. R. B. Dean (*Cambridge*) said: With regard to the mechanism suggested by Professor Krogh for the concentration of glucose by combination with a phosphorus compound in the presence of one enzyme and subsequent breakdown of this compound after diffusion in the presence of another enzyme I desire to call attention to the fact that, so far as I know, enzymes are true catalysts and will catalyse a reaction equally well in either direction. I do not see how the necessary energy could be put into the system.

If there is a flow of a substance such as oxygen across a membrane and this substance forms a complex permeable in the membrane with a substance to which the membrane is impermeable, *e.g.*, a hypothetical $[O_2Cl]^-$ complex, then such a mechanism will supply the necessary energy to transfer a substance against a concentration gradient.

Dr. Ancel Keys (*Rochester, Minn.*) said: The great majority of animal membranes are composed either of living cells or are inseparably associated with living cells. The fact that metabolic processes are taking place in these systems undoubtedly complicates their study, but that is not necessarily a good reason to exclude them from consideration as membranes. Membranes derive their claim to special and separate consideration chiefly because they represent morphological boundaries across which exchanges take place between two phases. From the biological point of view the membrane generally cannot be considered apart from these two phases and, in fact, many membranes such as the surface membranes in cells, can have no separate existence. It must be obvious, then, that of living organisms we usually have to deal with a "system" in which the membrane has a central but not an independent place.

Krogh emphasises the special virtues, for experimental study, of "exudation membranes," because they are themselves inert and are more or less independent of the liquid phases they separate. It will be granted

[5] See also the former publication of Handovsky and Uhlenbruck, *Klin. Wchn.*, 1925, p. 1401.

that the investigation of the properties and functions of these membranes may be less difficult than in other systems. At the same time it must be admitted that relatively few membranes of biological importance belong to this restricted " ideal " class of structures. From the biologist's viewpoint it would seem to be necessary to be ready to deal with any and all biological membranes, regardless of the fact that most of these membranes may not conform to the simplest first expectations of an ideal, passive equilibrium. Krogh's classification should help much in preventing oversimplifying assumptions ; I hope it will not daunt the worker who recognises the difficulties in non-ideal systems from continuing the experimental attack.

Professor E. Manegold (*Dresden*) said : In considering permeation through porous systems it is essential to bear in mind the question whether we are concerned with a capillary system or a space-energy system (*i.e.*, pores of molecular dimensions). If we take a membrane separating reservoirs having concentrations of C_i gm./c.c., on the inlet and C_o gm./c.c., on the outlet sides respectively, the permeability coefficient of a capillary system is defined by

$$\delta^*d = \frac{S}{Ft(c_i - c_0)} \text{ cm.}^2/\text{sec.},$$

where S is the mass, in grams, of matter which at the temperature of the experiment passes in t seconds through F cm.^{2} of a capillary system of thickness d cm.

If, however, we are concerned with a space energy system we take, in place of the difference in concentrations in the reservoirs, the concentration gradient between the inlet and outlet surfaces of the membrane ($c_i' - c_o'$). In the simplest case there is a relationship between the reservoir and the surface concentrations depending on a Henry distribution equation, so that we can write $C_i' - C_o' = \tau(C_i - C_o)$, where τ is the distribution coefficient. For the reduced permeability coefficient $(\delta^*d)_r$ we obtain thus :

$$(\delta^*d)_r = \frac{S}{F \,.\, t \,.\, \tau(c_i - c_0)} \text{ cm.}^2/\text{sec.}$$

For example, in the case of rubber the two coefficients are, at 25° C. :

CO_2	$(\delta^*d) = 0{\cdot}99 \times 10^{-6}$ cm.^{2}/sec.	$(\delta^*d)_r = 1{\cdot}04 \times 10^{-6}$ cm.^{2}/sec.
H_2	$= 0{\cdot}34 \times 10^{-6}$,,	$= 34{\cdot}4 \times 10^{-6}$,,

The order of the two dimensions is inverted in the two cases because τ for CO_2 is about 1 and for H_2 about 0·01. Similar results are obtained for permeation through glass membranes and dried collodion films.

Professor A. Krogh (*Copenhagen*) in reply (*communicated*) : Dr. Teorell's suggestion that a potential difference is the driving force in the active transport of charged particles through cells may be correct for some special cases, but more often the selectivity of the transport mechanism is too high to admit this suggested explanation.

The mechanism for breaking down the phosphorus compound of sugar in kidney cells is certainly not simply an enzymatic one, as suggested by Dr. Dean, because the breakdown requires the supply of a considerable amount of oxidation energy, and the same holds for the transport of anions against concentration gradients in plant roots and many animal " membranes."

I agree with Dr. Keys that all biologically important " membranes " should be studied and I am myself engaged upon an extensive study of certain types ; I wish, however, to emphasise that they should not be studied as problems in permeability until one is reasonably certain that the " membrane " selected for study is not organised as a highly specialised mechanism for doing transportation work of some kind.