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THE VITELLINE MEMBRANE OF THE UNFERTILIZED HEN'S EGG : ELECTROLYTE AND WATER TRANSPORT

T. RYMEN and J. STOCKX

Laboratorium voor Fysiologische Scheikunde, Rijksuniversiteit, Gent (Belgique)

SUMMARY

In vitro measurements of different electrolyte fluxes and membrane potentials point to the fact that the ovovitelline membrane is charged and asymmetrical. It's directional specificity to ion transport and accompanying volume flux is described for several electrolytes. Specific effects are induced by phosphate ions. A number of experiments, showing the dependence of the phosphate-membrane interaction on several variables such as concentration, temperature and duration of the phosphate pretreatment, suggest that this interaction represents more than just the result of the membrane's ion exchange behaviour and that it may involve an enzymatic mechanism.

INTRODUCTION

It is well known that the composition and physicochemical properties of egg white and yolk differ in many respects (PUCHER, 1927 ; ROMANOFF and ROMANOFF, 1949 ; NEEDHAM, 1950 ; ROMANOFF, 1960 ; GILBERT, 1971). As a consequence a great difference in osmotic pressure ($\simeq$ 1.8 atm.) exists between white and yolk of freshly laid eggs that persists for at least two months, diminishing slowly in value (WLADIMIROFF, 1926 ; RICE and YOUNG, 1928 ; KENZO, 1929). Moreover the concentration differences are different for almost each substance present (STRAUB and HOOGERDUYN, 1929). MOORS and STOCKX, 1968, and MOORS, 1969, found great differences in nucleolytic enzyme activities between white and yolk, the vitelline membrane acting as a barrier. These enzymes were shown to be subject to aggregation-dissociation equilibria, depending on concentration, temperature and ionic strength, the different aggregation forms showing different activities (MOORS, 1969 ; DE MOOR and STOCKX, 1969). MOORS, 1969, finds also several nucleolytic enzymes of yolk and white in the membrane itself, probably in a modified (bound) state. ETHEREDGE *et al.* (1971), HAALAND *et al.* (1971) and RHEA and ROSENBERG (1971) identified a soluble and a

membrane bound ATPase in the ovovitelline membrane. An ATPase of the white (mainly localized in thick white) was studied in this laboratory a few years ago (VAN MAELE, 1969). For all these reasons it is obvious that the ovovitelline membrane may play an active role in the morphogenesis of the chick. Despite earlier investigations (STRAUB and HOOGERDUYN, 1929 ; NEEDHAM, 1931 ; SMITH and SHEPHERD, 1931 ; ORRU, 1939, 1940 ; JORDANOV *et al.*, 1966 ; DE BOECK *et al.*, 1971) the most important aspects of its permeability properties are still obscure. A few results about the membrane of unfertilized chicken eggs will be described in this paper.

METHODS AND MATERIALS

The membranes were from unfertilized white Leghorn eggs, less than a week old and kept at 1°C. It was ascertained that ageing of the egg in these conditions does not interfere wich the membrane characteristics. After removal of the white and the chalazae, the blastodisc was cut out from the membranes, that were prepared by rinsing off adhering white and yolk with 0.9 p. 100 NaCl and a wash with redistilled water, in a way that white and yolk sides were known. (DE BOECK *et al.*, 1971). The most used experimental set-up consisted of two plexiglass cells equipped with water mantles interconnected so as to form one thermostatic unit (maintained at 20°C, unless stated otherwise). Both compartments communicated by a circular hole with a diameter of 1 cm and were provided with platinum electrodes, so as to form two cells for conductivity measurements. The membrane was pressed between them with the aid of Parafilm at the periphery.

J_s ([1]) was determined either by conductometry or colorimetry in samples taken at regular intervals.

J_v ([1]) was measured from the difference in levels read with a cathetometer. The electrical potential differences induced by NaCl were measured with Cl^- specific electrodes (Orion, model 92 17 00), yielding membrane potentials after correction for concentration and/or asymmetry potentials (junction potentials in the measuring circuit).

Experiments were performed at pH 5.8, 7.0 and 8.6.

All neutral salt solutions were buffered at pH 5.8 by 5×10^{-3} M cacodylate buffer and at pH 7.0 and 8.6 by 5×10^{-3} M *tris*. Prior to use the membranes were soaked for one hour in 0.1 M of the same buffer as used in the experiments to be performed. Subsequently they were washed three times during five minutes in 5×10^{-3} M of that buffer. When changing the concentration difference or reversing it the membranes were washed in the same way.

Phosphate or arsenate was adjusted with NaOH to the wanted pH, starting from the acid components. In this case, the electrolytes are good buffers themselves and the membranes were used without further pretreatment. Redistilled water was used for washing them.

RESULTS AND DISCUSSION

A. — *Permeability coefficient and NaCl or KCl activity*

According to Teorell's extended « fixed charge theory » ([2]) (TEORELL, 1953) and substituting concentrations by activities, the following equation holds for the flux

([1]) J_s : solute flux, in mmoles min^{-1} cm^{-2} ;
J_v : volume flux, in ml min^{-1} cm^{-2} ;
W → Y ; Y → W : from white side to yolk side and vice versa.

([2]) In Teorell's theory the fluxes of cations and anions (J_i^+ ; J_i^-) are expressed as electrical currents (coulomb min^{-1} cm^{-2}). They are related to J^+ and J^- (fluxes in gram ion min^{-1} cm^{-2}) by :

$$J^+ = \frac{J_i^+}{F} \quad \text{and} \quad J^- = \frac{J_i^-}{F}$$

of a single 1-1 valent electrolyte across a moderately positively (1) charged membrane, in the absence of electrical current and for $a_1 > a_2$:

$$J^+ = J^- = J_s = -\frac{RT}{\delta F}\,\frac{2\,uv}{u+v}\,(\bar{a}_2^+ - \bar{a}_1^+) - \left(\frac{\varphi_2 - \varphi_1}{50 \cdot 4}\right) \cdot \omega\overline{X} \qquad (a)$$

a_1, a_2 : electrolyte activities in the bathing solutions (mol · l^{-1}).
J_s : solute flux (mmol · min^{-1} · cm^{-2}).
u and v : absolute ion mobilities for positive or negative ions in the membrane (cm^2 · min^{-1} · v^{-1}).
a_1^+, a_2^+ : positive ion activities in compartments 1 and 2 (mol · l^{-1}).
$\bar{a}_1^+$, $\bar{a}_2^+$: positive ion activities in the membrane on boundaries 1 and 2 (mol · l^{-1}).
r : The Donnan distribution ratio ; $\bar{a}_1^+ = r_1 \cdot a_1^+$; $\bar{a}_2^+ = r_2 \cdot a_2^+$.
$\varphi_2 - \varphi_1$: internal membrane potential (mV).
$\omega\overline{X}$: constant membrane charge (equivalents · l^{-1}).
$\omega = +1$ for cations ;
$\omega = -1$ for anions.
δ : membrane thickness (cm).
R, T and F have their usual meanings.

For high values of the membrane charge one gets approximately :

$$J_s = -\frac{RT}{\delta F} \cdot \frac{2\,uv}{u+v}\,(\bar{a}_2^+ - \bar{a}_1^+) \qquad (b)$$

and when the membrane charge vanishes ($\omega\overline{X} = 0$) :

$$J_s = -\frac{RT}{\delta F} \cdot \frac{2\,uv}{u+v}\,(a_2 - a_1) \qquad (c)$$

or with

$$P = \frac{RT}{\delta F} \cdot \frac{2\,uv}{u+v}$$

$$J_s = -P\,(a_2 - a_1)$$

Il this case P should remain constant with increasing activity difference, provided that u and v are constant, *i. e.* for small concentration changes. Greater changes in concentration produce a decrease of u and v, resulting into a decrease of P.

However our experimental results show, at each pH, an increase of P (tables 1 and 2) with increasing activity differences. This can only be explained by assuming a charged membrane and is due to the variation of the Donnan distribution ratio with increasing concentration (LAKSHMINARAYANAIAH, 1969).

B. — *Membrane potentials in the presence of NaCl*

For a charged membrane the total potential (E_m), originating from concentration gradients, is composed of Donnan and diffusion potentials (TEORELL, 1953).

$$E_m = E_{Don_1} + E_{Don_2} + E_{diff}$$

(1) The assumption of positive membrane charges is arbitrary.

TABLE I AND 2

NaCl and KCl fluxes and permeability coefficients as a function of activity differences and pH. All NaCl diffusion experiments were performed with the same membrane and are thus completely comparable (idem for KCl).

Flux et coefficients de perméabilité pour NaCl et KCl en fonction de différences en activité et de pH. Dans tous les essais avec le NaCl on a fait emploi de la même membrane, de sorte que les résultats sont tout à fait comparables (idem pour KCl).

(1) C_2 was kept constant at $5 \cdot 10^{-4}$ M while C_1 varied.
C_2 égalait $5 \cdot 10^{-4}$ M, C_1 étant variable.

(2) Activities were calculated from the extended Debye-Hückel formula (GLASSTONE, 1946).
Les activités ont été calculées par la formule élargie de Debye et Hückel.

(3) $J_v = 0$ in all experiments.
$J_v = 0$ partout.

TABLE I

pH buffer	$C_1 - C_2$ (1) 10^{-4} mol · l^{-1}	$a_1 - a_2$ (2) 10^{-4} mol · l^{-1}	J_{NaCl} 10^{-4} mmol · min^{-1} · cm^{-2}		P_{NaCl} 10^{-4} cm · min^{-1}	
			Y→ W	W→ Y	Y→ W	W→ Y
$5 \cdot 10^{-3}$ M cacodylate pH 5.8	95	84.7	1.92 ± 0.03	1.92 ± 0.03	227 ± 4	227 ± 4
	195	168.2	4.30 ± 0.06	4.20 ± 0.06	256 ± 4	250 ± 4
	345	287.5	7.70 ± 0.14	7.30 ± 0.15	268 ± 5	254 ± 6
	495	401.7	10.6 ± 0.2	10.0 ± 0.2	264 ± 5	249 ± 5
$5 \cdot 10^{-3}$ M *tris* pH 7.0	95	83.9	1.56 ± 0.03	1.56 ± 0.03	186 ± 4	196 ± 4
	195	166.8	3.30 ± 0.12	3.25 ± 0.06	198 ± 7	195 ± 4
	345	285.9	6.60 ± 0.06	6.50 ± 0.11	231 ± 2	227 ± 4
	495	399.9	9.70 ± 0.13	9.40 ± 0.10	243 ± 4	235 ± 3
$5 \cdot 10^{-3}$ M *tris* pH 8.6	95	84.7	1.32 ± 0.02	1.14 ± 0.03	156 ± 3	135 ± 3
	195	168.2	2.70 ± 0.03	2.45 ± 0.06	161 ± 2	146 ± 4
	345	287.5	5.50 ± 0.03	5.40 ± 0.08	191 ± 1	188 ± 3
	495	401.7	8.45 ± 0.06	8.70 ± 0.14	210 ± 2	217 ± 4

TABLE 2

pH buffer	$C_1 - C_2$ (1) 10^{-4} mol · l^{-1}	$a_1 - a_2$ (2) 10^{-4} mol · l^{-1}	J_{KCl} (3) 10^{-4} mmol · min^{-1} · cm^{-2}		P_{KCl} 10^{-4} cm · min^{-1}	
			Y→ W	W→ Y	Y→ W	W→ Y
$5 \cdot 10^{-3}$ M cacodylate pH 5.8	95	84.5	2.28 ± 0.03	2.43 ± 0.02	270 ± 3	288 ± 3
	195	167.4	4.90 ± 0.06	5.15 ± 0.06	293 ± 4	308 ± 4
	295	246.3	7.95 ± 0.07	8.00 ± 0.06	323 ± 3	325 ± 3
	495	397.7	13.1 ± 0.09	13.4 ± 0.09	329 ± 3	337 ± 3
$5 \cdot 10^{-3}$ M *tris* pH 7.0	95	83.5	2.01 ± 0.03	2.03 ± 0.03	241 ± 4	243 ± 4
	195	166.0	4.10 ± 0.12	4.40 ± 0.05	247 ± 8	265 ± 3
	295	245.0	6.70 ± 0.07	6.95 ± 0.05	273 ± 3	284 ± 2
	495	395.9	12.3 ± 0.17	12.0 ± 0.17	311 ± 5	303 ± 5
$5 \cdot 10^{-3}$ M *tris* pH 8.6	95	84.5	2.06 ± 0.02	1.88 ± 0.04	244 ± 2	222 ± 5
	195	167.4	4.07 ± 0.04	4.07 ± 0.04	243 ± 3	243 ± 3
	295	246.3	6.45 ± 0.07	6.63 ± 0.03	262 ± 3	269 ± 1
	495	397.7	11.7 ± 0.1	11.8 ± 0.11	294 ± 3	297 ± 3

For our experimental conditions (1-1 valent electrolyte in 5 X 10^{-3} M buffer), and with the same assumptions as for the theoretical treatment of J_s, we find :

$$E_m = \frac{RT}{F} ln \frac{r_2}{r_1} - \frac{RT}{F} \frac{u - v}{u + v} ln \frac{\bar{a}_2^+ u + \bar{a}_2^- v}{\bar{a}_1^+ u + \bar{a}_1^- v}$$

For an uncharged membrane ($\omega \overline{X} = 0$) :

$$r_1 = r_2 = 1$$
$$\bar{a}_1^- = a_1^- \qquad \bar{a}_2^- = a_2^-$$
$$\bar{a}_1^+ = a_1^+ \qquad \bar{a}_2^+ = a_2^+$$

$$E_m = \frac{RT}{F} \frac{u - v}{u + v} ln \frac{a_1^+ u + a_1^- v}{a_2^+ u + a_2^- v}$$

or at 20°C and in mV :

$$E_m = 58 \frac{u - v}{u + v} \log \frac{a_1^+ u + a_1^- v}{a_2^+ u + a_2^- v}$$

In the latter case the graph E_m *vs.* $\log \frac{a_1^+ u + a_1^- v}{a_2^+ u + a_2^- v}$ should be a straight line with a slope $58 \frac{u - v}{u + v}$, giving zero potential for $a_1 = a_2$ and being independent on the orientation of the membrane in the concentration gradient.

The experimental curves, though being straight lines in the concentration range studied, never conform to all these requirements. Most of them are shifted to lower potential differences (fig. 1) and extrapolation shows that $E_m \neq 0$ for $a_1 = a_2$.

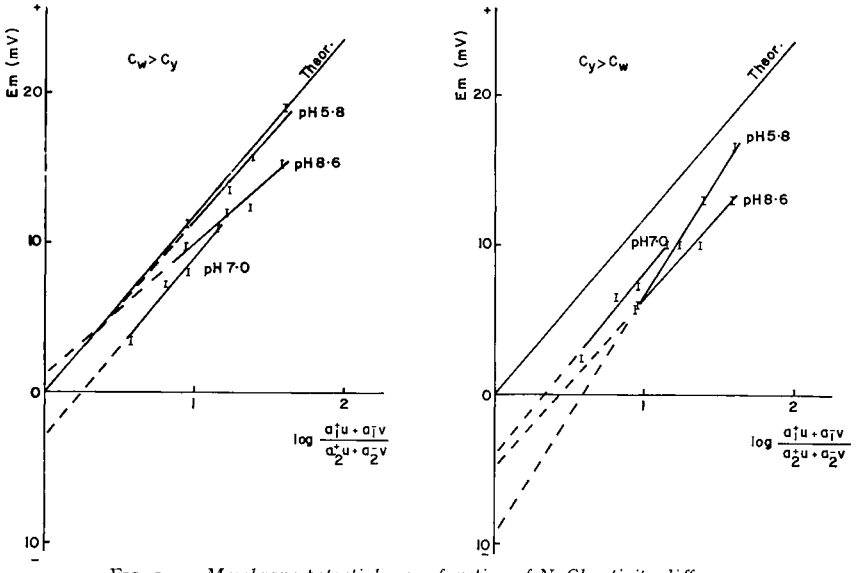


FIG. 1. — *Membrane potentials as a function of NaCl activity differences*

Potentiels membranaires en fonction des différences d'activité de NaCl

The + sign refers to the side with the highest concentration.
Le signe + indique le compartiment le plus concentré.

$E_{theor.}$ was calculated with the values of u and v in solution.
$E_{theor.}$ a été calculé en utilisant les valeurs de u et v en solution.

TABLE 3

Membrane asymmetry potentials : potential differences at equal NaCl concentrations on both sides. Correction is made for the junction potentials in the measuring circuit (see methods).

Potentiels résultant de l'asymétrie membranaire mesurés en présence d'une même solution de NaCl dans les deux compartiments. Les corrections pour les potentiels de jonction au long de la chaîne de mesure ont été effectuées (voir méthodes).

Asymmetry potential in mV (± 0,25 mV)
(+ for white side)

C mol/l	pH 5.8	pH 7.0	pH 8.6
10^{-3}	1	2	2.5
$5 \cdot 10^{-3}$	0.5	1.75	2
10^{-2}	0.25	1.50	1.75
$5 \cdot 10^{-2}$	0	0.75	1.25
10^{-1}	0.25	0.75	1
$5 \cdot 10^{-1}$	0.25	0.75	0.75

TABLE 4

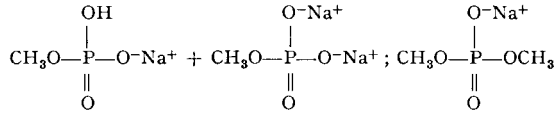
Direction and values of J_s in phosphate diffusion experiments at various pH values for $\Delta C = 0.0495$ M (C_1 = 0.05 M ; C_2 = 0.0005 M), *compared to other electrolytes.* For each salt, the experiments (in the two directions and at the different pH values) are performed with the same membrane, except for phosphate experiments, that require a statistical investigation (table 5).

Direction et valeurs de J_s phosphate à différentes valeurs de pH, pour $\Delta C = 0{,}0495$ M (C_1 = 0,05 M ; C_2 = 0,0005 M), *comparées à d'autres électrolytes.* Pour chaque électrolyte les essais (dans les deux directions, pour différentes valeurs de pH) ont été exécutés en faisant emploi de la même membrane, exception faite pour le phosphate, qui nécessite une analyse statistique (table 5).

(1) The percentages are calculated against the greatest flux representing 100 p. 100.
Les pourcentages sont calculés par rapport au plus grand flux qui représente 100 p. 100.

pH	J_s : phosphate	J_s : other salts tested (1)
5.8	$Y \rightarrow W > W \rightarrow Y$ 8.6 % (1)	$Y \rightarrow W \simeq W \rightarrow Y$
7.0	$Y \rightarrow W > W \rightarrow Y$ 68 %	
8.6	$Y \rightarrow W > W \rightarrow Y$ 42 %	

(1) NaCl ; KCl ; Na_2SO_4 ; Na_2HAsO_4 + NaH_2AsO_4 ;



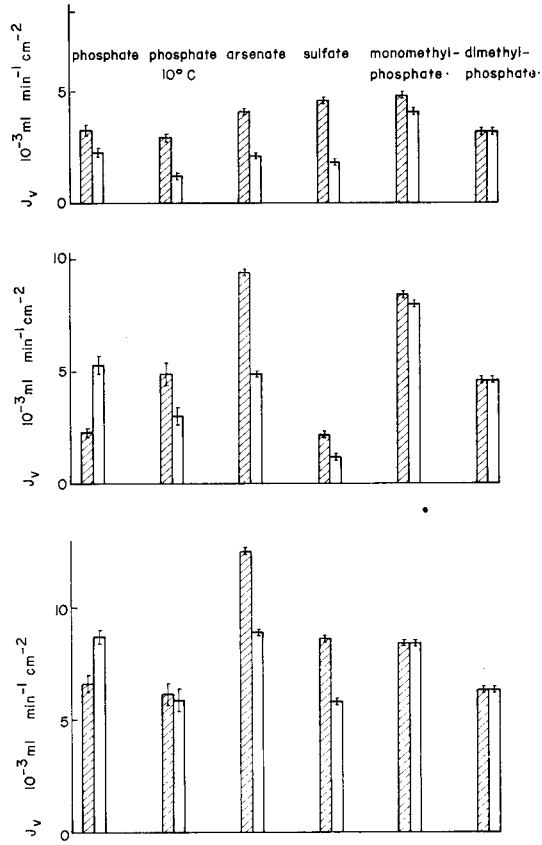


FIG. 2. — *Qualitative and quantitative characteristics of J_v for different electrolytes* (*) *at different pH values.*
The shaded areas refer to the direction W → Y. For each salt tested, except for phosphate, all experiments (in different directions and at different pH values) were performed with the same membrane.

Caractéristiques qualitatives et quantitatives de J_v pour des électrolytes différents (*) *en fonction du pH.*
Les aires hachurées ont trait à la diffusion dans le sens blanc → jaune. Pour chaque électrolyte, à l'exception des ions phosphate, la série totale des essais a été faite au moyen de la même membrane.

ΔC = 0.049 5 M (C_1 = 0.05 M ; C_2 = 0.000 5 M).

* For NaCl and KCl no volume fluxes could be detected at any pH or concentration difference tested.
Pour des solutions de NaCl ou KCl le flux de volume était nul dans toutes conditions de pH et de concentration.

Explanation is possible again on the basis of a charged membrane model where shifts are produced by the Donnan potentials. Moerover the shifts of the experimental curves, at the same pH, are dependent on the orientation of the membrane in the concentration gradient, thus revealing an asymmetrical membrane. This conception was supported by potential measurements with equal NaCl concentrations in the bathing solutions (table 3). These « membrane asymmetry potentials » increase from pH 5.8 to pH 8.6 and decrease with increasing concentration. In this case their origin

TABLE 5

Statistical investigation of J_s phosphate

Analyse statistique de J_s phosphate

ΔC = 0.0495 M (C_1 = 0.05 M ; C_2 = 0.0005 M).

R : regression coefficient for J_s vs. ΔC.
coefficient de régression pour J_s vs. ΔC.

σ_R : standard deviation of R.
erreur standard pour R.

P : probability according to the *t*-test of Student
probabilité selon le « *t*-test » de Student

ΔC 10^{-4} mol l^{-1}	J_s in 10^{-4} mmol · min^{-1} · cm^{-2}					
	pH 5.8		pH 7.0		pH 8.6	
	W→ Y	Y→ W	W→ Y	Y→ W	W→ Y	Y→ W
245	2.1	2.2	1.6	2.6	1.9	2.4
	3.1	2.1	1.2	2.6	1.7	2.8
495	5.7	6.5	3.6	10.4	3.2	5.0
	5.3	5.5	3.6	11.8	2.8	5.4
745	8.1	7.3	4.3	14.2	8.0	10.4
	6.9	9.1	4.0	12.0	6.4	9.6
995	11.6	9.7	8.3	15.3	7.8	12.2
	9.6	9.4	8.3	18.9	8.6	13.8
R 10^{-4} cm · min^{-1}	104	98	85	182	94	144
σR 10^{-4} cm · min^{-1}	8	12	11	27	12	10
P $R_{Y\to W} > R_{W\to Y}$	30 % < P < 40 %		P > 99 %		P > 99 %	

must be due to unequal Donnan distribution ratios at the two membrane boundaries. As, according to LAKSHMINARAYANAIAH and SIDDIQI (1971), the Donnan distribution is governed by both fixed charge density and porosity, we conclude that one of these factors or both are different for the inner and the outer layer of the ovovitelline membrane, whose structures were already shown, by electron microscopy and amino acid analysis, to be different (BELLAIRS *et al.*, 1963 ; BAIN and HALL, 1969).

C. — *Directional specificity of solute and volume fluxes for several electrolytes : the phosphate effect*

According to the results summarized in table 4 and fig. 2 the vitelline membrane exhibits particular properties with regard to phosphate ions between pH 7.0 and 8.6.

The phosphate flux is absolutely dependent on the membrane orientation in the concentration gradient. This contrasts with the behaviour of other electrolytes, and even with that of arsenate and monomethylphosphate which are close to phosphate and carry the same charge in the pH range studied. The asymmetry is small at pH 5.8 but evident at pH 7.0 and 8.6 where phosphate ions diffuse about twice as fast from Y → W than from W → Y (table 5). This may be relevant to the statement of HEVESY *et al.* (1938) that ^{32}P remains in white for a long period.

TABLE 6

Statistical investigation of J_v in phosphate diffusion experiments

Analyse statistique du flux de volume dans les essais avec le ion phosphate

ΔC = 0.0495 M (C_1 = 0.05 M ; C_2 = 0.0005 M).

$\bar{J}_v$: J_v averaged.
la moyenne de J_v.

$\sigma_{\bar{J}_v}$: standard deviation of $\bar{J}_v$.
erreur standard de la moyenne $\bar{J}_v$.

P : see table 5
voir tableau 5

	J_v in 10^{-3} ml · min^{-1} · cm^{-2}					
	pH 5.8		pH 7.0		pH 8.6	
	Y→ W	W→ Y	Y → W	W→ Y	Y→ W	W→ Y
	1.70	2.75	4.10	1.85	7.90	6.60
	2.75	3.80	5.30	2.45	8.75	5.30
	2.40	3.50	5.30	2.15	8.20	7.00
	1.80	3.90	6.50	2.70	9.55	7.95
	2.10	2.95	4.00	3.10	9.10	6.25
	2.85	2.45	6.50	1.50		
$\bar{J}_v$	2.25	3.25	5.30	2.30	8.70	6.60
$\sigma_{\bar{J}_v}$	0.20	0.25	0.40	0.20	0.30	0.40
	$P_{J_v}(W \rightarrow Y) > J_v(Y \rightarrow W) \simeq 99\ \%$		$P_{J_v}(Y \rightarrow W) > J_v(W \rightarrow Y) > 99{,}9\ \%$		$P_{J_v}(Y \rightarrow W) > J_v(W \rightarrow Y) > 99\ \%$	

The J_v-asymmetry, if present, has always the same direction for all electrolytes including arsenate and monomethyl-phosphate *i. e.* J_v (W → Y) > J_v (Y → W) The same holds for phosphate at pH 5.8, but between pH 7.0 — 8.6 J_v (Y → W) > J_v (W → Y) (table 6). These results may be relevant to the well known water

transport occurring from W → Y in the laid egg, that can be restrained if the outside layer of the vitelline membrane is protected by stabilization of the pH of the white (FROMM and GAMMON, 1968). Moreover only in phosphate solutions successive experiments with the same membrane are not reproducible; an irreversible change seems to occur. We may assume that phosphate ions interact somehow specifically with the ovovitelline membrane between pH 7.0-8.6, thereby changing its permeability properties. The following findings corroborate this assumption :

1. Conditioning the membrane a few hours in 0.05 M phosphate pH 7.0 (at this pH the differences in phosphate flux with regard to the direction are most evident) gives no longer a difference in J_s in a subsequent phosphate diffusion experiment. The J_v-asymmetry however persists.

2. Phosphate diffusion experiments in 0.5 M NaCl at any pH no longer result into an asymmetrical J_s (table 7). The volume fluxes are hardly measurable. High NaCl concentrations thus interfere with the phosphate-membrane interaction.

TABLE 7

Statistical investigation of J_s phosphate in 0.5 M NaCl

Analyse statistique de J_s phosphate en présence de 0,5 M NaCl

$\Delta C = 0.0495$ M ($C_1 = 0.05$ M ; $C_2 = 0.0005$ M)

$\bar{J}_s$, $\sigma_{\bar{J}_s}$, P : see tables 5 and 6
voir tableaux 5 et 6

	J_s phosphate in 10^{-4} mmol · min^{-1} · cm^{-2}					
	pH 5.8		pH 7.0		pH 8.6	
	Y→ W	W→ Y	Y→ W	W→ Y	Y→ W	W→ Y
	5.5	4.7	6.2	6.2	6.6	5.0
	5.9	6.6	5.4	6.4	5.0	6.9
	8.7	6.1	6.7	8.0	6.5	7.1
	7.6	6.1	6.0	5.9	7.2	4.9
	6.6	7.5	8.0	8.0	5.7	8.1
	6.0	7.5	6.3	5.7	6.4	5.5
$\bar{J}_s$ (1)	6.7	6.4	6.4	6.7	6.2	6.3
$\sigma_{\bar{J}_s}$ (2)	0.5	0.5	0.4	0.4	0.4	0.5
P_{J_s} (Y → W) = J_s(W→ Y) (3)	60-70 %		60-70 %		80-90 %	

3. Phosphate diffusion experiments at 10°C, with $\Delta C = 0.049\,5$ M, pH 7.0, no longer show the asymmetrical J_s found at 20°C and J_v (W → Y) > J_v (Y → W) by 38 p. 100, thus giving the same relative results as sulphate and arsenate diffusion (fig. 2 and table 8). The temperature dependance of the membrane-phosphate interaction is thus established.

TABLE 8

J_s and J_v in phosphate diffusion experiments at 10°C

J_s et J_v pour le ion phosphate à 10°C

ΔC = 0.0495 M (C_1 = 0.05 M ; C_2 = 0.0005 M)

Symbols : see tables 5 and 6
Symboles : voir tableaux 5 et 6

TABLE 8 *a*

	J_s in 10^{-4} mmol · min^{-1} · cm^{-2}					
	pH 5.8		pH 7.0		pH 8.6	
	Y→ W	W→ Y	Y→ W	W→ Y	Y→ W	W→ Y
	4.70	4.60	2.80	2.60	6.20	4.90
	4.60	5.90	3.20	3.80	4.10	3.20
	5.80	7.40	4.70	5.30	5.50	5.80
	3.30	4.90	5.80	4.90	3.00	5.40
	6.10	6.20	5.60	4.10	3.00	4.30
	6.60	3.60	3.30	4.90	4.40	3.00
$\bar{J}_s$	5.18	5.43	4.23	4.27	4.37	4.43
$\sigma_{\bar{J}_s}$	0.50	0.55	0.54	0.40	0.53	0.47

TABLE 8 *b*

	J_v in 10^{-3} ml · min^{-1} · cm^{-2}					
	pH 5.8		pH 7.0		pH 8.6	
	Y→ W	W→ Y	Y→ W	W→ Y	Y→ W	W→ Y
	1.70	3.31	2.55	5.09	6.07	5.30
	1.36	3.35	4.50	6.79	7.64	6.92
	0.85	2.12	3.48	4.16	6.28	8.15
	0.91	2.76	2.76	3.23	4.37	5.52
	0.85	3.06	1.91	4.58	6.24	6.03
	1.44	2.90	3.01	5.52	4.50	4.75
$\bar{J}_v$	1.19	2.92	3.04	4.90	5.85	6.11
$\sigma_{\bar{J}_v}$	0.14	0.18	0.37	0.49	0.5	0.5

TABLE 9

Phosphate effect on the J_v asymmetry in sulphate diffusion experiments at pH 7,0, ΔC = 0.049 5 M

Effet des ions phosphate sur l'asymétrie de J_v lors de la diffusion de sulphate à pH 7,0, ΔC = 0,049 5 M

Symbols : see tables 5 and 6.
Symboles : voir tableaux 5 et 6.

Conditioning	N° I		15 min ; 0.05 M phosphate pH 7.0 20°C II		15 min ; 0.05 M phosphate pH 7.0 4°C III	15 min ; 5.10^{-5} M phosphate pH 7.0 20°C IV
J_v-direction	Y → W	W → Y	Y → W	W → Y	Y → W	Y → W
$\bar{J}_v$ 10^{-3} ml min^{-1} cm^{-2}	1.8	3.5	4.6	3.7	1.5	4.6
Number of experiments	11	6	10	6	5	5
$\sigma_{\bar{J}_v}$	0.3	0.5	0.2	0.5	0.2	0.5
P probability in % (*t*-test)	$J_v^I(W \to Y) > J_v^I(Y \to W)$ 98-99 %		$J_v^{II}(Y \to W) > J_v^{II}(W \to Y)$ > 99.9 %	$J_v^{II}(W \to Y) = J_v^{II}(Y \to W)$ 60-70 %	$J_v^{III}(Y \to W) < J_v^{II}(Y \to W)$ > 99.9 % $J_v^{III}(Y \to W) < J_v^{I}(Y \to W)$ 50-60 %	$J_v^{IV}(Y \to W) = J_v^{II}(Y \to W)$ 90 % $J_v^{IV}(Y \to W) > J_v^{I}(Y \to W)$ > 99.9 %

4. When using membranes, previously conditioned in 0.05 M phosphate pH 7.0 and carefully washed in redistilled water, in sulphate diffusion experiments.

$$(\Delta C_{Na_2SO_4} = 0.049\,5\ M\ ;\ pH\ 7.0),$$

J_s has the same value in both directions but J_v (Y → W) shows a two to three fold increase while J_v (W → Y) remains unchanged so that qualitatively the same J_v- asymmetry obtains as for phosphate solutions (table 9).

The influence of previous conditioning in 0.05 M phosphate pH 7.0 on J_v (Y → W) in sulphate diffusion experiments at pH 7.0 and $\Delta C = 0.049\,5$ M, was followed as a function of time : the effect shows up rather rapidly (the shortest observation time was five minutes) and then remains rather constant for pretreatment times within two hours. Conditioning during longer periods decreases the effect gradually ; after ten hours no influence could be seen at all (fig. 3) ;

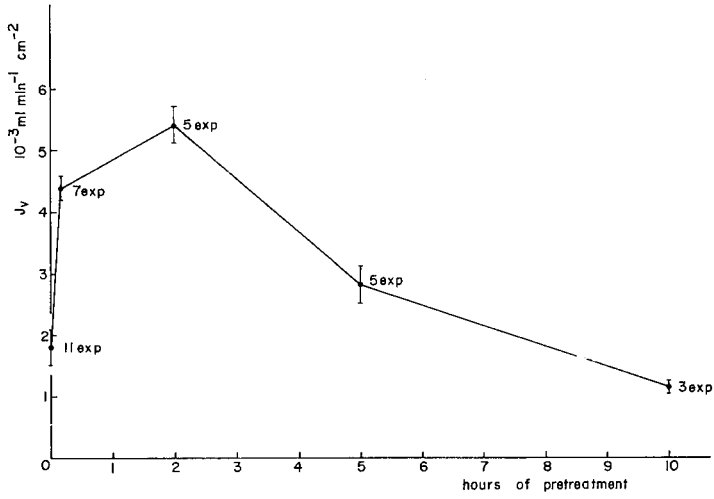


FIG. 3. — *The phosphate effect on J_v (Y → W) in sulphate diffusion experiments at pH 7.0 as a function of pretreatment time*

Effet des ions phosphate sur J_v (Y → W) dans des essais de diffusion de sulphate à pH 7,0, en fonction de la durée du traitement préalable

$\Delta C = 0.049\,5$ M (C_1 : 0.05 M ; C_2 : 0.000 5 M)

— concentration : the results are rather constant for all concentrations between 5×10^{-5} M and 5×10^{-2} M (table 9) ;

— temperature : after conditioning at 4°C in 0.05 M phosphate pH 7.0 and washing at 4°C, no increase in J_v (Y → W) is observed in subsequent sulphate diffusion experiments ($\Delta C = 0.049\,5$ M ; pH 7.0 ; 20°C) (table 9) ;

— ionic strength : when conditioning during 15 minutes in 0.01 M phosphate pH 7.0 in increasing NaCl concentrations, the effect disappears for $C_{NaCl} = 0.25$ M. In 0.05 M phosphate in stops at 0.1 M NaCl. These results make it highly improbable that the influence of increasing NaCl concentrations should be explained by assuming an ion exchange competition effect (table 10).

5. Phosphate determinations showed that membrane homogenates containing approximetely 6 membranes, treated during 4 hours with 0.05 M phosphate pH 7.0 at 25°C and washed with redistilled water until a conductivity of 10 μS, contained approximately 9 μg P more than untreated homogenates that contain only 2 μg P. Assuming an average dry weight of 5 mg for the ovovitelline membrane, only 0.3 μg P would be taken up per mg membrane. This would correspond to an average of one P atom per protein of MW 100 000, assuming that the membrane consists of 100 p. 100 protein. This is only a very rough estimate as amino acid analysis by BELLAIRS *et al.* (1963) points to a total of 66-78 p. 100 amino acid residues in different layers of the hen's ovovitelline membrane.

TABLE 10

Phosphate effect on J_v (Y → W) *in sulphate diffusion experiments at pH 7.0* (ΔC = 0.049 5 M) *as a function of NaCl concentration in the conditioning medium*

Effet des ions phosphate en fonction de la concentration de NaCl dans le milieu

Symbols : see table 6.
Symboles : voir tableau 6.

	J_v in 10^{-3} ml · min^{-1} · cm^{-2}					
	10^{-2} M phosphate				$5 \cdot 10^{-2}$ M phosphate	
C_{NaCl} mol · l^{-1}	10^{-2}	$5 \cdot 10^{-2}$	10^{-1}	$2.5 \cdot 10^{-1}$	$5 \cdot 10^{-2}$	10^{-1}
	5.70	4.75	4.65	2.80	3.80	0.55
	5.10	3.65	3.45	0.15	5.30	1.25
	5.35	5.15	5.70	0.35	6.20	0.55
	5.25	5.80	4.60	0.50	4.90	0.25
	5.25	6.30	4.65	1.35	5.45	0.70
	3.85		3.80	0.90	4.05	0.40
				0.50		
				0.85		
$\bar{J}_v$	5.10	5.15	4.50	0.95	4.96	0.60
$\sigma_{\bar{J}_v}$	0.2	0.4	0.3	0.3	0.4	0.15

CONCLUSION

The vitelline membrane of the unfertilized hen's egg is an asymmetrical ion exchange membrane. This asymmetry has no mentionable influence on J_s for most electrolytes but induces asymmetrical membrane potentials and volume fluxes.

Only the phosphate flux is direction dependent, probably as the result of a specific direction dependent phosphate-membrane interaction retarding the diffusion of this ion. This interaction also reverses the J_v-asymmetry, with regard to that

found for the other electrolytes, by enchancing J_v (Y → W). SCHULZ reported already in 1959 that the adsorption of diphosphate anions on the synthetic anion exchange membrane « Permaplex A-10 » reverses the direction of the electroosmotic water transfer by reversing the resin charge. Though the effect of the interaction vitelline membrane-phosphate anions possibly should be explained in the same way, its cause seems to be quite different from a simple ion exchange effect (non-reproducibility, concentration, temperature, ionic strength and time dependence).

The time dependence suggests that the interaction proceeds in several steps while non-reproducibility points to the fact that at least *in vitro* one of them should be irreversible. Bearing in mind the important influence of temperature, the participation of one or more enzymes does not seem impossible.

Investigations are in progress in order to get more information about the nature of this interaction that is supposed to be at the basis of a biologically mediated osmoregulating mechanism. An interacting possibility is the type of membrane protein phosphorylation reported by DEAMER and BASKIN (1972) and by MAKINOSE (1972) in sarcoplasmic reticulum membranes, consisting in a reversal of the membrane ATP-ase mediated ATP splitting reactions.

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RÉSUMÉ

LA MEMBRANE VITELLINE DE L'ŒUF DE POULE NON FÉCONDÉ : TRANSPORT DE L'EAU ET DES ÉLECTROLYTES

L'étude de la diffusion de plusieurs électrolytes à travers la membrane vitelline de l'œuf de poule non fécondé et de ses potentiels électriques mène à la conclusion qu'on se trouve en face d'une membrane chargée se comportant de façon asymétrique. Son asymétrie directionelle vis-à-vis du transport ionique et du flux de volume qui s'ensuit sont décrits pour plusieurs électrolytes. Le ion phosphate exerce un effet très spécifique. L'interaction membrane-phosphate dépend de divers facteurs (force ionique, température et durée). Elle semble bien plus complexe que si elle était due uniquement à un simple échange d'ions. Un mécanisme enzymatique n'est pas exclu.

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