INTERACTION OF IONS AND CONNECTIVE TISSUE*

by

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Certain features appear to be common to many physiological and pathological processes occurring in connective tissues. Thus, changes in state of the ground substance are suggested by altered uptake of intravital dyes, by modified solubility of glycoprotein components of the ground substance, and by changes in water and electrolyte content[1–5]. These reactions point to fundamental modifications of the colloidal structure of the ground substance of connective tissue. Recent studies of MEYER AND RAPPORT[6] have further suggested that part, at least, of the connective tissue matrix might act as a cation-exchange resin. This property would imply that electrolyte distribution depends on the state of charged colloidal aggregates of the tissue.

A direct study of the interaction of ions and tissues is facilitated by electrometric methods designed to determine selective ionic transference depending on the presence of immobile charges[7,8,9]. Such an approach was adopted by JOSEPH *et al.* in a study of the synovial membrane[10]. They measured the diffusion potentials of various ions in the knee joint cavity of dogs, and showed that the values approximated those found in aqueous solution, a result confirmed by EDLUND AND TEORELL in the rabbit[11]. Under the experimental conditions, the inner surface of the joint cavity thus represents tissues in which the apparent density of charged colloid is low.

In the present study, comparable electrometric techniques have been applied to the measurement of diffusion potentials in the *symphysis pubis* of the guinea pig. This material was chosen because 1) it represents a type of connective tissue which undergoes a wide variation in state, from a compact, tight structure in the normal animal to a soft, pliable structure in advanced pregnancy; 2) this sequence of events can be reproduced experimentally with relaxin[12]; 3) chemical and histo-chemical studies are available for comparison of the nature and quantity of certain components of the matrix[4,13]. Electrometric results indicated that in the normal structure the concentration of negatively charged immobile colloid is high, leading to selective cation permeability, while in relaxation, colloid concentration and cation selectivity are low. These studies have led to recognition of a continuous series of electrochemical states related to relaxation, which may be defined in terms of the apparent concentration of immobile colloid, and to a demonstration of a differential behaviour of potassium and sodium. A preliminary account of these findings has been given[14].

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METHODS AND MATERIALS

Details of the electrometric measurements as previously described for synovial tissues[10] were modified for application to the pubic symphysis. To form a reference junction with isotonic saline a 20 gauge hypodermic needle was inserted under the skin of the right leg, and secured with tape. The symphyseal region was shaved, and a 20 gauge needle, cut to ½ inch, was inserted by palpation into the underlying connective tissue of the interpubic region. In late pregnancy, when the symphysis is widely dilated, the needle was inserted fairly close to the right pubic bone. The position of the needle was maintained manually during the experiment.

Saturated KCl-calomel half-cells were used, and connected by means of saturated KCl bridges to the salt solutions perfusing the tissues. The junctions at the needles were small syringe barrels filled with saturated KCl in solidified agar. The circuit is represented schematically:

Hg	Hg_2Cl_2	KCl sat.	NaCl (0.15 M)	Subcut. tissue	Pubic symphysis	Salt solution I or II	KCl sat.	Hg_2Cl_2	Hg

Initially both needles were filled with isotonic (0.15 M) NaCl, and the baseline potential difference was measured. The fluid in the needle inserted at the symphysis was withdrawn by means of a syringe and a smaller needle, and was successively replaced by the following electrolytes: 0.015 M NaCl, 0.15 M KCl and LiCl, 0.075 M $CaCl_2$ and $MgCl_2$. The isotonic NaCl solution will hereafter be referred to as solution I; the 1/10 dilution or the substituted salt as solution II. The potential difference (p.d.) obtained with 0.015 M NaCl will be referred to as a "dilution potential"; those with other salts will be called "substitution potentials". In all cases they refer to the displacement of the observed potential when solution I is replaced by solution II.

Usually 2 or 3 flushes with electrolyte sufficed to yield a pair of steady values. Equilibrium was usually attained within 10–30 seconds. After each determination, the baseline potential was restored by withdrawing the fluid and flushing 2 or 3 times with isotonic NaCl. A series of determinations usually required about 20 minutes, after which the animal was allowed to recover. Usually intervals of at least 4 days separated successive studies on the same animal. The normal group comprised 8 adult females. Six females were castrated and tested at intervals after a 2 week recovery period. Twelve guinea pigs were obtained at various times after mating, mostly in the last trimester of pregnancy. These were tested at intervals and checked for day of delivery; there-after the mothers suckled their young, and were tested at intervals post-parturitionally. After their study as untreated castrates, 6 guinea pigs were injected with estrogen (1.0 gamma estradiol benzoate* in oil, daily, subcutaneously); after 4 days, 3 were tested as estrogenized (unrelaxed) controls, while 3 received 20 GPU** relaxin in water, administered subcutaneously. Six hours later these animals showed relaxation, and were studied electrometrically within the next two hours. Each animal of the first group of 3 estrogenized castrates was then given, over a 40 hour period, 5 injections of relaxin, a total of 20 GPU. All showed and maintained relaxation after 6–8 hours, and were tested as above 4–6 hours after the last injection. Relaxin-treated animals were allowed to recover, and were tested at 3 days, 1 week and 2 weeks after the last injection.

RESULTS

Summaries of the findings with the several groups of animals are presented, with statistical treatment in Table I. At this point, only certain qualitative features will be considered.

Dilution potentials

Normal adult females and castrates yielded similar results, with dilution potentials of + 18 to + 30 millivolts. Pregnant animals, which were generally within 2 or 3 weeks of term, showed lower dilution potentials. At near delivery times, these values tended to approach limiting values of approximately —5 to —8 mv. In one animal studied over

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* Estradiol benzoate was obtained from the Schering Corp., Bloomfield, N.J., courtesy of Dr Norman Hemenway.

** GPU, guinea pig units. Preparations from Chilcott Laboratories, Morris Plains, N.J., courtesy of Dr Robert L. Kroc, who also generously gave advice regarding relaxation diagnosis.

TABLE I

SUMMARY OF DILUTION AND SUBSTITUTION POTENTIALS*

Group	*No. Experiments*	*Dilution Potential (0.015 M NaCl)* E_d	*Substitution Potentials*** *(0.15 Equivalent)* E_B			
			KCl ($r = +0.88$)	*LiCl* ($r = +0.81$)	*CaCl*$_2$ ($r = +0.71$)	*MgCl*$_2$ ($r = +0.66$)
Normal	8	+ 24.0 ± 6.2	+ 5.4 ± 1.9	+ 8.2 ± 2.3	+ 9.5 ± 2.6	+ 11.6 ± 2.9
Castrate	9	+ 20.9 ± 4.0	+ 4.0 ± 1.2	+ 6.6 ± 1.4	+ 9.6 ± 2.7	+ 10.3 ± 2.2
Castrate + Relaxin	6	— 0.2 ± 2.9	— 1.0 ± 1.1	+ 2.5 ± 1.5	+ 5.3 ± 1.1	+ 7.7 ± 1.7
Post Relaxin (3 days)	3	+ 5.0 ± 2.8	+ 1.0 ± 0.8	+ 3.7 ± 1.3	+ 5.3 ± 1.0	+ 8.0 ± 1.4
Post Relaxin (7 days)	6	+ 19.0 ± 5.1	+ 3.2 ± 1.8	+ 5.7 ± 2.0	+ 8.8 ± 2.2	+ 10.0 ± 2.6
Pregnant (2–40 days pre-part.)	25	+ 1.2 ± 9.1	+ 0.1 ± 2.5	+ 4.0 ± 2.0	+ 6.9 ± 3.0	+ 8.5 ± 2.7
Post Partum (2–35 days)	16	+ 14.1 ± 7.8	+ 2.9 ± 2.2	+ 5.8 ± 2.7	+ 8.5 ± 3.1	+ 9.1 ± 3.1

* Values represent mean displacements from baseline in millivolts and standard deviations.
** Values of r are correlation coefficients between E_B and E_d for all experiments in column.

a longer period, the p.d. was slightly below the normal range at 6 weeks pre-partum, and well below at 5 weeks. Other animals showed lowered values at 4 weeks pre-partum. The change to negative dilution potentials seemed to occur at about 2 weeks pre-partum, when physical signs of relaxation were becoming apparent. After parturition, anatomic closure begins in 2 days, and p.d.'s approach normal values in 2–4 weeks. Some of the data on pregnancy and post-parturitional time relations are shown in Fig. 1.

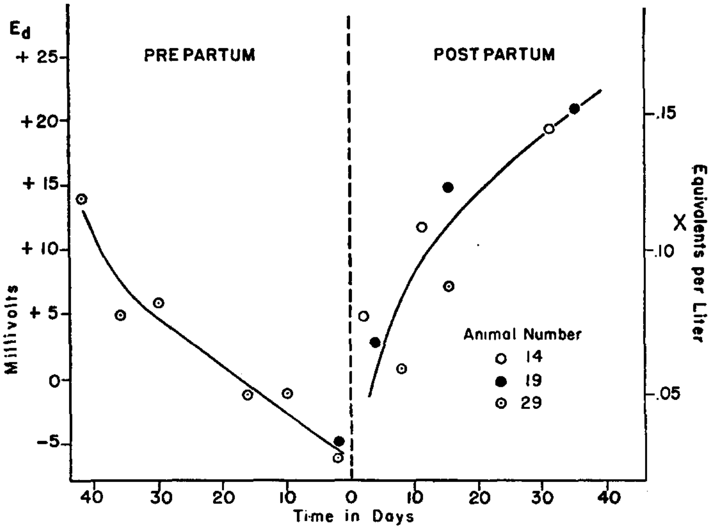

Fig. 1. Relation of the dilution potential to the time of pregnancy and to the post partum period. The right hand ordinate indicates the concentration of colloidal charge, x, in equivalents per liter.

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In castrated animals, the priming dose of estrogen, while sufficient to give vaginal opening in all cases, did not significantly affect the dilution potentials. The electrometric response to dosage with relaxin was present after 8–10 hours, and probably could have been detected earlier. These cases showed evidence of relaxation as determined manually. In the recovery period following relaxation produced by relaxin, it was not easy to determine the state of the symphysis by palpation. However, dilution potentials were found to be low after 3 days, and normal after 7 days.

Substitution potentials

Substitution potentials likewise showed systematic variations depending on the degree of symphyseal relaxation (Table I). On theoretical grounds, these variations should be correlated with those of the dilution potentials, with both depending on the electrochemical state of the symphysis. For this reason it is desirable to develop the electrochemical theory in some detail before proceeding to relate quantitatively either type of p.d. to the change of symphyseal state.

Theoretical relation of junction potential to electrochemical state

The following electrochemical treatment is based on two related principles: DONNAN's theory of membrane equilibrium in systems consisting of non-diffusible and diffusible ions[15], and HENDERSON's theory of liquid junction potentials[16]. General thermodynamic relations between free energy, ionic concentrations, and electrochemical work, developed by NERNST and PLANCK, are common to both theories, which deal with special cases. Systems have been studied in which non-diffusible electrically charged macromolecules cause unequal distributions of diffusible anions and cations[7,8]. The inequality has been shown to result in selective transference of the diffusible ion which is present in excess, for example, sodium ion, when it forms the salt of a macromolecular anion of low mobility. In such systems, it is necessary to consider simultaneously both DONNAN equilibrium and liquid junction potentials. A study of synovial connective tissue in the knee joint has shown that the potentials obtained with a number of ions may be approximately estimated from their relative aqueous mobilities[10,11]. This result represents a limiting case for boundaries at which the concentration of immobile charge is low. For cases in which selective transport is found, the treatment requires consideration of the immobile charges. Presumably the effective groups in connective tissue include glucuronic and sulphuric acid residues of the mucopolysaccharides, hyaluronic acid and chondroitin sulphuric acid, in addition to other base binding groups of the ground substance[6].

SCATCHARD[17] has presented a concise résumé of HENDERSON's theory of mixture boundary potentials, relating it to theories of interionic forces. The general expression includes a term for ideal strong electrolyte solutions and two correction terms which express non-ideal deviations arising from interionic forces. The correction terms are small, and tend to cancel. Far more important is the ideal term:

$$E' = \frac{RT\ \Sigma\ (m_i' - m_i'')u_i}{F\ \Sigma\ (m_i' - m_i'')u_i z_i} \ln \frac{\Sigma\ m_i'\ u_i z_i}{\Sigma\ m_i''\ u_i z_i} \qquad (1)$$

where, E' denotes the potential difference across a mixture boundary of two solutions, I and II, in which the molal concentrations of any ion, i, are respectively m_i' and m_i''; z_i and u_i are the valency and mobility respectively, of an ion, i; R, T, and F denote respectively the gas constant, the absolute temperature, and the Faraday electrochemical equivalent; ln refers to natural logarithms, and Σ indicates a summation. The summations include all the ionic species present in both solutions forming the mixture boundary. Both the charge z_i and the mobility u_i are taken positive for cations and negative for anions. In the following treatment, zero mobility will be attributed to non-diffusible or slightly diffusible macromolecules, including undissociated complexes formed with diffusible ions. This assumption differs somewhat from that of STEINBACH[18], who has assumed a definite low mobility for colloidal anions of scallop muscle.

For a mixture boundary between a solution of isotonic NaCl and an equivalent (0.15) concentration of an alkali or alkaline earth chloride, equation (1) becomes:

$$E_w = -26.8 \frac{u_{\mathrm{Na}} - \dfrac{u_{\mathrm{B}}}{z_{\mathrm{B}}}}{u_{\mathrm{Na}} - u_{\mathrm{B}}} \ln \frac{u_{\mathrm{B}} - u_{\mathrm{Cl}}}{u_{\mathrm{Na}} - u_{\mathrm{Cl}}} \tag{2}$$

E_w refers to the aqueous boundary potential between solutions I and II, and 26.8 is the value in millivolts of (RT/F) at 37° C. B denotes the cation of solution II, of valence z_{B} and mobility u_{B}.

If the mixture boundary occurs in a medium containing immobile negatively-charged colloid at a concentration of x equivalents per liter, equation (1) becomes for univalent cations:

$$E' = -26.8 \ln \frac{u_{\mathrm{B}}(0.15 + x/2) - u_{\mathrm{Cl}}(0.15 - x/2)}{u_{\mathrm{Na}}(0.15 + x/2) - u_{\mathrm{Cl}}(0.15 - x/2)} \tag{3}$$

This is obtained by using the following approximations for the ionic concentrations, m_i: $(\mathrm{Na}^+)' = 0.15 + x/2$; $(\mathrm{Cl}^-)' = 0.15 - x/2$; with similar relations for $(\mathrm{B}^+)''$ and $(\mathrm{Cl}^-)''$. These approximations are derived from a more exact quadratic equation[7,9], and satisfy the DONNAN equilibrium conditions for low values of x. They depend on the assumption that the ions do not form undissociated complexes with the charged colloid.

Equation (3) may be simplified by expansion of the natural logarithm as a polynomial, retaining only the linear term in x. This yields an approximation for small values of x:

$$E' = E_w - \frac{26.8\, u_{\mathrm{Cl}}(u_{\mathrm{Na}} - u_{\mathrm{B}})\, x}{0.15\,(u_{\mathrm{Na}} - u_{\mathrm{Cl}})(u_{\mathrm{B}} - u_{\mathrm{Cl}})} \tag{4}$$

or

$$E' = E_w + kx \tag{5}$$

where k is a constant which depends on the ionic mobilities of solutions I and II. E_w represents the limit of E' as x approaches zero, and is given also by equation (2). E_w and k are constants estimated from the standard mobilities, u_{Na}, u_{Cl}, and u_{B}. They apply to an ideal boundary in which x is independent of the nature of the perfusing ions, and in which the mobilities are proportional to their standard values in water.

The foregoing treatment is applied to systems in which a 0.15 M solution of an alkali chloride forms a boundary with connective tissue previously equilibrated with 0.15 M NaCl. For bivalent cations the treatment may be modified by the approximations: $(\mathrm{B}^{++})'' = 0.15 + 2x''/3$, and $(\mathrm{Cl}^-)'' = 0.15 - x''/3$, where the quantities refer to equivalents per liter. The expressions corresponding to (3) and (4) then lose simplicity. It is necessary also to correct for the difference between $(\mathrm{Cl}^-)'$ and $(\mathrm{Cl}^-)''$ as DONNAN

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potentials at the boundaries of I and II respectively. After applying this correction, the linear equation (5) remains a useful approximation, and k may be estimated from standard aqueous mobilities.

It is possible to relate boundary potentials to the electrochemical state of the tissue by using NaCl at a different concentration. If the concentration of NaCl is varied, diffusion potentials are obtained depending only on the mobilities of Na^+ and Cl^-, and on the concentration of immobile charge, x, expressed in equivalents per liter. In particular, if isotonic NaCl is compared with its $^1/_{10}$ dilution, (0.015 M NaCl), a considerable displacement from the baseline potential is often obtained. Theoretically, this is correlated with u_{Na} and u_{Cl} according to the general expression (1). The concentrations of diffusible ions depend on x, being determined by the DONNAN conditions. For values of x up to 0.20 equivalents per liter, an approximate linear relation applies:

$$E_d = -12.3 + 215\,x \tag{6}$$

where E_d denotes the dilution potential, a dilution of 0.015 M NaCl being always understood. This value is taken with reference to the baseline potential obtained with 0.15 M NaCl. The value — 12.3 millivolts represents the calculated value of the dilution potential at an aqueous boundary at 37° C. It is obtained from the HENDERSON equation, taking u_{Cl} as -1.5 and u_{Na} as 1.0 to agree with standard mobility data. It is also theoretically the limit of observed dilution potentials as x approaches zero. The proportionality factor, 215, similarly depends on standard mobilities of sodium and chloride ions, assuming that the ratio of mobilities is independent of x. Therefore, the value should be regarded as a first approximation, leading to tentative values of x.

Application of theoretical treatment to experimental results

The concentration of immobile negative charge, x, in the connective tissue may be estimated from the observed values of the dilution potentials according to equation (6). It ranges from values of the order of 0.03 equivalents per liter in the extreme relaxed state to 0.20 equivalents per liter in the tight symphysis (Figs. 1 and 2). These are based

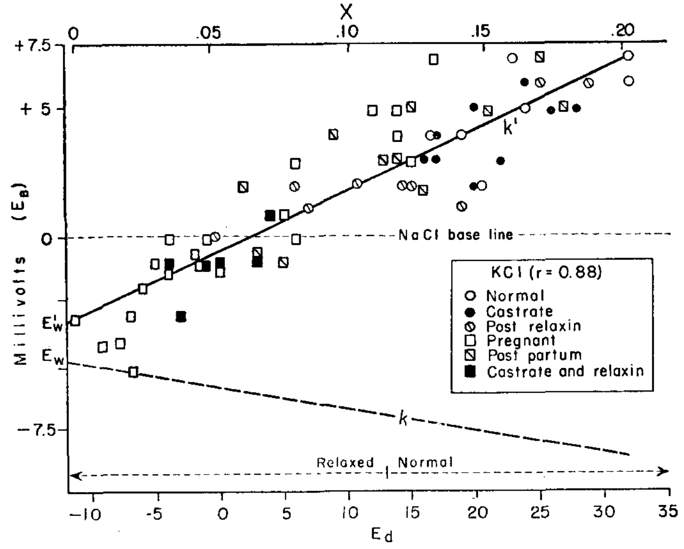

Fig. 2. Correlation of the substitution potential of potassium ion with NaCl dilution potential. Upper curve shows observed values, fitted with slope k' and intercept E_w'. Lower curve is calculated with ideal constants, k and E_w. E_w' approximates E_w.

on values of the dilution potential ranging from —5 to +30 millivolts, (Equation 6). Values of the substitution potentials, E_B, for cations are statistically correlated with dilution potentials, E_d, obtained with 0.015M NaCl, (correlation coefficients, $r = +0.88$

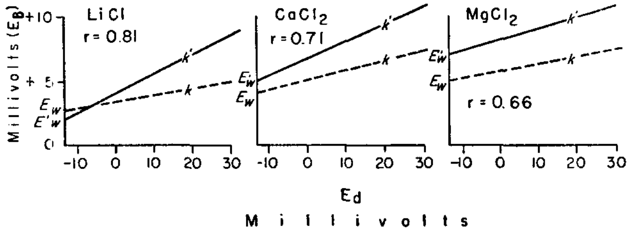

Fig. 3. Correlation of substitution potentials of lithium, calcium and magnesium ions with NaCl dilution potentials. Upper curves show observed values, fitted with slopes k' and intercepts E_w'. Lower curves are calculated with ideal constants, k and E_w.

for KCl to + 0.66 for $MgCl_2$). These data are plotted (Figs. 2 and 3) with dilution potential as abscissa against the cation substitution potentials. For potassium, a detailed plot of 60 individual experiments with 6 groups of animals is shown (Fig. 2). When the data for the several cations are fitted by the method of least squares, it is evident that they may be expressed in each instance by an equation of the form

$$E_B = E_w' + k'x \tag{7}$$

where x is calculated from E_d by equation (6). Equations (5) and (7) are similar in form. In Figs. 2 and 3, k' and E_w' are the slope and intercept, respectively, of the experimental curves, while k and E_w are corresponding constants of the ideal curves obtained from standard mobility data. The origin is taken as —12.3 millivolts, relating to the value of the dilution potential calculated for zero value of x (equation 6). A comparison of ideal slopes and intercepts with the observed values is presented in Table II.

TABLE II

COMPARISON OF OBSERVED AND CALCULATED CONSTANTS

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Ion	*Relative Aqueous Mobility*	u_B^0 *Extrapolated Mobility* ($x = 0$)	*Aqueous Junction Potential* E_w (*mv*)	*Extrapolated Potential* ($x = 0$) E_w' (*mv*)	*Knee Joint Potential* (*mv*)	*Ideal Slope* k	*Actual Slope* k'
Li	0.77	0.85	+ 2.8	+ 1.7	+ 3.3	+ 12	+ 37
K	1.49	1.31	— 4.9	— 3.2	— 3.4	— 18	+ 51
½ Ca	1.19	1.10	+ 4.2	+ 4.8	+ 5.4	+ 15*	+ 30
½ Mg	1.06	0.78	+ 5.0	+ 7.0	+ 8.0	+ 10*	+ 20
	Standard Mobilities referred to Na as 1.0.	Equation (2) Values of Col. 5.	Equation (2) Values of Col. 2.		Reference (10)	Equation (4,5) Values of Col. 2.	

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* DONNAN correction terms applied.

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Two criteria should be satisfied for ideal boundaries: 1) the substitution potential, E_w', of any cation extrapolated to zero value of x, the limit of relaxed states, should approach the value E_w calculated from standard data by equation (2); 2) k', the actual regression slope of E_B on x should agree with the ideal value, k, estimated from standard mobilities. It is convenient to refer all other ionic mobilities to that of Na^+, taking u_{Na} as 1.0 and u_{Cl} as -1.5. Values were computed from data in International Critical Tables. Calculations of Table II are based on these values, and indicate little deviation from the first criterion. The largest discrepancy between E_w and E_w', 2 millivolts, occurs with $MgCl_2$. The experimental value of E_w' for $MgCl_2$, 7.0 millivolts, also agrees well with the value 8.0 millivolts previously obtained for synovialis of the knee joint of dogs[10]. Similar agreement holds for the other ions (Table II). Thus in the highly relaxed symphysis, dilution and substitution potentials approximate the values calculated for ideal boundaries, and relative ionic mobilities are found to approximate to those in water.

On the other hand, comparison of ideal and observed slopes for the unrelaxed symphysis indicates that k' is greater than k, and the experimental p.d. is positive to that calculated from the ideal expressions (4) and (5). For Li, Ca, and Mg, the discrepancies are small, of the order of 5 millivolts for high values of x, as compared with 2 millivolts when x is small. With potassium, however, $k = -18$, while $k' = +51$, and the difference leads to an error of about 14 millivolts when x is at its upper limit, 0.20 equivalents per liter. Thus in this case, the second criterion is not satisfied.

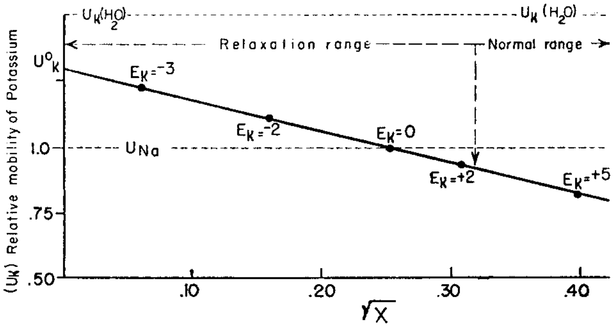

Fig. 4. Relation of apparent mobility of potassium to colloidal charge. Abscissa gives the square root of the concentration of colloidal charge. The change of sign of the substitution potential E_K with symphyseal relaxation is indicated.

Two assumptions are implicit in the ideal boundary treatment: 1) that the perfusing ions do not form undissociated colloidal complexes, and 2) that the relative mobilities of the ions are the same as in water, and independent of x, the colloidal charge. It may be concluded from the results with potassium that one or both assumptions should be modified, at least for this ion. Modification of the first fails to resolve the discrepancy. A much better approximation has been obtained by retaining the first assumption, and modifying the second to treat potassium mobility as a variable depending on the state of the tissue. An empirical correction has been introduced to give the ion a relative mobility, u_B, which decreases with the square root of x:

$$u_B = u_B^{\circ} - c\sqrt{x} \qquad (8)^*$$

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* This is of the same form as the KOHLRAUSCH-ONSAGER equation for dependence of equivalent conductance or mobility on concentration[19].

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where $u_B{}^\circ$ is the value of u_B extrapolated to zero value of x, and c is an empirically determined constant. A similar calculation is applicable to LiCl.

The results for two univalent salts, KCl and LiCl, are compared, (Table III). In each case, mobilities are estimated at several values of x to yield, when substituted into equation (3), the observed potentials. The apparent mobilities are then related to x by means of equation (8) with the empirically determined constants, $u_B{}^\circ$ and c. It is found that the values of $u_B{}^\circ$ are fairly close to the standard values of the relative mobilities. Thus only slight corrections are required when x is small. The values of c are 1.39 for K^+ and 0.50 for Li^+.

TABLE III

APPARENT RELATIVE MOBILITIES OF POTASSIUM AND LITHIUM IONS

Colloidal Charge Density (Eq. per liter)	*Dilution Potential (mv)*	*KCl*			*LiCl*		
x	E_d (*calc.*)*	u_K**	E_B (*calc.*)***	E_B (*obs.*)§	u_{Li}**	E_B (*calc.*)***	E_B (*obs.*)§
0.00	−12.3	1.31	−3.2	−3.2	0.85	+1.7	+1.7
0.05	−1.5	1.00	0.0	−0.6	0.74	+3.6	+3.5
0.10	+9.2	0.87	+2.1	+1.9	0.69	+5.2	+5.4
0.15	+20.0	0.77	+4.5	+4.4	0.65	+7.1	+7.2
0.20	+30.7	0.70	+7.0	+7.0	0.63	+9.1	+9.1

* Calculated, equation (6)

** Calculated, equation (8) with constants, $c = 1.39$, $u_B{}^\circ = 1.31$ for K, $c = 0.50$, $u_B{}^\circ = 0.85$ for Li.

*** Calculated, equation (3) from u_K or u_{Li}, values of preceding column.

§ Statistically estimated from data by equation (7). (Figs. 2 and 3).

Calculations for the bivalent ions, Mg and Ca, are complicated for reasons which have been indicated. It is evident, however, from the agreement of E' and E_B shown in Fig. 3, that empirical mobility corrections for these ions are of a smaller magnitude than that required for potassium.

When the value of x is 0.05 equivalents per liter, the calculated substitution potential of potassium ion becomes zero, ($u_B = 1.0$). This agrees well with the statistical observations (Fig. 2, Table III). At higher values of x, the apparent potassium mobility becomes less than 1.0, resulting in positive substitution potentials.

At low values of x, u_B exceeds 1.0, leading to negative substitution potentials. The value of $u_B{}^\circ$ is 1.31, compared with the standard value, 1.49, for aqueous solutions. Values of other ionic mobilities calculated from the values of $E_w{}'$, the extrapolated substitution potentials, are also found to be in fair agreement with the standard aqueous values, (Table II).

Cations such as Na, Li, Ca, and Mg have relatively low aqueous mobilities compared to K because of their greater degree of hydration. Migration is retarded by water molecules included in their electric fields[20]. The absence of a thick hydration layer surrounding potassium ions might possibly facilitate the formation of a retarding field of ground substance colloid, decreasing mobility in tissues. Similar explanations for selective adsorption of ions at biological interfaces, and in other colloidal systems have been cited[9, 21].

From equation (8) it can be seen that the mobilities of ions in tissues depend on their aqueous mobilities, on the state of the tissue, and on selectivity constants characteristic of the ions. The constant, *c*, is large for the least hydrated ion, K^+, and small for the highly hydrated ions of low aqueous mobility. An inversion of K^+ mobility with respect to Na^+, as observed by HODGKIN AND KATZ in the giant axon of the squid[22] may similarly depend on modification of ionic mobilities by varying colloidal states.

DISCUSSION

In attempting to explain the electrochemical behaviour of the pubic symphysis as its physical state changes from tightness to flexibility, it has been theoretically useful to postulate the presence of immobile, negatively charged aggregates. Substantial support for this concept derives from chemical and histochemical evidence. The symphysis comprises collagen fibers embedded in a glycoprotein ground substance[23]. As additional components of the extracellular tissue, the presence of hyaluronic acid and chondroitin sulphuric acid may be assumed. Immobile mucopolysaccharide and mucoprotein complexes have low isoelectric points, and at physiological pH would be expected to carry high negative charges. As a result, the structure tends to show high cation permeability, as indicated by NaCl dilution potentials. The characteristic change during relaxation is believed to be a depolymerization of the ground substance with formation of soluble fractions[13], and there is direct evidence of water uptake[4]. If in the unrelaxed state, the dry weight of 25% consists of polymerized macromolecules, then the electrometrically estimated density of immobile charge, 200 milliequivalents per liter, corresponds to an average base binding equivalent weight of 1250 at physiological pH. This figure is reasonable, since it lies between the value of hyaluronic acid, about 400, and values for proteins. Direct evidence for base binding of the estimated order of magnitude is provided by data of YIELDING AND ROSANO[5]. They have found that injection of hyaluronidase into the knee joint of dogs leads to a 70% increase of Na concentration in the fluid, and a 150% increase of potassium, and have shown that the increases were not caused by diffusion from blood. From these figures, a value of colloidal charge of about 100 milliequivalents per liter can be estimated, corresponding to values estimated for partially relaxed symphyses. From the electrometric results, the normal concentration of base binding residues of about 200 milliequivalents per liter in unrelaxed symphyses decreases to something of the order of 20–40 milliequivalents per liter in the highly relaxed state. This decrease implies a large uptake of water and dialyzable electrolyte, and is manifested by marked softening of the tissue.

The other significant finding has been the selective behaviour of the symphysis toward sodium and potassium, manifested by a low relative mobility of potassium in unrelaxed symphyses, with a progressive approach toward its standard aqueous mobility in highly relaxed symphyses. These shifts point to important properties of the connective tissue in determining water and electrolyte distribution. Further, these properties seem to be intimately related to the state of aggregation of the ground substance, which in the symphyseal system is under hormonal control.

Shifts of water and ions between blood and the ground substance of connective tissue raise questions concerning the maintenance of osmotic equilibrium. Addition of water (plus electrolyte) to a homogeneous colloidal system would decrease colloidal osmotic pressure and change the vapor pressure of the system, leaving it no longer in equilibrium with blood and other contiguous tissues. This would not be the case if ground substance represented a heterogeneous system in which the essential coexistent phases are a colloid-rich mixture, containing water and salt, in equilibrium with a water-rich mixture containing soluble colloids and salt. Formation of water-rich phase with uptake of dialysate might well leave osmotic pressure and vapour pressure undisturbed in the heterogeneous system. This is in accordance with GIBBS' phase rule that an increase in the number of coexistent phases in equilibrium decreases the number of degrees of freedom. Thus each phase of the heterogeneous system might become relatively invariant with respect to concentrations and osmotic pressure.

This concept agrees with the findings of OGSTON, PHILPOT AND ZUCKERMAN[3] in a study of sexual skin swelling in monkeys. They state that the sexual skin exudate may be regarded as the tissue fluid of the skin when it is swollen, and that the composition of the exudate does not vary much with the phase of swelling.

Application of the phase concept to the pubic symphysis would describe the unrelaxed state as containing a large fraction of colloid-rich phase (*e.g.* 0.20 equivalents per liter) in equilibrium with a small fraction of water-rich phase. As soluble fractions are formed in relaxation, dialysate is taken up from blood, forming a large fraction of water-rich phase. During the process, the *intensive* physicochemical properties, such as osmotic and vapour pressures, which depend only on concentrations, might remain relatively constant. In contrast, *extensive* properties, such as the total mass and volume of any phase, might vary widely. Plasticity, for example, depending on the relative quantities of water-rich and colloid-rich phases, would be a highly variable extensive property. At all stages of the reversible relaxation process, the system might remain iso-osmotic and isotonic with blood. Evidently, a heterogeneous equilibrium of this nature would contribute to physiological homeostasis in the face of considerable fluid shifts, involving profound mechanical changes.

As the pubic symphysis becomes highly relaxed, it tends to lose its selectivity with respect to ion mobilities, which approach standard aqueous values. However, in the unrelaxed symphysis it is not possible on this basis to make *a priori* predictions of potential differences, and corrections must be introduced for specific effects. The largest correction is required for potassium, the relative mobility of which may fall to less than one-half its value in water. This behaviour may be related to the low state of hydration of both the tissue and the ion, leading to a selective immobilization of potassium in the presence of a high content of colloid, and to a return to high mobility when relaxation occurs, with uptake of water. The colloid-rich phase in this sense seems to act as a cation-exchange resin[6]. An important corollary would seem to be that any hormonal action on connective tissue involving changes of state of ground substance or tissue would necessarily affect the distribution of sodium and potassium ions, as well as water and colloid content of the tissue.

SUMMARY

The interaction of ions with tissues was studied by measuring liquid junction potentials between tissues and 0.15 *M* KCl, and LiCl, or 0.075 *M* $CaCl_2$ and $MgCl_2$, as well as with one tenth isotonic NaCl. For baseline values, 0.15 *M* NaCl was used. In this way a series of substitution and dilution potentials was established.

As a connective tissue which varies in physicochemical state, use was made of the *symphysis pubis* of normal and castrate guinea pigs, and of guinea pigs in various stages of pregnancy. The former have tight, unrelaxed symphyses, while the latter show varying grades of relaxation. Experimentally, relaxation was also induced by the use of estrogens and relaxin in castrate guinea pigs.

Unrelaxed symphyses showed high positive dilution potentials (+ 20 to + 30 mv), while highly relaxed symphyses showed zero or negative dilution potentials, approaching in some cases values calculated for aqueous salt junctions (— 12.3 mv). These results are interpreted on the basis of a high concentration of negatively charged immobile colloid in tight connective tissue; following induction of the physiological change of relaxation, the potentials indicate low densities of immobile charged colloid. An estimate of 1250 for the average base binding equivalent weight of tissue colloids indicates a magnitude characteristic of mucopolysaccharides or mucoproteins.

Evidence from other work indicates that relaxation in the symphysis is accompanied by a disaggregation (depolymerization) of components of ground substance, and by an increase in water soluble fractions. In relaxed symphyses, where colloid density is low, mobility data indicate that the cations approach their behaviour in water. In tight symphyses, selective effects appear, related to the high concentrations of immobile charge. Potassium in particular showed a marked decrease of mobility to approximately one-half its value in water. Effects with other ions were much smaller. Thus connective tissue in a state of high aggregation appears to act selectively as an ion-exchange resin for potassium; with breakdown of aggregates and uptake of water, selectivity is lost.

From the NaCl dilution potentials, concentrations of immobile charges were estimated. Values ranged from 0.20 equivalents per liter in the tight symphysis to 0.05 equivalents per liter or less in the relaxed state. Values of this order of magnitude have been calculated from independent data.

RÉSUMÉ

L'interaction des ions avec des tissus a été étudiée par la mesure des potentiels de ponts liquides entre des tissus et les solutions suivantes: KCl et LiCl 0.15 *M*, $CaCl_2$ et $MgCl_2$ 0.075 *M*, NaCl un dizième isotonique. Pour les valeurs de base le NaCl 0.15 *M* a été employé. De cette façon une série de potentiels de substitution et de dilution a été établie.

Comme tissu conjonctif d'état physico-chimique variable les auteurs ont fait usage de symphyse pubienne de cobaye normal et castré et de cobayes à divers stades de grossesse. Les premiers ont des symphyses serrées non relâchées tandis que les seconds montrent divers degrés de relâchement. Le relâchement a aussi été induit expérimentalement chez les cobayes castrés à l'aide de substances oestrogènes et de relaxine.

Des symphyses non relâchées montrent des potentiels de dilution positifs élevés (+ 20 à + 30 mv), tandis que des symphyses très relâchées montrent des potentiels de dilution nuls ou négatifs qui s'approchent en certains cas de valeurs calculées pour des jonctions aqueuses de sels (−12.3 mv). Ces résultats sont expliqués par la présence dans le tissu conjonctif serré de colloïde immobile à charge négative en concentration élevée; après induction du changement physiologique du relâchement, les potentiels indiquent de faibles densités de colloïde immobile chargé. Le poids équivalent moyen de tissu colloïdal vis à vis des bases est évalué à 1250; cela indique une valeur charactéristique des mucopolysaccharides ou des mucoprotéines.

Des résultats obtenus au cours d'autres recherches indiquent que le relâchement de la symphyse est accompagné d'une désagrégation (dépolymérisation) de composantes de la matière de base et par une augmentation des fractions solubles dans l'eau. Dans des symphyses relâchées, où la densité en colloïde est faible, les données de mobilité indiquent que le comportement des cations s'approche de celui dans l'eau. Dans des symphyses serrées des effets sélectifs prennent naissance grâce à la concentration élevé de charge immobile. La mobilité du potassium en particulier était considérablement diminuée: elle n'était plus que la moitié environ de sa valeur dans l'eau. Pour d'autres ions les effets étaient beaucoup plus faibles. Ainsi, le tissu conjonctif serré à l'état d'aggrégation élevé semble agir sélectivement comme une résine échangeuse d'ions pour le potassium; lors de la dégradation et de l'absorption d'eau, la sélectivité disparaît.

A l'aide des potentiels de dilution de NaCl, les auteurs ont déterminé les concentrations des charges immobiles. Des valeurs allant de 0.20 équivalents par litre dans les symphyses serrées jusqu'à 0.05 et moins dans des symphyses à l'état relâché furent trouvées. Des valeurs de cet ordre de grandeur ont été calculées à partir de données indépendantes.

ZUSAMMENFASSUNG

Die Wechselwirkung zwischen Ionen und Geweben wurde durch Messung der Potentiale von Diffusionsbrücken zwischen Geweben und 0.15 *M* KCl und LiCl, oder 0.075 *M* $CaCl_2$ und $MgCl_2$ sowie auch ein Zehntel isotonischer NaCl-Lösung untersucht. Für Grundwerte wurde 0.15 *M* NaCl verwendet. Auf diese Weise wurde eine Reihe von Substitutions- und Verdünnungspotentialen ermittelt.

Als Bindegewebe mit wechselndem physiko-chemischen Zustand wurde Symphysis pubis von normalen und kastrierten Meerschweinchen und von Meerschweinchen in verschiedenen Stadien der Gravidität verwendet. Die ersteren haben feste, nicht erschlaffte Symphysen, während die Symphysen der letzteren mehr oder weniger erschlafft sind. Experimentell wurde die Erschlaffung auch durch Verwendung von Östrogenen und von Relaxin hervorgerufen.

Nicht erschlaffte Symphysen zeigten ein hohes positives Verdünnungspotential (+ 20 bis + 30 mv), während bei stark erschlafften Symphysen das Verdünnungspotential null oder negativ war und sich in manchen Fällen den für wässrige Salzverbindungen errechneten Werten (— 12.3 mv) näherte. Diese Ergebnisse werden auf Grund eines negativ geladenen, unbeweglichen, in hoher Konzentration im Bindegewebe vorkommenden Kolloides ausgelegt; nach Induktion der physiologischen Veränderung der Erschlaffung, zeigen die Potentiale niedrige Dichten des unbeweglichen geladenen Kolloides an. Das durchschnittliche Basenbindungsäquivalentgewicht von Gewebekolloiden wurde auf 1250 geschätzt, was auf eine für Mucopolysaccharide oder Mucoproteine charakteristische Grösse hinweist.

Ergebnisse anderer Arbeiten weisen darauf hin, dass die Erschlaffung der Symphyse von einer Desaggregation (Depolymerisation) von Bestandteilen der Grundsubstanz und von einer Zunahme der wasserlöslichen Fraktionen begleitet ist. In erschlafften Symphysen, wo die Kolloiddichte gering ist, weisen die Werte der Mobilität darauf hin, dass das Verhalten der Kationen sich hier demjenigen in Wasser nähert. In festen Symphysen treten selektive Effekte auf, im Zusammenhang mit den hohen Konzentrationen unbeweglicher Ladung. Insbesondere Kalium zeigte eine bedeutende Abnahme der Mobilität bis auf etwa die Hälfte des Wertes in Wasser. Bei anderen Ionen waren die Effekte viel geringer. Es zeigt sich also, dass Bindegewebe in hoch aggregierten Zustand selektiv wie ein Ionenaustauscher für Kalium wirkt. Bei Abbau der Aggregate und Wasseraufnahme geht die Selektivität verloren.

Aus den NaCl-Verdünnungspotentialen wurden die Konzentrationen der unbeweglichen Ladung ermittelt. Die Werte variierten von 0.20 Äquivalent pro Liter in fester Symphyse bis zu 0.05 oder weniger Äquivalent pro Liter im erschlafften Zustand. Werte dieser Grössenordnung wurden aus unabhängigen Zahlen errechnet.

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