Ion-Exchange Reactions Between Cartilage and Various Cations

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The mechanism of calcification of cartilage is considered to involve the formation of crystal nuclei upon which epitactic growth of the bone mineral takes place (Neuman & Neuman, 1958). Although the exact nature of the agent or agents responsible for the induction of the crystal nuclei is still unknown, proposals have been made about the nature of this mineralizing site. Strates, Neuman & Levinskas (1957) have shown that collagen can produce epitactic growth of hydroxyapatite crystals, and, as a consequence, it has been suggested that collagen or some molecule associated with it (i.e. mucopolysaccharide) may be responsible for crystal induction.

DiStefano, Neuman & Rouser (1953) have suggested that the mineralizing site is anionic and that a phosphorylated polysaccharide may be a factor in the formation of crystal nuclei. The presence of this compound correlated well with the ability of cartilage to calcify *in vitro*, but the implication of this compound in the calcifying mechanism is doubtful as it has been found in various non-calcifying rat tissues (Strates, 1956).

As a result of studies on the reversible inhibition of the calcification of rachitic-bone slices by cations, Sobel (1955) suggested that chondroitin sulphate is implicated as a component of the crystal-nucleation process. Subsequently, Bachra, Sobel & Stanford (1959) and Bachra & Sobel (1959) have shown that collagen reconstituted with chondroitin sulphate or other precipitating agents is capable of mineralizing *in vitro*.

Studies by Boyd & Neuman (1951) have indicated that the binding of cations by hyaline cartilage is largely dependent on the chondroitin sulphate content of the cartilage and that the process is one of ion exchange. However, they have found that such a cartilage preparation failed to induce crystal formation at physiological concentrations of calcium and phosphate ions. From this it is concluded (Neuman & Neuman, 1958) that if Sobel is correct in assigning a role to chondroitin sulphate in crystal nucleation, then the chondroitin sulphate of actively calcifying cartilage must differ either in structure or in its combination with collagen from that of the non-mineralizing hyaline cartilage.

In further investigations of the role of chondroitin sulphate in the calcification process, Dunstone (1959) found that the capacity of cartilage for bind-

ing various cations was relatively constant for Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} and Ba^{2+} ions, but higher capacities were observed for Be^{2+} and Cu^{2+} ions. The greater binding was attributed to the binding of multinuclear ions of these two elements. It was also thought that the greater binding of these two ions might be related to their powerful inhibition of calcification of rachitic-bone sections *in vitro*.

In order to study the mechanism of this inhibition of the calcification process by cations, the binding of these cationic inhibitors by cartilage has been investigated further. A study of the factors which influence the reactions between cartilage, saturated with these cations, and aqueous solutions of other cations has been made. The relative affinities of the cations for the cartilage have been measured and recorded in the form of exchange constants calculated from a Rothmund & Kornfeld (1918) equation.

Equations of this type have proved quite satisfactory in providing a quantitative measure of the relative affinities of ions for ion-exchange materials (Djurfeldt & Samuelson, 1957). Equation (1) is a modified version of the original Rothmund & Kornfeld equation for an exchange between ions of equal valency.

$$\frac{X_A}{X_B} = K \left(\frac{[A]}{[B]} \right)^p, \quad (1)$$

where X_A and X_B are the equivalent fractions of the ions A and B in the exchanger, $[A]$ and $[B]$ are their concentrations in the solution, and K and p are empirical parameters.

For an exchange between an exchanger, saturated with a univalent ion, and a bivalent ion from a solution, the equation is

$$\frac{X_N}{X_M^2} = K \left(\frac{[N]}{[M]^2} \right)^p, \quad (2)$$

where X_N and X_M are respectively the equivalent fractions of the bi- and uni-valent ions in the exchanger and $[N]$ and $[M]$ are their concentrations in the solution.

In the presentation of results equivalent fractions of a particular cation in the cartilage (X_c) and in the solution (X_s) are used. The equivalent fraction of a cation in the cartilage or solution phase is defined as the ratio of the number of equivalents (or m-equiv.) of that cation to the total number of

equivalents (or m-equiv.) of cations in the particular phase.

The calculation of constants from the equations (1) and (2) does not permit a direct comparison of the affinities of ions of unequal valency, although such constants do provide a good comparison between ions of the same valency. By plotting the equivalent fractions of the ions in the exchanger against their equivalent fractions in the solution, a direct comparison of the affinities of all ions, irrespective of valency, can be made (Kressman & Kitchener, 1949*a, b*; Bauman & Eichorn, 1947). This method has been used to compare the affinities of all ions for the cartilage.

EXPERIMENTAL

Preparation and analysis of the cation forms of cartilage. The various cation-saturated forms of the cartilage were prepared and analysed by the methods described by Dunstone (1959). All samples were analysed for both sulphate and cation.

Preparation of solutions. Cation solutions were prepared from the chlorides (A.R.) of the metals. With calcium and magnesium these chlorides were of uncertain composition and the solutions were analysed for the particular cation.

Analysis of solutions. Magnesium was determined by titration with ethylenediaminetetra-acetic acid (EDTA), Eriochrome Black T being used as indicator (Griswold & Pace, 1956). Calcium was determined by titration with EDTA, with murexide as indicator (Dunstone, 1957). Copper was determined by measuring the extinction of the copper-EDTA complex at a wavelength of 280 m μ in a Beckman model DU spectrophotometer (Dunstone, 1959).

Exchange reactions. Samples of cation-saturated cartilage (0.05–0.5 g.) were placed in glass-stoppered test tubes and measured volumes (5–100 ml.) of calcium chloride solution (6–12 m-equiv./l.) containing radioactive ^{45}Ca (40 $\mu\text{C}/\text{l.}$) were added. Shaking was carried out manually at intervals during several hours, after which time no further changes in the ion distributions occur (Dunstone, 1959). Exchange reactions involving other ions in the solution were prepared in similar fashion. No attempt has been made to control the temperature as ion-exchange reactions in general are little

affected by temperature changes (Patton & Ferguson, 1937; Magistad, Fireman & Mabry, 1944; Boyd, Schubert & Adamson, 1947.)

Radiochemical analyses. After equilibrium had been reached, portions (0.2 ml.) of the supernatant solutions were transferred to aluminium planchets (25 mm. diam.), 20 drops of an asbestos suspension (Francis, Mulligan & Wormall, 1954) were added to assist spreading and the planchets were dried under an infrared lamp. The radioactivity was then measured with an end-window Geiger-Müller tube (20th century Electronics Ltd., EW3H), coupled with a standard scaling and recording unit (Philips Electrical Industries Pty. Ltd.) No corrections were necessary for self-absorption of the β -emission of ^{45}Ca , owing to the small mass on the planchets (0.4–0.7 mg.), or for the physical decay, as comparisons of initial and final concentrations were made from planchets prepared at the same time. All planchets were prepared in triplicate and provided that sufficient counts were taken (not less than 5000) the activity of the replicates did not differ by more than 2%. As a check of this method a comparison of the initial and final concentrations were made by both chemical and radiochemical methods. Identical results were obtained by both methods for the exchange between sodium-saturated cartilage and Ca^{2+} ions.

Calculations. The ratio of the initial to the final count rate, which is a measure of the ratio of the initial to the final concentration of the calcium in the solution, together with a knowledge of the mass and capacity of the cartilage, and the volume and concentration of the added solution, are sufficient data for the calculation of the concentrations of both ions in both phases. It is assumed that an equivalent exchange takes place, that no extraneous ions such as H^+ take part and that insignificant amounts of chondroitin sulphate are removed from the cartilage during equilibration.

Table 1, containing a typical set of experimental details, is included to illustrate the calculation of the results in suitable form for graphical presentation.

Exchange capacity of cartilage. The sulphate content of cartilage has been taken as a measure of the exchange capacity for the ions Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} and Ba^{2+} , and the complex ions of copper and beryllium (Dunstone, 1959). It is assumed that the complex ions of beryllium and copper which take part in the exchange reactions are essentially bivalent in character.

Table 1. *Calculation of experimental results*

Samples of barium-saturated cartilage (sulphate content, 0.81 m-equiv./l.) were equilibrated with 25 ml. portions of $^{45}\text{CaCl}_2$ solution (11.72 m-equiv. of Ca^{2+} ion/l., 40 μC of $^{45}\text{Ca}^{2+}$ ion/l.). After 16 hr., 0.2 ml. portions were taken for radiochemical analysis. All results are expressed as mean values of triplicate experiments. Graphical presentation of results is made by plotting the equivalent fraction of the Ca^{2+} ion in the cartilage (X_c) against its equivalent fraction in solution (X_s). The parameters, K and p , have been calculated from equation (1).

Mass of cartilage (g.)	^{45}Ca activity (counts/min./ml.)	Concn. of Ca^{2+} in soln. (m-equiv./l.)	Equivalent fraction Ca^{2+} in soln. (X_s)	Ca^{2+} bound to cartilage (m-equiv.)	Total exchange capacity of cartilage (m-equiv.)	Equivalent fraction of Ca^{2+} in cartilage (X_c)
—	4835	11.72	1.000	—	—	—
0.1	3995	9.68	0.826	0.051	0.081	0.630
0.2	3560	8.63	0.736	0.077	0.162	0.475
0.3	3190	7.74	0.660	0.100	0.243	0.412
0.4	2970	7.20	0.614	0.113	0.324	0.349
0.5	2655	6.43	0.549	0.132	0.405	0.326

RESULTS

Reversibility of the exchange reactions. The reversibility of the exchange reactions was investigated by an indirect method. $^{45}\text{CaCl}_2$ solutions (12 m-

equiv./l.) were allowed to equilibrate with known masses of cation-saturated cartilage. The distribution of the ions between the phases was determined and the equivalent fraction of the Ca^{2+} ion in the cartilage phase were plotted against its equivalent

Fig. 1. Exchange reactions were carried out as described in the Experimental section. In each exchange reaction the distribution of the ions between the phases was determined (see Experimental section) and the equivalent fraction (X_c) of the Ca^{2+} ion in the cartilage phase was plotted against its equivalent fraction (X_s) in the solution phase. (a) Reversibility of exchange reactions. Samples (0.1–0.5 g.) of cation-saturated cartilage were equilibrated with 25 ml. portions of $^{45}\text{CaCl}_2$ soln. (12 m-equiv./l.). △, Barium-saturated cartilage; ▲, barium-saturated cartilage, 5 ml. of BaCl_2 soln. (12 m-equiv./l.) added after initial equilibration; ○, sodium-saturated cartilage; ●, sodium-saturated cartilage, 5 ml. of NaCl soln. (12 m-equiv./l.) added after initial equilibration. (b) Hysteresis effects in exchange reactions. Samples (0.1–0.5 g.) of cation-saturated cartilage were equilibrated with 25 ml. portions of cation solution (12 m-equiv./l.). ○, Sodium-saturated cartilage and $^{45}\text{CaCl}_2$ soln.; ●, calcium-saturated cartilage and NaCl soln. (c) Effect of solution concentration on the exchange reactions between cations and cartilage. Samples (0.1–0.5 g.) of cation-saturated cartilage were equilibrated with 25 ml. portions of $^{45}\text{CaCl}_2$ solutions of varying concentrations. ○, Sodium-saturated cartilage and $^{45}\text{CaCl}_2$ solution (7.5 m-equiv./l.); □, sodium-saturated cartilage and $^{45}\text{CaCl}_2$ solution (12.7 m-equiv./l.); ●, sodium-saturated cartilage and $^{45}\text{CaCl}_2$ solution (18.7 m-equiv./l.); △, magnesium-saturated cartilage and $^{45}\text{CaCl}_2$ solution (12.7 m-equiv./l.); ▲, magnesium-saturated cartilage and $^{45}\text{CaCl}_2$ solution (18.7 m-equiv./l.). (d) Effect of dilution on the exchange reactions between cations and cartilage. Samples (0.1–0.5 g.) of cation-saturated cartilage were equilibrated with 25 ml. portions of $^{45}\text{CaCl}_2$ solutions (12.7 m-equiv./l.). ○, Sodium-saturated cartilage; ●, sodium-saturated cartilage, 15 ml. of water added after initial equilibration; △, barium-saturated cartilage, 5 ml. of water added after initial equilibrium had been attained; ▲, barium-saturated cartilage, 25 ml. of water added after initial equilibration.

fraction in the aqueous phase. A second series of identical experiments were set up, and, after equilibration, portions of solutions of the cation originally present in the cartilage (12 m-equiv./l.) were added, and the system was again allowed to equilibrate. The distribution of ions between the phases was determined and the equivalent fractions were plotted on the same axes as those used for the experiments without the cation additions. Typical results are shown in Fig. 1 (a), which indicates that, under these experimental conditions, the exchange reactions are reversible. However, there does appear to be some slight deviation in the exchange between Ca^{2+} ions and sodium-saturated cartilage.

A second method was used to investigate further the exchanges between uni- and bi-valent ions. A solution of a bivalent ion (12 m-equiv./l.) was equilibrated with cartilage saturated with the univalent ion, and a solution of the univalent ion (12 m-equiv./l.) was equilibrated with cartilage saturated with the bivalent ion. The distributions of the ions between the phases were determined and the equivalent fractions plotted in the manner previously described. Fig. 1 (b) shows the results for such experiments with sodium as the univalent ion and calcium as the bivalent ion. It is obvious that different ion distributions are obtained in each case. Similar results have been found for exchanges between magnesium and sodium but with slightly greater differences between the curves.

Effect of the concentration of the solution. This effect was investigated by equilibrating $^{45}\text{CaCl}_2$ solutions of different concentrations with known masses (0.1–0.5 g.) of cation-saturated cartilage. The results of exchange reactions with sodium and magnesium as the saturating ions are shown in Fig. 1 (c). With decreases in solution concentration, no changes in the distribution curves were observed for the exchange between ions of equal valency, but there was an increased uptake of the bivalent ion by the cartilage for the exchanges between ions of unequal valency.

A second method of investigating this effect was also employed. Solutions of $^{45}\text{CaCl}_2$ were equilibrated with known amounts of cation-saturated cartilage, the distribution of the ions between the phases was determined and the respective equivalent fractions were plotted in the manner previously described. A second series of identical experiments were set up and, after equilibration, water was added to alter the solution concentrations. After further equilibration the ion distributions were determined and the equivalent fractions plotted on the same axes as those used for experiments without the water additions. The results shown in Fig. 1 (d) confirm the findings of the first method.

Affinity of cations for cartilage. The results of experiments carried out by equilibrating $^{45}\text{CaCl}_2$

solutions with cartilage saturated with the cations under investigation are shown in Fig. 2, in which the equivalent fractions are plotted as described above.

Fig. 2 clearly indicates that the order of increasing affinity for cartilage is $\text{K}^+ \approx \text{Na}^+$, Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Be^{2+} (probably a complex ion) and Cu^{2+} (probably a complex ion). In most experiments the mass of the cartilage was varied in preference to varying the volume of solution because a wider range of equivalent fractions in both phases could be obtained. However, identical curves were obtained when the volume of the solution was varied.

Equations (1) and (2) have been applied to the experimental data and the values for the parameters K and p are tabulated in Table 2.

The values show that the parameter p is approximately unity, that the affinity series for the bivalent ions is essentially the same as that deduced from Fig. 2 but that sodium has a slightly greater affinity for the cartilage than has potassium.

Exchange reactions were carried out in unbuffered solutions, the pH being measured at the beginning

Fig. 2. Comparison of affinities of cations for cartilage. Exchange reactions were carried out and the distribution curves drawn as described in Fig. 1. Samples (0.1–0.5 g.) of cation-saturated cartilage were equilibrated with $^{45}\text{CaCl}_2$ soln. (11.7 m-equiv./l.). $\circ$, Sodium-saturated cartilage; $\bullet$, potassium-saturated cartilage; $\triangle$, magnesium-saturated cartilage; $\blacktriangle$, calcium-saturated cartilage; $+$, strontium-saturated cartilage; $\blacksquare$, barium-saturated cartilage; $\square$, beryllium-saturated cartilage; $\odot$, copper-saturated cartilage.

and end of each experiment. The initial pH was 7.0 in all experiments. In exchange reactions involving cartilage saturated with Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} and Ba^{2+} ions, the final pH varied between 6.8 and 7.2. In exchanges involving copper-saturated and beryllium-saturated cartilage, the final pH values were respectively 5.7 and 4.8.

DISCUSSION

The curves in Figs. 1–2, which have been used to represent the experimental results, may be either convex or concave. Those curves which are convex (reaction: $\text{Ca}^{2+} + \text{Na}^+$ –cartilage, Fig. 2) indicate that the binding of the cation for which the equivalent fraction has been calculated (in this case Ca^{2+} ion) is stronger than that of the other reacting cation. Conversely, a concave curve (reaction: $\text{Ca}^{2+} + \text{Ba}^{2+}$ –cartilage, Fig. 2) indicates that the binding of the cation for which the equivalent fraction has been calculated (in this case Ca^{2+} ion) is weaker than that of the other reacting cation. For two reacting cations that have equal strengths of binding the curve becomes a straight line joining the points (0, 0) and (1.0, 1.0). Thus if a common cation is caused to react with cartilage saturated with various cations, a series of cation-binding affinities can be determined by a study of the relative positions of the ion-distribution curves.

The curves illustrated in Figs. 1 and 2 are similar to those derived theoretically by Bauman & Eichorn (1947) and those obtained practically by Kressman & Kitchener (1949*a, b*) for other exchange materials. However, the curves obtained with cartilage differ from those obtained by the above-mentioned workers in two respects. First, for the exchange between a bivalent ion in solution and a cartilage-bound univalent ion the curve appears to approach a maximum value of 0.9 and not 1.0 for the equivalent fraction in the cartilage. Secondly, the curve for the reaction between Ca^{2+} ions and calcium-saturated cartilage does not follow the expected curve for an exchange involving cations of equal cation-binding affinity. These differences indicate that a fraction of the cations initially bound to the cartilage is not capable of exchanging under these experimental conditions.

It is evident from Fig. 1 (*a*) that the exchange reactions are reversible with only slight deviations in the exchange between Ca^{2+} ions and sodium-saturated cartilage.

This finding appears to be contradicted by two facts, namely: (i) the ion distribution obtained when a cartilage-bound univalent ion is exchanged for a bivalent ion is different from the ion distribution obtained when a cartilage-bound bivalent ion is exchanged for a univalent ion (Fig. 1*b*); (ii) the parameter, K , for the exchange between calcium-

saturated cartilage and Ca^{2+} ions, is 0.62 (where $p \neq 1$), and not unity, as would be expected from equation (1).

These results also suggest that some of the ions initially bound to cartilage are bound in such a way as to prevent their ready exchange and that those ions which exchange readily do so reversibly. Similar hysteresis effects have been observed for other naturally occurring exchange materials by Vanselow (1932).

The experimental data used in Fig. 2 to show the exchange between calcium-saturated cartilage and Ca^{2+} ions, coupled with the fact that the value of K for this reaction should be unity (for $p = 1$), should enable the amount of calcium bound 'irreversibly' to the cartilage, under these experimental conditions, to be determined. Such a calculation has been made and 18 % of the initially bound Ca^{2+} ions have been found to be bound 'irreversibly'.

The curves for exchanges in which univalent ions are initially bound to cartilage indicate that the fraction of 'irreversibly' bound ions lies in the range 10 (Figs. 1*a*, 1*c*, 1*d*, Fig. 2) to 20 % (Fig. 1*b*).

Fig. 3 has been obtained by recalculating the experimental data of Fig. 1 (*b*) assuming that 20 % of the cations initially bound to cartilage are bound 'irreversibly'. It can be seen that the data which previously defined two separate curves now define a single curve of the type expected for a thermodynamically reversible exchange reaction (Bauman & Eichorn, 1947; Kressman & Kitchener, 1949*a, b*). An identical result has been found for the sodium-magnesium exchange on cartilage. These facts further support the hypothesis that a fraction of cartilage-bound cations are bound 'irreversibly' under these experimental conditions.

Fig. 3. Recalculation of the experimental data from Fig. 1 (*b*) on the basis that 20 % of initially bound cations are bound 'irreversibly'. $\circ$, Sodium-saturated cartilage and $^{45}\text{CaCl}_2$ soln.; $\bullet$, calcium-saturated cartilage and NaCl soln.

Table 2. *Exchange parameters for reactions between cartilage and cations*

The parameters were calculated by applying equations (1) and (2) to the experimental data.

Reaction	Parameters calculated on the assumption that all initially bound ions are exchangeable		Parameters calculated on the assumption that 82% of the cations initially bound are exchangeable	
	<i>K</i>	<i>p</i>	<i>K</i>	<i>p</i>
Ca ²⁺ + K ⁺ -cartilage	0.09	0.86	0.10*	0.98*
Ca ²⁺ + Na ⁺ -cartilage	0.05	0.98	0.05*	1.05*
Ca ²⁺ + Mg ²⁺ -cartilage	0.83	1.03	1.35	1.06
Ca ²⁺ + Ca ²⁺ -cartilage	0.62	0.97	1.00	1.06
Ca ²⁺ + Sr ²⁺ -cartilage	0.60	0.96	0.82	1.03
Ca ²⁺ + Ba ²⁺ -cartilage	0.41	0.80	0.50	1.03
Ca ²⁺ + Be ²⁺ -cartilage	0.12	0.91	0.18	0.94
Ca ²⁺ + Cu ²⁺ -cartilage	0.09	0.95	0.11	1.03

* From Fig. 2, the exchangeable fraction in reactions involving Na⁺ and K⁺ ions approximates to 90%, and this fraction has been used in the recalculation of the results for these reactions.

By the use of this hypothesis and the experimental data of Fig. 2, the exchange parameters have been recalculated and are included in Table 2.

The estimation of the fraction of the cations bound 'irreversibly' can be regarded only as approximate, hence the recalculated parameters must also be regarded as approximate. However, these recalculated parameters may be considered as representing thermodynamically reversible reactions. The recalculation has conferred more uniformity on the parameter *p*, which can be considered as unity. Thus equations (1) and (2) become equivalent to mass-action equations, the parameter *K* representing the equilibrium constant.

Evidence that equations such as (1) and (2) can be used to describe these exchange reactions is found in Figs. 1 (c) and 1 (d), where the equation predicts and the experiment confirms that a decrease in solution concentration favours an increase in the selectivity of the cartilage for bivalent ions.

The cation-binding function of cartilage probably depends on the carboxyl and sulphate groups in the chondroitin sulphate (Dunstone, 1959). Changes of pH might be expected to modify the behaviour of such groups towards cations, but in order to avoid the introduction of competing ions the reaction mixtures were not buffered. However, the pH remained relatively constant for all exchanges except those involving beryllium and copper, where the decrease in pH was probably due to the hydrolysis of the displaced cation. In these two cases the H⁺ ion liberated in the hydrolysis may constitute a third competitive ion in the exchange reaction. No estimate of the possible contribution made by the H⁺ ion could be made, but it would appear from Fig. 2 that the contribution would have to be considerable to alter the positions of beryllium and copper relative to those of the other ions in the affinity series.

The series of elimination concentrations for cations reported by Simkiss & Tyler (1958) for cation-binding by egg-shell sulphated mucopolysaccharide is similar to the affinity series found for cartilage, but the positions of strontium and calcium, copper and beryllium are here reversed. These authors claim correlation between their elimination concentration series and the order of stability constants of the metal-chelate compounds. It is doubtful if such a correlation exists with cartilage as the order of affinity found for the alkaline-earth cations, except beryllium, is directly opposite to the order of stability of their chelate compounds (Martell & Calvin, 1952). However, the ions most strongly bound to cartilage, namely copper and beryllium, do form more stable chelates than those ions which are less strongly bound to the cartilage.

The cation-affinity series for cartilage bears some resemblance to the order of calcification-inactivating power of cations reported by Sobel (1955). The cations which inhibit the calcification process to the greatest extent, namely beryllium and copper, are among the ions most strongly bound to cartilage, whereas those which least inhibit calcification, namely sodium and potassium, show a low affinity for cartilage. With the affinity series as a basis, strontium would be expected to inhibit calcification to a greater extent than does magnesium, but Sobel (1955) has shown that its inactivating power lies somewhere between the powers of sodium and potassium. The differences observed between these two series may be due to the chondroitin sulphate of this hyaline-cartilage preparation differing structurally, or in its combination with protein, from that in the calcifying tissue used by Sobel (1955) or it may be that the process of calcification-inhibition is not one of simple ion exchange involving the 'reversible' binding sites of cartilage. The cartilage

preparation used represents a special case and samples taken from other sources and subjected to different chemical treatments might yield a different affinity series. Consequently, strict comparisons of results with those obtained by other workers should not be made.

Up to this point only the possible significance of the affinities for cartilage of 'reversibly bound' cations has been discussed. It is possible that the relative affinities of the 'irreversibly bound' cations might be of importance in the study of the mechanism of calcification, but the experimental data at present available do not permit discussion of this possibility.

SUMMARY

1. Some of the factors which influence exchange reactions between cations and cartilage have been investigated.

2. It has been found that a fraction of the cations initially bound to cartilage does not exchange readily with other cations. Those cations which do exchange readily do so reversibly.

3. The affinities of some cations for cartilage have been measured and recorded in the form of exchange constants, calculated from a Rothmund & Kornfeld (1918) equation. The order of increasing affinity for cartilage is K^+ , Na^+ , Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Be^{2+} (probably a complex ion) and Cu^{2+} (probably a complex ion).

4. A comparison of this affinity series with those obtained in studies on sulphated mucopolysaccharides and on the mechanism of calcification has been made.

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Purification of β -N-Acetylglucosaminidase from the Pig Epididymis

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In previous papers it has been shown that the richest source of β -N-acetylglucosaminidase in different mammalian species is the epididymis, and that the activity in this organ is exceptionally high in the pig (Conchie, Findlay & Levvy, 1959a, b).

It is probable that this enzyme, like β -glucuronidase, is concerned in the ultimate degradation of hyaluronic acid and chondroitin (Linker, Meyer & Weissmann, 1955). Pig epididymis, like other pig tissues, displays a remarkably low β -glucuronidase