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CONTRIBUTION OF CARBOHYDRATES TO THE CATION-EXCHANGE SELECTIVITY OF AQUATIC HUMUS FROM PEAT-BOG WATER

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ABSTRACT

A sample of soluble humic acid from peat-bog water was a glycoconjugate containing 46% of a glycuronoglycan moiety and 54% of a dark-brown chromophore. These accounted for 37% and 63%, respectively, of the titratable acidity of the polymer. Cation-exchange capacities, and cationic selectivity coefficients relative to magnesium ions (K_{Mg}^{Me}), were measured on the humic acid for Pb^{2+}, Cu^{2+}, Zn^{2+}, Ba^{2+}, Ca^{2+}, and Sr^{2+}, and compared with those of extractive-free *Sphagnum* and other mosses, their chlorite holocelluloses, and two soluble fragments of *Sphagnum* holocellulose, prepared by acidic and alkaline degradation, respectively. The humic acid showed considerably higher K_{Mg}^{Me} values than most of the control materials, the enhancement being especially marked for Pb^{2+}, Cu^{2+}, and Ca^{2+}. Scatchard plots showed that both parts of the glycoconjugate contributed to its selectivity, and that the selectivity of the carbohydrate part was greater in the humic acid than in the holocellulose or its soluble fragments. The results are explained by assuming that there are enhanced possibilities for cross-linking in the colloidal humic-acid complexes.

INTRODUCTION

The high cation-exchange capacities of *Sphagnum* and other mosses[1], and of peat[2–5] and aquatic humus from peat[6], are well documented, and there are reports of high selectivity in the binding of alkaline-earth and heavy-metal ions by these materials[2–6]. The profound significance of this selectivity for agriculture and fisheries is universally recognised[7].

The present work represents an attempt to trace the origin of the cation-exchange selectivity of a sample of soluble humic acid isolated from *Sphagnum* peat-bog water. An effort was made to establish whether its selectivity was similar to that of any component of the living *Sphagnum* moss, or whether entirely new selectivities appeared as a result of chemical changes occurring during humification.

It had been established that the total ion-exchange capacity of the living moss can be accounted for almost entirely by that of its holocellulose[1], and that the aquatic humus contains a glycuronoglycan moiety similar in composition to a soluble

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fragment of the holocellulose, prepared in the laboratory by autohydrolysis[8]. In addition, strong evidence was obtained that the non-carbohydrate moiety had originated by acid-catalysed dehydration and partial decarboxylation of residues of D-*lyxo*-5-hexosulopyranuronic acid (5-keto-D-mannuronic acid, 5KMA) present in the holocellulose[8].

Cationic selectivity coefficients (K_{Mg}^{Mc}) were therefore measured on the fresh, extractive-free moss, its chlorite holocellulose, soluble glycuronoglycans prepared from the holocellulose, and the whole humic acid. The results are presented as Scatchard plots[9], which allow conclusions to be drawn about the contributions of the separate parts of the macromolecules to the total selectivity. Some data for four other mosses that are believed to contribute importantly to the production of aquatic humus are included.

EXPERIMENTAL

Materials. — Bog water (20 L) was made mM with respect to copper sulphate, and the precipitate was collected by decantation and centrifugation, suspended in water (200 mL), and dialysed exhaustively against 0.1M ethylenediamine tetra-acetic acid (Na^+ salt, pH 7) and then against water. The solution was centrifuged and freeze-dried, to yield a coffee-coloured solid (*A*, 1.5 g).

Freshly harvested *Sphagnum quinquefarium* (Braithw.) Warnst. was dried in a current of air at 60°, milled to pass a 20-mesh sieve, and extracted with acetone and methanol until the extracts were colorless. It was then air-dried, to yield a light-brown powder (*B*). Chlorite delignification was carried out as described by Whistler and BeMiller[10], to yield pure, white holocellulose (*C*).

The holocellulose was converted into its free-acid form by washing with 0.05M hydrochloric acid, followed by distilled water until the washings were neutral. A portion (5 g) was then suspended in distilled water (1 L) and heated at 98° in a current of nitrogen. At daily intervals for 10 days, the fibrous residue was filtered-off, suspended in fresh distilled water, and heated again. The combined filtrates were concentrated *in vacuo* at 30° to 500 mL, dialysed against distilled water, and freeze-dried. The yield of light-brown polymer (*D*) was 2.5 g.

The free-acid form of the holocellulose (5 g) was suspended in propylene oxide (100 mL), kept at room temperature for 48 h, and then collected by filtration, washed with acetone, and air-dried. The resulting propylene glycol ester was shaken overnight under nitrogen with 2M potassium hydroxide at 20°. The mixture was then filtered, and the filtrate was neutralised with acetic acid, dialysed against distilled water, and freeze-dried. The yield of light-brown polymer (*E*) was 2.0 g.

Analytical methods. — For qualitative sugar analysis, samples (20 mg) were heated overnight in 0.5M sulphuric acid (2 mL) at 98°. The solutions were then neutralised with barium carbonate, filtered, concentrated, and subjected to t.l.c. on cellulose (ethyl acetate–acetic acid–pyridine–water, 5:3:1:4; detection with alkaline silver nitrate[11]).

For quantitative sugar analysis, samples (10 mg) were heated in sealed tubes with 0.5M hydrochloric acid (3 mL) at 98° for 3, 6, 9, 12, 18, and 24 h, respectively, and then cooled. Portions (2.5 mL) of the hydrolysates were mixed with 0.2M sodium acetate (3 mL), the pH was adjusted to 6 with 0.5M sodium hydroxide (2.4 mL), and the solutions were then diluted appropriately for analysis.

D-Glucose was determined with D-glucose oxidase[12]; hexuronic acid by the carbazole method with D-galacturonic acid as standard[13]; xylose and arabinose (combined) by the phloroglucinol method[14]; rhamnose by the thioglycolic acid method[15]; and mannose and galactose by Dische's "secondary" cysteine reaction[16], a correction being applied for the contribution of glucose, measured separately. The results were plotted against time. In the determination of glucose, the true glucose content was taken as the point of intersection of the initial and final slopes of the plots. In the other assays, the plots showed decay only, and were extrapolated to zero time.

5-Keto-D-mannuronic acid (5KMA) was determined[17] by warming the unhydrolysed samples with an excess of aqueous 0.1% phenylhydrazine at 60° for 2 h. The orange-coloured complexes were collected by centrifugation, washed with ethanol, dried, and then decomposed by shaking overnight at room temperature with conc. hydrochloric acid. After filtration and appropriate dilution, the liberated phenylhydrazine in the solutions was measured by its absorbance at 275 nm.

For the measurement of selectivity coefficients[18], portions (15–25 mg) of material were dissolved or suspended in water (2–5 mL) and enclosed in small cellophane dialysis-sacs. These were shaken with 50-mL portions of 0.2M solutions of the mixed chlorides or nitrates of magnesium and the test metal. The ratio of Mg^{2+} to test ion in these solutions was varied from 0.1 to 2000, depending upon the selectivity and the degree of saturation desired. The solutions were replaced at daily intervals for 5 days, to ensure that the concentrations at equilibrium in the solution phase were of the pre-determined ratio. The dialysis sacs were then dialysed exhaustively against distilled water to remove all unbound salts, and against 0.2M hydrochloric or nitric acid to displace the bound cations. The mole fractions, X_{Me} and X_{Mg}, of the cations originally bound on the polymer phase were then measured by using these solutions and a Perkin–Elmer Model 560 atomic absorption spectrophotometer. The selectivity coefficients were calculated from the formula $K_{Mg}^{Me} = (X_{Me}.C_{Mg})/(X_{Mg}.C_{Me})$, where C_{Mg}/C_{Me} is the predetermined ratio of cations in the ambient solution. The ion-exchange capacities were obtained by plotting $(X_{Me} + X_{Mg})$ against X_{Me}, and extrapolating to $X_{Me} = 1$ (or to $X_{Me} = 0$ for magnesium ions).

RESULTS

Isolation and examination of humic acid (A). — Water was collected from a hole dug in a *Sphagnum* peat bog, shortly after a heavy shower that had been preceded by a long, dry period. This provided a conveniently high concentration of

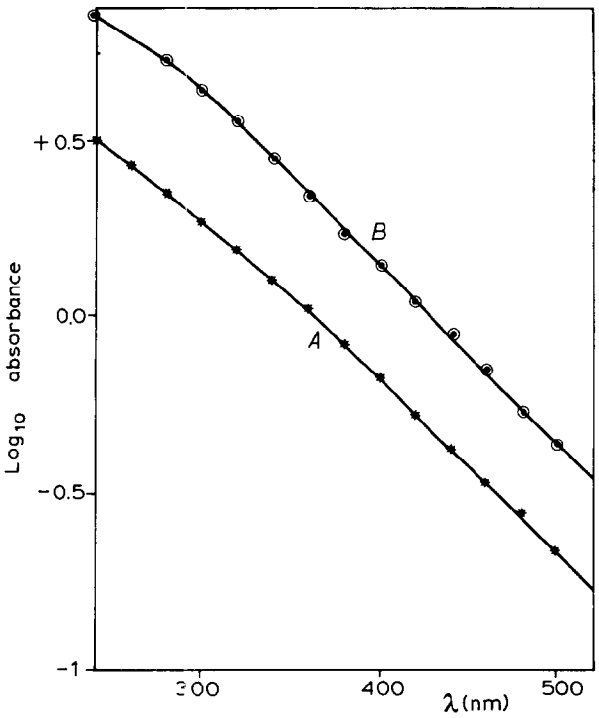

Fig. 1. Absorption spectra of solutions (10 μg/mL) in 25mM sodium borate buffer (pH 10). Curve *A*, aquatic humus; curve *B*, chromophore isolated from aquatic humus by acid hydrolysis.

humic acid, and some assurance that it had not been exposed to oxygen for long. The humic acid was almost completely insoluble below pH 2, and was purified by reprecipitation several times at this pH. Between pH 2 and 10, it existed in solution as colloidal aggregates that bound irreversibly to chromatographic and electrophoretic support media. At pH 8, free boundary electrophoresis indicated that most of the material migrated as a single peak, but the high absorbance of the solution would have obscured the presence of minor components. At higher pH values, irreversible chemical changes, leading to an increase in titratable acidity, were noted.

TABLE I

DISTRIBUTION OF IONIC GROUPS IN THE HUMIC GLYCOCONJUGATE *(A)*[a]

Moiety	A (%)	*Acidic groups (mequiv./g of* A)	*N (%)*
Carbohydrate	46	0.543	—
Chromophore	54	0.923	4.65
Total	100	1.466	2.51

[a]Free-acid form.

TABLE II

SUGAR COMPOSITIONS OF TEST MATERIALS (MOLES %)

Material	*GalA*	*5KMA*	*Rha*[a]	*Pentoses*[b]	*Glc*	*Gal*	*Man*	*Total*
Carbohydrate moiety of *A*	8	16	26	10	15	3	23	100
Holocellulose *(C)*	21	9	6	14	38	3	6	97
Fragment *D*	25	27	19	6	7	10	4	98
Fragment *E*	22	—	24	3	10	4	24	87[c]

[a]Includes traces of fucose. [b]Includes arabinose and xylose. [c]Preparation contains unsaturated derivatives of uronic acids formed by β-elimination under the conditions of extraction with alkali; these are lost upon acid hydrolysis.

The sodium salt contained 48.1% of C and 2.43% of N, and its absorption spectrum (Fig. 1, curve A) was characteristically[2-6] monotonal. Potentiometric titration (Fig. 2, curve A) showed one, main equivalence point at pH 8.3, corresponding to an equivalent weight of 682, and a minor one at pH ~5.1, correspond-

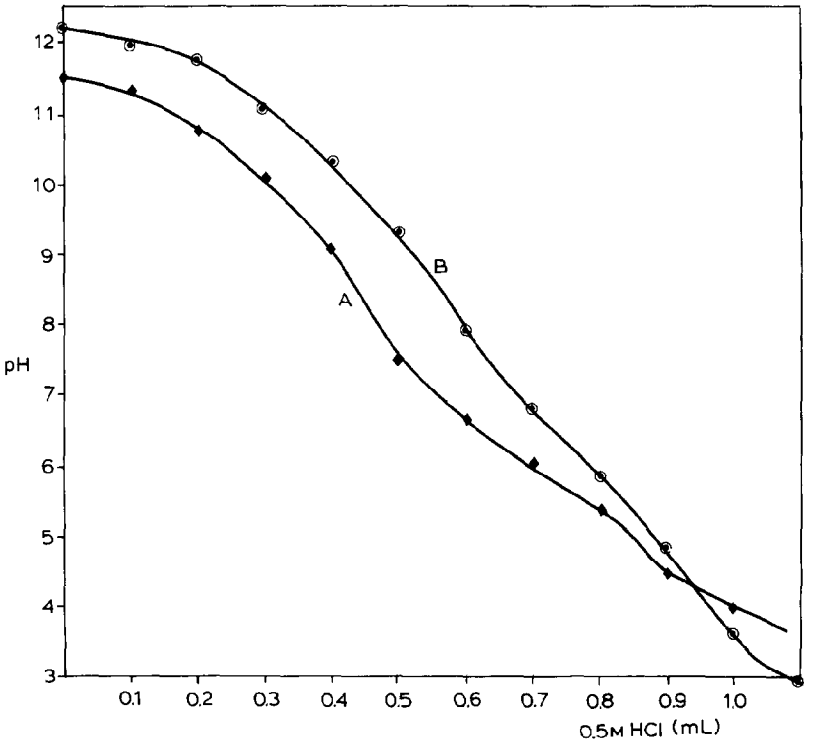

Fig. 2. Potentiometric titration of *A*, aquatic humus (188 mg); and *B*, isolated chromophore (97 mg). Samples were dispersed in 0.5M sodium hydroxide (1.0 mL) and back-titrated with 0.5M hydrochloric acid.

ing to an equivalent weight of ~1560. The latter was not considered to be due to a different kind of functional group (see Discussion).

On hydrolysis with 0.5M sulphuric acid at 98° for 12 h, the humic acid liberated galacturonic acid, galactose, glucose, mannose, arabinose, xylose, and rhamnose, and 58.5% of an almost black, acid-insoluble chromophore having an equivalent weight (Fig. 2, curve B) of 425. The sodium salt contained 57.4% of C and 1.60% of N. Its absorption spectrum (Fig. 1, curve B) was almost parallel to that of the original glycoconjugate, and, on the assumption that hydrolysis had not changed its absorbance, indicated that its content in the latter was ~52%.

The glycuronoglycan moiety was isolated in 46% yield by chlorite-bleaching[10] and dialysis. It contained some particulate matter that appeared to be degraded cellulose and hemicelluloses, but ~90% of it was freely soluble in acid. The sugar composition of the whole carbohydrate moiety is given in Table II. Its equivalent weight was 847, and it contained no nitrogen.

Since it is established that chlorite-bleaching does not modify glycans significantly, whereas the residues of 5KMA in the carbohydrate moiety would have been converted into chromophore under the conditions of acid hydrolysis used to isolate the latter[8], it was concluded that the distribution of acidic groups in the glycoconjugate is most reliably represented by the data in Table I.

Quantitative sugar analysis. — The principal difficulties were incomplete release of sugars, because of the initial insolubility of the holocellulose and the formation of acid-resistant aldobiouronic acids, and the decay of sugars by dehydration reactions[19]. The decay of 5KMA was rapid and complete, but it could be determined for unhydrolysed samples by making use of its reactivity with phenylhydrazine[17]. This method would not determine 5KMA residues substituted at position 5, of which there appear to be a small proportion in the holocellulose[17].

For the other sugars, the adopted methods of extrapolation (see Experimental) probably gave an accuracy of ±10%. Its is noteworthy that the traditional

TABLE III

ION-EXCHANGE CAPACITIES (MEQUIV /G) OF TEST MATERIALS

Cation	*Humic acid* (A)	Sphagnum *moss* (B)	*Holocellulose* (C)	*Fragment* D	*Fragment* E
Na^+	1.47 ±0.1	0.80 ±0.1	1.60 ±0.1	2.45 ±0.05	1.55 ±0.05
Mg^{2+}	1.47 ±0.1	1.00 ±0.05	1.60 ±0.1	2.50 ±0.15	1.60 ±0.1
Ca^{2+}	1.35 ±0.1	0.94 ±0.1	1.44 ±0.15	2.45 ±0.2	1.60 ±0.1
Sr^{2+}	1.28 ±0.2	0.94 ±0.1	1.44 ±0.15	2.45 ±0.15	1.60 ±0.1
Ba^{2+}	1.60 ±0.2	1.00 ±0.2	1.60 ±0.2	2.50 ±0.2	1.60 ±0.2
Zn^{2+}	2.06 ±0.2	1.00 ±0.2	1.60 ±0.2	2.66 ±0.1	1.60 ±0.2
Cu^{2+}	2.44 ±0.2	1.23 ±0.1	1.70 ±0.1	3.10 ±0.15	1.60 ±0.1
Pb^{2+}	2.45 ±0.2	1.36 ±0.1	1.76 ±0.1	2.78 ±0.1	1.60 ±0.1

TABLE IV

ION-EXCHANGE CAPACITIES (MEQUIV./G)[a] OF OTHER MOSSES CONTRIBUTING TO THE PRODUCTION OF AQUATIC HUMUS

Cation	Hylocomium splendens	Rhytidiadelphus loreus	Polytrichum commune	Rhacomitrium lanuginosum
Na^+	0.76	0.81	0.74	0.65
Mg^{2+}	0.50	0.52	0.44	0.40
Ca^{2+}	0.55	0.52	0.44	0.40
Sr^{2+}	0.50	0.52	0.46	0.40
Zn^{2+}	0.75	0.66	0.60	0.65
Pb^{2+}	0.88	0.77	0.75	0.75
Cu^{2+}	1.00	0.94	0.82	0.97

[a]After extraction with acetone. Probable error, ±0.1 mequiv./g.

methods of colorimetric analysis, depending as they do upon the use of concentrated acids and the measurement of dehydration products instead of the sugars themselves, are well suited for coping with the problems noted above. The results are presented in Table II.

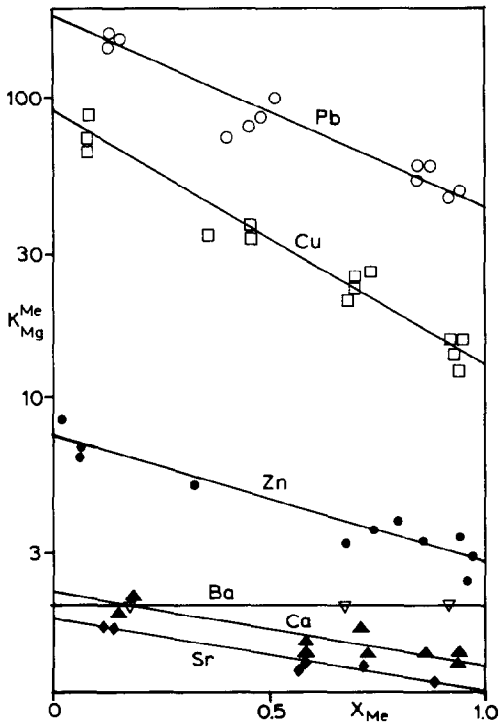

Fig. 3. Whole *Sphagnum* moss: semi-log plots of selectivity coefficients (K^{Me}_{Mg}) against mole fraction (X_{Mc}) of test cation in the polymer phase.

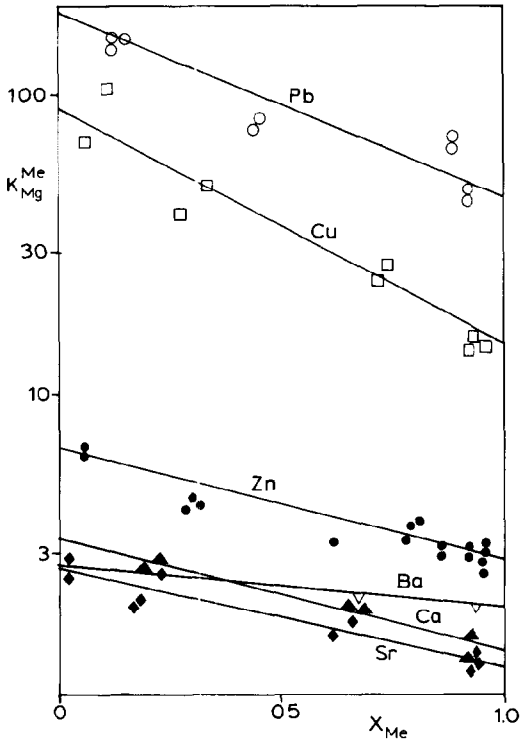

Fig. 4. *Sphagnum* holocellulose: semi-log plots of K^{Me}_{Mg} against X_{Me}.

Cation-exchange capacities. — Table III presents data for the ion-exchange capacities of the five main test-materials. All except *E* bound significantly more Cu^{2+} and Pb^{2+} than the other ions, and the humic acid also showed an enhanced

TABLE V

SELECTIVITY COEFFICIENTS (K^{Me}_{Mg}) AT HALF-SATURATION FOR FOUR MOSSES[a] AND THEIR CHLORITE HOLOCELLULOSES (HC)

Cation	H. splendens		R. loreus		P. commune		R. lanuginosum	
	Moss	*HC*	*Moss*	*HC*	*Moss*	*HC*	*Moss*	*HC*
Ca^{2+}	2.5	1.6	2.5	1.8	1.5	1.7	1.0	1.5
Sr^{2+}	1.3	1.3	1.9	1.0	2.0	1.4	1.9	1.0
Zn^{2+}	7	5	7	5	6	8	8	10
Pb^{2+}	200	100	150	100	150	70	200	70
Cu^{2+}	700	50	1000	50	200	20	500	25

[a]After extraction with acetone.

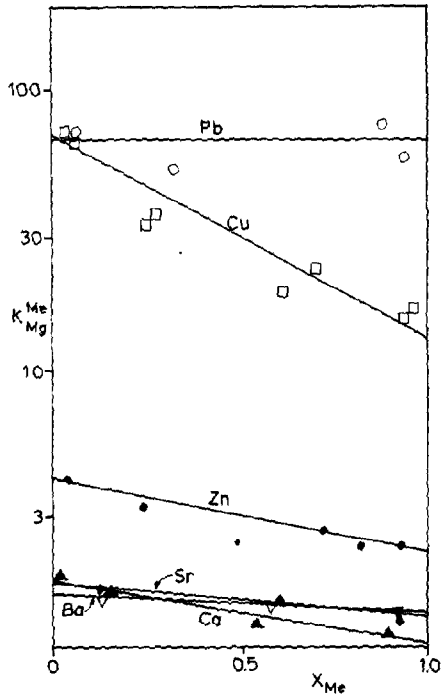

Fig. 5. Fragment (*D*) of *Sphagnum* holocellulose: semi-log plots of K^{Me}_{Mg} against X_{Me}.

capacity for Zn^{2+}. Some ion-exchange capacities for extractive-free samples of four other mosses are given in Table IV.

Cation-exchange selectivities. — Figs. 3–7 show Scatchard plots for the five main test-materials, respectively. The corresponding plots for the other four mosses and their holocelluloses were all closely similar to those of *Sphagnum* and its holocellulose, respectively, and the results are therefore presented concisely in Table V, as the K^{Me}_{Mg} values at half-saturation ($X_{Me} = 50\%$).

THEORY

Significance of intercepts in Scatchard plots. — Consider a polymer containing different, independent binding-sites, 1, 2, 3, *etc.*, with mole fractions F_1, F_2, F_3, *etc.*, and selectivity coefficients $(K^A_B)_1$, $(K^A_B)_2$, $(K^A_B)_3$, *etc.* Let the molar ratio (C_B/C_A) of the reference ion B to the test ion A in the solution phase be R, and let

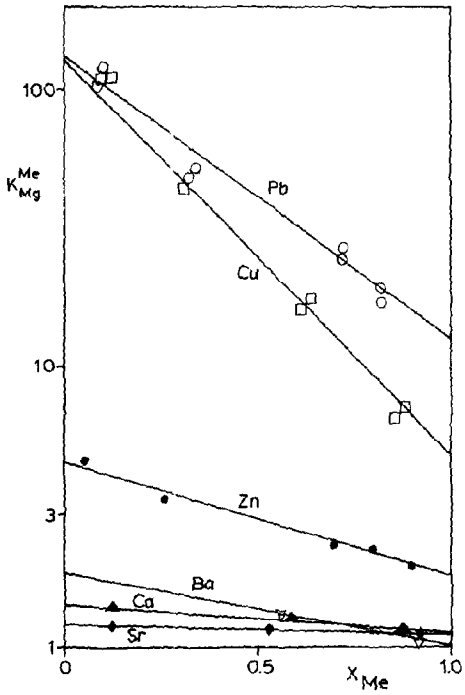

Fig. 6. Fragment (*E*) of *Sphagnum* holocellulose: semi-log plots of K_{Mg}^{Me} against X_{Me}.

X_1^A, X_2^A, X_3^A, *etc.*, be the separate degrees of saturation of the different binding sites. Then,

$$K_B^A = \frac{RX^A}{(1 - X^A)}; (K_B^A)_1 = \frac{RX_1^A}{(1 - X_1^A)}; (K_B^A)_2 = \frac{RX_2^A}{(1 - X_2^A)}, \textit{etc.}$$

Hence,

$$K_B^A = \frac{R[F_1X_1^A + F_2X_2^A + F_3X_3^A + \cdots]}{1 - F_1X_1^A - F_2X_2^A - F_3X_3^A - \cdots}$$

$$= \frac{F_1(1 - X_1^A)(K_B^A)_1 + F_2(1 - X_2^A)(K_B^A)_2 + \cdots}{1 - F_1X_1^A - F_2X_2^A - F_3X_3^A - \cdots}. \qquad (1)$$

Whence,

$$\text{Lim}_{(X^A \to 0)}\, K_B^A = F_1(K_B^A)_1 + F_2(K_B^A)_2 + F_3(K_B^A)_3 + \cdots.$$

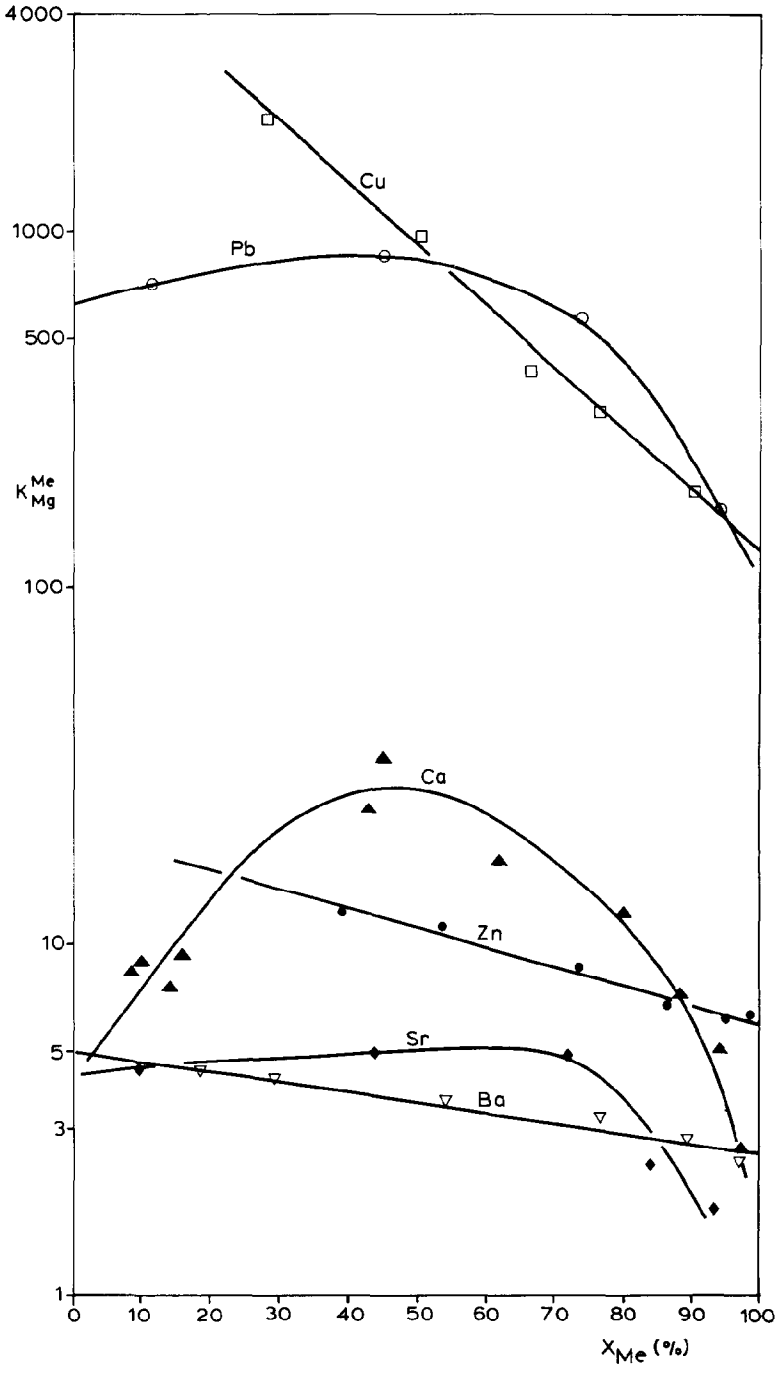

Fig. 7. Aquatic humus: semi-log plots of K^{Me}_{Mg} against X_{Me}.

When the binding sites of type 1 are the weakest, they will be the last to be filled, and hence $\mathrm{Lim}_{(X^A \to 1)} (1 - X^A) = F_1(1 - X_1^A)$, which, upon substitution in Eq. *1*, gives:

$$\mathrm{Lim}_{(X^A \to 1)} K_B^A = (K_B^A)_1,$$

because the other terms in the numerator vanish.

DISCUSSION

The humic acid was collected very close to its origin, and there can be little doubt that it originated almost entirely from *Sphagnum* moss. It is possible, however, that some of it could have run off from higher terrain, inhabited by other mosses, and data were therefore collected for four species that grow prolifically in the region (Trøndelag, Norway). There was very little other vegetation in the area.

The solubility properties of the humic acid prevented a satisfactory physical characterisation, but the ready solubility of the glycuronoglycan moiety at low pH, after removal of the chromophore by chlorite-bleaching, left little doubt that the two parts of the macromolecule were covalently linked.

The two points of inflexion in the potentiometric titration curve of the humic acid (Fig. 2, curve A), and, less clearly, in its isolated chromophore (Fig. 2, curve B) have been repeatedly noted by other workers[2,5], and attributed by some to the presence of nitrogen[5]. We found, however, that *Sphagnum* holocellulose and its soluble fragment (*D*), which contained no nitrogen, behaved similarly, and it seems likely that the transition at pH ~5 corresponds to a change in conformation or solubility, leading to the co-operative release of protons. Interestingly, the soluble fragment (*E*), which contained no 5KMA, did not show this behaviour.

The most striking feature of the data for cation-exchange capacity (Tables III and IV) is the higher capacity for Cu^{2+} and Pb^{2+} shown by all of the materials except fragment *E*. The only monomer found in these that was not found in *E* was 5KMA (Table II). At present, it is not possible to supply a definitive explanation, but it may be noted that 5KMA can be expected to isomerise readily to an analogue of ascorbic acid[17], whose ability to form complexes with heavy-metal ions is well known.

The Scatchard plots (Figs. 3–6) for the four main control materials are strikingly similar, and indicate that the living *Sphagnum* moss contains no component, other than holocellulose, that contributes importantly to its cation-exchange selectivity. This is consistent with the observation that the titratable acidity of the moss was also fully accounted for by that of its holocellulose component alone[1].

This is not true of the other mosses, however, whose non-carbohydrate components seem to contribute an exceptional selectivity for Cu^{2+} and Pb^{2+} (Table V). The higher nitrogen content of these mosses (2–3%, compared to 0.5% for *Sphagnum*) may be especially significant in this respect. The *Sphagnum* mosses are

unique in containing a high proportion of empty cells (hyalin cells, or leucocysts) whose function is simply to imbibe water[20], and hence it is to be expected that their protein content would be low.

With all of the control materials, there was a monotonal decline in selectivity with increasing degree of saturation of the polymer phase (X_{Me}), indicating the presence of more than one kind of binding site (Eq. *1*). This may be due simply to the presence of two different kinds of uronic acid residue (partly or wholly modified by β-elimination in *E*), but the presence of ring substituents could modify the selectivity of each, leading effectively to a multiplicity of binding sites.

Because of the similarity of *D* and *E* to some of the more complex pectins of higher plants[21], it is surprising that their selectivity coefficients for Ca^{2+}, Sr^{2+}, and Ba^{2+} are considerably lower. Values at $X_{Me} = 0.5$ of 7.0, 9.6, and 10.1, respectively, have been reported for apple and sunflower pectins[22]. The important effects of solubility upon selectivity must be recognised, however. Possibly because of their heavy substitution with neutral sugar residues, which could prevent the chains from associating closely enough for cross-linking, *D* and *E* were soluble in all the present experiments, whereas typical pectates would not be. With a fragment of alginate, rich in L-guluronic acid residues, it was found that K_{Mg}^{Ca} at $X_{Ca} = 0.5$ decreased from ~50 to ~5 when the chains were prevented from associating by dispersion in an agarose gel[23]. Likewise, the activity coefficient of Ca^{2+} in solutions of oligoguluronides decreased sharply as soon as the chains became long enough to form insoluble complexes[24].

Chelate-bridge formation of this type can be co-operative when the binding sites are adjacent in the chains, and is manifested in Scatchard plots as a positive slope at low degrees of saturation, followed by a negative slope at higher X_{Me} values, where all the possibilities for cross-linking have become exhausted, and only "unpaired" binding sites remain [23,25]. The theoretical treatment of this kind of system is more difficult[25], but the limiting value of K_{Mg}^{Me} as $X_{Me} \rightarrow 1$ would still be expected to correspond to the weakest of the "unpaired" binding sites.

Turning to the results for humic acid (Fig. 7), it is evident that both parts of the glycoconjugate contribute not only to the ion-exchange capacity, but also to the selectivity. This is particularly clear for the linear plots for Cu^{2+}, Zn^{2+}, and Ba^{2+}, which can be extrapolated to $X_{Me} = 1$, and compared with the corresponding values for the control materials. The contribution of the carbohydrate moiety to the selectivity is evidently greater in the glycoconjugate than in *C*, *D*, or *E*. Moreover, the slopes of these plots are not markedly greater than those for the control materials. It is therefore not possible to attribute the higher absolute values of the selectivity coefficients to the contribution of the non-carbohydrate moiety alone.

Co-operativity in the binding is very clear for Ca^{2+}, and less so for Pb^{2+} and Sr^{2+}. This implies cross-linking of the chelate-bridge type, and, indeed, the humic acid–metal ion complexes were insoluble in all experiments. As noted above, even the sodium salt did not form true solutions. This provides the most likely explana-

tion for the upward shift in selectivity noted for all the cations, and it is likely that a special study of the binding of Cu^{2+}, Zn^{2+}, and Ba^{2+} at low degrees of saturation would reveal co-operativity in these cases also.

It is not clear which parts of the glycoconjugate cross-link with which. Haworth[26] envisaged the humic acid molecule as a central core of aromatic material, to which glycan and polypeptide chains are attached at the periphery. This suggests a comparatively important role for these two parts. It should be noted that the observed N content of 2.51% in the humic acid studied in the present work would correspond to ~28% of polypeptide in the non-carbohydrate moiety.

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REFERENCES

1 T. J. PAINTER AND N. A. SØRENSEN, *Carbohydr. Res.*, 66 (1978) C1–C3.
2 M. M. KONONOVA, *Soil Organic Matter*, Pergamon, Oxford, 1961.
3 M. SCHNITZER, in M. SCHNITZER AND S. U. KHAN (Eds.), *Soil Organic Matter*, Elsevier, Amsterdam, 1978, pp. 1–64.
4 W. FLAIG, H. BEUTELSPACHER, AND E. RIETZ, in J. E. GIESEKING (Ed.), *Soil Components*, Vol. 1, Springer-Verlag, Berlin, 1975, pp. 1–211.
5 C. H. FUCHSMAN, *Peat–Industrial Chemistry and Technology*, Academic Press, New York, 1980.
6 E. T. GJESSING, *Physical and Chemical Characteristics of Aquatic Humus*, Ann Arbor Science Publishers, Ann Arbor, 1976.
7 D. POVOLEDO AND H. L. GOLTERMAN, *Proc. Int. Meet. Humic Substances, Nieuwersluis, 1972*, Pudoc, Wageningen, 1975.
8 T. J. PAINTER, *Carbohydr. Res.*, 124 (1983) c22–c26.
9 D. J. R. LAURENCE, in B. CARROLL (Ed.), *Physical Methods in Macromolecular Chemistry*, Vol. 1, Dekker, New York, 1972, pp. 91–184.
10 R. L. WHISTLER AND J. N. BEMILLER, *Methods Carbohydr. Chem.*, 3 (1963) 21–22.
11 W. E. TREVELYAN, D. D. PROCTER, AND J. S. HARRISON, *Nature (London)*, 166 (1950) 444–445.
12 I. D. FLEMING AND H. F. PEGLER, *Analyst (London)*, 88 (1963) 967–968.
13 T. BITTER AND H. M. MUIR, *Anal. Biochem.*, 4 (1962) 330–334.
14 Z. DISCHE, *Methods Carbohydr. Chem.*, 1 (1962) 487–488.
15 M. N. GIBBONS, *Analyst (London)*, 80 (1955) 268–276.
16 Z. DISCHE, *Methods Carbohydr. Chem.*, 1 (1962) 489–490.
17 T. J. PAINTER, *Carbohydr. Res.*, 124 (1983) c18–c21.
18 O. SMIDSRØD AND A. HAUG, *Acta Chem. Scand.*, 22 (1968) 1989–1997.
19 O. THEANDER, *Acta Chem. Scand.*, 8 (1954) 989–1000.
20 E. V. WATSON, *British Mosses and Liverworts*, Cambridge Univ. Press, Cambridge, 1955.
21 G. O. ASPINALL, *Polysaccharides*, Pergamon, Oxford, 1970, pp. 119–129; also in W. PIGMAN AND D. HORTON (Eds.), *The Carbohydrates*, 2nd edn., Vol. IIB, Academic Press, New York, 1970, pp. 515–521.

22 A. HAUG AND O. SMIDSRØD, *Acta Chem. Scand.*, 24 (1970) 843–854.
23 O. SMIDSRØD AND A. HAUG, *Acta Chem. Scand.*, 26 (1972) 2063–2074.
24 R. KOHN AND B. LARSEN, *Acta Chem. Scand.*, 26 (1972) 2455–2468.
25 I.-L. ANDRESEN, O. SKIPNES, O. SMIDSRØD, K.ØSTGAARD, AND P.-C. HEMMER, *ACS Symp. Ser.* 24 (1977) 361–381.
26 R. D. HAWORTH, *Soil Sci.*, 111 (1971) 71–79.