Cependant, le raccourcissement apparent des fibres fusoriales ne s'accompagne d'aucune augmentation de leur épaisseur ou de leur densité. La zône centrale de la cellule qui apparaissait uniformément transparente en métaphase subit des transformations profondes: au début du mouvement anaphasique aucun changement n'est perceptible, l'espace qui sépare les chromosomes est non absorbant (Fig. 1). Au fur et à mesure que les deux groupes s'éloignent l'un de l'autre dans leur répulsion symétrique, l'espace intermédiaire devient progressivement plus dense (Fig. 4). Le raccourcissement apparent des fibres chromosomiques ne s'accompagnant d'aucune augmentation de densité (donc de masse) pourrait s'expliquer par une destruction totale ou partielle de la fibre au point d'attache du chromosome progressant en même temps que le chromosome lui-même. Cette manière de voir trouve une confirmation dans le fait que la disparition progressive du fuseau au cours du cheminement des chromosomes s'accompagne d'une augmentation parallèle de la densité dans la zône centrale qui les sépare.

L'analyse microradiographique confirme l'opinion émise par STICH[4]. L'auteur pense qu'il y a destruction progressive des fibres fusoriales au cours de la migration des chromosomes: le centromère provoquerait la désintégration locale des hétéroprotéides en son point d'attache et arriverait jusqu'au pôle par destruction progressive de la fibre.

L'étude microradiographique de l'évolution du fuseau au cours de la méiose de *Listera ovata* permet d'aboutir aux conclusions suivantes:

1° Le fuseau apparaît en métaphase formé par les fibres individuelles de chaque chromosome convergeant vers les pôles et constituées par des substances de densité relativement élevée.

2° Les fibres chromosomiques disparaissent au fur et à mesure de la progression des chromosomes vers les pôles.

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[4] H. STICH, Chromosoma *6*, 199 (1954).

Zusammenfassung

Mikroradiographische Untersuchungen an der Spindel während der Meiose von *Listera ovata* führen zu folgenden Ergebnissen:

1. Die Spindel erscheint in der Metaphase aus den individuellen, stark absorbierenden Fasern jedes einzelnen Chromosoms gebildet.

2. Die Spindelfasern verschwinden während der Polwanderung der Chromosomen.

The Sodium and Potassium of Bone Mineral

The object of the present investigation was to study the nature of the sodium and potassium of bone by exhaustive acid extraction, using some of the bone materials and techniques which were used in previous work on the magnesium of bone mineral[1]. In the first series of experiments 5 g samples of 300 mesh (B. S.) fat-free oxi-bone powder were extracted with 12 successive 150 ml portions of 0·01 *N* hydrochloric acid by shaking for 15 min followed by centrifugation. Cortical bone from calcium-depleted hens[2] was treated in the same way. Samples of the undissolved residues and aliquots of the extracts were analysed for sodium and potassium at each stage of the fractionation with an EEL flame photometer.

A single 5-g sample of each type of bone was extracted once with 150 ml 0·01 *N* nitric acid and the amount of chloride extracted was determined. Virtually all the chloride present was extracted by this procedure.

The amounts of sodium, potassium, and chloride dissolved in the twelve extractions and the total amounts dissolved are given in Table I, together with the amounts originally present in the samples. The preferential release of sodium and potassium during the first extraction was very marked, particularly with the hen-bone. Almost 90% of the total potassium present in this bone appeared in the first extract, together with 38% of the total sodium. If,

[1] T. G. TAYLOR, J. agric. Sci. *52*, 207 (1959).
[2] T. G. TAYLOR and J. H. MOORE, Brit. J. Nutr. *10*, 250 (1956).

Table I

Weights (mg) of sodium, potassium, and chloride dissolved from 5 g ox- and hen-bone in 12 successive extractions with 150 ml 0·01 *N* hydrochloric acid and weights present in the original bone samples

Extract No.	Ox-bone		Cl	Hen-bone		
	Na	K		Na	K	Cl
1	7·00	3·43	3·50	13·43	21·30	5·40
2	4·25	0·80	—	3·86	2·06	—
3	2·02	0·30	—	1·90	0·32	—
4	0·90	0·12	—	1·08	0·15	—
5	0·65	0·07	—	0·97	0·14	—
6	0·65	0·07	—	0·96	0·13	—
7	0·55	0·07	—	0·77	0·11	—
8	0·50	0·07	—	0·70	0·11	—
9	0·45	0·07	—	0·70	0·11	—
10	0·40	0·07	—	0·65	0·09	—
11	0·38	0·07	—	0·60	0·07	—
12	0·38	0·07	—	0·60	0·07	—
Total 1–12	18·13	5·21	3·50	26·22	24·67	5·40
Contained in 5-g original bone	30·20	5·40	3·50	35·00	25·00	5·40
Weight sodium equivalent to Cl present	2·30			3·50		
Weight soluble sodium	12·70			14·35		
Weight insoluble sodium	17·50			20·65		

Table II

Percentage composition of the ash of ox- and hen-bone before and after twelve extractions with 0·01 *N* hydrochloric acid

	Ox-bone				Hen-bone			
	Ca	P	Na	K	Ca	P	Na	K
Before extraction	37·1	17·1	0·89	0·16	36·3	17·6	1·37	0·78
After extraction	38·2	17·9	0·39	0·03	37·9	18·0	0·56	0·04

as seems probable, all the chloride extracted by the 0·01 *N* nitric acid was derived from tissue fluid remaining in the bone samples, it may be calculated that, in each case, less than half of the sodium dissolved in the first extraction could have been associated with this chloride. (Table I). Further evidence that only a small proportion of the total bone sodium was derived from the tissue fluid was obtained by removing the organic matter from a sample of the original ox-bone with ethylene diamine (80% v/v) according to the method of WILLIAMS and IRVINE[3]. When this was done, it was found that only 5% of the sodium, together with 25% of the potassium and 100% of the chloride was extracted. The percentage of sodium and potassium present in the ash of the two samples of bone before and after the twelve extractions is given in Table II. It will be seen that the extracted materials were very low in potassium, but they still retained subtantial amounts of sodium.

It appears from these experiments that the sodium of bone occurs in two forms, one relatively soluble and the other relatively insoluble in acid, a conclusion similar to that reached for the magnesium of bone[1]. If the amounts of sodium remaining in the bone powders after twelve acid extractions are taken as measures of the acid-insoluble fractions, which are released only when the bone crystals themselves are dissolved, it can be calculated that 42% and 41% of the total sodium was originally present in this form in the ox- and hen-bone respectively. About 60% of the total bone sodium was freely soluble in dilute acid and it would seem reasonable to suggest that this represents the exchangeable fraction of EDELMAN, JAMES and MOORE[4], and BAUER[5] as determined by the use of radioactive sodium, and that the fraction which does not exchange *in vivo* is that portion which cannot be dissolved preferentially by acid. This exchangeable sodium is probably located in the hydration layer of the bone crystals and as surface-bound ions[6].

In an attempt to effect a more complete removal of sodium from bone, a second extraction technique was employed, using boiling dilute lactic acid as extractant. Three different samples of bone were treated in this way, the hen-bone used previously, and two samples of calf bone, one cortical and the other cancellous, which had been treated with ethylene diamine to remove the organic matrix[3]. The percentage composition of the ash of the bone samples before and after extraction is given in Table III. Whilst this extraction technique was more effective in removing sodium than the previous one, substantial amounts remained in the bone materials. The removal of magnesium from the bone was far more complete and it is clear that a fraction of the sodium was even more firmly combined than the magnesium. If the relatively insoluble fraction of the magnesium occurs as Mg^{2+} ions this is the opposite of what would be expected, since this ion, by virtue of its small radius (0·065 mμ) and its double positive charge, would be expected to be more strongly adsorbed than the larger Na^{+} ion (0·098 mμ radius) with its single charge. It would appear, therefore, that whereas the magnesium is present wholly in the hydration shell of the bone crystals and as ions adsorbed on the crystal surfaces, a substantial part of the sodium replaces surface calcium ions of the crystal lattice as suggested by HARRISON[7] and HENDRICKS[8].

Table III

Percentage composition of the ash of bone samples before and after lactic acid extraction

Bone	Before or after extraction	Ca	Mg	Nv	K	P
Hen . . .	before	36·3	0·84	1·37	0·78	17·6
Cortical . .	after	38·7	0·16	0·38	0·05	18·1
Calf . . .	before	35·9	0·55	1·21	0·29	17·0
Cortical . .	after	39·1	0·02	0·25	0·06	18·6
Calf . . .	before	36·0	0·55	1·23	0·42	17·1
Cancellous	after	39·1	0·06	0·28	0·08	18·6

The potassium of bone is so highly soluble that it seems probable that all of it is in the hydration layer. Potassium ions would be expected to be less strongly adsorbed than sodium ions owing to their greater ionic radii and this is reflected in the higher solubility of potassium in these experiments. The fact that the samples of cortical and cancellous calf-bone contained 0·29 and 0·42% potassium respectively after the complete removal of the organic matrix makes it appear certain that potassium is a true constituent of the bone mineral and not simply a constituent of the bone cells.

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Zusammenfassung

Fraktionierungsexperimente an Knochenpulver zeigten, dass das Kalium in verdünnter Säure leicht und fast vollständig löslich ist. Ein wesentlicher Teil des Natriums bleibt aber, sogar nach stärkster Behandlung mit Säure, ungelöst.

[3] J. B. WILLIAMS and J. W. IRVINE, Science *119*, 771 (1954).

[4] I. S. EDELMAN, A. H. JAMES, and F. D. MOORE, Trans. Macy Conference on Metabolic Interrelations *4*, 240 (1952).

[5] G. C. H. BAUER, Acta physiol. scand. *31*, 334 (1954).

[6] W. F. NEUMAN and M. W. NEUMAN, *The Chemical Dynamics of Bone Mineral* (University of Chicago Press, 1958).

[7] H. E. HARRISON, J. biol. Chem. *120*, 457 (1937).

[8] S. B. HENDRICKS, Trans. Macy Conference on Metabolic Interrelations *4*, 246 (1952).