Full Papers

The histones of *Dictyostelium discoideum*

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Summary. The histones of the cellular slime mould *Dictyostelium discoideum* have been separated by electrophoresis using both acid urea and sodium dodecyl sulphate systems, and the gel pattern compared with that of histones from *Physarum polycephalum* and calf thymus. *Dictyostelium* is found to possess a full complement of H1, H2A, H2B, H3 and H4.

Key words. Dictyostelium; slime mould; histone; electrophoresis.

Dictyostelium discoideum, the cellular slime mould, is an extensively studied organism. Its easy growth and culture, the existence of axenic strains of free living amoebae, such as AX-2, which may be cultured indefinitely in liquid suspension cultures, and its small genome size, combine to make the organism interesting and important in the context of biochemistry and genetics. Work on the genome of *Dictyostelium* has focused attention on its chromatin, and many laboratories have attempted separation and characterization of its histones[1–8].

Studies on *Dictyostelium* histones are of interest since this organism is a primitive eukaryote and there has been some doubt about whether such organisms have the full complement of histones as found in higher eukaryotes. The paths of histone evolution are also interesting, especially since core histone H3 and H4 are the most highly conserved of all proteins whilst H1 is less strongly conserved and, at least in higher organism, is coded for by a small multi gene family.

Since the genome of this organism is small, the yield of histones is also small, and identification of histones by comprehensive comparison of the amino acid composition with that of other lower eukaryotes such as *Physarum,* has not as yet proved possible. We have, therefore, sought to achieve good separation of *Dictyostelium* histones with both triton/acetic acid urea gels and sodium dodecyl sulphate gels, and also to use a two-dimensional gel electrophoresis separation, somewhat similar to that advocated by Bonner et al.[9] and thus to proceed to a more accurate identification of these histones than has been previously obtained. In addition, our histones have been extracted from nuclei isolated by the use of digitonin, a natural plant saponin, rather than with nonidet P40. We have already shown that, using a number of criteria, such nuclei are superior to those isolated with NP-40[10] and, in particular, that their DNA is not substantially degraded.

Materials and methods

Growth conditions. Dictyostelium discoideum strain AX-2 cells were grown in suspension culture as previously described[10] and nuclei were isolated from such cells using the 0.1 mg/ml digitonin method described in the same paper.

Isolation of histones. Basic nucleoproteins were extracted from isolated nuclei by one of two methods. 1) The acid extraction of Bakke and Bonner[4]. 2) The method of Mohberg and Rusch using the modification of Coukell and Walker[11] the suspension of nuclei being heated in 1 M $CaCl_2$ at 80 °C for 15 min.

In both methods basic nucleoproteins were precipitated by adding 4 vol. of ethanol at −20 °C, and allowing precipitation to proceed for 24 h.

Electrophoresis of histones. Histones were analysed on polyacrylamide slab gels using either the acid/urea/triton system of Bonner et al.[9] or the SDS system described by Laemmli[12]. Acid urea gels for comparative studies were run as described by Panyim and Chalkley[13].

Two-dimensional slab gels were run with the acid/urea/triton systems in the first dimension, the gel being briefly stained with Coomassie Brilliant Blue, the required lane cut out of the gel, and equilibrated against 2.3% SDS, 10% glycerol, 5% 2-mercaptoethanol and 62.5 mM Tris pH 6.8 for 1 h. The gel slice was then embedded in 1% agarose in the same buffer on top of the second dimension gel, which was a 12.5% SDS gel as used for 1-dimensional separation.

Electrophoresis was continued until the dye front reached 1 cm from the bottom of the gel, and the gel was finally stained with Coomassie Brilliant Blue.

Scanning of gels. Gels were scanned at 530 nm in a Joyce Loebl Chromoscan 3 gel scanner.

Results

Sodium dodecyl sulphate gels. As seen in figure 1 A(b), a scan of *Dictyostelium* histones run on SDS gels reveals a pattern in which one or more bands of variable intensity run most slowly, which we identify as histone H1. As expected the yield of this histone is particularly dependent on the state of the chromatin from which histones are extracted. Any mistreatment of the chromatin will result in a reduction of the yield of H1, and although the H1 band is expected to be one half of the intensity of the core histone bands, its intensity on stained gels is often less than expected. Although we call this histone H1, it has been shown to differ markedly in amino acid composition from the H1 histone of higher eukaryotes[8]. An even slower band, which has caused confusion in some earlier publications, we can firmly identify as actin, a protein which is abundant in these amoeboid cells[14]. The amount

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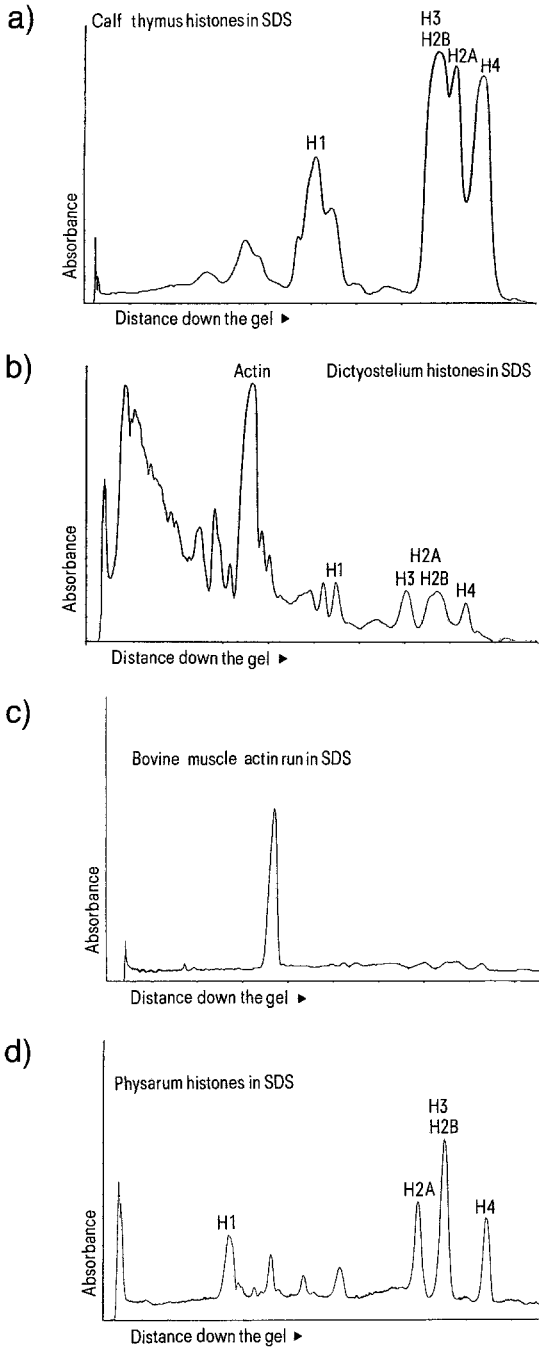

Figure 1A. Scans of the gel electrophoretic separation of histones shown in figure 1B. *a* Calf thymus histone. *b Dictyostelium* histone. *c* Actin. *d Physarum* histone.

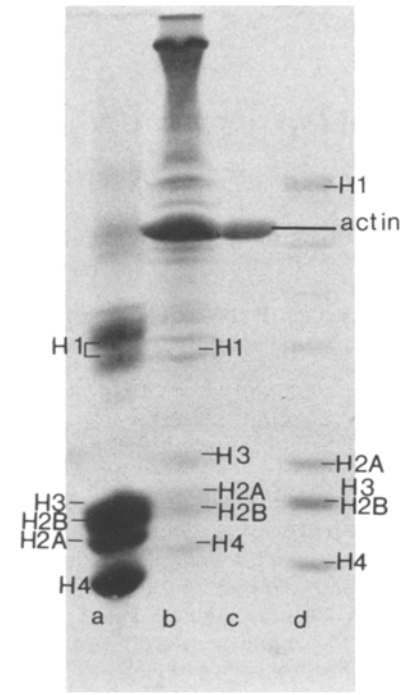

Figure 1B. Separation of histones on 12.5% polyacrylamide SDS gels. Lane (a) calf thymus histone, (b) *Dictyostelium* histone, (c) bovine muscle actin, and (d) *Physarum* histone. Notice that in the *Dictyostelium* histone separation, two bands run close together in the lane, opposite to the calf thymus H1 bands. Both of these may be *Dictyostelium* H1 bands; sometimes only one such band is resolved. Note that this gel separation is not optimal for all the lanes, but it is technically difficult to run all four samples in parallel and obtain ideal banding patterns from each. Histones extracted using method 2 (see materials and methods).

of actin retained in histone preparations is variable and may be close to zero in some. By running similar gels with histone mixed with exogenous actin, and actin alone, the identity of this band as actin can be confirmed (see fig. 1 A(c)). Histone H1 runs well behind the core histones, which tend to be clustered. This is especially so with histones H2A, H2B and H3. Histone H4 runs most rapidly, and as we discuss later, can be readily resolved into minor bands in other gel systems, depending on its state of comparative acetylation. Since the identity of H2A, H2B and H3 is easiest on acid/urea/triton gels we have used two-dimensional electrophoresis, as shown in figure 2, to verify the order of these core histones on the gel. It seems likely that the order is H3, H2A, H2B in SDS, and in some gel runs the H3 and H2A peaks coincide, or alternatively the H2A may merge substantially with H2B. But the convincing and wide separation of H2A and H2B histones in acid/urea/triton gels permits their identification on SDS.

Acid/urea/triton gels. Dictyostelium histones, run in these conditions on electrophoretic gels, show a markedly different pattern of banding from those run on SDS gels. As seen in figure 3 (a), H2A is now the slowest peak, followed by H1, H3 and H2B in fairly close proximity. The H2A peak appears small, chiefly, we suggest, because it is somewhat diffuse and so has a broad base in the scan as shown. H1 and H2B show some tendency to distribute as shoulders of the central H3 peak in some gels. H4 is the fastest running band and is usually quite clear. As can be seen in figure 3(b), when cells have been grown and extracted in the presence of 10 mM sodium butyrate, the acetylated forms of H4 are evident, having been protected from deacetylation by the action of butyrate on the deacetylase enzyme[15]. Mono-, di-, tri- and tetra-acetylated peaks can be seen, running respectively more slowly than the small peak of non-acetylated H4. In figure 3(c) two peaks of calf thymus histone are labeled as representing H3. In this we simply follow the convention adopted by Mende et al.[16]. We think there is some uncertainty about the designation of the slower running band as H3 and many authors simply fail to label it.

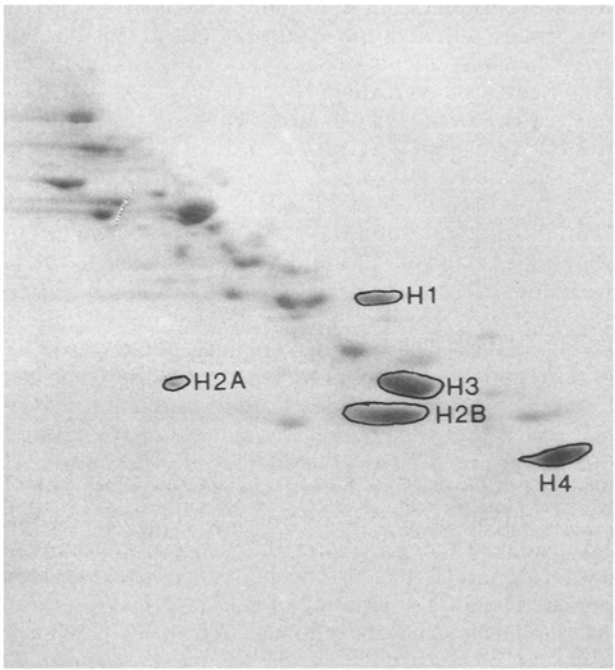

Figure 2. Two-dimensional electrophoresis of *Dictyostelium* histones. Histones were run in 15% polyacrylamide acid urea triton in the first dimension (left to right across the gel), the strip cut out and laid on top of the slab, and run in the second dimension down the gel in 12.5% polyacrylamide with SDS buffers, followed by staining with Coomassie Brilliant Blue. The assignment of spots to histones on this figure is tentative and based on the assignment of *Physarum* histones on 2D gels by Mende et al.[16] and on the distribution of the *Dictyostelium* histones in the differing ID gels.

Acid urea gels. Figure 4 shows scans of both calf thymus (4a) and *Dictyostelium* (4b) histones on an acid-urea gel. It can be seen that the calf thymus bands run slightly ahead of the corresponding *Dictyostelium* bands. The second peak shows that two of the bands run as a doublet. The order of mobility on the acid urea gel is the same as that on an SDS gel except that the relative mobility has altered. Thus *Dictyostelium* H1 is here slower than calf thymus H1 whereas on SDS gels it is vice versa: other *Dictyostelium* bands run more slowly than calf thymus on both SDS and acid urea gels.

Two-dimensional gels. These gels have been run with acid/urea/triton in the first dimension, the gel strips being cut out and laid onto a slab to run in the second dimension with SDS; results are shown in figure 2. This system is particularly effective in identifying the closely running bands of H2A, H2B and H3 in SDS. It seems quite clear that, in our gels, H2A is invariably slower than H2B in SDS, and that H3 is only marginally slower than H2A and may often merge with it in one dimension. But the two-dimensional separation of H2A and H2B is apparently unambiguous and indeed all the histone bands are clearly resolved in this separative situation.

Discussion

Histones from calf thymus nuclei are often used as a standard for comparison, but histones have also been carefully characterized from *Physarum polycephalum*, the acellular slime mould[16] and we have, therefore, been able to make comparisons with material from both sources. *Dictyostelium* histone gives a gel pattern closely similar to that of *Physarum* and quite distinct from calf thymus. For example, in SDS, calf thymus histones migrate in an order (slowest to fastest) of H1, H3, H2B, H2A and H4, whilst *Physarum* histones migrate as H1 (also much slower than calf thymus H1), H2A, H3, H2B and H4. The *Dictyostelium* histone pattern resembles *Physarum* in all respects except the position of H3, which is slightly slower than H2A in SDS gels of *Dictyostelium*. The *Physarum* pattern in acid/urea/triton is in an order (slowest

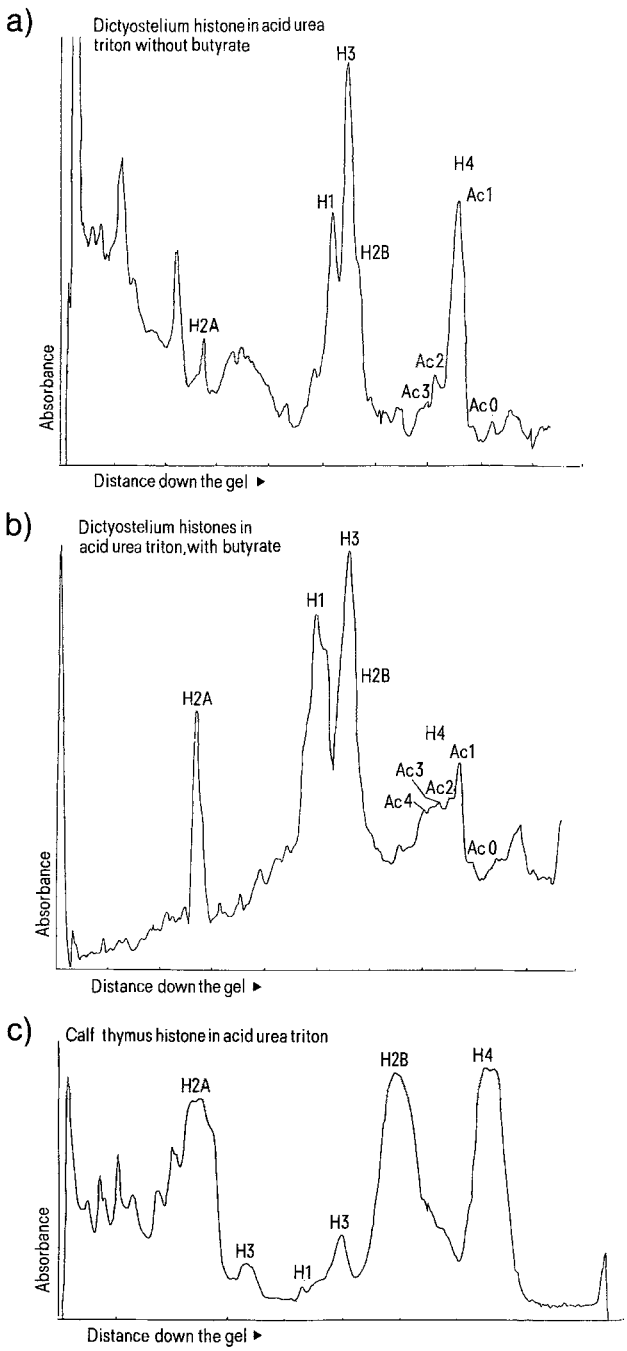

Figure 3. Scans of histones run in 15% polyacrylamide acid urea triton gels. *a Dictyostelium* histones run without butyrate. *b Dictyostelium* histones run with 10 mM Na butyrate, from cells grown in the presence of butyrate. *c* Calf thymus histones run without butyrate. Histones extracted using method 1 (see materials and methods).

to fastest) of H2A, H1, H3, H2B and H4, whilst calf thymus is H2A, H3, H1, H2B and H4. The *Dictyostelium* pattern in AUT is similar to *Physarum*. The histones which show most divergence of migration characteristics are firstly H1, (and as shown by Parish and Schmidlin[8] this histone is very divergent in *Dictyostelium*) and H2A, which in both *Physarum* and *Dictyostelium* is slightly slower than H2B, especially in *Physarum* where it may run slower than H3 in SDS. H2A from both slime moulds is also slower in AUT gels than H2A from calf thymus. On acid urea gels (without triton) the corresponding *Dictyostelium* and calf thymus histones migrate differently; the only bands that run together are the calf thymus and *Dictyostelium* H1 and the dimer (possibly H3 and H2A) These results agree with the acid urea results of Bakke and Bonner[1] but differ from those of Charlesworth and Parish[2] who suggest that two bands comigrate with calf thymus. These two bands are also different from the one that comigrates on our gels. The reason for this could be due to the fact that Charlesworth and Parish ran rod gels compared to our slab gel. In rod gels minor differences may arise in each gel and, therefore, it is difficult to compare one gel with another with complete certainty: slab gels on the other hand, can be assumed to provide uniform conditions for multisamples run in one slab.

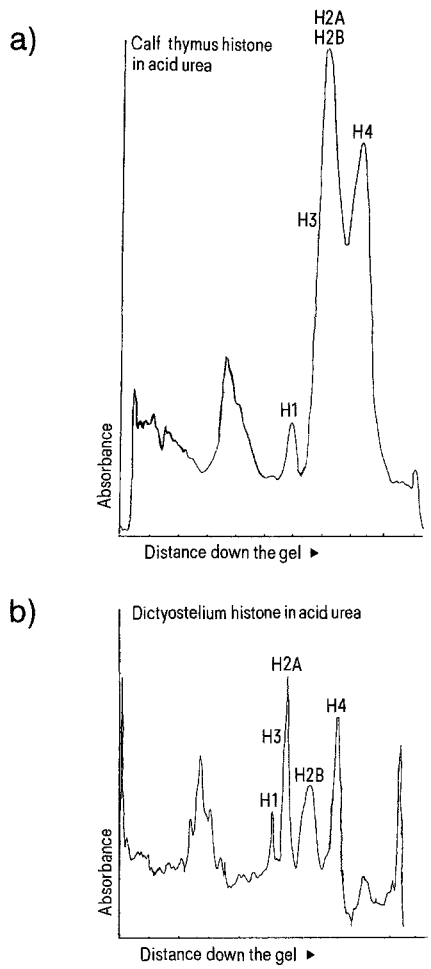

Figure 4. Scans of histones run in 15% polyacrylamide acid urea gels. *a* Calf thymus histones. *b* *Dictyostelium* histones. Histones extracted using method 1 (see materials and methods).

It now seems that both slime moulds have a standard set of histones, although the identity of H1, at least in *Dictyostelium*, rests on its similar position to calf thymus H1 in our gels, and on its high lysine content[8]. As in *Physarum*, we can resolve two adjacent bands in the presumptive H1 region and these may be distinct H1 histones. Up to 5 separate H1 types can be resolved in mammals[17] and these have been categorized as H1a, H1b, H1c, H1d and H1e. In addition, other H1-like histones occur in higher vertebrates, namely H1° and H5. H1° has been shown to partially replace H1 in cells which are highly differentiated[18] H5 may partially or largely replace H1 in highly condensed chromatin, as in nucleated erythrocytes of birds and amphibians. There is as yet no evidence that lower eukaryotes possess any histones resembling H1° or H5, and it seems likely that the number of H1 variants (and therefore, perhaps, the number of genes in the H1 gene family) in lower eukaryotes is only two.

At one time there was uncertainty about the existence of H1 histone in yeast and other fungi. However Sommer[19] has located an H1-like histone in *Saccharomyces* which is present in half the amount of the core histones, but, curiously, is low in lysine and insoluble in 5% perchloric acid. A similar protein is identifiable in *Neurospora crassa*[20]. There seems some uncertainty about the number of H1 types in yeast, however, since Pastink et al.[20] find two proteins resembling H1 which run on either side of rat liver H1 in terms of electrophoretic mobility. Neither, however, shows the characteristics of perchloric acid insolubility or low lysine content reported by Sommer[19]. Brandt et al.[21] find two H1-like proteins in yeast, although at least one of these lacks the high positive charge of most histones and may actually be an HMG protein. The situation regarding fungal H1 can thus be seen to be somewhat confused, but one and perhaps two H1-like proteins are probably present. In terms of SDS mobility, both peaks of *Dictyostelium* H1 migrate close to the position adopted by calf thymus H1 and are certainly substantially faster than *Physarum* H1. It is therefore apparent that there is considerable divergence of amino acid composition between the H1 histones of the two slime moulds, and perhaps, as the eukaryotic genome has enlarged in both mass and complexity during the course of evolution, selective pressure has arisen which has favoured the further duplication and divergence of the H1 histone genes from a possible minimum of two to a possible maximum of seven (if H1° and H5 are classified as being similar to H1). It may yet turn out, as more work is carried through on fungal H1 proteins and the genes which code for them, that one of these H1s is the ancestral protein for vertebrate H1s of types a–e, while the other fungal and slime mould H1 may be the ancestral protein for vertebrate H1° and H5.

The H2A and H2B histone mobilities of *Dictyostelium* histones appear to resemble those of *Physarum* both in SDS and acid urea triton gels, and to be dissimilar to calf thymus H2A and H2B, in that the mobilities of these two histones from calf thymus are reversed from those found in the two slime moulds. These histones have been found to diverge markedly in mobility and amino acid composition when yeast histones are compared to those of higher animals and plants[22] and again yeast and both slime moulds betray a similar pattern. Interestingly, the H2A

and H2B genes of yeast are adjacent on the genome[23] whereas in calf thymus and other higher orgnism the two genes are repeated and present on different chromosomes.

H3 and H4 histones are the most conserved of all known proteins, and they also are the most similar as between calf thymus, *Physarum* and *Dictyostelium* as judged by electrophoretic mobility. However, even with these highly conserved histones, the mobility of H3 is slightly variable, being slower in SDS when derived from *Dictyostelium* than from *Physarum*. Both yeast and *Neurospora* have closely similar H3 and H4 histones, and in both organisms the single copy genes are known to be linked and, uniquely amongst histone genes, to possess introns[23].

Conclusion

Although the cellular and acellular slime moulds *Dictyostelium* and *Physarum* are probably not closely related, both are simple eukaryotes. Their histones, as judged by electrophoretic separation, are closely similar, only H1 showing any considerable divergence, and H2A rather less. Both slime moulds yield histones with substantially different migration patterns from calf thymus. H3+H4 are the most strongly conserved in evolution and, therefore, it is not surprising that they show minimal charge differences. H2A and H2B are somewhat less conserved and here differences from the calf thymus pattern are detectable, especially with H2A. The non-core particle histone H1 is the least conserved and shows considerable differences in charge both as between the two slime moulds, and between either slime mould and calf thymus.

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Uptake and toxic effects of heavy metal ions: Interactions among cadmium, copper and zinc in cultured cells

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Summary. Absorption of metal ions by KB, HeLa and L-59 cells has been analyzed by atomic absorption spectrophotometry in the course of culture. Ions of the elements of the fourth period in the periodic chart such as Fe(II), Cu(II), Zn(II), Mn(II) and Ni(II) were not taken up, but those of the higher periods, such as Cd(II), Pb(II), Hg(II) and Ag(I) were were taken up easily. The uptake behavior by the cultured cells was in accordance with the characteristic features of metals, that metals in the fourth period are essential elements, and most of the elements of the fifth and the sixth periods are non-essential or toxic elements.

The initial rate of Cd(II) uptake and the Cd(II) concentration has a sigmoidal relationship. Cd(II) was absorbed homotropically through cell membranes. The uptake of Cd(II) was specifically inhibited by Cu(II), but was affected little by Zn(II). The toxicity of Cd(II) to KB cells was greatly enhanced in the presence of Cu(II). On the contrary, the toxicity of Cd(II) was reduced by the addition of Zn(II) at several concentrations of Cd(II). The toxicity of Cd(II) did not depend on the amount of Cd(II) absorbed in the cells, but was determined by cofactors such as Cu(II). The interaction between Cd(II) and Cu(II) may be important for Itai-itai disease.

Key words. Cd uptake; heavy metal uptake; essential and toxic metal; metal interaction; heavy metal toxicity.