10–20% can be readily released, and only after a strong stimulus (severe haemorrhage)[12]. The ultimate capacity of the neurohypophysis is apparently even higher, as documented in the works referred to above, and because of the neurohypophysial accumulation of the endogenous AVP, the percentage of the releasable hormone might be raised and account for an observed peak. If this is true, the question arises as to how a large amount of vasopressin could be released from the neurohypophysis during a very short time period. Two explanations may be considered. First, the release could be stimulated by a sudden drop of the steady and high plasma level of the hormone. Second, the blood flow in pituitary vessels might decrease during the increase of AVP plasma concentration, and rapidly increase when this concentration in the peripheral blood falls; in such a case, outflowing blood would have a high AVP concentration. At present, direct evidence for any particular mechanism of this abnormal AVP elimination is lacking.

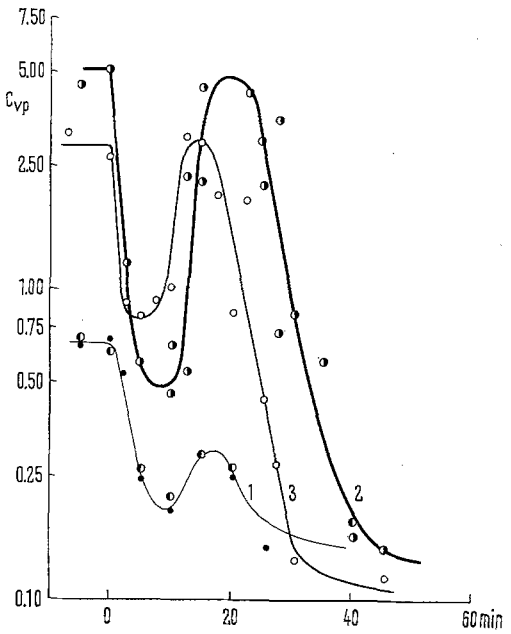

Fig. 2. Changes of plasma concentration of arginine vasopressin (c_{vp}, nmol/l) after cessation of i.v. infusion (zero time). Female dogs: 15.7 kg, AVP-infusion 0.36 nmol/h/kg (●); 9.7 kg, AVP-infusion 0.46 nmol/h/kg (◐); 14.0 kg, AVP-infusion 1.65 nmol/h/kg (○); 15.0 kg, AVP-infusion 0.74 nmol/h/kg (◑).

Zusammenfassung. Die Plasma-Konzentration sinkt beim hydrierten Hunde nach Beendigung einer VP-Infusion in 10 min auf 10–20% der Gleichgewichtskonzentration ab, um dann in ungefähr 20 min auf 40–100% anzusteigen. Nach 2 Stunden war die Konzentration wieder vermindert. Dieser abnorme Zeitverlauf wird mit der Depotfunktion der Neurohypophyse für Vasopressin in Zusammenhang gebracht.

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Cadmium as a Trace Element and Cadmium Binding Components in Human Cells

The rapid expansion of industrial technology has introduced into our environment increasing quantities of cadmium[1]. It is well known that this element is highly toxic. Apart from this, cadmium has teratogenic and tumorigenic properties and it may also play a role in the pathogenesis of hypertensive cardiovascular disease[2–6]. Absorption of Cd^{++} by gastrointestinal tract is poor, nevertheless, an average person absorbs approximately 2 μg Cd/day[7]. For unknown reasons the absorbed Cd is poorly excreted and accumulates in the tissue[6,8,9]. The presence of cadmium binding protein has been demonstrated in equine and human kidneys[10,11]. Similar cadmium binding protein (Cd-BP) appears also in the organs of rats[12,13]. Exposure of rats to $CdCl_2$ by ingestion or s.c. injection induces the synthesis of Cd-BP[13]. Other ions such as Zn^{++}, Hg^{++}, Co^{++}, Cu^{++}, Ni^{++} did not produce this effect. In vivo incorporation of ^{14}C from uniformly labeled cystine-^{14}C into Cd-BP was shown to be increased after exposure of the animals to Cd^{++}. The labeled Cd-BP undergoes a continuous catabolism and resynthesis which is accompanied by only minor losses of intracellular ^{109}Cd[13]. Since in vivo the Cd-BP is confined to intracellular compartment it was of interest to study the interaction of Cd^{++} and Zn^{++} with isolated cells.

Materials and methods. HeLa cells, monkey kidney epithelial cells and human embryonic fibroblasts derived from skin and muscle as well as from lungs were cultured in milk dilution bottles according to Embil et al.[14]. Equimolar mixture of $^{109}CdCl_2$ and $^{65}ZnCl_2$ or carrier free $^{109}CdCl_2$ with radiochemical purity better than 99% were added to culture media and aliquots from the media were drawn periodically for assay of the isotopes. After incubation the cells were washed with Eagles diploid medium and trypsinized[14]. Surviving cells were lysed and the lysate centrifuged at 2000 × *g* for 1 h. The supernatant was separated at +10 °C on Sephadex G-75 column (1.5 × 83 cm) using *Tris*-HCl buffer 0.001 *M*; pH 8.6. Fractions collected from the column were assayed for radioactivity of the isotopes in Nuclear Chicago dual channel gamma scintillation spectrometer.

Results and discussion. Human fetal skin and muscle fibroblasts cultured as monolayer for 8 days in 15 ml of Eagles diploid medium containing ^{109}Cd and ^{65}Zn (1.3 n*M*/ml of each) showed a continuous uptake of ^{109}Cd without detectable change in ^{65}Zn concentration

Removal of ^{109}Cd from human fibroblasts (skin and muscle) after trypsinization

Cell culture	No. 1 (cpm ^{109}Cd)	No. 2 (cpm ^{109}Cd)	No. 3 (cpm ^{109}Cd)
Trypsin resistant cells	67.8×10^3	97.4×10^3	46.3×10^3
Trypsin solution	11.9×10^3	9.7×10^3	8.0×10^3

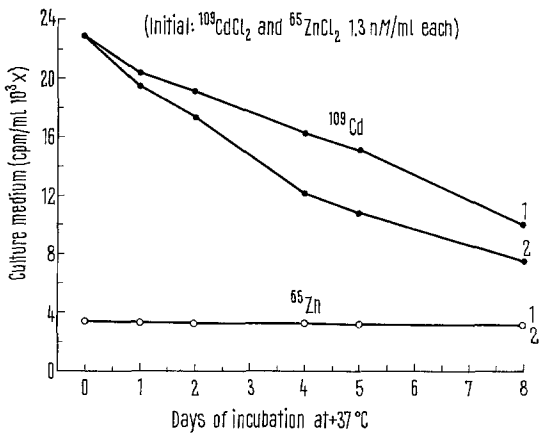

Fig. 1. Changes in ^{109}Cd and ^{65}Zn in culture media (1, 2 individual human fibroblast cell cultures).

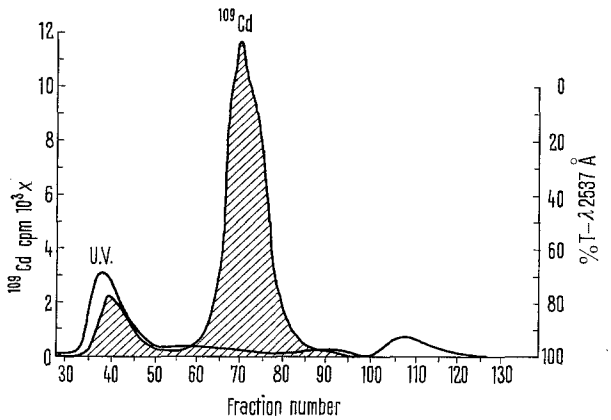

Fig. 2. Supernatant of human fibroblast lysate. Fractionation of ^{109}Cd binding components on Sephadex-G75 (1.5 × 83 cm flow rate 7.7 ml/h).

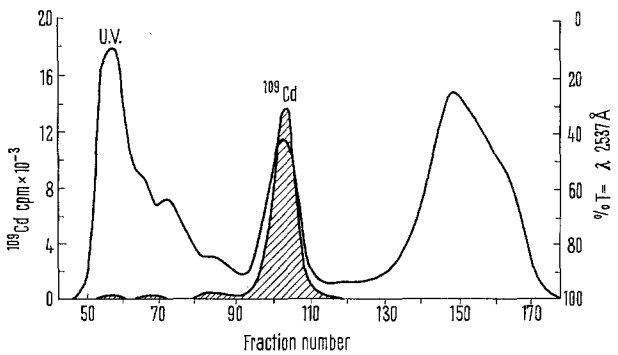

Fig. 3. Separation of Cd-BP from rat liver soluble fraction on Sephadex-G75 column (2.5 × 90 cm). The animal was treated with 0.01 m*M* $CdCl_2$/kg by daily injections for 9 days. A marked rise in Cd-BP is evident (Ve/Vo = 1.8). The UV-absorption in Cd-BP parallels the protein concentration.

(Figure 1). After incubation the cells adhering to the glass surface were washed with 10 ml of fresh Eagles diploid medium and trypsinized. As shown in the Table the trypsin resistant cells contained most of the ^{109}Cd and no detectable ^{65}Zn. Pooled trypsin resistant cells containing 2.13×10^5 cpm ^{109}Cd were lysed with H_2O and the lysate was centrifuged. The sedimented cell debris contained 21.7% ^{109}Cd and the supernatant accounted for 78.3% of ^{109}Cd. The supernatant (5 ml) containing 1.60×10^5 cpm ^{109}Cd was applied on Sephadex G-75 column with continuous flow of 7.7 ml/h. Effluent from the column was monitored by UV ($\lambda = 2537$ Å) and 1.3 ml fractions were collected by an automatic fraction collector. The changes in UV-transmission and in ^{109}Cd content in the collected fractions are shown in Figure 2. The radioactivity of ^{109}Cd was associated with 2 fractions; one at the exclusion limit of Sephadex G-75 and the other major fraction having elution factor Ve/Vo = 1.85. In its properties on Sephadex the latter component was similar to Cd-BP found in rat liver cells. Figure 3 shows the separation of rat liver 'soluble fraction' ($105{,}000 \times g$ supernatant) from an animal exposed to $CdCl_2$. In vitro uptake of ^{109}Cd was also demonstrable with human embryonic fibroblasts derived from lungs as well as with HeLa cells and monkey kidney epithelial cells. Apparently the mammalian cells are endowed with a specific mechanism for sequestration of this biologically alien element. Further characterization of Cd-BP in these cells is in progress.

Zusammenfassung. Fötale menschliche Fibroblasten wurden in einem ^{109}Cd und ^{65}Zn enthaltenden Kulturmedium gezüchtet. ^{109}Cd wurde in allen Zellen kontinuierlich absorbiert. Mit Gel-Filtration wurde der Zellsaft in 2 Komponenten aufgetrennt, die beide ^{109}Cd enthielten. Ähnliche, ^{109}Cd bindende Komponenten wurden auch in Rattenleber-Homogenaten gefunden.

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