

Department of Basic Veterinary Sciences

**A novel assay method for measuring added
plasma caesium and its application in the
measurement of short-term kinetics**

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ACADEMIC DISSERTATION

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Contents

1. Abstract	5
2. List of original articles	6
3. Abbreviations	7
4. Introduction	8
5. Review of the literature	10
5.1. Caesium chemistry and physics	10
5.2. Caesium biology	12
5.3. Caesium assay methods	18
6. Aims of the study	20
7. Materials and methods	21
7.1. Animals	21
7.2. Assay method for added stable caesium	21
7.3. Plasma caesium kinetics	23
7.4. Tissue caesium distribution	23
7.5. Statistical analysis and curve fitting	24
8. Results and discussion	25
8.1. Assay method for caesium	25
8.2. Short-term plasma kinetics of caesium	30
8.3. Short-term distribution of caesium	32
8.4. Effect of exercise on plasma caesium	33
8.5. Future prospects	34
9. Conclusions	35
10. Acknowledgements	37
11. References	38
12. Original articles	49

1. Abstract

The aim of this thesis was to develop a new simple method for measuring added caesium (Cs) in blood plasma and to use it in the study of short-time plasma kinetics.

The new method, based on isotopic dilution, is analogous to radioimmunoassay. The dispersive complex salt ammonium-iron(III)-hexacyanoferrate(II) (AFCF) has a very high affinity towards Cs^+ . In a mixture of plasma, AFCF and added radioactive Cs tracer (^{134}Cs), the amount of AFCF-bound activity depends on the amount of stable Cs in plasma. This bound activity is separated from soluble activity by co-precipitating AFCF with plasma proteins using trichloroacetic acid and measured using a standard gamma counter. Using standard samples prepared from plasma collected prior to an intravenously given Cs dose, the Cs concentration in plasma samples after the dose can be determined. The qualitative detection limit for the method is around $0.2 \mu\text{mol l}^{-1}$, and the practical limit for quantitative results around $1 \mu\text{mol l}^{-1}$.

Using this method, short-term plasma kinetics after an intravenous dose of Cs was measured in both goats and horses. The values measured for goats were very similar to those using ^{134}Cs as a tracer in another study.

The rate constant for the removal of Cs from the bloodstream was initially above 0.1 min^{-1} , but decreased within 40 min to a value below 0.02 min^{-1} . From 2 to 40 min, the plasma concentration can be approximated with a biphasic exponential decay curve. Exercise speeds up the rate of Cs removal from blood. Between 30 and 40 min after the start of exercise, the rate of Cs removal was twice as high in exercising individuals compared to resting individuals (0.06 min^{-1} vs. 0.03 min^{-1}). Stimulation of muscle sodium-potassium pumps (Na, K-ATPase) is a plausible explanation for the increased removal of Cs from blood during exertion.

At 30 min after dosing, the tissues with the highest ^{134}Cs content were the gastrointestinal tract (22% of original dose), skeletal muscle (14% of dose) and the kidney (13%). A likely location for the large unrecovered portion of the original dose (38%) was connective tissue. Incorporation in one or several of these tissues probably explains the rapid initial removal of Cs from circulation.

2. List of original articles

This thesis is based on the following original articles, which are referred to in the text by their Roman numerals:

I. Kaikkonen M., Lehto J. 2000. Coprecipitating ammonium–iron(III)–hexacyanoferrate(II) from aqueous dispersion with albumin and trichloroacetic acid. **Analyst**, 125: 855–859.

II. Kaikkonen M., Lehto J. 2006. A simple assay method for added stable caesium in blood plasma. **Radiochimica Acta**, 94: 47-52.

III. Kaikkonen M., de Gritz B., Eriksson L. 2005. Short-term distribution of ¹³⁴Cs in relation to ⁵¹Cr-EDTA after intravenous dose in goats. **Acta Physiologica Scandinavica**, 183: 321–332.

IV. Kaikkonen M., Hyypä S. 2005. Comparison on plasma caesium kinetics in goats and horses with special emphasis on exercising horses. **Acta Physiologica Scandinavica**, 185: 335–340.

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3. Abbreviations

AAS	atomic absorption spectrometry
AES	atomic emission spectrometry
AFCF	ammonium ferric cyanoferrate ($\text{NH}_4\text{Fe}[\text{Fe}(\text{CN})_6]$)
ALI	annual oral limit of intake
B_R	relative binding
Cl	chloride
Cs	caesium
E	extracellular fluid fraction
F_{Cs}	fraction of Cs dose accumulated in an organ
Hb	blood haemoglobin content
I	apparent intracellular ^{134}Cs relative concentration
ICP-MS	inductively coupled plasma mass spectrometry
ISF	interstitial fluid
K	potassium
MS	mass spectrometry
Na	sodium
NAA	neutron activation analysis
Na,K-ATPase	sodium-potassium pump
NMR	nuclear magnetic resonance
PB	Prussian blue
PCV	packed cell volume
R	relative concentration
Rb	rubidium
RIA	radioimmunoassay
SID	strong ion difference
TCA	trichloroacetic acid
WBC	white blood cell count

4. Introduction

Due to the central role of radioactive caesium (Cs) isotopes in nuclear fall-out, the long-term distribution and kinetics of Cs in animal tissues have been studied intensely, especially in the 1950s and 1960s (Stara et al., 1971). These studies, mostly involving experimental animals, concentrated on the medium-term (days) and long-term (weeks) distribution following chronic oral administration. The data collected have been useful in the assessment of risks caused by food and feed contaminated with nuclear fall-out (Howard & Howard, 1997; Voigt, 1997).

Another biologically interesting aspect of Cs is its interaction with cellular potassium (K) transport proteins, which have been elucidated mainly using different *in vitro* techniques (Draber & Hansen, 1994; DiFrancesco, 1995; Ussing et al., 1996; Schornack et al., 1997; Thompson et al., 2000). These studies, among others, have been helpful in elucidating the transport processes of both K and Cs.

Blood plasma, together with interstitial fluid (ISF), is the connecting element between all cells in the body. As a small ion, Cs^+ moves readily between plasma and ISF. Thus, processes transporting Cs^+ in and out of cells are likely to be mirrored as changes in plasma Cs concentration. Since the same transport processes are involved in the transport of K, following the kinetics of plasma Cs might be useful in the study of K transport processes *in vivo*. It must be remembered, however, that the atomic mass of Cs (132.9 u) is more than three times larger than that of K (39.1 u). The ionic radius of Cs^+ is also considerably larger than that of K^+ (Table 1). Differences in the kinetic behaviour of the two alkali metal ions are therefore to be expected.

Although the long-term kinetic behaviour of Cs can generally be explained using cell-level mechanisms, insufficient information exists to explain all peculiarities involved in kinetics on the whole-animal level. Studies on the short-term kinetics of Cs, i.e. the development of tissue concentrations within an hour or so after application, are therefore warranted. This information is needed for the estimation of the rate of entry of Cs into tissues, whereas long-term changes reflect better the balance between entry and exit of Cs. This thesis presents results from the studies of the short-term kinetics of Cs in goats and horses. In the latter species, the effect of exercise on Cs plasma kinetics was also investigated.

To examine plasma Cs kinetics, several traditional methods are available. On a purely technical basis, gamma-emitting radioactive isotopes of Cs are ideal since they are easy to measure and their low Cs carrier concen-

tration is certain not to cause gross interference with physiological processes. However, radiohygienic considerations limit the use of long-lasting and accumulating radioactive isotopes in animal experiments, especially in non-terminal experiments with larger animals. The toxicity of stable Cs (^{133}Cs) is relatively low, with an LD_{50} in the range of 10 mmol kg^{-1} (Johnson, et al., 1975; Pinsky & Bose, 1984). Thus dosing and measurement of trace levels of stable Cs could be another way of obtaining information about the biological behaviour of Cs. However, the measurement of stable Cs, especially in the micromolar region, involves equipment and know-how not available in standard biological or biochemical laboratories. Here, a novel, simple and inexpensive method for the assay of added stable Cs in blood plasma is presented and used in practical studies. The method is based on the competitive binding of stable and radioactive Cs isotopes into ammonium ferric cyanoferrate (AFCF), a complex salt with a high specific affinity towards Cs (Giese & Hantzsch, 1970; Arnaud et al., 1988; Giese, 1988; 1989).

5. Review of the literature

5.1. Caesium chemistry and physics

The term “caesium” comes from the Latin word “caesius”, meaning sky blue. This name is due to the intense blue colour ($\lambda = 456 \text{ nm}$), emitted by the element when heated in a gas flame.

Like other alkali metals, Cs is very electropositive, existing in aqueous solution only in its most positive oxidation state (+I). Among the stable alkali metals, Cs has the largest crystal ionic radius (**Table 1**). However, the mobilities of K, Rb and Cs in an electric field in aqueous solution are almost the same (**Table 1**), indicating that the size of the hydrated ions of these elements is similar.

Table 1. Crystal ionic radius (Shannon, 1976) and mobility (Bell & Lott, 1972) of alkali metal cations.

	Li ⁺	Na ⁺	K ⁺	Rb ⁺	Cs ⁺
Crystal ionic radius^a (pm)	76	102	138	152	167
Mobility (m²s⁻¹V⁻¹ x 10⁻⁹)	40	52	76	79	80

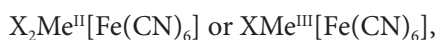
^aco-ordination number VI

Cs has only one stable isotope (¹³³Cs). General literature presents 49 radioisotopes with a mass number between 112 and 151 (Anonymous, 2005). Naturally, most of these have a rapid decay rate. Only three radioactive Cs isotopes have a half-life of over two weeks:

Nuclide	Half-life (years)	Energies of major emitted particles (keV)	
		β^- (max.)	γ
¹³⁴ Cs	2.1	658	605, 796
¹³⁵ Cs	2.3 x 10 ⁶	269	-
¹³⁷ Cs	30.2	514	662

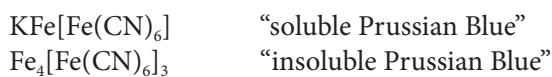
Of these, ^{137}Cs and ^{135}Cs are accumulated in large quantities (ca. 6%) in the beta decay processes following fission of the common fissile nuclides ^{235}U and ^{239}Pu . Both of these Cs isotopes are therefore found in nuclear waste and fallout from nuclear explosions. ^{135}Cs is a pure beta-emitter with a specific activity some five orders of magnitude less than ^{137}Cs . Because activity measurement of ^{135}Cs is difficult and its activity in samples is negligible compared with that of ^{137}Cs , the nuclide is usually ignored in radiochemical measurements. Although ^{134}Cs is also a fission product, its yield is very low (ca. 0.01%). However, considerable amounts accumulate in fissile nuclear fuel, due to the activation of ^{133}Cs by slow neutrons. The activity ratio $^{134}\text{Cs}/^{137}\text{Cs}$ in the fall-out of the Chernobyl accident, for example, was around 0.55 (Mück et al., 2002). The annual oral limit of intake (ALI) for ^{134}Cs is 1 MBq and for ^{137}Cs 2 MBq. For dose estimation following oral intake 19 nSv Bq $^{-1}$ for ^{134}Cs and 13 nSv Bq $^{-1}$ for ^{137}Cs are used (International Commission on Radiological Protection, 1991).

The binary salts of Cs, like those of other alkali metals, are highly soluble in water. However, certain insoluble complex hexacyanoferrates have proven to be specific agents for the removal of Cs from solution (Streat & Jacobi, 1995). These complex salts have the general structure of



where X denotes a monovalent entity (alkali metal or ammonium), Me^{II} a metal with an oxidation state of +II and Me^{III} a metal with an oxidation state of +III. Cs cations tend to replace the monovalent entity in the complex, rendering Cs insoluble. For example, insoluble potassium cobalt hexacyanoferrate ($\text{K}_2\text{CoFe}(\text{CN})_6$) has been used to decontaminate coolant waters in atomic power plants (Lehto & Harjula, 1999).

Especially interesting from the viewpoint of this thesis are the salts in which Me^{III} is an iron. These salts are often referred as “Prussian Blue”-compounds. Unfortunately, there is some ambiguity regarding this nomenclature. Traditionally, the term “Prussian Blue” (PB) has been used for two complex compounds:



The latter lacks potassium ions and does not readily form colloidal dispersions, unlike the former. These dispersions are not, however, true solutions. The colloidal crystals can be separated from the dispersion using, for example, ultrafiltration.

Because of their affinity towards Cs and lack of toxicity (Pearce, 1994), PB compounds have been used as a feed additive to reduce the contamination of radioactive Cs from nuclear fall-out in animals. Nigrovic (1963)

found that $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$ given to rats orally both reduced the incorporation and accelerated the decorporation of ^{137}Cs . Richmond and Bunde (1966) also observed an increased decorporation of ^{137}Cs in rats receiving PB. The authors did not publish the structure of the PB that they used, but since it was given together with drinking water, it is reasonable to assume that the compound was “soluble” PB ($\text{KFe}[\text{Fe}(\text{CN})_6]$). Müller et al. (1974) reported that this “soluble” PB was more effective in the decorporation of ^{137}Cs than “insoluble” PB ($\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$). Giese & Hantzsch (1970) compared the effect of different soluble PB compounds and found out that, among others, ammonium–iron(III)–hexacyanoferrate(II) ($\text{NH}_4\text{Fe}[\text{Fe}(\text{CN})_6]$) was significantly more effective in binding ^{137}Cs in the alimentary tract than traditional soluble PB ($\text{KFe}[\text{Fe}(\text{CN})_6]$). Moreover, the authors noticed that, unlike other PB-compounds containing monovalent ions, $\text{CsFe}[\text{Fe}(\text{CN})_6]$ does not readily form colloidal dispersions. $\text{CsFe}[\text{Fe}(\text{CN})_6]$ was also not very effective in binding ^{137}Cs . The authors proposed the existence of a “solubility trap”: when ^{137}Cs undergoes exchange with a monovalent ion in the PB-compound, a less dispersive complex forms, which reduces desorption of bound ^{137}Cs .

After the nuclear accident in Chernobyl, ammonium–iron(III)–hexacyanoferrate(II) was approved as a feed additive for reducing the incorporation of radioactive Cs from contaminated feed (Giese, 1989). In the literature, the abbreviation AFCF is often used for the compound, short for ammonium ferric cyanoferrate (Arnaud et al., 1988). Due to the manufacturing process, the commercial product contains approximately 30% (by weight) ammonium chloride (NH_4Cl).

5.2. Caesium biology

No biological function has been found for Cs. To a certain degree, however, its cellular behaviour resembles that of K. Thus, it makes sense to compare these two elements when reviewing Cs biology.

Like K, the absorption of soluble Cs from the gastrointestinal tract is fast and almost quantitative, and it most likely occurs through paracellular transport (Nedergaard et al., 1999). However, certain feedstuffs, particularly non-digestible fibre, adsorb Cs and can reduce its absorption (Giese, 1971). In blood plasma, Cs exists as a free ion, just like potassium and sodium. This has been shown experimentally; the ultrafiltration of ^{137}Cs -containing plasma resulted in an ultrafiltrate with 94% of the original activity (Liubashevskii, 1968).

The membrane potential results in the passive flow of all permeable cations into the cell, until the Nernst equilibrium is reached. For univalent cations, like Cs^+ , this equilibrium means a transmembrane concentration ratio near that of K, with an intracellular concentration some 20 to 40 ti-

mes higher than extracellular concentration. In rats, tissue/plasma ratios of this magnitude were reached in most internal organs within 2 h of intravenous administration of ^{134}Cs (Gregus & Klaassen, 1986). This passive transport is facilitated by the numerous different kinds of potassium channels on cell membranes. Cs permeability varies depending of the channel type. According to Leggett et al. (2003), the Cs/K selectivity ratio for K channels ranges from less than 0.02 to approximately 0.2. In fact, Cs is often used as a potassium channel blocker in in vitro work (Clay & Shlesinger, 1984).

In addition to passive transport by the electrochemical gradient, Cs is pumped actively into cells by Na,K-ATPase, along with K. In general, this potassium transport protein discriminates Cs less than potassium channels. According to a review by Leggett et al. (2003), the typical Cs/K selectivity ratio for Na,K-ATPase is 0.25. Experiments with Na,K-ATPase inhibitors indicate that Na,K-ATPase is almost solely responsible for the inward cellular transport of Cs in the rat heart (Schornack et al., 1997), frog sartorius muscle (Beauge & Sjodin, 1968) and frog skin epithelium (Ussing & Lind, 1996). This may not be universal, however, as in rat soleus muscle as much as 28% of Cs uptake remained after addition of ouabain, a common Na,K-ATPase inhibitor (Dorup & Clausen, 1994). In the long run, the tissue/plasma ratio exceeds that of K in just about all soft tissues (Relman et al., 1957; Brefeld et al., 1968; Kernan, 1972). Obviously, the inward active potassium transport processes of the cell membrane discriminate caesium less than the outward passive transporting processes.

Due to the release of radioactive Cs into the environment in nuclear atmospheric explosions, the whole-body retention of ingested Cs by different animals received much attention in the 1950s and 1960s. Usually, a three-component exponential function is needed for the exact description of body retention (Stara et al., 1971). Each of these components has a characteristic half-life. For animals with similar feed and gastrointestinal physiology, the half-life of the slowest component is a function of body mass. On a double logarithmic scale (log-log), the plots of half-lives and typical body weights of non-ruminants fall practically on one line. The values for ruminants form another line, with their half-lives being considerably shorter than those of non-ruminants (Stara et al., 1971). In cows, for example, the half-life of the slowest compartment is around 20 days, whereas in humans it is around 110 days. Pigs are an exception, since their plot falls on same line as ruminants. In some studies concerning radioactive Cs retention, a single value for half-life is used to describe the time required for excretion of 50% of a single dose. The following values were collected by Shannon et al. (1965):

Species	Half-life (days)
Mouse	1.2
Rat	6.5
Dog	25
Pig	26
Goat	31
Human	110

The different ways of interpreting the term “half-life” must be kept in mind when analysing data from the literature. However, since the effective half-life of Cs in larger animals is measured in weeks, excretion is not likely to be a significant factor when studying the short-term kinetics of Cs in plasma.

Due to differences in cell types and blood flow, marked variation of Cs uptake and release in different organs exists. Leggett et al. (2003) developed a biokinetic model (henceforth the “Leggett model”) for the retention of Cs in human organs and tissues, in which they used the following data for each organ or tissue:

Steady state Cs content in humans (fraction of total body Cs)
 Blood flow (fractional cardiac output)
 Tissue specific extraction fraction of Cs

The *extraction fraction* is defined as the fraction of atoms extracted by the organ/tissue in passage from arterial to venous plasma. Exact data for this quantity were unavailable, so the authors made well-founded estimations based on indirect data for Cs, K and Rb. Their estimates for the extraction fractions for different organs/tissues were as follows:

Kidneys, gastrointestinal tract, heart	0.2
Liver, skin	0.05
Brain	0.002
All other tissues	0.1

Based on these estimates, the authors calculated the transfer coefficients for Cs between different organs/tissues and plasma, both for uptake and release. In general, the predictions based on the Leggett model seem to agree fairly well with empirical observations of Cs kinetics in different tissues (Leggett et al., 2003).

Some of the estimates based on the Leggett model are presented in **Table 7**. As can be seen, in kidneys the rate of both uptake and release of Cs is very high. In muscle, on the other hand, the difference between the transfer coefficients is huge, which is consistent with the observed high proportion (80%) of total body Cs in muscle in a steady-state situation. It is therefore not surprising that muscular dystrophies significantly decrease Cs retention in humans (Lloyd et al., 1968, 1973). Guinea pigs suffering from hereditary muscular dystrophy accumulate significantly less ^{134}Cs in their muscles than healthy control animals (Szentkuti et al., 1976). This also holds true also for swine with a deficiency of selenium and vitamin E (Szentkuti, 1976)

Cs is excreted through both urine and faeces. In carnivores, the majority of Cs is excreted in urine (Hood & Comar, 1953; Mraz et al., 1958). In humans, the reported urinary fractions (ratio of cumulative urinary Cs to cumulative total Cs excretion) have varied from around 0.7 to more than 0.9, with an average value of approximately 0.86 (Leggett et al., 2003). However, considerable reabsorption of filtered Cs occurs in the kidneys. According to the Leggett model, the transfer coefficient from the kidneys to the urinary bladder is only 1.7 day^{-1} , compared with 32 day^{-1} from the kidneys to blood plasma. Due to the high degree of reabsorption, the excretion rates of Cs are modest. Lloyd et al. (1973) measured urinary clearance values for Cs ranging from 2.8 to 4.6 plasma volumes d^{-1} in five male subjects. The values according to the Leggett model in the same circumstances were between 3.5 and 3.9 plasma volumes d^{-1} (Leggett et al., 2003). Aldosterone, which stimulates the excretion of K, also increases the rate of removal of Cs from rats (Alli-Balogun, 1975). Adrenalectomy, on the other hand, increases the retention of ^{134}Cs (Feurer, 1976). Lowered aldosterone levels have also been proposed to be the reason behind the increased body half-life of ^{134}Cs at high altitudes (Rundo & Richmond, 1970).

Although carnivores excrete less in faeces than in urine, considerable circulation of Cs occurs between the gastrointestinal tract and plasma. This can be inferred from the observation that oral application of insoluble Cs binders (e.g. Prussian Blue) considerably reduces the retention of body Cs. Melo et al. (1998) concluded that oral administration of PB reduced the long-term retention half-life by an average of 69%. However, there are many kinds of secretory and absorptive processes in the gastrointestinal tract, and the exact pathways for this circulation between the plasma and the gastrointestinal tract have not yet been elucidated. In the Leggett model, the gastrointestinal tract is assumed to receive Cs from saliva, gastric juices, cells sloughed from the gastrointestinal tract walls, secretions through the walls of the small and large intestines, pancreatic juices, bile and Brunner's gland secretions (Leggett et al., 2003). Using the normal assumptions pertinent to the model, the calculation of transfer coefficients between plasma and the contents of the stomach, small intestine and gas-

gastrointestinal tract results in figures of 4.5, 1.0 and 0.02 day⁻¹, respectively. The transfer coefficient in the other direction, from small intestine to plasma, was 28 day⁻¹. When this transfer coefficient was assumed to be zero, the long-term retention half-life reduced by 60%, which is similar to the effect reached when using effective Cs binders.

Feed fibre also binds Cs. Increasing the feed fibre content considerably increases the proportion of Cs excreted in faeces compared to that excreted in urine (Mraz & Patrick, 1957a; 1957b). This difference in the structure is a likely explanation for the high ratio of faecal to urinary excretion in ruminants compared with non-ruminants. These aspects have been extensively discussed by Giese (1971).

Little data exists in the literature on plasma Cs kinetics, especially short-term kinetics. Ekman (1961) followed the plasma kinetics of ¹³⁷Cs in goats for 14 days following a single oral dose. However, during the first day only six samples were taken and measured. Possibly due to differences in gastrointestinal absorption, the time of maximum plasma concentration in different animals varied from 30 minutes to 6 hours. Thus, the experiment did not shed much light on the short-term plasma kinetics of Cs. Poe (1972) compared the plasma kinetics of ¹³¹Cs and ⁴²K in dogs after an intravenous dose for 3 h, with sample frequency varying from 5 to 20 min. The author found the plasma concentration curve to be multiphasic, containing three basic components. At 2 min after the dose, 42% of the administered ¹³¹Cs was still in the blood, whereas at 60 min only 3.8% remained. ⁴²K vanished much faster from the circulation, with 22% and 1.6% of the original dose remaining in blood at 2 and 60 min, respectively.

Cellular intake is not necessarily the only way to incorporate Cs. Using autoradiographic techniques, Nelson et al. (1961) found that following an intravenous injection the initial uptake of Cs in murine cartilage was very pronounced. The authors suggested ion exchange as a mechanism for this uptake. There are also indications that the tendinous fraction from beef muscle has a much greater affinity to ¹³⁴Cs than muscle homogenate as a whole (Szentkuti & Giese, 1974).

Within cells, Cs tends to distribute unevenly. In many different types of cells, a large fraction of Cs was found within the nucleus or fixed to the nuclear membrane 24 hours after an oral dose (Giese and Rekowski, 1970). Using autoradiography, in myofibrils, ¹³⁴Cs was shown to attach at distinct distances similar to those between isotropic and anisotropic bands Szentkuti & Giese (1973). With the help of transmission electron microscopy, Edelmann (1980) confirmed that Cs, together with K and Rb, accumulates preferentially in the A-band and, to a lesser extent, the Z-disc of myofibrils. Edelmann's results have been used to champion the association-induction hypothesis as an explanation for biological electrochemical gradients (Ling, 2005). This hypothesis has not, however, gained wide acceptance in the scientific community (Edelmann, 2005).

In a two-week chronic feeding experiment with rats, muscles rich in red fibres (type I) accumulated significantly more Cs than muscles containing predominantly white fibres (type II) (Kernan, 1969). The K^+ efflux in electrically stimulated type I muscles is considerably less than in type II muscles (Clausen et al., 2004). Thus the difference in net Cs accumulation between the two muscle types may be due to differences in the rate of Cs^+ efflux.

The concentration of endogenous stable Cs (^{133}Cs) in biological samples is extremely low. In human soft tissue, the concentration of Cs is generally in the range of 2-50 ppb (Yamagata, 1962; Iyengar et al., 2004). In blood plasma, the concentration is even lower, around 1 ppb (Versieck et al., 1977). Since the LD_{50} of Cs is approximately 1g/kg (Johnson et al., 1975; Pinsky & Bose, 1984), toxic amounts of Cs can not be ingested from natural sources. However, some cancer patients treat themselves with large amounts of CsCl, which can lead to symptoms of poisoning (Saliba et al., 2001; Lyon & Mayhew, 2003; Dalal et al., 2004). This unofficial self-treatment is based on some reports published in the 1980s indicating Cs to have tumor-repressing activity (El-Domeiri et al., 1981; Sartori, 1984a; Tufte et al., 1984). However, no well-founded clinical tests on the anti-cancer-properties of Cs are available. Moreover, the professional qualifications of one researcher proposing Cs therapy for cancer have been challenged (Samadani & Marcotte, 2004). Brewer (1984) and Sartori (1984b) speculated that the tumour-repressing activity was due to a specific rise of intracellular pH in cancer cells, which slows down their rate of metabolism. Guns et al. (2005) recently confirmed experimentally the rise of pH in certain tumour cells following Cs-treatment. The physiological or chemical basis for this shift was not, however, discussed by the authors. One explanation for the effect could be that Cs^+ as a strong base cation tends to increase the cytoplasmic strong ion difference (SID). This, in turn, decreases the cytoplasmic hydrogen ion concentration (Lindinger et al., 2005).

The toxic effects of Cs include ventricular arrhythmia (Jones et al., 2001; Saliba et al., 2001), salivation, diarrhoea and inhibition of motor activity (Bose & Pinsky, 1984; Fujii & Nomoto, 1987). In bone marrow of mice, high doses of caesium have been shown to have clastogenic effects (Ghosh et al., 1991) as well as induce micronucleation in polychromatic erythrocytes (Santos-Mello et al., 2001). There are also reports about the developmental toxicity of high doses of Cs (Messiha, 1994).

5.3. Caesium assay methods

Because of the low concentration of endogenous Cs in tissue and plasma samples, its analysis requires sensitive assay methods. Neutron activation analysis (NAA) is based on the activation of stable isotopes with a neutron flux and measurement of specific gamma emission of the daughter nuclide with a gamma spectrometer. Due to its exceptional sensitivity, NAA has been used widely in the study of Cs content in biological samples (Yamagata, 1962; Hock et al., 1975; Versieck et al., 1977; Kasperek et al., 1979; Demmel et al., 1982; Gawlik et al., 1989; Kumar et al., 1989; Scholz, 1990; Steiner, 1990; Iyengar et al., 2004). However, the method requires an experimental nuclear reactor which reduces considerably its practical applicability.

Mass spectrometry (MS) is another highly sensitive assay method, which utilizes the separation of accelerated ions in a magnetic field. Schulten et al. (1978) developed a method based on field desorption MS to investigate Cs in physiological fluids. Inductively coupled plasma mass spectrometry (ICP-MS), a more recent method, has largely replaced NAA in the study of endogenous stable Cs in biological material (Vandecasteele et al., 1990; Corrigan et al., 1991; Krachler et al., 1999; Iyengar et al., 2004).

In atomic absorption spectrometry (AAS), the sample is atomized in a flame or some other heating device. Specific hollow-cathode lamps, plated with the metal under investigation (e.g. Cs), emit light of specific wavelengths, which is guided as a beam through the atomized sample. This light is absorbed by the atoms of the same metal in the atomized sample. One drawback of the method is that each element requires a specific lamp, complicating multi-elemental analysis. Generally, AAS-applications are also far less sensitive than methods based on NAA or MS. AAS has been used mainly when studying the effects of added Cs in biological systems, either in basic research (Guns et al., 2005) or in cases of poisoning (Centeno et al., 2003). AAS-applications have also occasionally been used in the study of endogenous Cs (El-Yazigi et al., 1988).

In principle, Cs concentration can also be investigated by using atomic emission spectrophotometry (AES). However, due to their relatively high detection limits, these methods have seldom been used in the study of biological samples. Kernan (1969, 1972) used flame photometry to determine the concentration of caesium in plasma and skeletal muscle following chronic feeding of Cs. Earlier, Relman et al. (1957) had also used the method in a similar experiment, together with radioactive tracers (^{134}Cs). In these experiments, the typical tissue Cs concentrations were well above 10 mmol l^{-1} ($1 \text{ mmol} = 133 \text{ mg}$).

Like photometry, nuclear magnetic resonance (NMR) is not a very sensitive method, but it has a distinct advantage; it can be used to measure both extracellular and intracellular ^{133}Cs in intact tissue and cells (Davis et al., 1988; Li et al., 1995). The method has been used in many experiments to study potassium transport mechanisms across cell membranes (Shehan et al., 1993; Schornack et al., 1997; Wellard & Adam, 2002).

Radioactive tracers are frequently used in the study of the biological properties of Cs. At the beginning of the nuclear era, ^{137}Cs ($T_{1/2} = 30$ years), separated from fission products was the main tracer isotope used in such research. It was later replaced by ^{134}Cs ($T_{1/2} = 2.1$ years), which can be produced by neutron activation of ^{133}Cs . Both are strong gamma-emitters and are hence easily measured using gamma-spectroscopy. Cs isotopes with a short half-life, including ^{129}Cs ($T_{1/2} = 32$ h), ^{131}Cs ($T_{1/2} = 9.7$ days) and $^{134\text{m}}\text{Cs}$ ($T_{1/2} = 30$ h), have been used as potassium analogues in experimental scintigraphy (Rothman et al., 1974; Nishiyama et al., 1976; Turner et al., 1976; Gustafson et al., 1977). These experiments have not, however, lead to widespread use of radioactive Cs isotopes in practical scintigraphy.

In autoradiography, a radioactive tracer in the sample exposes a photographic film pressed close to the sample. From the developed film, body and tissue localization of the tracer can then be determined. Nelson et al. (1961) used autoradiography to study whole-body distribution of radioactive Cs in mice. Autoradiography has also been used to examine the intracellular distribution of Cs (Giese & Rekowski, 1970; Szentkuti & Giese, 1973; Edelmann, 1980).

Cs is an electron-dense element and therefore tends to be visible as such in transmission electron micrographs. Edelmann (1980) used this property to study the distribution of Cs in Cs-loaded myocytes.

6. Aims of the study

- To develop a simple, inexpensive method to investigate the short-term kinetics of plasma Cs. (I, II)
- To gather information about the short-term kinetics of plasma Cs in goats and horses using the method developed (IV)
- To gather information about the short-term distribution of Cs in order to explain its short-term plasma kinetics (III).

7. Materials and methods

7.1. Animals

The study protocols were approved by the Ethics Committee for animal experimentation at the University of Helsinki. The goats used were non-lactating Finnish Landrace females, aged between 7 and 12 years and weighing between 43 and 56 kg. The horses were Standardbred mares and geldings aged between 3 and 12 years and weighing between 408 and 524 kg.

7.2. Assay method for added stable caesium

The general assay procedure (II, IV) is based on competitive binding and is thus analogous to radioimmunoassay (RIA) (Yalow & Berson, 1960). In brief, ^{134}Cs is added as a label to the sample containing stable Cs. Ammonium-iron(III)-hexacyanoferrate(II) (AFCF) is then added. After incubation, AFCF-bound Cs is precipitated together with plasma proteins using trichloroacetic acid (TCA). Free Cs (both stable and radioactive) stays in solution. The precipitate is separated by centrifugation, and its ^{134}Cs content is measured using a gamma-counter.

An essential element of the assay method is the eventual separation of AFCF-bound Cs and freely soluble Cs. Thus, the co-precipitation of AFCF and AFCF-bound Cs with plasma proteins should be quantitative. In addition, a minimum amount of free Cs should be precipitated in this process.

To study these aspects, the behaviour of different mixtures of AFCF, albumin and TCA were investigated (I). Following mixing of components, precipitation and centrifugation, the supernatant was decanted to another vial. Both precipitate and supernatant were studied for their AFCF-content.

The AFCF was of commercial grade ("Giese salt"), which contains 30–35% ammonium chloride (by weight) in the dry product. The albumin solution was prepared from commercial dried bovine serum albumin. The solutions of TCA were prepared daily from a concentrated stock solution.

The concentration of AFCF was measured in two different ways. For concentrations above 10 mg l^{-1} , absorption spectroscopy at 685 nm was used. For the measurement of lower concentrations, ^{134}Cs -labelled AFCF (^{134}Cs -AFCF) was prepared by incubating a dispersion of AFCF (10 mg l^{-1}) with radioactive ^{134}Cs tracer solution for three days. A standard gamma-counter was used for quantitative measurement.

In Study I, the magnitude of the following parameters was varied in order to evaluate their effect to the precipitation process:

TCA concentration (2-50 g l⁻¹)
AFCF concentration (0.5-10 mg l⁻¹)
Albumin concentration (0.002-20 g l⁻¹)
Temperature (0, 20, 37 °C)
Centrifugation speed and time (1000-4000 g, 5 – 20 min)
Washing of the precipitate
Order and delay of adding components
Cation concentration (0.01 and 0.1 Eq l⁻¹, ions H⁺, NH₄⁺, Li⁺, Na⁺, K⁺, Cs⁺, Ca⁺, Mg⁺)

Based on the results of Study I, an assay procedure was developed (II) and tested (IV). The assay involves adding the following components:

a) Sample: heparinized blood plasma	0.1 ml
b) Standard: Cs solution ([CsCl] = 0-10000 μmol l ⁻¹)	0.1 ml
c) Tracer: ¹³⁴ Cs (7300 kBq l ⁻¹ , [Cs] = 1 μmol l ⁻¹)	0.1 ml
d) Buffer: acidic calcium phosphate (0.1 mol l ⁻¹ , pH 2.1)	0.5 ml
e) Binding agent: AFCF (12.5 mg l ⁻¹)	0.2 ml

Standard samples were prepared from plasma containing no added plasma Cs. *Zero samples* were standard samples with plain water ([CsCl] = 0) added at Stage b. *Unknown samples* contained an unknown amount of Cs from the beginning; hence, plain water was added at Stage b, as well. In *blank samples*, plain water was added instead of binding agent (AFCF) at Stage e.

All plasma samples were taken from the animals within a short time period (1-2 h). Standard, zero and blank samples were prepared from plasma taken from animals before they received a Cs-dose. The unknown samples were collected after this dose.

After incubating the mixture for 24 h, AFCF and bound Cs were co-precipitated with plasma proteins by adding 1 ml of TCA (0.3 mol l⁻¹). After centrifugation (2500 g, 15 min), the supernatant was discarded and the count rate of the precipitate measured. The count rate of the blank sample was subtracted to reach the *blank-corrected count rate*. Dividing the blank-corrected count rate of a sample with blank-corrected count rate in the zero sample gave the *relative binding* (= B_R) of Cs. This value was between 0 and 1, with zero indicating no binding and one indicating maximum binding.

When studying the interference of monovalent ions, other salt solutions were used instead of CsCl (Stage b). In addition, in order to eliminate the effect of endogenous plasma cations, a pure albumin solution (70 g l⁻¹), instead of plasma, was used to precipitate AFCF (Stage a).

7.3. Plasma caesium kinetics

The five experimental goats in Study IV were all non-lactating females. The 12 experimental horses, including both mares and geldings, were divided randomly into two groups (Group I and Group II), both with six individuals. Group I rested (standing) during the whole experiment. Group II trotted for 40 min on a treadmill (inclination 3°, speed 5 m s⁻¹).

From all animals, blood was evacuated for standard plasma samples, followed by an intravenous dose of CsCl (5 µmol kg⁻¹ of live weight). Blood samples were collected at regular intervals into heparinized vials. In the group of running horses, heart rate was constantly monitored. In all horses, haematological parameters (Hb, PCV, WBC) and lactate concentration was analyzed from whole blood samples. The concentrations of K, Na, Cl and Cs were determined from plasma. Plasma Cs was assayed using the method described above (Section 7.2).

The Cs concentration (µmol l⁻¹) was divided by both 1.03 kg l⁻¹ (density of plasma) and 5 µmol kg⁻¹ (Cs dose) to reach a unitless value, henceforth called *relative concentration* (R). A two-phased exponential decay curve was fitted to the data for plasma Cs relative concentration as a function of time, (see Section 7.5).

7.4. Tissue caesium distribution

In Study III, six goats intravenously received a solution containing ¹³⁴Cs as a Cs tracer and ⁵¹Cr-EDTA as an extracellular marker. Blood samples were collected at 2, 4, 6, 8, 10, 12, 15, 20, 25 and 30 min post-injection, and plasma was separated from cells by centrifugation. After 30 min, the animals were euthanized and tissue samples collected.

The count rate of plasma and tissue samples was measured with a gamma-counter. Applying the appropriate corrections, the specific activities (Bq g⁻¹) of both ¹³⁴Cs and ⁵¹Cr in each sample were determined. These were divided by the specific dose (total dose activity / animal weight) to reach a value for the relative concentration (R) of these isotopes, as described above (Section 7.3). In Study III, the values are referred as *relative activity concentration*.

⁵¹Cr-EDTA does not enter cells. Dividing the relative concentration of ⁵¹Cr-EDTA in a tissue sample by its extracellular relative concentration, approximated by its terminal plasma relative concentration, gives the approximate apparent *extracellular fluid fraction* (E) in tissue. Using the parameters R and E, the *apparent intracellular ¹³⁴Cs relative concentration* (I) in sample tissues was approximated. In addition, the total *fraction of Cs dose* accumulated in an organ (F_{Cs}) at termination was calculated.

7.5. Statistical analysis and curve fitting

A t-test was used to determine significance of differences between average values of two sets. In Study III, analysis of variance was used to test the significance of differences in Cs concentration in different muscle types.

In the assay of Cs (II, IV), the data for B_R vs. Cs concentration were fitted on a curve of the form

$$B_R = 1/(1+10^{H(\log C - \log L)}) \quad (\text{Eq. 1})$$

B_R = relative binding of Cs (between 0 and 1)

H = constant ('Hill slope')

C = Cs concentration

L = Cs concentration at 50 % response, i.e. when $B_R = 1/2$

In Study II, the concentration of all samples was known. For each concentration, there were six parallel samples. In Study IV, the Cs concentration of unknown samples was resolved using their B_R and the standard curve according to Eq 1.

In the study of plasma kinetics (III, IV), the following equations were applied:

$$R = K_1 \exp(-k_1 t) + K_2 \exp(-k_2 t) \quad (\text{Eq. 2})$$

$$R = K' \exp(-k' t) \quad (\text{Eq. 3})$$

R = relative concentration of Cs^+

t = time elapsed after injection of dose (min)

K' , K_1 and K_2 = spans of different phases

k' , k_1 and k_2 = rate constants of different phases

Eq. 2 was applied to data collected during a longer time span, starting from injection (III, IV), whereas the simpler Eq. 3 was used in Study IV to determine segmental rate constants during shorter periods.

The *apparent rate of removal* of Cs at instant t (k_t) was estimated using the following equation:

$$k_t = \frac{-dR/R}{dt} = \frac{k_1 K_1 \exp(-k_1 t) + k_2 K_2 \exp(-k_2 t)}{K_1 \exp(-k_1 t) + K_2 \exp(-k_2 t)} \quad (\text{Eq. 4})$$

R = relative concentration of Cs^+

t = time elapsed after injection of dose (min)

K_1 and K_2 = spans of different phases resolved using Eq. 2

k_1 and k_2 = rate constants of different phases resolved using Eq. 2

All curve fitting was done using the computer program GraphPad Prism, version 3.03 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

8. Results and discussion

8.1. Assay method for caesium

As described earlier (Section 7.2), the method is analogous to RIA. As the concentration of stable Cs increases, less ^{134}Cs is bound by AFCF. The quantitative separation of Cs bound to AFCF and free Cs in solution is an essential element of this new assay method. The separation is achieved by co-precipitating AFCF and Cs bound to it together with plasma proteins, using TCA. The chemical background for this process is not clear. As speculated in Study I, the mutual affinity might be due to the different charges of AFCF and protein precipitate in acidic surroundings.

The optimization of the precipitation process was the main aim of Study I. This involved, among others, experimenting with different concentration of components. Unless otherwise mentioned, the concentrations below refer to *initial concentrations*, i.e. the analytical concentrations of individual components in dispersion after mixing all components.

Initial TCA concentrations of the order 50 g l^{-1} are typical when precipitating proteins from blood plasma in clinical chemistry (Henry & Szustkiewicz, 1974). However, when precipitating AFCF-bound Cs^+ ions, it is reasonable to keep the hydrogen ion concentration as low as possible to minimize the concentration of cations competing with Cs^+ . In a system where the initial concentrations of albumin and AFCF were 0.5 g l^{-1} and 50 mg l^{-1} , respectively, an initial TCA concentration of 16 g l^{-1} was sufficient to reduce the absorption due to AFCF (685 nm) to well below 1% compared with the situation with no TCA. Increasing the TCA-concentration did not significantly improve precipitation. However, below a TCA concentration of 16 g l^{-1} , the precipitate no longer separated with simple centrifugation. Thus, in the actual assay, an initial concentration of 25 g l^{-1} (0.3 mol l^{-1}) was used to err on the side of caution.

In the actual assay (II, IV), the incubation concentration of AFCF was set at 2.5 mg l^{-1} . At this level, 50% of tracer is absorbed in zero samples, which is the preferred level in RIA applications (Skelley et al., 1973). Using a ^{134}Cs -labelled AFCF with an initial concentration of 2 mg l^{-1} , the fraction of activity remaining in supernatant after precipitation was around 1.6% (I). The separation of the Cs-AFCF complex and soluble Cs is thus adequate also in the environment used in actual assay.

An initial albumin concentration of 0.1 g l^{-1} was found to be sufficient to quantitatively bind AFCF at a level of 10 mg l^{-1} . In the assay method, 0.1 ml of plasma was initially added. At precipitation, the volume was 2 ml . Hence, a 20-fold dilution of plasma occurred, indicating that the protein concentration lies between 3 and 4 g l^{-1} . This concentration is more than adequate for the binding of AFCF, but it has one minor drawback: this amount of precipitated protein binds around 3 % of total water and dissolved free Cs (I). However, since all plasma samples in an assay were collected from the same animal over a short period, the plasma protein concentration is not likely have varied much. The fraction of free Cs in precipitate is thus also similar in all samples, reducing the error caused by this “unspecific binding”.

Increasing or decreasing the incubation temperature from room temperature did not increase the yield of precipitated ^{134}Cs -AFCF. Room temperature was therefore used in the actual assay procedure. Centrifugal accelerations above 2000 g and centrifugation times exceeding 10 min did not significantly improve the separation of ^{134}Cs -AFCF from dispersion. To be on the safe side, values of 2500g and 15 min were selected for the actual assay procedure.

Washing the precipitate once with TCA (20 g l^{-1}) removed less than 0.5% of the ^{134}Cs . How much of this was desorbed ^{134}Cs and how much intact ^{134}Cs -AFCF was not known. In any case, the amount was so low, that a precipitate wash during the actual assay procedure was deemed unnecessary.

When ^{134}Cs -AFCF, albumin and TCA were added together just prior of centrifugation, around 1% of ^{134}Cs activity remained in supernatant. When TCA was added 1 h later to a mixture of ^{134}Cs -AFCF and albumin, the residual supernatant activity was somewhat higher, 1.6% (I). As before, it is difficult to ascertain the form of the activity (^{134}Cs -AFCF or ^{134}Cs) in the supernatant. In the actual assay procedure Cs is bound to AFCF within an incubation period of 24 hours. Following precipitation, the curve of bound Cs vs. AFCF concentration had a typical sigmoid shape (**Figure 1** in II). When the AFCF concentration was 50 mg l^{-1} , more than 98% of Cs was in the precipitate. Obviously, this long “delay” due to incubation in the actual assay procedure did not prevent quantitative removal of AFCF from dispersion.

In Study II, $0.2 \text{ } \mu\text{mol l}^{-1}$ was the lowest sample concentration giving a significantly different count rate from zero samples ($P < 0.05$, **Table 2**). In RIA, this lower detection limit is often used as a measure of *sensitivity*. This concentration also served as the lower cut off value for further data processing.

Table 2. Count rates and relative binding of tracer ^{134}Cs in precipitated AFCF after incubation with added Cs ($n = 6$ for each concentration) at the plasma concentrations indicated. The coefficient of variation (CV) of relative binding at each concentration indicates the precision for the measurements of relative binding at that concentration. P shows the statistical significance of the difference in count rate compared with the count rate in samples without added Cs (II)

Added Cs plasma concentration (mmol l ⁻¹)	Count rate (cpm)	Relative binding of ^{134}Cs tracer (average \pm sd)	CV (%) (sd/average)	P
0	10293 \pm 80	1 (by definition)	0.9	-
0.05	10179 \pm 96	0.988 \pm 0.010	1.0	0.049
0.1	10136 \pm 271	0.983 \pm 0.029	3.0	0.204
0.2	10067 \pm 154	0.976 \pm 0.017	1.7	0.01
0.5	9516 \pm 152	0.916 \pm 0.016	1.8	<0.0001
1	8845 \pm 124	0.844 \pm 0.013	1.6	<0.0001
2	7929 \pm 150	0.745 \pm 0.016	2.2	<0.0001
5	6038 \pm 90	0.540 \pm 0.010	1.8	<0.0001
10	4678 \pm 47	0.393 \pm 0.005	1.3	<0.0001
20	3407 \pm 47	0.256 \pm 0.005	2.0	<0.0001
50	2373 \pm 35	0.144 \pm 0.004	2.6	<0.0001
100	1861 \pm 44	0.089 \pm 0.005	5.3	<0.0001
200	1549 \pm 32	0.055 \pm 0.003	6.2	<0.0001
500	1269 \pm 25	0.025 \pm 0.003	10.9	<0.0001
1000	1174 \pm 19	0.015 \pm 0.002	13.5	<0.0001
2000	1110 \pm 22	0.008 \pm 0.002	30.1	<0.0001
5000	1060 \pm 28	0.002 \pm 0.003	121	<0.0001
10000	1038 \pm 21	0.000 \pm 0.002	1373	<0.0001
Blank	1037 \pm 9	0 (by definition)		
Total	19122 \pm 170			
Background	175			

At low relative binding values (higher concentrations), their coefficient of variation of relative binding tends to increase, increasing error (**Table 2**). For further data processing, the cut off limit was set at 0.1 (10 %), thus excluding all sample concentrations above 200 $\mu\text{mol l}^{-1}$.

Between the two cut off values (0.2-200 $\mu\text{mol l}^{-1}$), the fit of the data ($\log C$ vs. B_R) to Eq. 1 (Section 7.5) was excellent; the coefficient of determination (R^2) for the standard curve was 0.9989, with 56 degrees of freedom. Based on the resolved equation parameters, a concentration estimate for each data point was made. For parallel samples, the mean and the coefficient of variation were calculated (**Table 3**). The difference between the mean of estimates and actual concentration was used as a measure of *accuracy* at that concentration. The coefficient of variation of the estimates of parallel samples was used as an estimate of *precision*.

Table 3. Comparison of actual and estimated Cs incubation concentrations using non-linear regression analysis (n = 6 for each concentration). The difference between mean of estimates and actual concentration indicates the accuracy of the method. The coefficient of variation (CV) for concentration estimates indicates their precision (II)

Actual concentration ($\mu\text{mol l}^{-1}$)	Mean of estimates ($\mu\text{mol l}^{-1}$)	Difference between mean of estimate and actual concentration (%)	CV of estimates (%)
0.2	0.22	9.9	33.9
0.5	0.52	4.7	17.9
1	1.00	-0.4	9.8
2	1.87	-6.7	8.8
5	5.03	0.6	4.4
10	9.89	-1.1	2.5
20	20.7	3.7	3.2
50	49.1	-1.8	3.7
100	98.5	-1.5	7.3
200	200.2	0.1	9.5

As can be seen from **Table 3**, the precision of the method drops rapidly below a concentration of $1 \mu\text{mol l}^{-1}$. The most precise and accurate concentration range is 5-50 $\mu\text{mol l}^{-1}$. When applying the method, the dose of Cs should be chosen to ensure that the plasma concentration is within the most reliable range.

In Study III, the plasma kinetics of intravenously dosed ^{134}Cs was followed for 30 min. As can be seen in **Table 4**, the results are comparable with the results of Study IV, where stable Cs was used.

Table 4. Mean relative concentrations (R) (\pm SEM) of plasma Cs⁺ following an intravenous dose of Cs⁺ in different groups of goats and horses (III, IV)

Minutes after Cs dose	Goats				Horses (IV)			
	¹³⁴ Cs tracer (III)		Stable Cs (IV)		Resting		Exercising 0-40 min	
	R	n	R	n	R	n	R	n
2	8.9 \pm 0.4	6	9.3 \pm 1.3	4	7.7 \pm 0.4	6	6.8 \pm 0.2	6
4	5.6 \pm 0.2	6	6.3 \pm 0.7	5	5.7 \pm 0.2	6	5.1 \pm 0.2	6
6	4.4 \pm 0.2	6	4.7 \pm 0.5	5	4.5 \pm 0.2	6	4.0 \pm 0.1	6
8	3.5 \pm 0.2	6	4.0 \pm 0.4	5	4.1 \pm 0.1	6	3.2 \pm 0.1	6
10	3.1 \pm 0.2	6	3.2 \pm 0.4	5	3.5 \pm 0.2	6	2.7 \pm 0.1	6
12	2.7 \pm 0.2	6	2.8 \pm 0.3	5	n/a		n/a	
15	2.3 \pm 0.1	6	2.4 \pm 0.3	5	2.6 \pm 0.1	6	1.8 \pm 0.1	6
20	1.8 \pm 0.1	6	2.0 \pm 0.3	5	2.2 \pm 0.1	6	1.3 \pm 0.1	6
25	1.6 \pm 0.1	6	1.6 \pm 0.2	5	1.8 \pm 0.1	6	0.96 \pm 0.06	6
30	1.4 \pm 0.1	6	1.5 \pm 0.2	5	1.5 \pm 0.04	6	0.73 \pm 0.07	6
40	n/a		1.2 \pm 0.2	5	1.2 \pm 0.1	6	0.41 \pm 0.03	6
50	n/a		1.1 \pm 0.2	5	1.0 \pm 0.1	6	0.39 \pm 0.05	6
60	n/a		0.93 \pm 0.14	5	0.97 \pm 0.08	6	0.30 \pm 0.05	6
80	n/a		0.78 \pm 0.09	5	n/a		n/a	
100	n/a		0.71 \pm 0.11	5	n/a		n/a	

The ions H⁺, NH₄⁺, Li⁺, Na⁺, K⁺, Cs⁺, Ca²⁺ and Mg²⁺ did not disturb the precipitation of AFCE (50 g l⁻¹), even at the relatively high concentration of 0.1 Eq l⁻¹ (I). However, many monovalent cations interfere with the binding of Cs with AFCE (II). The ion concentration resulting in half-maximum binding of tracer (Cs⁺ 0.1 μ mol l⁻¹) increased as follows: Cs⁺ > Tl⁺ > Rb⁺ > NH₄⁺ > K⁺ > Na⁺, indicating a decreasing affinity. This order of affinity is the same as the order of crystal ionic radii (**Table 1**). The range in the binding affinity was extensive, with Cs having a half-maximum binding concentration at 0.45 μ mol l⁻¹ and Na at 160 mmol l⁻¹. Li⁺, Mg²⁺ and Ca²⁺ had no apparent affinity towards AFCE, even at concentrations around 1 mol l⁻¹.

The half-maximum binding concentration value for Cs was about 30% higher in plasma than in pure albumin solution. The most likely reason is plasma Na, which reduces the relative binding of ¹³⁴Cs by around 20% under the experimental conditions. Rb plasma concentrations between 1 and 11 μ mol l⁻¹ are reported in the literature (Versieck et al., 1977). The highest value would reduce the relative binding of ¹³⁴Cs by approximately 10%

under the experimental conditions. However, since all plasma samples, including those used to prepare the standard samples, were taken from the same animal within a short period, the concentration of these interfering ions is not likely to have varied, thus not causing significant bias.

8.2. Short-term plasma kinetics of caesium

Intravenously dosed stable Cs disappears from blood plasma very rapidly, as illustrated in **Table 4**. Between 2 and 30 min after intravenous dose, the extracellular Cs level decreased by more than 80% in both goats and horses. During the next 30-min time span (30-60 min after injection) the reduction in plasma Cs concentration was around 40%. Between 60 and 100 min after injection, the plasma Cs concentration dropped by 25 %. These results are in general accordance with graphical data on dogs presented by Poe (1972).

At 30 min, around 14% of the original Cs dose is estimated to remain in the extracellular space (III). Assuming a rapid equilibrium between plasma and interstitial fluid, the fraction of the dose remaining in extracellular fluid at different instances can be estimated from the plasma concentration data in Study IV. These estimates are presented in **Table 5**.

Table 5. Estimated fraction of Cs in goat extracellular fluid and the apparent rate (Section 7.5) for removal of Cs from blood plasma following an intravenous dose of Cs.

Minutes after intravenous Cs dose	% of Cs dose in extracellular space	Apparent rate (k_i) for removal of Cs from plasma (min^{-1})
2	87	0.121
10	30	0.085
20	19	0.040
30	14	0.019
40	11	0.013
50	9.8	0.011
60	8.7	0.011
80	7.3	0.011
100	6.6	0.011

By applying Eq. 2 (Section 7.5) to data from **Table 4**, different kinetic parameters can be resolved for both goats and horses. These parameters were fairly similar for the two species, with significant differences only in the value of K_1 (**Table 6**). Using goat data from the whole measured time span (2-100 min) resulted in slightly different kinetic values ($K_1 = 8.3 \pm 1.0$, $K_2 = 1.9 \pm 0.3$, $k_1 = 0.15 \pm 0.3 \text{ min}^{-1}$, $k_2 = 0.011 \pm 0.0023$). Applying Eq. 4 (Section 7.5) to these values, the apparent rate of removal of Cs (k_t) at any instat can be estimated. As shown in **Table 5**, this rate falls rapidly during the first 30 min. Rate constants with a small absolute value can be interpreted as the fraction of removed component per unit time. For example, a rate constant of 0.01 min^{-1} means that the concentration of the component reduces approximately 1% each minute.

Table 6. Mean kinetic parameters (\pm SEM) for the curve describing the removal of plasma Cs⁺ in resting horses and goats. The parameters were calculated by applying Eq. 2 (Section 7.5) to relative concentration data between 2 and 60 min.

	Goats	Horses	p
K_1	9.6 \pm 0.8	6.5 \pm 0.5	0.0052
K_2	2.4 \pm 0.2	2.3 \pm 0.3	0.95
k_1 (min^{-1})	0.2 \pm 0.02	0.15 \pm 0.002	0.11
k_2 (min^{-1})	0.016 \pm 0.002	0.016 \pm 0.003	0.89

8.3. Short-term distribution of caesium

The rapid initial removal of plasma Cs raised the question: where does it go? As discussed before (see Section 5.2), the excretion of Cs is too slow to affect significantly the short-term kinetics of Cs. Thus, the drop in plasma Cs is almost solely due to distribution to different tissues. The overall organ distribution of ^{134}Cs at 30 min after an intravenous dose (III) is displayed in **Table 7**. For comparison, the same table presents the plasma-organ and organ-plasma transfer coefficients used in the Leggett model (Leggett et al., 2003).

Table 7. Comparison of the distribution of ^{134}Cs in major organs in goat 30 minutes after dose and estimated transfer coefficients of Cs in different organs of man.

	Cs content ^a (% of total dose)	Estimated transfer coefficient from plasma to organ ^b	Estimated transfer coefficient from organ to plasma ^b
Kidney	13.0	67	32
Skeletal muscle	13.8	30	0.075
Gastrointestinal tract tissue	13.8	53	8.2
Gastrointestinal tract contents	7.9	5.5 ^c	28 ^d
Liver	4.7	19	2.2
Heart	3.3	14	8.1
Lungs	2.2	4.4	1.5
Salivary gland	1.8	n/a	n/a
Pancreas	0.8	1.8	1.7
Spleen	0.5	5.3	5.0
Total recovered	62.1		

^aStudy III

^bLeggett et al.(2003)

^csum of values for stomach, small intestine and large intestine contents

^dsmall intestine contents

After 30 minutes, the gastrointestinal tract, kidneys and skeletal muscle have incorporated a large fraction of the injected dose. The same tissues also have the greatest difference between the plasma-organ and organ-plasma transfer coefficients in the Leggett model (Leggett et al., 2003). These locations are thus good candidates when contemplating the destiny of rapidly removed Cs during the first 20-30 min after dosing. However, since a large fraction (38%) of the initial dose was not recovered quantitatively after 30 minutes (**Table 7**), other possibilities must be considered. According to Study III, the specific activity of most connective tissue samples 30 min after the intravenous dose of ^{134}Cs is more than twice as high as that of most skeletal muscle samples. Ubiquitous connective tissue is therefore a good candidate for the unrecovered fraction of the initial Cs dose

in Study III. Its proteins and glycosaminoglycans have a negative charge in physiological surroundings. Thus, it is likely that the initial mechanism of incorporation of Cs into connective tissue is extracellular ion exchange, rather than transport into intracellular space. This mechanism has been suggested earlier to explain the high initial affinity of cartilage towards Cs (Nelson et al., 1961). Leggett et al. (2003) did not include this phenomenon in their pharmacokinetic model due to insufficient quantitative information (personal communication).

8.4. Effect of exercise on plasma caesium

Exercise affects plasma Cs kinetics, as can be seen in **Table 4**. As presented in Study IV, the rate constant k_2 is twice as high in the exercising group ($0.057 \pm 0.005 \text{ min}^{-1}$) as in the resting group ($0.027 \pm 0.003 \text{ min}^{-1}$). The differences in other parameters are not significant. Exercise thus seems to have an effect only on the slower compartment when applying a two-compartment model to the data. This is in accordance with the findings that non-muscular tissue in goats is responsible for more than 80% of the removal of Cs from the bloodstream during the first 30 min (**Table 7**). The larger rate constant k_1 , which is unaffected by exercise, is likely to reflect the rate of fast incorporation, for which mainly non-muscular tissue is responsible. With increased intracellular concentration, the rate of incorporation decreases and the rate of decorporation increases. As non-muscular tissues approach steady state with respect to Cs, the slower incorporation of Cs into skeletal muscle starts to play a major role in gross plasma kinetics.

The mechanism responsible for the increase of k_2 during exercise remains obscure, but a plausible explanation is the increase in the activity of muscle Na,K-ATPase. Na,K-ATPase is known to be the major transporter of Cs into muscle cells (Beauge & Sjodin, 1968; Dorup & Clausen, 1994). Exercise is also known to be a very effective stimulant of muscle Na,K-ATPase (Clausen, 2003).

In study IV, the rate of Cs removal from plasma returns to resting level within 10 min after the end of exercise (**Figure 1** in IV). Assuming that Na,K-ATPase is the major remover of Cs from blood, this would mean that this active transport protein responds rapidly to changes in muscle activity, just like suggested by Lindinger (1995). However, in Study IV the level of plasma K dropped significantly from an already hypokalaemic level even later than 10 min after cessation of exercise (**Figure 2** in IV). These differences in post-exercise plasma kinetics of Cs and K would be an interesting topic for future research.

8.5. Future prospects

As indicated above, determining the rate of Cs removal from plasma by applying added Cs might be one means of gaining information about Na,K-ATPase in-vivo. Measurement of plasma kinetics using numerous time points from the beginning of application of Cs might be superfluous. A few measurements between 20 and 40 minutes and applying Eq. 3 (Section 7.5) could be enough to get an estimate for the segmental rate constant k' , which has a value very close to k_2 (Study IV).

One drawback in such experiments is the use of exogenous stable Cs. Although modern assay methods allow for doses that have absolutely no physiological effect, the dosing of an exogenous substance might cause ethical problems, especially when investigating human biology. These problems might be circumvented by measuring the level of endogenous Cs in plasma before and after procedures that increase or decrease the activity of Na,K-ATPase. Needless to say, for these experiments, the assay method described in this thesis (Section 8.1) is not sensitive enough. But with appropriate modern assay methods, like ICP-MS, more information about the short-term kinetics of Cs in different physiological states can be gained. As mentioned above, this might be useful in the study of Na,K-ATPase activity in vivo.

9. Conclusions

In a mixture of water, caesium ions (Cs^+) and the colloidal Cs binder ammonium-iron(III)-hexacyanoferrate(II) (AFCF), the amount of AFCF-bound Cs after a set incubation period is a function of Cs concentration in the system. AFCF and the Cs bound to it can be precipitated together with albumin and other plasma proteins using trichloroacetic acid, leaving the unbound Cs fraction in the supernatant. If radioactive Cs (^{134}Cs) is added to the incubation mixture, the activity of the precipitate depends on the total incubation concentration of Cs. Thus the concentration of added Cs in plasma samples can be determined by adding fixed amounts of ^{134}Cs and AFCF, incubating them for a set period (24 h), co-precipitating AFCF-bound Cs with proteins and measuring the radioactivity of the precipitate. For this new assay method, the lower concentration limit of quantitative measurements is around $1 \mu\text{mol l}^{-1}$.

The method is limited to the measurement of added Cs in blood plasma, and due to its low sensitivity is not suitable for the measurement of endogenous Cs in blood or other tissues. However, it provides a simple way of measuring short-term plasma kinetics of Cs following non-toxic Cs doses. Thus, it may be useful to researchers who do not have access to more sensitive methods, such as nuclear activation analysis or mass spectrometry. Here, the new method developed was used to determine the short-term kinetics of Cs of blood plasma in goats and horses.

In both horses and goats, the rate of removal of Cs from blood plasma decreases very rapidly after an intravenous dose. The apparent rate initially exceeds 0.1 min^{-1} both in goats and horses, but drops to a value well under 0.02 min^{-1} at 40 min after dosing. Between 2 and to 40 min, the plasma kinetics of Cs can be approximated with a two-phase exponential decay curve. According to the literature, the sodium-potassium pump (Na, K-ATPase) has a central role in the uptake of Cs from the extracellular space into cells. Tissues with known high Na, K-ATPase activity were found to contain a high concentration of Cs 30 min after dosing. Exercise, a known stimulant of muscle Na,K-ATPase, also increased considerably the removal of Cs from blood.

During the first 30 min after the intravenous dose, connective tissue, especially cartilage, incorporates Cs at a faster rate than most skeletal muscle. Connective tissue consists mostly of extracellular components, which contain a large amount of negatively charged groups. Thus it is reasonable to assume that ion-exchange processes contribute to the rapid initial phase of Cs removal.

The connection between Na,K-ATPase activity and short-term plasma Cs kinetics may open up possibilities for the use of intravenously dosed Cs in the study of Na,K-ATPase activity *in vivo*.

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