THE UPTAKE, ACCUMULATION AND RETENTION OF 137-CAESIUM BY SALMONID FISH IN FRESH WATER.

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To my Mum and Dad

- without their support I'd never

have got this far.

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ABSTRACT

The Chernobyl disaster on 26th April 1986, caused contamination over much of the U.K. with radiocaesium, principally via rainfall in upland areas such as N. Wales and S.W. Scotland. 137-Caesium was of particular concern in the freshwater environment as it has a long physical half-life (30yr) and previous studies had shown that 137-caesium accumulated in freshwater animals and that the levels of accumulation increased with trophic level up the food chain. This thesis presents the results of studies on the uptake, accumulation and retention of 137-caesium in salmonid fish in fresh water.

A number of previous studies both *in vitro* and *in vivo* have observed biochemical similarities of caesium to potassium. The uptake of 137-caesium and 86-rubidium (as a tracer for potassium) by erythrocytes of the rainbow trout (*Oncorhynchus mykiss* Walbaum) was studied. The total caesium influx was much smaller than that of potassium (14.4 and 756.0nmoles min^{-1} ml^{-1} packed cells respectively), at an external concentration of 3mM. Potassium influx was significantly inhibited by caesium and *vice versa*, at concentrations >0.1mM. The results indicated that caesium behaved as potassium in a qualitative but not quantative manner. This conclusion, together with evidence from the literature, was used to justify biochemical comparisons of the two elements later in the thesis.

The accumulation of 137-caesium was studied in the early stages of development of Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.). The

accumulation of the isotope in the eggs of brown trout was relatively small until a few days prior to hatching, when the 137-caesium concentration factor (C.F.) increased rapidly.

The accumulation of 137-caesium in juveniles of Atlantic salmon and brown trout followed a first order rate equation, ie. the rate of increase of accumulation decreased until a constant, equilibrium C.F. was reached. 137-Caesium accumulated several times above the concentration in water, reaching equilibrium after 4-6 months at C.F.s of approximately 10-12 at "normal" pH (≈7.4). The uptake was greatest in gills, muscle, liver and kidney. The majority of the radiocaesium was however, deposited in muscle tissue and this had consistently the longest biological half-life ($t_{b\frac{1}{2}}$). Accumulation was significantly reduced at "low" pH (≈5.0) in both species. This was attributed primarily to an inhibition of caesium uptake by protons in a manner similar to that recorded in the literature for other group-I metals. The elimination of 137-caesium from juvenile Atlantic salmon was best described by a single exponential equation and was little affected by increased acidity.

The accumulation of 137-caesium in alevins of the two species also followed first-order kinetics. The accumulation was much more rapid however, and reached much greater C.F.s (>50) at equilibrium. This difference between the stages of development was attributed to the greater metabolic rate (MR) of the alevins and/or their extra dependence on water as a source of ions. No consistently significant differences were recorded between the two species.

The branchial and intestinal influx of 137-caesium was measured in a perfused, whole-body preparation of the rainbow trout (*O. mykiss*). The *in vitro* caesium fluxes recorded in these experiments were in line with values in the literature. Branchial influx displayed saturation kinetics, with J_{max} and K_m values of 1.17μmoles Cs kg^{-1} fish h^{-1} and 3.93mM respectively, and was therefore concluded to be a mediated process dependent upon a limited number of carriers or active sites. In contrast, intestinal influx was not saturable but was directly proportional to the mucosal concentration of caesium and was thought to occur via paracellular, "leak" pathways. Intestinal influx was consistently greater than branchial influx; this difference increased with caesium concentration as the branchial influx approached saturation. Reduction of ambient pH had no significant effect on short-term branchial influx.

Caesium binding to intracellular muscle protein in rainbow trout (*O. mykiss*) was relatively weak and the majority of the caesium remained "free" at *in vivo* caesium concentrations. It was therefore concluded that intracellular binding was not a significant factor in the long-term retention of radiocaesium seen in nature. It was suggested that caesium transport occurred via ion-specific potassium channels and that the relatively slow uptake and long retention of radiocaesium was due to its large size with respect to such channels.

The results of this thesis were used to speculate on the consequences of a single input of 137-caesium into a previously uncontaminated lake. Initially, direct branchial uptake would be the greater source of radiocaesium in fish, to which larval forms would be the most susceptible. As the dissolved concentration of the isotope decreased

however, due to flushing and loss to sediments, the radiocaesium concentrations in food organisms would gradually increase. Food would then become the primary source of radiocaesium in fish, although the uptake would obviously vary significantly depending on the diet.

TABLE OF CONTENTS

CHAPTER 1: GENERAL INTRODUCTION

The need to study radioisotopes in the environment.

The harmful effects that radiation can produce in living tissue were recognised soon after the discovery of X-rays and radioactivity at the end of the nineteenth century. The potential benefits to medicine were similarly recognised however, and the use of radiation as a medical tool was rapidly developed.

After the first controlled nuclear chain reaction in 1942 the amount of radioactive material generated for industrial, military and power generation purposes increased enormously. Whilst knowledge and techniques in radiological safety have also expanded greatly the biological hazards of radiation remain (Coggle, 1971).

Any environmental contamination with radioactive substances therefore, is of immediate concern. The principal problem is one of human health; that exposure to either individuals or populations does not exceed acceptable limits and moreover, is minimised as much as possible. The exposure of the environment itself and of organisms not directly involved with humans is of secondary importance but nevertheless is worthy of considerable attention.

The study of the fate of radionuclides in organisms and the environment, radioecology, has therefore been the subject of much research. A number of comprehensive reviews of the radioecology of the aquatic environment have been

compiled (Mauchline and Templeton, 1964; Polikarpov, 1966; Pentreath, 1977; Woodhead, 1984); only a brief synopsis follows. Mauchline and Templeton (1964) defined radioecology thus: " Radioecology or radiation ecology is the branch of ecology which concerns itself with the dispersion and interaction of radionuclides in and with the physical, chemical and biological environment." Once the presence of such radioactive pollution has been determined within an ecosystem, further investigation (into the fate of that radionuclide) often requires the use of physiological and biochemical procedures. Such techniques are the basis of this thesis but as such are considered to be an integral part of radioecology. Radiological units are shown in Appendix A.

Radionuclides in the environment.

Natural radionuclides.

Natural radionuclides can be divided into two types:

a) Primordial radionuclides ie. those that have existed since the formation of the Earth, and their decay products (daughters) eg. 238-uranium, 232-thorium, 222-radon, and 40-potassium.

b) Radionuclides resulting from interactions between cosmic rays and elements in the earth or atmosphere eg. 14-Carbon, tritium (^{3}H) and 22-sodium. The environmental levels of natural radionuclides are obviously outwith human control and as such are of lesser importance in radioecological studies.

Artificial radionuclides.

Ever since the explosion of the first destructive nuclear device at Alamogordo,

New Mexico on July 16th, 1945, artificially-produced radionuclides have been introduced into the environment. In terms of total activity released, this has been principally via atmospheric testing of nuclear weapons. As a result, a number of radionuclides could be detected globally eg. ^{55}Fe, $^{95}Zr/^{95}Nb$, ^{144}Ce, ^{106}Ru and ^{90}Sr (Pentreath, 1977), and high levels of contamination of long-lived isotopes persisted in the vicinity of the detonation site.

Controlled discharges of radionuclides from the nuclear industry, especially power stations and fuel reprocessing plants, are another source of artificial radionuclides in the environment. Although these discharges are relatively low-level ($<10^3$ Ci yr^{-1}), they occur repeatedly from a point source and hence the final concentrations recorded in the local environment are often greater than those resulting from weapons testing. These discharges of radioactivity are usually released with water drawn from local, natural supplies and hence the activity is released primarily to the aquatic rather than the terrestrial environment. After the U.S. and the U.S.S.R. agreed to ban atmospheric and underwater nuclear explosions, under the Limited Test Ban Treaty of 1963, this became the major source of artificial radionuclides in the aquatic environment.

Artificially-produced radionuclides are of two types, direct fission products and neutron-activation products (formed by the bombardment of stable atoms by energetic neutrons). Weapons testing and the nuclear industry produce both types of products, but environmental contamination from the latter source has largely been of fission product radionuclides. Overall, a large number of different radionuclides have been released into the aquatic environment from these sources. Pentreath (1977) listed over

50 artificially-produced radionuclides which have been detected in the marine environment.

A third source of artificial radionuclides is the input from nuclear accidents. Although these contribute much less in terms of total activity, local contamination may be considerable and present a significant human health hazard. Such incidents therefore receive much publicity and are well-documented eg. Windscale, 1957; Three-Mile Island, 1979.

The Chernobyl disaster.

One of the worst nuclear disasters occurred on 26th April, 1986 when an explosion in reactor 2 at the Chernobyl Nuclear Power Plant in the Ukraine, U.S.S.R. released approximately 2×10^{18} Bq (5.4×10^{7} Ci) of radioactivity into the atmosphere over a period of ten days (Petersen *et al*., 1986), some 1600 and 22 times more than Windscale (Beattie, 1981) and Three-Mile Island (Fabrikant, 1979) respectively. This formed clouds of radioactive material containing a number of radionuclides including fission products such as ^{103}Ru, ^{106}Ru, ^{132}Te and the actinides, which spread over much of Europe (Devell, *et al*., 1986; Pringle *et al*., 1986; Thomas and Martin, 1986; Webb *et al*., 1986; Beentjes *et al*., 1987; Simmonds, 1987). Much initial concern was directed towards ^{131}I which may concentrate in fresh milk from animals grazing contaminated pastures. It may then accumulate in the thyroid gland in humans and irradiate the body. This radionuclide has a relatively short half-life of 8 days however, and as levels declined attention was switched to radiocaesium

Caesium is a group-1 alkali metal two places below potassium in the periodic table (Table 1.1). Radiocaesium has 21 known isotopes including the series from caesium-123 to caesium-144, except caesium-124 (Finston and Kinsley, 1961). The principal caesium fission product and therefore the main environmental pollutant is caesium-137 (^{137}Cs). Caesium-134 (^{134}Cs) has a low fission yield but is readily manufactured by neutron bombardment of stable caesium or barium and has therefore been widely used as a biological tracer. These are the two most widely used isotopes of radiocaesium. Both emit both β and γ radiation to produce isotopes of barium. The physical half-lives of the isotopes are 2.1 years and 30.0 years for ^{134}Cs and ^{137}Cs respectively:

$$^{137}Cs \rightarrow {}^{137}Ba + \beta + \gamma$$

$$^{134}Cs \rightarrow {}^{134}Ba + \beta + \gamma$$

(For more detail, see Wilson, 1966).

Although it has no known physiological or biochemical function caesium is readily soluble in water and is known to accumulate in biological systems (Davis, 1963). In the aquatic environment the problem is generally greater in fresh water where the greatest concentrations of radiocaesium in fish have been recorded. 137-Caesium is of particular concern due to its long half-life.

Radiocaesium was already present in the environment in the U.K. prior to the Chernobyl disaster from both weapons testing and discharges from nuclear installations. For example, 137-Caesium was easily detectable in the Irish Sea due to discharges from the Sellafield Nuclear Fuel Reprocessing Plant (Hunt, 1986). As a result of the Chernobyl fallout however, the Ministry of Agriculture Fisheries and Food (MAFF)

Table 1.1. Ionic radii of group-IA metal ions (pm). Naked ionic radii are taken from Kaye and Laby (1986). Hydrated radii are values quoted by Marcus (1985) for a single hydration shell measured by X-ray diffraction of an aqueous solution of the chloride salt. N.D. = no data.

cation	atomic number	atomic weight	ionic radii (pm)	
			naked	hydrated
Li	3	6.94	59	208
Na	11	22.99	102	242
K	19	39.10	138	280
Rb	37	85.47	149	N.D.
Cs	55	132.91	170	315
Fr	87	(223)	N.D.	N.D.

instigated a monitoring programme to study the fate of the fallout radionuclides in the aquatic environment, with particular emphasis on 137-caesium (Camplin *et al.*, 1986). The Chernobyl fallout significantly increased the radiocaesium levels present in freshwater ecosystems. Pollution occurred mainly via rainfall as the radioactive cloud passed over Britain on 2nd/3rd May, 1986, especially in upland areas eg. N. Wales, Cumbria and S.W. Scotland (Clark, 1986; Smith and Clark, 1986). The full consequences of Chernobyl in the Scottish uplands were not appreciated for over a year however, as no detailed aerial survey of the fallout distribution using a gamma-camera was carried out, although the technology was available at the Scottish Reactor Centre, East Kilbride. The MAFF monitoring programme was, however, concerned primarily with potential hazards to human health and only the environmental concentrations of 137-caesium were examined, no experimental studies were carried out.

A number of previous investigations have studied the fate of 137-caesium in various species of fish principally as a result of environmental pollution via discharge from nuclear power stations or fuel reprocessing plants (see following review). Most of these however have been concerned with the marine environment, fewer data are available for freshwater species. A review of the literature was therefore undertaken to determine those subjects most in need of further investigation concerning the uptake and accumulation of 137-caesium in freshwater fish.

THE UPTAKE, DEPOSITION AND EXCRETION OF RADIOCAESSIUM IN AQUATIC ORGANISMS; A REVIEW.

This section presents a review of the behaviour of radiocaesium in aquatic organisms with particular reference to freshwater studies, although relevant information from marine investigations is included.

Environmental levels of caesium.

The vast majority of the studies on the fate of caesium in the aquatic environment have been concerned with the ecological consequences of radiocaesium pollution and therefore little attention has been paid to stable or inactive caesium (133-caesium). When the physiological consequences of caesium accumulation, and the biochemical similarities between caesium and other group-I metals are examined however, the total caesium concentration, including radioactive and stable forms, is important.

Caesium can be detected in a wide range of animals, both vertebrate and invertebrate (Bertrand and Bertrand, 1949). The concentration of natural caesium in sea water has generally been given as approximately $2\mu g\ l^{-1}$ (15nM) although more accurate methods gave a value of $0.8\mu g\ l^{-1}$ (6nM) (Davis, 1963). Bryan (1961) quoted caesium concentrations in sea water as $0.4\text{-}1.3\mu g\ l^{-1}$ (3.0-9.8nM). In fresh water few figures are available for stable caesium concentrations. The preliminary results of Kolehmainen *et al.* (1966b) gave inactive caesium concentrations in Finnish lakes as "$<5\mu g\ l^{-1}$" (<38nM). Eyman and Kevern (1975) recorded $0.021\mu g\ l^{-1}$ (0.15nM) for "Wintergreen

Lake" but this was said to be "guanotrophic"; hypereutrophic owing to considerable input of of wildfowl excreta which contained significant concentrations of stable caesium. The concentration of 0.025μg l^{-1} (0.19nM) recorded in the Clinch river by Nelson (1967) was however, very similar. Stable caesium concentrations in fish ranged from 3.4μg kg^{-1} (bluegill, *Lepomis macrochirus*, Raf.) to 16.0μg kg^{-1} (white bass, *Roccuss chrysops*, Raf.). Kolehmainen (1972) reported stable caesium concentrations in bluegill (*L. macrochirus*) from White Oak Lake, Tennessee of 8.9-10.1μg kg^{-1} fresh weight but no water concentrations were quoted. Hewett and Jefferies (1976) recorded 0.005μg l^{-1} (0.04nM) stable caesium concentrations in water obtained from Trawsfynydd Reservoir, Wales. Stable caesium concentrations in muscle of brown trout (*Salmo trutta*) ranged from 5.1-39.0μg l^{-1}.

A number of studies have measured the levels of radiocaesium found in freshwater fish and their natural environment. These were often prompted by public health concerns to determine possible doses received by humans via consumption of fish. In a number of pre-Chernobyl studies in Scandinavia and Northern U.S.A. the levels of radiocaesium recorded were quite similar; 0.3-15 nCi kg^{-1} fresh weight (Häsänen and Miettinen, 1963, Gustafson *et al.*, 1966, Rickard and Eberhardt, 1971; Carlsson and Liden, 1978).

In the U.K. Preston *et al* (1967) recorded 137-caesium levels in brown trout (*S. trutta*) flesh of 2.5-15 nCi kg^{-1} wet weight from a large numbers of sites. 137-Caesium concentrations of the waters involved were also measured such that concentration factors (C.F.s) could be calculated.

$$C.F. = \frac{^{137}Cs\ g^{-1}\ fish\ (wet\ weight)}{^{137}Cs\ ml^{-1}\ water}$$

Concentration factors are widely used in studies of the accumulation of radioisotopes in aquatic organisms as a quick and useful method of comparing results, for example in the same species from different sites of contamination.

Caesium-137 concentration factors varied from 0.5-5.0 x 10^3 over a range of waters and from 3.5-4.5 x 10^3 within one water studied in greater detail; Lake Trawsfynydd, Wales. This lake received low-level discharge from Trawsfynydd Nuclear Power Station, hence the greater concentration factors. Kolehmainen *et al* (1966a, 1966b) recorded 137-caesium concentrations in perch (*Perca fluviatilis*) and pike (*Esox lucius*) of approximately 0.20-20.0nCi kg^{-1} from different types of limnological lakes, the greatest concentrations being recorded in oligotrophic waters. More recently, post-Chernobyl, Brittain *et al* (1991) recorded radiocaesium activities in brown trout from a Norwegian subalpine lake, up to a peak of 7KBq kg^{-1} (190nCi kg^{-1}) in June 1986, but this had fallen to 4KBq kg^{-1} by June 1988.

A number of studies noted an inverse relationship between 137-caesium concentrations in fish and potassium concentration in water and suggested that caesium behaves as a potassium analogue due to its similar chemical properties (Häsänen and Miettinen, 1963, Kolehmainen *et al* , 1966b, Kevern and Spigarelli, 1971, Kolehmainen, 1972). Similarities in behaviour between caesium and other group-I metals, especially potassium, have been observed since Ringer (1882) studied the effects of potassium,

rubidium and caesium on the action of the frog (*Rana temporaria*) heart. Sjodin (1959) found that with respect to its effect on the resting potential of the frog sartorius muscle, caesium behaved more like sodium at low temperature but more like potassium at room temperature. Twenty mM caesium blocked steady-state potassium conductance in cardiac Purkinje fibers (Isenberg, 1977).

Much of the early work relating caesium to potassium was reviewed by Davis (1963). One of the main conclusions of this review was that caesium ions can replace potassium ions in a number of tissues. The extent to which caesium was able to replace potassium was however very variable. In some studies close to 100% replacement occurred whilst in others caesium only partially replaced potassium. The mechanism(s) by which this took place were not discussed.

Preston *et al* (1967) quantified the relationship between 137-caesium concentration factors in fish and potassium concentrations in water thus:

$$^{137}\text{Cs C.F.} = 3.19\ [K^+]^{-0.71}$$

In unpolluted waters total conductivity which was proportional to potassium concentration, could be used as an indicator of the concentration factor of 137-caesium in fish (Kolehmainen *et al*., 1966b). Gustafson *et al*. (1966) cited the much lower Cs:K ratio as the probable reason for lower 137-caesium levels in marine fish. Bryan (1961) and Bryan and Ward (1962) treated caesium in a similar manner to potassium in decapod crustaceans but found that the former was taken up and lost more slowly, and

was concentrated to a greater degree. Similar results were obtained by McNeil and Trojan (1960). Whicker *et al.* (1967) found no clear-cut relationship however, between high caesium-137 concentrations in game fish from Colarado, U.S.A. and low environmental potassium concentrations. Wrenn *et al.* (1971) examined fish from the Hudson River estuary and found no difference in caesium-137 levels in fish over the range 2-30mg l^{-1} of potassium. The physiological implications of this similarity between caesium and potassium will be discussed later in this review.

Another phenomenon commonly noted in field surveys was a general increase in radiocaesium concentrations up the food chain ie. an increase with trophic level (Gustafson *et al.*, 1966; Gustafson, 1967; Kolehmainen *et al.*, 1966b; Wrenn *et al.*, 1971; Carlsson and Liden, 1978). Pendleton *et al.* (1965) described an increase in bioaccumulation of approximately three times for an increase of one trophic level in the food chain - the "increase ratio". A similar value of 3.26 was recorded by Whicker *et al.* (1972). Häsänen and Miettinen (1963) found that when a dividing line of 2nCi kg^{-1} wet weight was applied to their data, those species above that concentration were at least partially piscivorous eg. pike (*Esox lucius*), whereas those below eg. grayling (*Thymallus thymallus*) fed lower down the food chain on insects, insect larvae and plankton. Differing diet was also used to explain the considerable interspecific variation in the radiocaesium concentrations of fish living in the same lake (Hannerz, 1968). Kolehmainen *et al.* (1966a) concluded that food played a much greater role than direct uptake from water in the radiocaesium accumulation of freshwater fish. An exception to this trend was reported by Nelson (1967), where "no clear trophic level increase of ^{137}Cs was found". A similar conclusion was reached by Anderson *et al.* (1973) studying

plants and arthropods in a streambed community.

One other consistent result from field surveys was the importance of sediments as a sink of radiocaesium (Ritchie *et al.*, 1974; Alberts and Muller, 1979; Alberts *et al.*, 1979; Stanners and Aston, 1982; Conkie *et al.*, 1988) especially on micaceous soils (Francis and Brinkley, 1976). Lerman and Tanaguchi (1971) identified two mechanisms of 137-caesium transport to sediments; adsorption on settling particles and via diffusion into interstitial water. Wrenn *et al.* (1971) found 137-caesium concentrations in sediments to be approximately three orders of magnitude higher than in water. In a hypereutrophic lake 97% of the 137-caesium was associated with sediments (Eyman and Kevern, 1975). Gustafson (1967) recorded 3.4-18.0nCi kg^{-1} in the top 5cm of bottom sediment in fresh water lakes compared to a maximum of 3.62nCi kg^{-1} in fish, of which bottom feeding species generally had the highest concentrations. When input of new fallout 137-caesium was approaching zero, the sediments were estimated to account for 94% of the 137-caesium in fish, including indirect uptake via food. The loss of 134-caesium to sediments was more rapid under acid conditions (Schindler *et al.*, 1980).

Experimental studies of radiocaesium accumulation.

In order to understand how the concentrations of 137-caesium measured in environmental monitoring programmes came about, more detailed study of the uptake, accumulation and excretion processes is required. This necessitates laboratory experiments where conditions such as the environmental concentration of the radionuclide can be carefully controlled. There are two main pathways by which any dissolved ion, including radiocaesium can be taken up from the environment by fish:

directly from the surrounding water or indirectly via the ingestion of radioactive food.

Uptake from water

Ichikawa (1960) found that radiocaesium accumulation in juvenile salmon (*S. salar*) was directly proportional to the concentration in water. This result was confirmed for unicellular algae (Williams and Pickering, 1961; King, 1964) and for the water flea, *Daphnia pulex* (King, 1964). In contrast, no change in whole-body concentration of 134-caesium by plaice (*Pleuronectes platessa*) was recorded over a range of sea water concentrations of 1×10^{-3} - 16×10^{-1} μCi ml^{-1} (Morgan, 1964). Over the range of dissolved concentrations in the aquatic environment that have been reported however, it is assumed that radiocaesium uptake is indeed proportional to the concentration in the water (Jefferies and Hewett, 1971; Hewett and Jefferies, 1976; Evans, 1988).

Williams and Pickering (1961) placed bluegill (*Lepomis macrochirus*) in fresh water containing 137-caesium at 400nCi l^{-1} for 48h after which time the uptake was 200nCi kg^{-1}; a concentration factor of approximately 0.5 (assuming all the body was available to ^{137}Cs uptake). The length of the experiment was very short however, and it was very unlikely that the fish had reached an equilibrium concentration of 137-caesium (indicated by a flattening-off of a C.F. vs. time curve). King (1964) concluded that uptake of 137-caesium from water by bluegill fingerlings was negligible, but again the exposure time (24h) was very short.

Hewett and Jefferies (1976) placed brown trout (*Salmo trutta*) in 1μCi l^{-1} 137-

caesium for 420 days. Individual trout were then dissected and 137-caesium C.F.s were calculated for various organs, the highest of which were gut (8.92), muscle (8.00) and liver (5.74). In terms of intake $g^{-1} d^{-1}$ ("flux") the order was gut, gill, kidney, liver. An important conclusion to this experiment was that the gill was the major site of 137-caesium entry from water as drinking rate in freshwater teleosts is only approximately 1% of total water flux (Evans, 1969; Motais *et al*., 1969). Of all the organs studied, only the gut had a higher daily intake of 137-caesium from drinking, than from branchial uptake.

A number of similar studies have been carried out with marine species. Bryan (1963a) recorded whole-body C.F.s for 137-caesium of 1.2-14.0 in various invertebrate phyla. Radiocaesium penetrated rapidly into body fluids but uptake into tissues was much slower; this limited the rate of accumulation. Further evidence for the inverse relationship between radiocaesium and potassium concentrations was provide by C.F.s of the former in a euryhaline isopod, *Sphaeroma* in 100% and 2.5 % sea water; =7 and 2-300 respectively. This was contradicted by Morgan (1964) who found concentration factors of 134-caesium after 28 days at 10°C were higher in marine teleosts than freshwater species by approximately one order of magnitude (1.6-9.2 and 0.3-0.8 respectively). The comparatively slow uptake by fresh water teleosts was attributed to their pattern of osmotic regulation, but the above values did not represent equilibrium concentrations.

A whole-body C.F. for 137-caesium of approximately 10 was recorded in post-larval flounder (*Paralichthys dentatus*) after 90d accumulation (Baptist and Price, 1962).

The common goby, *Acanthogobius flavimanus*, placed in 10μCi l^{-1} ^{137}Cs reached a concentration factor of 4.2 in 28 days (Kimura, 1984). Jefferies and Hewett (1971) studied the uptake of 134-caesium (1-6μCi l^{-1}) in plaice (*P. platessa*) and rays (*Raia clavata*) and measured C.F.s in various organs after 780 days. The values were 2-3 times greater in plaice but were of a similar magnitude to the results of earlier work eg. gut C.F. = 14.58 (plaice), 5.81 (rays). Using a drinking rate for plaice of 4% body weight d^{-1} (Evans, 1969) it was estimated that only 50% or less of the 137-caesium uptake from water was via the gut; it was assumed that the other 50%+ entered via the gills.

A major factor affecting radiocaesium concentration factors was the size of individual fish. Morgan (1964) found the uptake per unit weight of 134-caesium to be related to $W^{-0.22}$. The steady-state (equilibrium) concentration factor and uptake g^{-1} d^{-1} of 137-caesium in brown trout under laboratory conditions decreased as weight increased as $W^{-0.23}$ (Hewett and Jefferies, 1976). In natural populations however, larger fish had a higher radiocaesium concentration factor (Spigarelli, 1971; Hewett and Jefferies, 1976; Carlsson and Liden, 1978). This was presumed to be due to a change in diet as larger fish were more piscivorous and hence the effect of trophic level was seen.

Other factors that influenced radiocaesium concentrations in aquatic organisms included temperature and growth rate. The uptake of 137-caesium was less for *Daphnia* and bluegill fingerlings at 8°C compared to 22°C (King, 1964). In contrast, the C.F. of

137-caesium in fresh water shrimps (*Paleomontes paludus*) fell from 620 at 20°c to 380 at 32°C. This was attributed to more frequent losses via moulting at increased temperature - the exoskeleton accounted for 57% of 137-caesium in whole shrimps (Harvey, 1971). Growth rate affected accumulation of radiocaesium when fish were growing rapidly - the total accumulation decreased when growth rate exceeded uptake by new tissues, a "biological dilution" (Baptist and Price, 1962; Spigarelli, 1971). This could have led to seasonal changes in whole-body concentration factors in natural populations as feeding and growth tended to be at a maximum in summer (Rickard and Eberhardt, 1971).

All of the above studies involved physically mature animals; no work on the uptake of radiocaesium by eggs or larvae was found in the literature. Gravid females of tench (*Tinca tinca*) and pike (*Esox lucius*) in which eggs accounted for 10-20% of body weight however, lost approximately 11% of their body-burden of 137-caesium in spawning (Kulikov *et al.*, 1971).

Uptake from food

Baptist and Price (1962) simulated uptake of 137-caesium from food in a number of marine fish by pipetting radioactive water or placing solid capsules containing the isotope directly into the throat or stomach. After a single oral dose, accumulation was rapid in internal organs but was much slower in muscle. All tissues concentrated 137-caesium to a higher level than blood

Hewett and Jefferies (1978) made a detailed study of the uptake of 137-caesium from contaminated food in plaice (*P. platessa*) and brown trout (*S. trutta*). The input of 137-caesium from food was manipulated to be equal to the intake from water only ie. 4% body weight day^{-1} (Hewett and Jefferies, 1976) and the relative contributions were compared for various organs. Those organs involved in digestion and excretion had a higher input of 137-caesium from food eg. gut, liver and kidney. Skin and muscle however, received the majority of their 137-caesium from water. The overall input of 137-caesium from food was estimated as 42% for plaice and 67% for trout. For plaice, this result showed good agreement with an estimation made in the natural environment by Pentreath and Jefferies (1971) off the Windscale (Sellafield) Nuclear Reprocessing Plant. Using measured 137-caesium concentrations in sea water and in plaice and a simple model, the input of 137-caesium from food was approximately 50%. For trout however, Hewett and Jefferies (1978) attributed 90% of the 137-caesium in fish in the environment (Lake Trawsfynydd) to intake from food. These fish were presumed to be feeding at an optimal rate of 10% body weight day^{-1}.

Kimura (1984) fed the common goby (*A. flavimanus*) on radioactive polychaete worms (*Nereis spp.*). From a single ingested dose absorption of 137-caesium (activity remaining after 3 days) was 80% on average. If a feeding rate of 4% body weight day^{-1} was assumed, the accumulation of 137-caesium from food would have been 24 times that direct from water. The value for 137-caesium absorbed from food for a fresh water species, bluegill (*L. macrochirus*) was however, much lower, 13-14% of the total (King, 1964).

Retention and excretion of radiocaesium.

There are two commonly-used concepts in retention studies of radioisotopes that require brief explanation. The first is the biological half-life ($t_{0.5}$), the time taken for the activity of an isotope in an organism or part of an organism to decrease to half its original concentration. This is a function of the rate of elimination from the organism and should not be confused with the physical half-life of the isotope concerned. The second is that the overall rate of excretion can be divided up into a number of "components", each described by a separate exponential function. The proportion of the total activity represented by each function at zero time and the biological half-life of that proportion can also be determined.

Baptist and Price (1962) studied the retention of 137-caesium in post-larval flounder (*P. dentatus*) and mature Atlantic croaker (*Micropogon undulatus*) under natural conditions. Two rate functions were identified for the flounder:

a) 34% of ^{137}Cs at t = 0; $t_{0.5}$ = 5.3 days.

b) 66% of ^{137}Cs at t = 0; $t_{0.5}$ = 36.9 days.

The latter was thought to include the influence of muscle, the most massive tissue.

In general, whole-body excretion of 137-caesium by fish followed a two-exponent equation; typically a "fast" component comprising 10-20% of the total activity at t=0 with a short $t_{0.5}$, and a "slow" component comprising 80-90% of the activity with a long $t_{0.5}$ (Nelson, 1967; Kolehmainen and Miettinen, 1967). For rainbow trout (*Oncorhynchus mykiss*) at 15°C the "slow" component comprised 66-76% of the total activity with a $t_{0.5}$ of 25-80 days, both parameters increasing with size of the fish. In

perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) the "slow" component comprised 88-96% of the total (Häsänen *et al.*, 1966). Kimura (1984) however, described only a single function for the whole-body excretion of 137-caesium from the common goby (*A. flavimanus*) with a biological half-life of 18-39 days.

Hewett and Jefferies (1978) measured the $t_{0.5}$ for various organs of brown trout (*S. trutta*) after 137-caesium uptake from food as follows:

gut, 25.6 days

kidney, 38.6 days

muscle, 126.4 days.

The whole-body biological half-lives following 137-caesium accumulation from food and water were almost identical: 63 and 64.7 days respectively. Individual tissues can themselves be described by more than one rate function. In croaker (*M. undulatus*), muscle had two with biological half-lives of 34.8 and 94.7 days representing 35 and 61% of the total 137-caesium at t = 0 respectively. In contrast, liver was described by four functions, including 61% of 137-caesium at t = 0, $t_{0.5}$ = 0.7 days (Baptist and Price, 1962).

The excretion of 137-caesium was dependent upon temperature and fish size. Häsänen *et al.* (1966) demonstrated a 2-3 fold decrease in $t_{0.5}$ for a fall in temperature of 10°C and a general increase in $t_{0.5}$ with age of the fish. Gallegos and Whicker (1971) produced an equation to quantify the effects of temperature and weight on the retention of radiocaesium by rainbow trout (*O. mykiss*):

$$\ln R_r = 0.09 - 0.01056T + 0.0397 \ln (W_f/T_p)$$

where R_r = retention of radiocaesium; W_f = weight (g) at any time; T; T_p = temperature of water (°C) during the test interval. Hence, retention increased with weight of the fish but decreased with rise in temperature.

In the environment however, where radiocaesium input may occur over long periods, the biological half-life of the isotope concerned is often much greater. Gustafson (1967) recorded whole-body biological half-lives in excess of two years for juvenile walleye (*Stizostedion vitreum*, Mitchill) and yellow perch (*Perca flavescens*, Mitchill) of approximately 10g weight. Brittain (1991) estimated half-lives in trout (*S. trutta*) in a Chernobyl-polluted subalpine lake to be 3.0 and 1.3 years for 137-caesium and 134-caesium respectively.

The effects of radiocaesium accumulation.

Radiological effects.

The general consensus of early work on the concentration of radiocaesium in fish was that no significant doses could be obtained by man from eating contaminated fish, even from regular consumption of freshwater species (Miettinen *et al*., 1963; Häsänen and Miettinen, 1963; Gustafson *et al*., 1966; Preston *et al*., 1967).

Considerable work has been done on the effects of acute doses of radiation on various stages of development in the fish themselves (Engel, 1967; Ward *et al*., 1971). Doses of 0.0025 and 0.005 Gy h^{-1} (equivalent to approximately 190μCi l^{-1} ^{137}Cs) over

two years given to breeding guppies (*Lebistes reticulatus*) reduced the number of progeny surviving to maturity by 50% (Purdom and Woodhead, 1973). Angelovic *et al* (1967) and Neuhold and Sharma (1967) found that acute doses damaged the osmoregulatory capabilities of mature fish, particularly with respect to sodium.

The doses received by individuals in natural populations are however, much lower than those in the above studies. Woodhead (1970) estimated that the dose to plaice (*P. platessa*) eggs from 137-caesium in the North Irish Sea was 1.77×10^{-4} μGy h^{-1}. The total dose from artificial radionuclides was 8.5×10^{-4} μGy h^{-1} compared to 2.98×10^{-3} μGy h^{-1} from natural 40-potassium. It was therefore concluded that radioactive pollutants were extremely unlikely to have any adverse effects on plaice embryos in that area. Fedorov *et al* (1964) however, found evidence of damage to plaice eggs at environmental concentrations of 10^{-11} Ci l^{-1} of 137-caesium, an estimated dose of 5×10^{-3} μGy h^{-1}. Polikarpov (1966) reported an increase in abnormal larvae of various Black Sea fish after incubation of eggs in water containing 10^{-10} Ci l^{-1} $^{90}Sr/^{90}Y$. The only study found that reported the effects of chronic exposure to low-level radiation for fresh water fish noted no deaths among eggs of sea trout (*S. trutta*) and Atlantic salmon (*S. salar*) when exposed to 10^{-9} Ci l^{-1} $^{90}Sr/^{90}Y$ (Brown, 1962).

Physiological effects.

Potassium channels have been described on a number of cells and epithelia (Latorre and Miller, 1983) and therefore a number of authors have suggested that caesium uptake occurs via potassium channels. Sjodin (1961) described the uptake of

potassium, rubidium and caesium ions by frog sartorius muscle using a model which assumed a limited number of sites at the cell surface and noted that "The ions were found to compete for the postulated sites in various bi-ionic mixtures." The order of decreasing site affinity was Rb>K>Cs (Bolingbroke *et al.*, 1961; Sjodin, 1961). In voltage clamped squid axons, Bezanilla and Armstrong (1972) found that internally-perfused caesium did not permeate out of the axon and interfered with outward current through potassium pores.

Caesium may also replace potassium in biochemical reactions: a number of enzymes/enzyme systems have been isolated which are dependent on potassium but may be partially activated by caesium, eg. bovine-liver propionyl-CoA carboxylase (Giorgio and Plaut, 1967); bovine-liver fructokinase (Parks *et al*., 1957) and rat-liver tyrosine enzyme (Holley *et al.*, 1960). Probably the best-known potassium-dependent enzyme is the ubiquitous sodium plus potassium-adenosine triphosphatase (Na^++K^+ ATPase). This has also been found to be partially activated by caesium (Bader and Sen, 1966; Jørgensen and Petersen, 1982). The concentration of ions that produced half-maximal activation of Na^++K^+ ATPase from the gills of seawater-adapted eels (*Anguilla anguilla*) was approximately 2mM for potassium but 13mM for caesium (Bell *et al*., 1977). A number of other enzymes are known to be potassium-dependent (Webster, 1966; Edwards and Keech, 1968) and it is possible that these may also be partially-activated by caesium.

It is therefore possible that accumulation of radiocaesium from the environment may cause some disturbance of normal physiological and biochemical functions. The

concentrations of radiocaesium that have been recorded in body tissues are however, very small (<10pmoles kg^{-1}, Camplin *et al.*, 1986) compared to the intracellular concentration of potassium and therefore any such effects are likely to be trivial.

Conclusions.

Considerable work has been done on the uptake, deposition and excretion of radiocaesium in aquatic organisms. The principal conclusions regarding freshwater species were:-

i) Radiocaesium accumulates to concentrations above those in the surrounding water.

ii) All tissues/organs are available for radiocaesium accumulation.

iii) Radiocaesium C.F.s increase as the potassium concentration in the surrounding water decreases.

iv) Radiocaesium C.F.s increase with trophic level up the food chain.

v) There are two main routes of radiocaesium uptake: directly from water (primarily via the gills) and indirectly via food. In the natural environment, food is the major source of radiocaesium.

vi) Radiocaesium is retained in the tissues. Excretion from the whole body or an individual tissue can be divided into a number of components with different biological half-lives, and comprising different proportions of the total radiocaesium burden.

The majority of the studies referred to in this review concerned the behaviour of 137-caesium in mature animals, primarily in marine species. The patterns of uptake, deposition and excretion in eggs, larvae and juveniles therefore require further investigation for comparative purposes. In addition, the physiological behaviour of

radiocaesium is not fully understood and the actual mechanisms regulating the fluxes of radiocaesium remain unidentified for both uptake and excretion. These areas of uncertainty, together with aspects of the biochemistry of radiocaesium are the subject of the following experimental investigations.

CHAPTER 2: THE UPTAKE OF CAESIUM AND POTASSIUM BY ERYTHROCYTES OF THE RAINBOW TROUT *(ONCORHYNCHUS MYKISS*, WALBAUM).

INTRODUCTION

In the general introduction to this thesis (Chapter 1) it was noted that a number of investigations, both in the laboratory and in the field, proposed a close biochemical relationship between caesium and potassium (Häsänen and Miettinen, 1963; Gustafson *et al*., 1966; Kohlemainen *et al*., 1966b; Kevern and Spigarelli, 1971). Preston *et al* (1967) related the 137-caesium concentration factor to the potassium concentration in water, $[K^+]$ as follows:

$$^{137}Cs \text{ Concentration factor} = 3.19\ [K^+]^{-0.71}$$

These conclusions were however, based on correlative rather than direct data and were therefore circumstantial evidence for such a relationship. Moreover, Nelson and Whicker (1967) found no clear-cut relationship between high levels of 137-caesium in game fish from Colarado, U.S.A. and low environmental potassium concentrations. Similarly, no difference occurred in 137-caesium levels in fish from the Hudson River estuary over the range 2-30mg l^{-1} of potassium (Wrenn *et al*., 1971).

A short series of experiments was designed therefore, to examine the

biochemical similarity of caesium to potassium directly, by measuring the uptake of each in erythrocytes of the rainbow trout (*Oncorhynchus mykiss*, Walbaum) and studying the inhibitory effect of potassium on caesium uptake, and vice versa. These experiments were also used to investigate the behaviour of 137-caesium at the cellular level. Although erythrocytes are not typical of vertebrate cells in general, those of fish are somewhat unusual in that they possess both nuclei and mitochondria, they consume oxygen, and Greaney and Powers (1978) found that they are able to incorporate radio-labelled amino acids into acid-precipitable protein. The erythrocyte is however, a convenient cell in which to study ionic transport as it is easily obtained and isolated and both the internal and external milieu can be varied as required.

The fluxes of the major ions eg. sodium, chloride and potassium have been well-documented in the trout erythrocyte (Bourne and Cossins, 1984; Romano and Passow, 1984; Cossins and Richardson, 1985; Borgese *et al.*, 1986; Tufts *et al.*, 1988; Nikinmaa and Tufts, 1989). Bourne and Cossins (1984) described three kinetically distinct mechanisms of potassium influx in trout erythrocytes:

i) The ouabain-sensitive Na^+ K^+ pump.

ii) The specific furosemide-sensitive route.

iii) A route with passive, non-specific permeability characteristics.

These three fluxes accounted for 50, 46 and 4% of the total potassium influx respectively. Bourne and Cossins (1984) concluded that in possessing these mechanisms "... trout erythrocytes seem little different to those of other vertebrates."

Hence, the uptake by erythrocytes provided a straightforward and well-documented method with which to compare the biochemical behaviour of caesium and potassium and also provided valuable indicators of the membrane transport of these ions in other types of cells.

MATERIALS AND METHODS

Chemicals and Radioisotopes

137-Caesium (as CsCl in 1M HCl; 0.25μg Cs MBq^{-1} ^{137}Cs) and 86-rubidium (as RbCl in aqueous solution; 24.7μg Rb MBq^{-1} ^{86}Rb) were purchased from Amersham International plc., Amersham, England. All other chemicals were of AnalaR grade where available, from BDH Chemicals, Poole, U.K. or FSA Laboratory Supplies, Loughborough, U.K.

Animals

Rainbow trout (*Oncorhynchus mykiss*, Walbaum) of 0.25-0.50kg weight were obtained from College Mill Trout Farm, Almondbank, Perthshire. These were kept indoors in large (576l capacity), fresh water aquaria containing dechlorinated Stirling tapwater. The major ion concentrations in the water were typically (μmoles l^{-1}): [Na^+] = 120; [Ca^{++}] = 200; [K^+] = 6.4. Temperature was ambient and varied between 4 and 10°C and photoperiod was maintained at 10h light: 14h dark. The fish were fed daily on a diet of commercial trout pellets and kept for at least two weeks before blood sampling.

Blood sampling and preparation

The experimental procedures were based on those of Bourne and Cossins (1984). The fish were stunned by a sharp blow to the head and blood was withdrawn by cardiac puncture into cooled, heparinised syringes and shaken to prevent clotting. Samples from five fish were pooled in heparinised vials (to eliminate variation in blood parameters between individuals) and stored on ice. The haematocrit (% packed cells) was measured in a Gelman-Hawksley Microhaematocrit Centrifuge (8min at 10,000g). The cells were then washed four times by centrifugation (5min 400g) in a bench centrifuge followed by re-suspension with ice-cold trout saline, discarding the thin surface layer of white blood cells (the buffy coat) and supernatant after each spin. The saline was made up to an osmolarity of 300mM as follows (mM): NaCl, 131; KCl, 6; $CaCl_2$, 1; $MgCl_2$, 1; D-glucose, 5; imidazole-HCl, 15; pH 7.60 at 20°C. The erythrocytes were finally re-suspended in the saline at the original haematocrit and kept overnight at 4°C such that they were fully equilibrated with the saline and were in a non-catecholamine-stimulated state (Bourne and Cossins, 1982). The density (no ml^{-1}) and appearance of the cells were checked before and after storage to ensure that no haemolysis occurred during overnight incubation.

Measurement of ionic uptake

The uptake of 137-caesium into the trout erythrocyte was measured at four external caesium concentrations (including 137-caesium at 5.0μCi ml^{-1} = 1.2μM): 50μM, 100μM, 250μM and 3mM when $[K^+] = 0$. The effect of potassium on caesium uptake was studied by repeating the experiment with external concentrations of potassium of 0.1 and 1.0mM.

Aliquots of 50µl of the washed cell suspension were added to Eppendorf tubes containing 0.9ml modified trout saline. Potassium chloride was initially omitted from the saline and caesium and potassium chloride were added to achieve the required concentrations for each incubation. The balance of the caesium/potassium was made up by addition of mannitol as an osmotic filler. The Eppendorf tubes containing the final saline were equilibrated to 10°C (±0.5°C) in a water bath. The reaction was started by the addition of 5.0µCi (0.19MBq) 137-caesium (final activity = 5.0µCi ml^{-1}). The tubes were shaken at regular intervals during the incubation.

Samples of 200µl were taken in quadruplicate after 2.5, 5, 10 and 15 minutes from separate tubes. The reaction was stopped by rapidly washing the cells four times in 0.5ml ice-cold isotonic $MgCl_2$ solution in an Eppendorf micro-centrifuge (Sarstedt MH2) for 5s at 5000g. The final supernatant was discarded and the final pellet was lysed with 0.5ml distilled water. The resulting suspension was transferred, together with a further washing of 0.5ml distilled water, to counting-vials for assaying of 137-caesium activity in a gamma-counter (Canberra-Packard A500C).

The influx of potassium and the effect of caesium upon it was similarly studied by following the uptake of 86-rubidium. Bourne and Cossins (1984) demonstrated that the behaviour of 86-rubidium and 42-potassium were identical in the trout erythrocyte. Gardaire *et al* (1991) used 86-rubidium as the radiotracer in a study of potassium transport across the gills of rainbow trout (*O. mykiss*). The total concentrations of the ion used in the present experiments were 50µM, 100µM, 250µM and 3mM with only 1.0µCi (37kBq) 86-rubidium added to each tube (final specific activity = 1.0µCi ml^{-1}

= 10.6μM). The experiments were carried out at caesium concentrations of 0, 0.1 and 1.0mM. The experimental procedure was similar to that outlined for the uptake of caesium except that 5ml scintillant (Packard Hionic Fluor) was added to the final lysed cell suspension and 86-rubidium activity was measured in a Canberra Packard TriCarb 200CA liquid scintillation counter. 86-Rubidium is a strong β-emitter and therefore the colour quench of the lysed erythrocytes was minimal. The counts were corrected for quench however, when this exceeded 5%.

The data were initially plotted as mean cpm 137-caesium or 86-rubidium (per 200μl sample) versus time for each concentration of the influx ion; plotting the three (potential) inhibitor ion concentrations on the same graph. Straight lines were fitted to the data by computer (STATGRAPHICS) and the influx was calculated from the slope of the lines and the specific activity of the isotope used in each incubation. The flux values were expressed as μmoles min^{-1} ml^{-1} packed cells.

The calculated influxes were used to plot double-reciprocal or Lineweaver-Burke plots ie. 1/v vs 1/[s], where v = rate of reaction (influx) and [s] = external concentration of the influx ion. This plot is a transformation of the Henri-Michaelis-Menten equation into a linear (y = ax + b) form. The y-intercept of the double-reciprocal plot gives $1/V_{max}$ (V_{max} = maximum rate of reaction) whilst the x-intercept gives $-1/K_m$ (K_m = concentration of substrate (ion) which gives a reaction rate of 0.5 V_{max}). [These parameters should be referred to as "apparent K_m" and "apparent V_{max}" as both are likely to be the sum of a number of separate constants, derived from separate pathways of ionic uptake. In this chapter however, the basic terms "V_{max}" and "K_m" will be used].

The final double-reciprocal plots each displayed three straight lines (fitted by computer); one for each concentration of the (potential) inhibitor ion.

RESULTS

Uptake plots of cpm vs time for both 137-caesium and 86-rubidium were linear over the experimental period of 15min (Figs. 2.1.a and b). The uninhibited influx of caesium into the rainbow trout erythrocyte was much smaller than that of potassium; 0.014 and 0.756 μmoles min^{-1} ml^{-1} packed cells respectively at an external concentration of 3.0mM. The K_m of caesium uptake was considerably smaller than that of potassium which suggested that caesium had the higher affinity for the pathway(s) involved (Table 2.1). The value of V_{max} was however, much greater for potassium than for caesium, as was the ratio of V_{max}/K_m. The latter value demonstrated that the influx of potassium increased more quickly than the influx of caesium as the external ion concentration was increased.

The increasing slope of a Lineweaver-Burke plot as the concentration of the potential inhibitor is increased shows a reduction in uptake of the primary substrate and is evidence for inhibition. This can be seen on both Fig. 2.2 and Fig. 2.3; hence the presence of potassium inhibits the uptake of caesium and *vice versa*, in the trout erythrocyte. The relative inhibition of caesium uptake by potassium was greater than that of potassium by caesium: at [S] = 0.05mM a potassium concentration of 1mM caused a 78% decrease in caesium uptake, whereas 1mM caesium only caused a 47% decrease in potassium uptake.

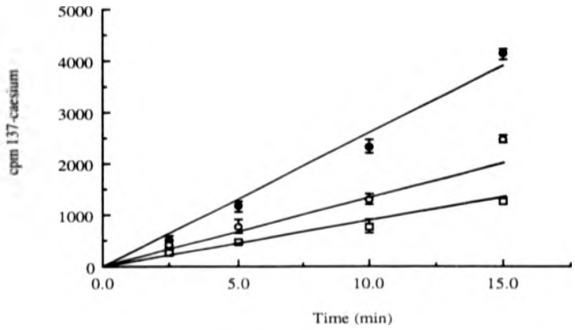

Fig. 2.1.a. Uptake of 137-caesium (Cs = 0.1mM) by rainbow trout erythrocytes (O. mykiss) at three potassium concentrations (mM); 0 (closed circles); 0.1 (open circles) and 1.0 (squares).

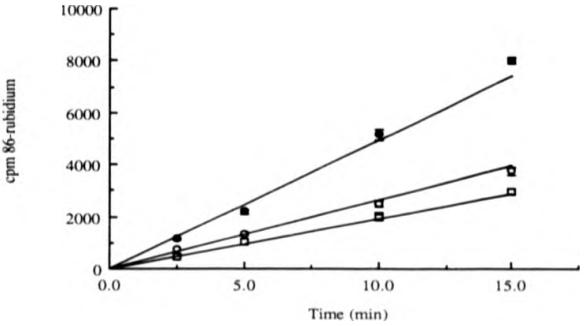

Fig. 2.1.b. Uptake of 86-rubidium (Rb = 0.1mM) by rainbow trout erythrocytes (O. mykiss) at three caesium concentrations (mM); 0 (closed circles); 0.1 (open circles) and 1.0 (squares).

Table 2.1. Michaelis-Menten parameters (from Lineweaver-Burke plots) of uninhibited potassium and caesium uptake by erythrocytes of rainbow trout (*O. mykiss*).

parameter	potassium	caesium
K_m(mM)	7.42	2.45
V_{max}(μmoles min^{-1} ml^{-1} cells)	3.85	0.0241
V_{max}/K_m	0.52	0.010

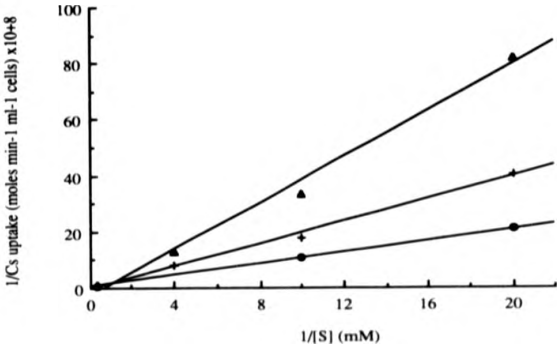

Fig. 2.2 Lineweaver-Burke plot of caesium uptake by erythrocyte of rainbow trout (O. mykiss) at three potassium concentrations (mM): 0 (circles); 0.1 (crosses) and 1.0 (triangles).

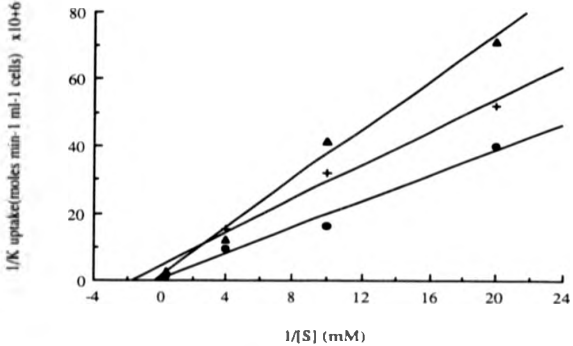

Fig. 2.3. Lineweaver-Burke plot of potassium uptake by rainbow trout erythrocytes (O. mykiss) at three caesium concentrations (mM): 0 (circles); 0.1 (crosses) and 1.0 (triangles).

DISCUSSION

The potassium influx recorded in this experiment was approximately three times larger than that recorded by Bourne and Cossins (1984) at the same external concentration (3mM). Sodium and potassium influx in the fish erythrocyte is known to be highly dependent on the presence of catecholamines (Baroin *et al*., 1984; Cossins and Richardson, 1985; Borgese *et al*., 1987; Heming *et al*., 1987) and is also affected by other factors such as relative cell volume and oxygen tension (Cala, 1977). Although the cells in these experiments were prepared in a non-catecholamine-stimulated condition no attempt was made to measure cell volume, hence this could explain some of the difference in potassium influx between these results and those of Bourne and Cossins (1984). The blood sampling methods were however, designed to minimise haemolysis or changes in cell volume (Korcock *et al*., 1988).

Bourne and Cossins (1984) described three kinetically distinct mechanisms for potassium influx in trout erythrocytes. A saturable ouabain-sensitive sodium-potassium pump (K_m = 1.5mM; V_{max} = 0.22mmol min^{-1} l^{-1} cells), a saturable furosemide-sensitive route (K_m = 25mM; V_{max} = 1.0 mmol min^{-1} l^{-1} cells) and a third route with the characteristics of passive, non-specific permeability. These pathways accounted for 50, 46 and 4% respectively of the total potassium influx.

It is not possible however, to ascertain which pathway(s) are involved in the uptake of caesium. The caesium influx was relatively small (<2% of that of potassium) and could have taken place entirely via any one of the three routes. From the inhibitory

effects of potassium ions however, especially at the lower concentration of 100μM, it appears that some active transport occurs. If the uptake of caesium were entirely passive the presence of 100μM potassium would not have reduced the caesium influx.

The ratio of V_{max}/K_m was greater for potassium, which is an indication that the efficiency of the transport process was greater for potassium than for caesium, or that potassium was the "better" of the two substrates (Segel, 1976). The greater affinity of caesium for the pathway(s) of uptake (as indicated by its lower K_m value: Table 2.1) was unusual as one would expect any enzymes or carriers involved to be adapted to the usual ion taken up ie. potassium, which would therefore have the greater affinity. "Natural" systems would never be exposed to caesium other than in trace amounts however, so it is very unlikely that there would be any specific adaptation to discriminate in favour of or against caesium. The values of K_m were derived from Lineweaver-Burke plots: one criticism of these plots is that small errors in v are magnified when reciprocals are taken. The K_m value of total potassium uptake (7.42mM) was however, in line with the two values recorded by Bourne and Cossins (1984) for the ouabain-sensitive and furosemide sensitive routes of potassium influx in the trout erythrocyte - 1.5 and 25mM respectively

The most important aspect of these experiments is the result concerning the relationship between caesium and potassium. The presence of potassium inhibits the uptake of caesium and vice versa in the rainbow trout (*O. mykiss*) erythrocyte (Figs. 2.2 and 2.3). The form of the graphs indicates that this inhibition is either competitive or non-competitive as opposed to uncompetitive inhibition as in the latter, the slope

remains constant as the concentration of the inhibitor is increased. The precise type of inhibition, either competitive or non-competitive, is determined by the relative positions of the intercepts of the lines. Each line, in the presence of different fixed concentrations of a competitive inhibitor has the same y-intercept (ie. the same V_{max}) but different, negative x-intercepts (x-intercept = $-1/K_m$). In the presence of a non-competitive inhibitor K_m is unchanged but V_{max} decreases with inhibitor concentration. Each line on the double-reciprocal plot therefore has a different y-intercept but the same (negative) x-intercept. Unfortunately this distinction could not be made with certainty from the graphs as in these experiments three potential pathways of potassium/caesium transport exist and hence the specificity of the uptake parameters would be reduced.

However, caesium and potassium ions are very similar, both chemically and physically and caesium has been shown to interact with potassium sites in a number of systems (Sjodin, 1961; Bezanilla and Armstrong, 1972; Beauge *et al.*, 1973). Caesium is also well-known as a blocker of potassium movement in neurophysiological studies (Adelman and Senft, 1966; Adelman *et al.*, 1971; Blatz and Magleby, 1984; Matteson and Swenson, 1986). It is likely therefore, that inhibition of caesium uptake by potassium in trout erythrocytes was due to competition for binding and/or carrier sites of potassium channels and is likely to be the factor determining caesium uptake.

The results of these short preliminary experiments demonstrate that caesium behaves as potassium in a qualitative but not quantitative way, and will therefore be used to justify the comparison of caesium biochemistry with that of potassium in

salmonids in fresh water in later sections of this thesis.

CHAPTER 3: THE ACCUMULATION AND EXCRETION OF 137-CAESIUM IN THE EARLY STAGES OF DEVELOPMENT OF ATLANTIC SALMON (*SALMO SALAR* L) AND BROWN TROUT (*SALMO TRUTTA* L).

INTRODUCTION

Chapter one presented the reasons for studying the fate of radiocaesium in aquatic ecosystems and reviewed the previous work regarding the accumulation and excretion of 137-caesium in fish. The Chernobyl disaster on the 26th April 1986 caused widespread, low-level contamination with a mixture of radioisotopes, including 137-caesium, over much of Europe. Interest in the uptake, accumulation and excretion of 137-caesium in fish was therefore renewed.

In the U.K. pollution from Chernobyl occurred mainly via rainfall in upland areas eg. N. Wales and S.W. Scotland (Clark, 1986; Smith and Clark, 1986) where the typical freshwater species are salmonids, especially brown trout (*Salmo trutta*, L.). A further feature typical of these upland areas was low environmental pH due to acidic soils in the catchment area and/or acid rain. The effects of acid stress on fish physiology have been extensively reviewed (Muniz and Leivestad, 1980; Fromm, 1980; Haines, 1981; Spry *et al.*, 1981, Brown, 1982; Leivestad, 1982; Wood and McDonald, 1982; McDonald, 1983; Howells *et al.*, 1983; Howells, 1984; McDonald *et al* , 1988;

Wood, 1988) and include severe disruption of normal ionic regulation.

A conclusion of the review in Chapter one was that there is a lack of information concerning the behaviour of radiocaesium in the early stages of the life-history of fish. The present chapter therefore presents experiments on the accumulation from freshwater, and elimination, of 137-caesium by eggs, larvae and juveniles of Atlantic salmon (*Salmo salar* L) and brown trout (*Salmo trutta* L). The experiments with the juvenile fish were carried out at "normal" and "low" pH (=7.4 and 5.0 respectively) to study the effect of increased acidity on the rate and magnitude of 137-caesium accumulation and elimination.

MATERIALS AND METHODS

Chemicals and radioisotopes

137-Caesium (as CsCl in 1M HCl; 0.25μg MBq^{-1} ^{137}Cs) was purchased from Amersham International plc., Amersham, U.K. MS 222 (ethyl 3-aminobenzoate methanesulphonate) was purchased from the Sigma Chemical Company Ltd., Poole, U.K. All other chemicals were of AnalaR grade where available, from BDH Chemicals, Poole, U.K. or F.S.A. Laboratory Supplies, Loughborough, U.K.

Animals

Brown trout (*S. trutta* L): unfertilised eggs and milt, and juveniles at the first-feed stage (immediately after absorbtion of the larval yolk-sac) of 0.10-0.20g initial weight, were obtained from the Howietoun Fish Farm, Sauchieburn, Stirling.

Atlantic salmon (*S. salar* L): eyed eggs, and juveniles of 0.40-0.70g initial weight,

were donated by the Aquatic Vaccine Unit, Institute of Aquaculture, University of Stirling. Alevins of both species were hatched from developing eggs at ambient water temperature (3-8 °C) within the department.

Experimental design

A number of experiments were carried out:

1. The accumulation of 137-caesium from water in eggs of the brown trout, (*S. trutta*).

2. The accumulation of 137-caesium from water by alevins of Atlantic salmon (*S. salar*) and brown trout (*S. trutta*).

3. The accumulation of 137-caesium from water at "normal" (pH = 7.4) and "low" (pH = 5.0) pH by juvenile Atlantic salmon (*S. salar*) and brown trout (*S. trutta*).

4. The elimination of 137-caesium at the two acidities from juvenile Atlantic salmon (*S. salar*).

The procedure was similar for all of the experiments. The animals were kept in suitable covered trays/tanks containing dechlorinated Stirling tapwater to which 137-caesium was added to give a concentration of 0.11-0.19 MBq l^{-1} (3-5μCi l^{-1}). The water was moderately soft, typical major ion concentrations being, Ca, 8mg l^{-1} (200μM); Na, 2.7mg l^{-1} (120μM) and K, 0.25mg l^{-1} (6.4μM). Each of the experiments used totally separate stages of development of the animals:

Experiment 1. Approximately 500 eggs of brown trout were fertilised for 20min, rinsed, and immediately laid down in radioactive water, on a metal-mesh egg basket in a fibre-glass hatching tray (70 x 80cm). The depth of water covering the eggs was maintained at approximately 15mm. The eggs were kept in darkness and sampled

until hatching (approximately 50d). The accumulation of 137-caesium in water-hardened eggs was studied by placing "clean" eggs in radioactive water after the first 10 days of normal development.

Experiment 2. Approximately 200 14-day alevins of each species were placed in water at a depth of 10cm in separate, blackened glass tanks (40 x 25 x 25cm) containing a number of small, rounded pebbles as a protective substrate. The accumulation of 137-caesium was followed for approximately 50 days. Light feeding with a commercial crumbed pellet commenced at t=28d.

Experiment 3. Approximately 100 juveniles of each species were placed in two separate, glass tanks (40 x 25 x 25) containing water at a depth of 20cm, 4-5 weeks after the first-feed stage (approximately 90d post-hatching). One tank of each species was maintained at "normal" pH (7.4 ± 0.2) whilst the other tanks were maintained at "low" pH (5.0 ± 0.5) by the continuous, slow input of 0.25M HCl via a Harvard syringe infusion/withdrawal pump. The accumulation of 137-caesium was followed for 2-5 months.

Experiment 4. The elimination of 137-caesium from the juvenile salmon was studied by transferring the fish remaining at the end of the accumulation experiment to clean, non-radioactive water and sampling at suitable intervals as before (see below) over a period of 90 days.

In all experiments the water was aerated, and recirculated via a centrifugal pump (Eheim). All experiments were conducted at a constant temperature of 10°C (±1.0°C). The juvenile fish were fed *ad lib* on a diet of crushed commercial brand pellet. Any uneaten food was removed with a wide-mouthed pipette in order to minimize uptake

of 137-caesium via contaminated food. The water in all experiments was changed as required (approximately weekly) to prevent an accumulation of toxic waste products in the water. (A biological filter was found to be unsuitable as it absorbed a large proportion of the radioisotope).

Sampling procedure

Samples were taken at appropriate intervals depending on the total duration of the individual experiment. Typically, this was every 2-3 days during the first two weeks and subsequently at one to two-weekly intervals (except during the uptake of 137-caesium in brown trout eggs which was also examined in detail over the first four hours post-fertilisation).

For each sample, five eggs or fish were taken. Eggs and alevins were removed using a fire-polished, wide-mouthed pipette; juveniles with a small aquarium net. The animals were transferred to a nylon tea-strainer and rinsed as follows.

Eggs: Rinsed for 20s in tapwater, agitated continuously.

Fish: Rinsed in three wash-baths;

i) Tapwater, 5min, agitated intermittently.

ii) CsCl solution (=10mM), 5min, agitated intermittently.

iii) Tapwater, 1min, agitated constantly.

The final bath contained a lethal dose ($0.25g\,l^{-1}$) of anaesthetic (MS 222). The eggs and alevins were dabbed dry with tissue paper and placed individually into pre-weighed vials. The vials were immediately re-weighed and placed in a gamma-counter (Canberra-Packard A5000C) for 10min each to measure the 137-caesium activity.

In experiments three and four, the accumulation of 137-caesium was monitored in both species and the excretion of 137-caesium from salmon only was monitored in a number of individual tissues/organs in addition to the whole-body. The tissues analysed were blood (whole), gills (filaments plus arches), liver, kidney gut (posterior to the stomach), muscle and bone. Blood was collected immediately after rinsing the fish, from the caudal vein by caudal section. The whole carcass was analysed and the other tissues were removed, rinsed briefly (2 or 3s) in distilled water, dried, weighed and assayed as before.

Data analysis

All data were standardised for weight and expressed as concentration factors (C.F.s).

$$C.F. = \frac{\text{c.p.m. } ^{137}Cs \text{ g}^{-1} \text{ animal/tissue}}{\text{c.p.m. } ^{137}Cs \text{ g}^{-1} \text{ water}}$$

The results were plotted as mean C.F. (± S.D.) versus time. Accumulation curves were fitted to the whole-body data for alevins and to the whole-body and tissue data for juveniles by computer (Enzfitter, Elsevier Biosoft, Cambridge; STATGRAPHICS, STSC, Rockville, U.S.A.) based on a first-order rate equation:

$$Q = Q_{eq}(1-e^{-kt})$$

Where Q = C.F. at time t,

Q_{eq} = C.F. at equilibrium.

K = rate constant or "turnover" of ^{137}Cs (d^{-1}).

t_{eq} = time taken to reach equilibrium (d). This was estimated from extrapolations of the first-order curves.

This equation was based on a linear differential equation:

$$dQ/dt = I - KQ \qquad \text{(Jefferies and Hewett, 1971)}$$

Where I = influx

K, Q = as above.

At equilibrium, $Q = Q_{eq}$:

$$dQ/dt = 0$$

$$\Rightarrow \quad I = K.Q_{eq}$$

The influx of 137-caesium could therefore be calculated easily from the predicted parameters of the fitted first-order curves. The units of influx were the same as those of K ie. d^{-1}, as Q_{eq} was a ratio and therefore had no units. Normal units of influx (μmoles kg^{-1} h^{-1}) could be calculated from the specific activity of the water in the experiment(s).

The biological half-life, $t_{b_{\frac{1}{2}}}$ could also be calculated assuming that the depletion of the radioisotope could be described by a simple exponential function:

$$Q = Q_0 e^{-kt} \qquad \text{(see also below)}$$

Where Q_0 = C.F. at time t = 0, and all other symbols were as before.

When $t = t_{0.5}$, $Q = 0.5Q_0$

$\Rightarrow \quad 0.5Q_0 = Q_0 e^{-Kt}$

$\Rightarrow \quad 0.5 = e^{-Kt}$.

Taking natural logs (ln):

$\Rightarrow \quad 0.693 = Kt$

$\Rightarrow \quad t_{0.5}(d) = 0.693/K$ (Jefferies and Hewett, 1971).

Whole-body accumulation data were compared statistically using one of two methods. In experiment three the effect of pH on the accumulation of 137-caesium was analysed using two factor analysis of variance (factors = time and pH) for both species. This statistic calculates the variation due to both factors compared to inherent variation (error), and determines whether any interaction occurs (ie. whether the effect of one factor depends on the level of the other). This method however, requires that samples were taken at the same time points for the two acidities. Therefore, some of the data for the accumulation of 137-caesium in brown trout at pH 7.4 were omitted for the purpose of this test. When insufficient corresponding points were available for analysis of variance, accumulation was compared by transforming the data by taking natural logs (ln):

$$Q = Q_{eq}(1-e^{-kt})$$

$$Q_{eq} - Q = Q_{eq} e^{-kt}$$

$$\ln(Q_{eq}-Q) = \ln Q_{eq} - k.t$$

$$y = a - b.x$$

A plot of ln(Qeq-Q) vs time therefore produced a linear relationship. A regression line was fitted to the transformed data by computer (Minitab) and the homogeneity of the slopes and/or the elevations of the lines were compared using t-distribution analysis (Zar, 1984). This method was used in experiment two, and to compare accumulation of 137-caesium between species at the same pH in experiment three. This analysis however, cannot determine whether the difference in 137-caesium accumulation at the two acidities changes with time (ie. whether any interaction occurs).

The depletion data from experiment four were also plotted as C.F.s versus time. The biological half-lives derived from the accumulation experiments above assumed that the depletion of 137-caesium from juvenile salmonids could be described accurately by a single exponential function. A number of studies have shown however, that the depletion of a radioisotope may be divided into a number of "components", each described by a separate exponential function, such that each component has its own rate constant and accounts for a separate proportion of the total radioactivity at t = 0. The overall depletion of the radioisotope is therefore described by a multiple exponential function (Baptist and Price, 1962; Hüsänen *et al.*, 1966; Kolehmainen and Miettinen, 1967; Nelson, 1967). This may be expressed as:

$$Q_t = Q_{01}e^{K1t} + Q_{02}e^{K2t} \ldots + Q_{0n}e^{Knt}$$ (Baptist and Price, 1962)

Where Q_t = C.F. at time t, $Q_{01} \ldots Q_{0n}$ and K1 ...Kn = initial C.F.s and rate constants respectively of n individual components of the total depletion.

Three models were fitted to the data for 137-caesium depletion from juvenile

Atlantic salmon (*S. salar*). A simple linear model; a single component exponential model and a two-componenet exponential model. In all cases a single exponential model was found to be the best fit (ANOVAR, STATGRAPHICS). This confirmed the validity of the biological half-lives calculated from the accumulation curves in experiment three. The value of K and therefore $t_{0.5}$ obtained from the elimination experiments should be the same as those obtained from the accumulation experiments.

In order to compare statistically the elimination of 137-caesium from salmon at the two acidities, the data was transformed by taking natural logs (ln):

$$Q_t = Q_0 e^{-Kt}$$

$$\ln Q_t = \ln Q_0 - Kt$$

$$y = a - bx$$

A plot of $\ln Q_t$ versus time therefore produced a linear relationship The homogeneity of the slopes and/or the elevations of the regression lines fitted to the transformed data (Minitab) were compared using t-distribution analysis (Zar, 1984).

RESULTS

137-Caesium accumulation in eggs.

The first sample was taken 24h after the newly-fertilised eggs of brown trout (*S. trutta*) were laid down in the radioactive water. By that time however, a small but significant burden of 137-caesium had accumulated (C.F. = 0.4) which showed no

further increase until hatching was imminent, at which point the 137-caesium concentration factor of the eggs increased rapidly (Fig. 3.1). Eggs which were incubated in clean water for the first ten days of development and were subsequently transferred to active water, also accumulated 137-caesium to a C.F. = 0.4 within 24h and showed no further increase over a period of 11 days.

When the uptake of 137-caesium into the eggs was examined in detail over the first 4h post-fertilisation, an initial, rapid uptake was observed which peaked at a C.F. of approximately 1.0 after 20min (Fig. 3.2). The C.F. then fell to around 0.4 after one hour and remained at that level throughout the incubation. No significant trends were noted in Fig.s 3.1 and 3.2, therefore the points were simply joined rather than fitting a single line.

The early embryos were sampled by puncturing the outer shell or chorion with a capillary tube and removing a small amount of embryonic fluid by capillary action. This confirmed that the 137-caesium accumulated was in fact, present internally and not merely bound to the surface of the eggs. Analysis of "eyed" eggs by removing the chorion and blotting away the perivitelline fluid (pvf) clearly demonstrated that the majority of the 137-caesium was located within the pvf rather than the embryo itself (Table 3.1).

137-Caesium accumulation in the alevins.

Both species of alevins accumulated 137-caesium to a level many times greater

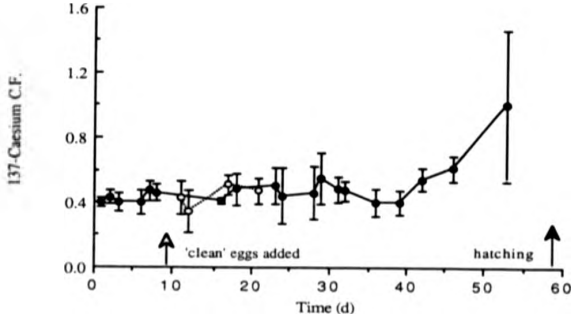

Fig. 3.1. 137-Caesium accumulation in the eggs of the brown trout (*S. trutta*). Data are shown as mean C.F. ± 1 S.D. Filled circles are eggs placed in 137-Cs immediately post-fertilisation, open circles are "clean" eggs placed in 137-Cs after 10d normal development.

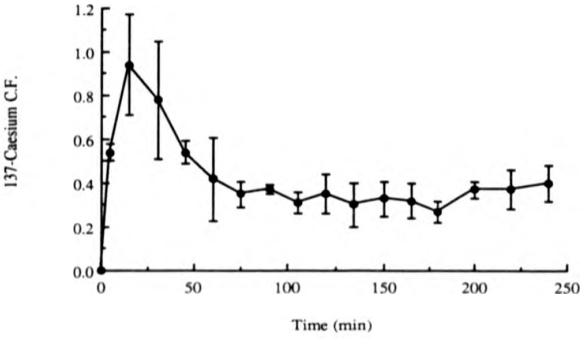

Fig. 3.2. 137-Caesium accumulation in the eggs of the brown trout (S. trutta) over 4h immediately post-fertilisation. Data are mean C.F. ± 1 S.D.

Table 3.1. Location of 137-caesium accumulation in the perivitelline fluid and embryo of eyed eggs of brown trout (*S. trutta*): mean activity ± 1 S.D. cpm g^{-1} (n=5).

time (d)	Perivitelline fluid	Embryo
39	4457 ± 1715	299 ± 217
42	3686 ± 1365	250 ± 275
46	3666 ± 893	210 ± 72
49	3580 ± 268	644 ± 280

than that present in the water. The rate of accumulation of 137-caesium in both species of alevins decreased with time as an equilibrium concentration factor was approached. A first-order rate equation was a good fit to the data for Atlantic salmon and brown trout; R^2 = and 0.808 and 0.856 respectively, $p<0.001$ (ANOVAR) in both cases (Fig. 3.3). The curves fitted to the data were somewhat misleading however, in that they predicted times to reach equilibrium (t_{eq}s) of 140 and 190 days for Atlantic salmon and brown trout respectively (Table 3.2). An examination of the accumulation data in Fig. 3.3 however, showed that no increase in mean C.F. was recorded in either of the two species after t = 30 days. This illustrates a danger in fitting theoretical curves to experimental data and the predicted parameters should therefore be treated with a degree of caution. These reservations notwithstanding, the influx of 137-caesium and the C.F. at equilibrium were considerably greater in the brown trout than in the salmon (Table 3.2). The rate of 137-caesium accumulation of the two species however, was not significantly different (t-distribution analysis; $0.50>p>0.20$).

137-Caesium accumulation in the juveniles.

The accumulation of 137-caesium in both species was asymptotic at the two acidities (Fig.s 3.4 and 3.5). First-order rate kinetics fitted the data well in all cases; R^2 values = 0.851 - 0.945, $p<0.001$ (ANOVAR). 137-Caesium accumulated to a level several times that in the surrounding water. The C.F.s achieved at "normal" pH (=7.4) in the juvenile fish were however, significantly lower than those previously recorded in alevins for both species (t-distribution analysis; $p<0.001$).

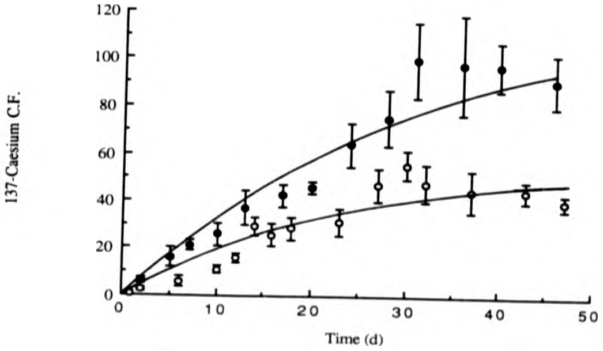

Fig. 3.3. 137-Caesium accumulation in the alevins of brown trout, S. trutta (filled circles), and Atlantic salmon, S. salar, (open circles). Data are mean C.F. ± 1 S.D.

Table 3.2 Parameters of 137-caesium accumulation in alevins of brown trout (*S. trutta*) and Atlantic salmon (*S. salar*). (See Materials and Methods for an explanation of symbols).

	Brown trout (*S. trutta*)	Atlantic salmon (*S. salar*)
$K(d^{-1})$	0.0294	0.0398
Q_{eq}	129.6	58.2
$K.Q_{eq}(d^{-1})$	3.81	2.32
$t_{eq}(d)$	190	140

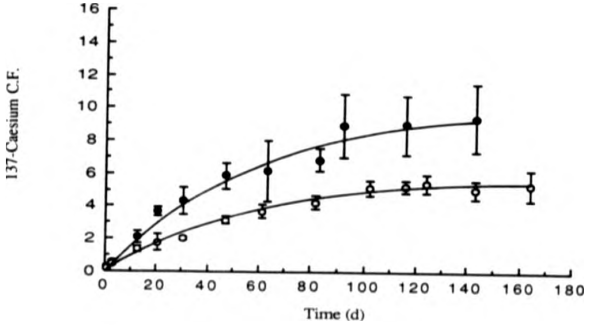

Fig. 3.4. 137-Caesium accumulation in juvenile Atlantic salmon (S. salar) at two acidities; pH ~ 7.4 (filled circles) and pH ~ 5.0 (open circles). Data are mean C.F. ± 1 S.D.

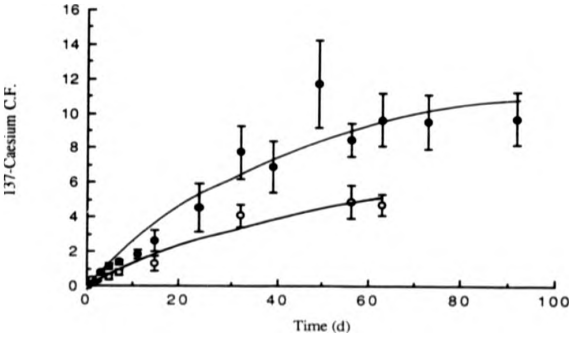

Fig. 3.5. 137-Caesium accumulation in juvenile brown trout (S. trutta) at two acidities; pH ~ 7.4 (filled circles) and pH ~ 5.0 (open circles). Data are mean C.F. ± 1 S.D.

The accumulation of 137-caesium in Atlantic salmon at pH=7.4 approached an asymptote at 143 days (Fig. 3.4). The calculated rate constant was $0.0181d^{-1}$ and equilibrium was reached at a C.F. of 9.95 after 220d (Table 3.3). These gave a influx value of $0.180d^{-1}$ and a biological half-life of 36.4 days. At low pH (=5.0) the accumulation of 137-caesium was significantly reduced (ANOVAR, $p<0.001$) and this difference increased with time ($p<0.001$). The rate constant and hence the biological half-life were little affected ($0.0168d^{-1}$ and 41.3d respectively), but equilibrium was reached after a longer time and at a much smaller C.F. (250d and 5.57 respectively) resulting in an influx of $0.099d^{-1}$. Assuming a 137-caesium concentration in water of 0.13 MBq l^{-1} (0.105µg Cs l^{-1}) these equilibrium C.F.s would result in whole body concentrations of 1.29 and 0.72MBq $^{137}Cs\ kg^{-1}$ (1.04 and 0.58µg Cs kg^{-1}) for pH=7.4 and pH=5.0 respectively.

The rate constant, equilibrium C.F. and influx were also greater at pH=7.4 than at pH=5.0 for brown trout. The time taken to reach equilibrium and the biological half-life were greater at low pH (Table 3.3). The effect of pH was found to be statistically significant (ANOVAR, $p<0.001$) and to increase significantly with time ($p<0.001$). The equilibrium C.F.s for brown trout equate to whole-body concentrations of 1.54 and 0.89MBq $^{137}Cs\ kg^{-1}$ (1.25 and 0.72µg Cs kg^{-1}) for pH=7.4 and pH=5.0 respectively.

Although the rate constant, equilibrium concentration factor and influx were greater for brown trout than for Atlantic salmon at both acidities, only the rate constant was significantly smaller at reduced pH (t-distribution analysis, $p<0.01$).

Table 3.3. Parameters of 137-caesium accumulation in the whole-body of juvenile brown trout (*S. trutta*) and Atlantic salmon (*S. salar*) at two acidities. (See Materials and Methods for an explanation of symbols).

	Brown trout (*S. trutta*)		Atlantic salmon (*S. salar*)	
	pH=7.4	pH=5.0	pH=7.4	pH=5.0
$K(d^{-1})$	0.0256	0.0214	0.0181	0.0168
$t_{0.5}(d)$	27.1	32.4	36.4	41.3
Q_{eq}	11.87	6.85	9.95	5.87
$K.Q_{eq}(d^{-1})$	0.30	0.147	0.180	0.099
t_{eq}	160	180	220	250

Tissue analysis

137-Caesium was found in all tissues analysed. The 137-caesium activity in the various tissues was not analysed frequently enough in the brown trout at normal pH (the initial experiment) to fit lines to the data as was done with the other three treatments in experiment three. The C.F.s in the tissues of brown trout at t = 57d were therefore compared with the C.F.s in the tissues at the nearest sample for the other three treatments (Table 3.4). Brain was not initially included in the analysis and therefore no data were available for this tissue in the salmon at "low" pH. The patterns of accumulation of 137-caesium in the tissues were less obvious than those in the whole-body owing to considerable variation in C.F.s recorded in different individuals within samples. The 137-caesium accumulation curves however, generally approximated to first-order rate kinetics (Fig. 3.6), but the parameters resulting from such curve fitting (Q_{eq} etc.) were only accepted if $p<0.05$ (ANOVAR) given the relevant degrees of freedom. This value was only exceeded in blood of brown trout at "low" pH. In all cases however, rate equations fitted the data better than did straight lines.

In all treatments, muscle followed by liver and kidney had the greatest C.F.s of 137-caesium, whilst blood and bone consistently displayed the lowest (Table 3.4 and 3.5). An apparent anomaly was present in Table 3.5 in that the whole-body C.F. in the salmon at both pHs was greater than that of any of the component tissues. There was no satisfactory explanation for this; it was a result of taking predicted parameters from curves fitted to data with considerable variation. Gills had a relatively low C.F. of 137-caesium. These were analysed whole however, including both the filaments and the bony arches. It is possible that had the gill epithelium been measured separately

Table 3.4. 137-Caesium concentration factors (Q) in tissues of brown trout (*S. trutta*) and Atlantic salmon (*S. salar*) at two acidities. Data are mean C.F. ± 1 S.D. N.D. = no data, t = sampling time (d), * indicates significantly reduced accumulation at pH=5.0 (Student's t-test, $p<0.05$).

	Brown trout (*S. trutta*)		Atlantic salmon (*S. salar*)	
	pH=7.4	pH=5.0	pH=7.4	pH=5.0
Whole-body	8.50 ± 0.95	4.76 ± 0.61*	6.13 ± 1.85	3.71 ± 0.39*
Gills	4.20 ± 1.67	2.11 ± 1.19*	3.88 ± 0.96	0.81 ± 0.41*
Liver	4.64 ± 1.69	3.07 ± 0.77	4.18 ± 1.04	1.03 ± 0.49*
Kidney	7.34 ± 2.73	2.93 ± 0.61*	3.00 ± 0.77	3.18 ± 0.60
Gut	4.25 ± 1.32	0.83 ± 0.52*	3.29 ± 1.07	0.14 ± 0.16*
Muscle	10.03 ± 0.92	5.28 ± 1.99*	5.52 ± 1.20	2.19 ± 0.12*
Bone	0.26 ± 0.16	0.62 ± 0.14	4.24 ± 1.19	0.34 ± 0.13*
Brain	N.D.	1.62 ± 0.38	4.08 ± 0.63	2.27 ± 1.05*
Blood	1.44(0.80)	0.70(0.17)	1.24(0.89)	0.38(0.31)
	n=5	n=4	n=5	n=5
	t=57	t=63	t=63	t=61

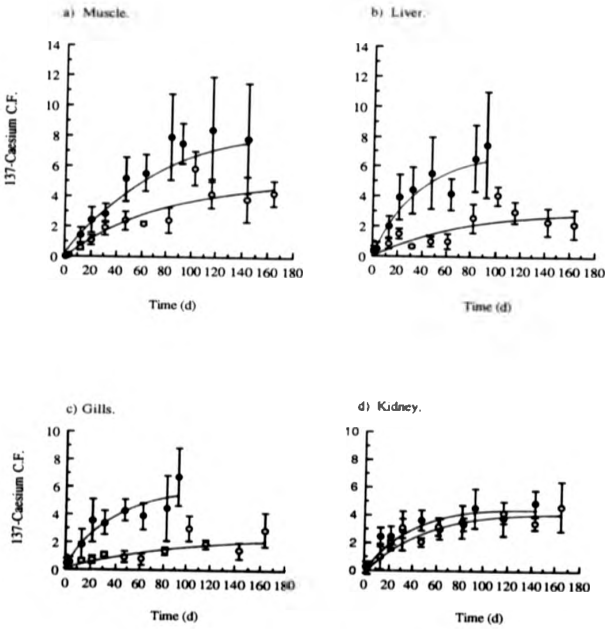

Fig. 3.6. 137-Caesium accumulation in various tissues of Atlantic salmon (S. salar) at two acidities; pH~7.4 (filled circles) and pH~5.0 (open circles). Data are mean C.F. ± 1 S.D.

Table 3.5. 137-Caesium concentration factors at equilibrium (Q_{eq}) in tissues of brown trout (*S. trutta*) at pH=5.0 and Atlantic salmon (*S. salar*) at pH=7.4 and 5.0. Q_{eq} ± S.E. N.D. = no data, * indicates rejection of parameter owing to inaccuracy of fitted curve (see text).

	Brown trout (*S. trutta*)	Atlantic salmon (*S. salar*)	
	pH=5.0	pH=7.4	pH=5.0
Whole-body	6.85 ± 1.18	9.95 ± 0.73	5.87 ± 0.20
Gills	0.91 ± 0.13	5.44 ± 0.65	2.02 ± 0.43
Liver	3.41 ± 1.46	7.11 ± 1.58	2.92 ± 0.69
Kidney	3.60 ± 1.10	4.12 ± 0.26	4.20 ± 0.31
Gut	1.28 ± 0.44	4.82 ± 0.34	2.28 ± 0.92
Muscle	9.99 ± 11.50	8.84 ± 1.61	4.98 ± 1.15
Bone	1.04 ± 0.13	5.72 ± 1.30	1.87 ± 0.40
Brain	3.33 ± 2.55	5.21 ± 0.51	N.D.
Blood	*	1.81 ± 0.33	1.35 ± 0.23

greater C.F.s would have been recorded. The 137-caesium C.F.s were significantly lower in the majority of tissues at reduced pH in both species as would be expected from the lower whole-body burden (Tables 3.4 and 3.5).

The turnover of 137-caesium was greatest in kidney, gills and gut, as indicated by their greater K values (Table 3.6) and biological half-lives (Table 3.7). The turnover in muscle and bone was consistently low and therefore these tissues had the longest biological half-lives; in excess of 40d at "normal" pH. The influx ($K.Q_{eq}$) was greatest for kidney, liver and gills, and was lowest for blood, bone and brain; muscle had an intermediate value (Table 3.6). The accumulation of 137-caesium in the tissues of both species was reduced at "low" pH (Tables 3.4 and 3.6).

137-Caesium depletion from juvenile Atlantic salmon (*S. salar*).

The whole-body depletion of 137-caesium from juvenile Atlantic salmon (*S. salar*) was best described by a single exponential function (Fig. 3.7):

$$Q_t = Q_0 e^{-Kt}$$

The R^2 values of this model were 0.657 ($p<0.001$) at pH=7.4 and 0.839 ($p<0.001$) at pH=5.0; greater than either simple linear or two-component exponential functions (ANOVAR). The values of K and $t_{0.5}$ obtained from these depletion experiments should theoretically, have been the same as those from the accumulation experiments (above). K and $t_{0.5}$ were $0.0076d^{-1}$ and 91.2d, and $0.015d^{-1}$ and 45.6d at pH=7.4 and pH=5.0 respectively (Table 3.8). These values showed good agreement

Table 3.6. Rate constants (K) and influx ($K.Q_{eq}$) of 137-caesium accumulation in tissues of brown trout (*S. trutta*), and Atlantic salmon (*S. salar*) at two acidities. K in $d^{-1} \pm 1$ S.E.; $K.Q_{eq}$ in d^{-1}. N.D. = no data, * indicates rejection of parameter owing to inaccuracy of fitted curve (see text). § Indicates significantly reduced accumulation at pH=5.0 (ANOVAR, $p<0.05$).

	Brown trout (*S. trutta*)		Atlantic salmon (*S. salar*)			
	pH=5.0		pH=7.4		pH=5.0	
	K	$K.Q_{eq}$	K	$K.Q_{eq}$	K	$K.Q_{eq}$
Whole-body	0.021 ± 0.006	0.147	0.018 ± 0.003	0.180	0.017 ± 0.002§	0.099
Gills	0.082 ± 0.029	0.075	0.034 ± 0.010	0.183	0.015 ± 0.004§	0.030
Liver	0.017 ± 0.011	0.059	0.026 ± 0.008	0.184	0.016 ± 0.005§	0.048
Kidney	0.036 ± 0.015	0.131	0.041 ± 0.008	0.169	0.024 ± 0.005	0.100
Gut	0.014 ± 0.006	0.018	0.046 ± 0.011	0.221	0.004 ± 0.002§	0.008
Muscle	0.008 ± 0.002	0.079	0.014 ± 0.003	0.127	0.013 ± 0.003§	0.063
Bone	0.012 ± 0.003	0.013	0.016 ± 0.008	0.094	0.004 ± 0.001§	0.008
Brain	0.012 ± 0.001	0.040	0.028 ± 0.008	0.146	N.D.	N.D.
Blood	*	*	0.021 ± 0.006	0.038	0.023 ± 0.006§	0.031

Table 3.7. Biological half-lives ($t_{0.5}$) of 137-caesium elimination from tissues of brown trout (*S. trutta*) at pH=5.0, and Atlantic salmon (*S. salar*) at pH=7.4 and 5.0. $T_{0.5}$ in d ± S.E. N.D. = no data, * indicates rejection of parameter owing to inaccuracy of fitted curve (see text).

	Brown trout (*S. trutta*)	Atlantic salmon (*S. salar*)	
	pH=5.0	pH=7.4	pH=5.0
Whole-body	33.0 ± 9.4	38.3 ± 6.4	41.3 ± 4.9
Gills	8.5 ± 3.0	20.4 ± 6.0	46.2 ± 12.3
Liver	40.8 ± 26.4	26.7 ± 8.2	43.3 ± 13.5
Kidney	19.3 ± 8.0	16.9 ± 3.3	28.9 ± 6.0
Gut	49.5 ± 21.2	15.1 ± 10.6	36.9 ± 18.5
Muscle	86.6 ± 21.7	49.5 ± 7.1	53.3 ± 12.3
Bone	57.8 ± 14.5	43.3 ± 21.7	66.6 ± 16.7
Brain	57.8 ± 4.9	N.D.	N.D.
Blood	*	33.0 ± 10.4	30.1 ± 7.9

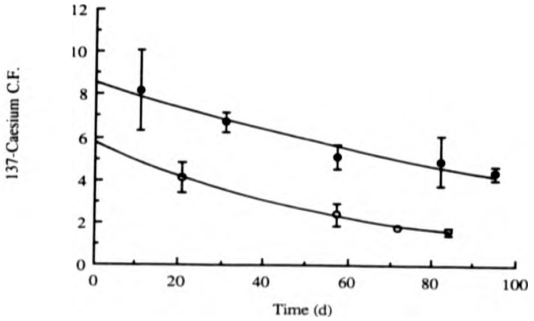

Fig. 3.7. 137-Caesium excretion from juvenile Atlantic salmon (S. salar) at two acidities; pH~7.4 (filled circles) and pH~5.0 (open circles). Data are mean C.F. ± 1 S.D.

with those obtained from the accumulation of 137-caesium at pH=5.0 but not at pH=7.4 (Tables 3.6 and 3.7). The rate constant of whole-body elimination of 137-caesium was not, however, significantly different at the two acidities ($0.10>p>0.05$, t-distribution analysis). The C.F.s at any time during excretion were significantly greater at pH=7.4 ($p<0.05$, t-distribution analysis) simply as a result of the higher initial C.F.

Tissue analysis

The excretion of 137-caesium from the tissues at both acidities was best described by single exponential rate functions. The data for some of the tissues were however, much more variable than those for the whole-body and therefore the curves fitted were less accurate. The values of the rate constant K, and the resulting values of $t_{0.5}$ were again rejected if the critical value of the correlation coefficient, r exceeded $p = 0.05$ (Table 3.8). The agreement between accumulation and depletion-derived estimates of the same parameters was in many cases poor, reflecting the large scatter of the tissue C.F. data in general (compare Tables 3.6 and 3.7 with 3.8). Where such disagreement occurred, the accumulation parameter estimates were used as these were derived from much larger data sets. The elimination-derived parameters were therefore not analysed in as much detail as the accumulation-derived parameters.

DISCUSSION

The pattern of 137-caesium accumulation in newly-fertilised brown trout eggs (Fig. 3.2) was similar to that observed for 24-sodium in freshly-stripped eggs of salmon (*S. salar*) by Rudy and Potts (1969). They noted an increase in activity of the eggs

Table 3.8. Rate constants (K) and biological half-lives ($t_{0.5}$) of 137-caesium elimination from tissues of Atlantic salmon (*S. salar*) at two acidities. K in d^{-1} ± S.E.; $t_{0.5}$ in d ± S.E.). * Indicates rejection of parameter owing to inaccuracy of fitted curve (see text).

	pH=7.4		pH=5.0	
	$K(d^{-1})$	$t_{0.5}$(d)	$K(d^{-1})$	$t_{0.5}$(d)
Whole-body	0.008 ± 0.001	91.2 ± 11.4	0.015 ± 0.002	45.6 ± 6.8
Gill	0.010 ± 0.002	70.0 ± 14.0	0.031 ± 0.025	22.4 ± 18.1
Liver	0.017 ± 0.004	40.8 ± 9.6	0.014 ± 0.005	49.5 ± 17.7
Kidney	0.016 ± 0.003	43.3 ± 8.1	0.010 ± 0.004	69.3 ± 27.7
Gut	0.013 ± 0.004	53.3 ± 16.4	0.026 ± 0.008	26.7 ± 8.2
Muscle	*	*	0.020 ± 0.004	34.7 ± 6.9
Bone	*	*	0.010 ± 0.004	72.9 ± 29.2
Brain	0.008 ± 0.003	92.4 ± 34.7	0.030 ± 0.009	23.1 ± 6.9
Blood	0.022 ± 0.003	31.5 ± 4.3	0.016 ± 0.015	43.3 ± 40.5

over 1.5h followed by a slight decrease 137-Caesium reached a maximum more rapidly and the subsequent decrease was more marked. It is possible that this decrease was due to the loss of 137-caesium bound to mucus on the surface of the egg which was subsequently shed; it was noted that the newly-fertilised eggs were "sticky" but that this condition only lasted briefly.

These results can be related to the ionic regulation of the eggs during development. When salmonid eggs are shed into fresh water, water is absorbed and the eggs swell and become turgid - a process known as water-hardening. During this process the vitelline membrane surrounding the yolk becomes increasingly impermeable and the perivitelline fluid (pvf) is formed as high molecular weight colloidal substances are secreted from the yolk into the perivitelline space. The outer chorion (zona radiata) remains freely permeable to water, ions and small molecules. The colloids in the pvf are however, too large to permeate the chorion and hence the pvf exerts an outward pressure and the eggs become hardened (Eddy, 1974).

The sodium exchange in freshly-stripped salmon eggs was confined to the pvf and chorion. In fresh water, sodium was in fact concentrated by the perivitelline colloids which possessed a net negative charge (Rudy and Potts, 1969; Shephard and McWilliams, 1989). Eddy *et al.* (1990) observed that the chorion exhibited little selectivity to monovalent cations and was unable to distinguish between Na^+ and K^+. It seems therefore, that Cs^+ behaved in a similar manner. Very little radioactivity was recorded in the embryo of brown trout eggs (Table 3.1). In contrast, significant activity was present in the pvf and it is possible that the activity recorded in the embryos was

in fact due to contamination with pvf which was very difficult to avoid. The C.F. of 137-caesium in the pvf itself was approximately 1.5 (Table 3.1; cpm g^{-1} water = 2500). This is significantly lower than the maximum C.F. recorded for sodium (C.F. =9.5) in the pvf of Atlantic salmon eggs by Rudy and Potts (1969). This suggested either that the chorion did, in fact, select against caesium compared to sodium or that caesium was not bound and concentrated to the same extent by the perivitelline colloids.

Brown trout eggs incubated in 'clean' water over the first 10d of development and subsequently transferred to water containing 137-caesium, equilibrated within 24h to a C.F. identical to that of the eggs that had been placed in radioactive water immediately post-fertilisation. This provides further evidence that the chorion remains permeable to ions and that only a limited fraction of the egg is available to ionic uptake. Shephard and McWilliams (1989) proposed that salmon embryos accumulate sodium in the later stages of development. This suggests that changes in the permeability of the vitelline membrane take place and may explain the sudden increase in 137-caesium C.F. in the brown trout eggs immediately prior to hatching.

The accumulation of 137-caesium in brown trout and Atlantic salmon alevins followed first-order rate kinetics (Fig. 3.3). Whether or not the accumulation from water occured entirely via the gills was uncertain. The alevins of fresh water salmonids have a fully-developed and functional branchial system in the later stages of development and are therefore assumed to osmo/iono regulate in a similar way to post-larval fish (Rombough and Garside, 1984; Lacroix *et al*., 1985; Reader *et al*., 1988). Petersen and Martin-Robichaud (1986) found that salmon (*S. salar*) alevins suffered

sodium loss at low pH - a result that has been well-documented in mature salmonids. Newly-hatched alevins may however, utilise the integument for osmotic and ionic regulation for the first few days post-hatching (Talbot *et al*., 1982; Petersen and Martin-Robichaud, 1986). Extrabranchial chloride cells are known to develop on the embryo prior to hatching and these could be utilised before switching to juvenile branchial and renal function. The timing of appearance and location of such chloride cells is however, not well known and appears to vary greatly between species (Alderdice, 1988). The timing of the transition to juvenile osmotic and ionic regulation is similarly little known.

The C.F.s achieved in the alevins (Fig. 3.3) were much larger than those of juvenile fish (Fig. 3.4) and the rate of accumulation was significantly lower in the latter. No obvious change in the pattern of accumulation of 137-caesium was seen during larval development however, and it seemed unlikely therefore, that the excessive 137-caesium C.F.s in the alevins were due to changes in the regulatory mechanisms involved. Previous studies have found that the accumulation of radiocaesium was inversely proportional to size (Morgan, 1964; Hewett and Jefferies, 1976 - see below). The C.F.s reached by the alevins compared to the juveniles in the present experiments were however, greater than would be expected if the difference were due to size alone.

One significant difference between the alevins and juveniles however, was that the former could not initially utilise food as a source of ions. Approximately 65% of the sodium, 45% of the potassium and 75% of the calcium required by the alevin prior to the commencement of exogenous feeding must be absorbed directly from the

environment via the skin and/or the gills if present (Rombough and Garside, 1984). Given the postulated similarity in chemical behaviour between potassium and caesium (Chapter 2) this extra demand for dissolved ions could explain the excessive accumulation of 137-caesium in brown trout and Atlantic salmon alevins. It was noted that the C.F.s in the alevins appeared to reach equilibrium soon after the start of feeding.

Alevins also have a greater metabolic rate than do juvenile fish. Metabolic rate (MR) is related to weight as:

$$MR = aW^b$$

Where W = weight

a,b = constants.

The value of b has often been quoted as 0.8 after Winberg (1956) such that larger fish have a smaller MR per unit mass (Fry, 1967; Brett and Groves, 1979; Rombough, 1988). Moreover, Winberg's value of the exponent b was based on juvenile and adult fish and this value decreased as development proceeded (Rombough, 1988) such that the relative MR of larval fish was increased further. Kamler (1976) found that in carp, b = 0.97 in feeding larvae whilst that of postlarvae was 0.8.

Odum and Golley (1963) proposed that the elimination of radioisotopes, in particular 65-zinc, could be used as a measure of metabolic rate. The evidence to support this is, however, somewhat conflicting. Edwards (1967) found that elimination of 65-zinc from juvenile plaice (*Pleuronectes platessa*) was proportional to metabolic rate. In contrast, Hoss *et al* (1978) concluded that the study of radioisotope elimination

was not a useful tool for estimating metabolism of fish. Investigations of metabolism in a number of other animals have however, used this method, including the use of 137-caesium elimination from leaf beetles, *Chrysomela knabi* Brown, by Crossley (1966). If the turnover of a radioisotope increases with MR in a similar manner, then it is possible that the higher MR of the alevins in experiment two contributed to their greater accumulation of 137-caesium.

137-Caesium accumulated to a level several times greater than that in the surrounding water in juvenile Atlantic salmon and brown trout at both acidities (Figs 3.4 and 3.5). The accumulation of 137-caesium at "normal" pH (≈7.4) in Atlantic salmon and brown trout was of a similar magnitude to that recorded for juvenile fish of other freshwater species (Williams and Pickering, 1961; King, 1964). When compared to larger fish, the accumulation of 137-caesium recorded was faster and achieved greater C.F.s. In this experiment the time to reach a C.F. of one was only three days for brown trout and five days for Atlantic salmon. Morgan (1964) recorded a time of 30-40d for *S. trutta*, average weight 7g, to reach a C.F. of one for 134-caesium.

Hewett and Jefferies (1976) studying 137-caesium accumulation in four sizes of brown trout (*S. trutta*) from 9-710g recorded rate constants (K) of 0.0073-0.0184d^{-1} and times to reach (t_{eq}) equilibrium of 180-300d. In the present experiment the values for brown trout at pH=7.4 were 0.0256d^{-1} and 160d respectively. Hewett and Jefferies (1976) also observed an inverse relationship between size and equilibrium concentration factor: C.F. decreased as weight increased as $W^{n.23}$. The results from the present

experiment fit that trend:

Mean weight of brown trout (g)	137-Caesium C.F. at equilibrium	
710	1.8	
178	3.6	Hewett and Jefferies
24	4.4	(1978)
9	5.5	
0.9	11.7	This report

Low environmental pH significantly reduced the rate of 137-caesium accumulation in both Atlantic salmon and brown trout. No data concerning radiocaesium accumulation at different acidities were found in the literature. The effects of acid stress on fluxes of other Group-I metal ions are however, well-documented. It has been established, for example, that at reduced pH potassium is lost from the fish (Booth *et al.*, 1982, 1987) and that the majority of this loss is via the gills (McDonald and Wood, 1981; McDonald, 1983).

Sodium has been studied more extensively. At low pH sodium influx across the gills decreases and sodium efflux increases (Packer and Dunson, 1970; McWilliams and Potts, 1978; McWilliams, 1980; Potts, 1980) resulting in a decrease in plasma sodium concentration (Lacroix and Townsend, 1987). During prolonged exposure, uptake does not recover but passive efflux falls back to approximately control levels resulting in net branchial ion losses (Booth *et al.*, 1987). In the present investigation, "low" pH

decreased the whole-body influx of 137-caesium by approximately 50% in both brown trout and salmon (Table 3.6) whereas the whole-body rate constants were much less affected by increased acidity.

The mechanisms by which this occurs are not certain. McWilliams (1980) suggested that changes in the transepithelial potential (TEP) of the gill increased sodium efflux; in acid exposed brown trout (*S. trutta*) gill potentials became more positive (McWilliams and Potts, 1978) increasing the workload of the sodium pump. If Na^+ were exchanged for H^+ (Maetz, 1973; Heisler, 1988) an increase in external H^+ concentration would increase the gradient against which H^+ had to be expelled, further inhibiting sodium uptake (Potts, 1980). Wood (1988) concluded that in the light of recent research however, changes in TEP were unlikely to explain these results and that "the blockade of sodium influx by acid is almost certainly due to a direct competition of H^+ with Na^+ for the transport sites and/or access channels to the carrier". It is possible that increased external acidity reduces 137-caesium accumulation by inhibition of Cs^+ uptake in a similar manner. Further research is required however, to ascertain the mechanisms involved.

The biological half-life of 137-caesium in salmon and brown trout, 36.4 and 27.1d respectively, was similar to that of the long component of 137-caesium excretion in juvenile rainbow trout, *O. mykiss*; 25d, (Hüsänen 24 *et al*., 1967) and in post-larval flounder, *Paralichthys dentatus*; 36.9d, (Baptist and Price, 1962). The biological half-life of the slow component of 137-caesium excretion in 2-3yr-old perch (*Perca fluviatilis*), roach (*Leuciscus rutilus*) and rainbow trout (*O. mykiss*) was 175, 55 and 80d

respectively (Häsänen *et al*., 1967). Increased acidity had little effect on whole-body biological half-lives (Table 3.7).

The accumulation of 137-caesium was considerably greater in brown trout than in Atlantic salmon both in alevins and in the juveniles at the two acidities. This difference was only significant however, in the juveniles at low pH. Whilst it is possible that the differences were due to the difference in average weight between the juvenile Atlantic salmon and brown trout, a difference (although not significant) was also apparent between alevins of the two species, which had the same average weight. Parry (1960) observed that the osmoregulatory ability of Atlantic salmon parr was greater than that of brown trout when these were transferred to hyperosmotic media. This was confirmed by Talbot *et al.* (1982) who noted that sodium uptake in 100% seawater was markedly lower in Atlantic salmon alevins when compared to brown trout. It was suggested however that this difference was simply due to size and therefore surface area : volume ratio. In fresh water no differences were seen. It remains a possibility however that differences occur in the osmoregulatory mechanisms between Atlantic salmon and brown trout in the early stages of development (before smoltification) but the present experiments provide no conclusive evidence to support this.

137-Caesium was found in all tissues analysed, indicating a general availability to caesium uptake (Table 3.4 and 3.5). The turnover of 137-caesium was most rapid in kidney, gill and gut. All of these tissues have important roles in osmotic and ionic regulation. Whilst the loss of solutes via the urine is well-known in freshwater fish one

would expect the gills and the gut to be the sites of active ion uptake and therefore have the greatest turnover of 137-caesium. This was in fact the case at "normal" pH. This does however present the possibility that food may have played a significant role in the 137-caesium accumulation experiments in spite of the experimental design. Imbibed water, both from drinking and ingestion of food (Tytler *et al.*, 1990) may also have contributed to the influx of 137-caesium via the gut.

The greatest 137-caesium C.F.s were consistently recorded in muscle (Tables 3.4 and 3.5). Muscle is the most massive tissue/organ in salmonids. Bainbridge (1960) found that in trout (*O. mykiss*) with a body length of 7cm, locomotory muscle comprised 55% of the body mass. Using Bainbridge's value, juvenile brown trout in this experiment (pH=7.4) would have had approximately 80% of the 137-caesium accumulated at equilibrium located in muscle tissue. The rate constants (K) of muscle were the lowest recorded in any of the tissues (Table 3.6) and hence the biological half-lives were relatively long (Table 3.7). The influx of 137-caesium in muscle was not extreme. This suggests that the large accumulation of 137-caesium in muscle is due to slow rate of elimination rather than a rapid influx. This provides further, if circumstantial, evidence for the similarity in behaviour between caesium and potassium as all muscle is relatively rich in potassium. Conway (1957) found the intracellular potassium concentration in frog skeletal muscle to be approximately 55 times greater than the plasma concentration whilst in humans muscle had approximately 20% greater potassium concentrations than other soft tissues (Kernan, 1980). Similarly bone, which had low 137-caesium C.F.s, was also low in potassium (Kernan, 1980).

Low pH simultaneously reduced 137-caesium influx and increased biological half-lives in the majority of tissues studied (Tables 3.6 and 3.7). The relative effect of increased acidity was however, greater on the former than on the latter. This confirms that it is an inhibition of influx rather than a stimulation of efflux that is the cause of reduced 137-caesium accumulation at low pH.

Similarities between the effects of low pH on the behaviour of caesium and other Group I metals could be seen. Packer and Dunson (1970), McWilliams and Potts (1978) and McWilliams (1980) all noted significant decreases in muscle and plasma sodium concentrations at low pH. In contrast to sodium however, potassium losses at reduced pH do not originate from the plasma but from the intracellular fluid volume (ICFV), and indeed plasma potassium concentrations may increase (Fugelli and Vislie, 1980; Wood, 1980). The majority of this loss was from muscle tissue (McDonald and Wood, 1981; Stuart and Morris, 1985) which reflects the large relative mass of this tissue. The expected increase in plasma, and hence blood, 137-caesium concentration however, was not apparent.

The elimination of 137-caesium from the whole-body and the tissues of juvenile Atlantic salmon was described by a single exponential equation. Most studies of whole-body radiocaesium excretion described a two-component exponential function in which the "slow" component comprised the majority of the radioactivity (Baptist and Price, 1962; Hüsänen *et al*., 1967; Kolehmainen and Miettinen, 1967; Nelson, 1967). These components do not necessarily represent excretion from different compartments. The "slow" component is usually seen as a linear "tail" on a depletion curve of $\log_{10}$ % 137-

caesium remaining vs time (Baptist and Price, 1962). Such a "tail" was not apparent in the present experiments although it is possible that further samples would have produced this feature. The whole-body excretion of 137-caesium from the common goby (*Acanthogobius flavimanus*) however, followed a single function (Kimura, 1984). The elimination of 65-zinc from plaice, *Pleuronectes platessa*, (Pentreath, 1973) and inorganic 203-mercury from rays, *Raja clavata*, (Pentreath, 1976) were also best described by single exponential functions.

The elimination of 137-caesium from juvenile Atlantic salmon was not significantly affected by increased acidity. The significantly greater C.F.s at pH=7.4 during the elimination experiment were merely a result of the increased accumulation before the fish were moved to non-radioactive water (Fig. 3.7.). This fits the proposed theory (Booth *et al.*, 1987) that during prolonged exposure to increased acidity branchial influx of Group-1 cations is inhibited but efflux recovers to control levels.

The values of K and $t_{0.5}$ obtained for the various tissues in salmon from accumulation and elimination curves should theoretically, have been equal whilst in fact, they differed greatly. At "normal" pH, the rate constants obtained from the accumulation experiments were consistently greater than those derived from elimination, but at "low" pH no clear pattern was apparent. The vast majority of the curves fitted to both sets of data were however, significant at p=0.05. This therefore presented a problem as to which set of tissue rate constants and biological half-lives were "correct". Although it might be expected that those for which elimination was measured directly would be used, the accumulation-derived parameters are presented here as "correct"

owing to the much larger data sets to which the curves were fitted. The elimination of 137-caesium from the tissues of Atlantic salmon is therefore only discussed with respect to the accumulation experiments (above).

CHAPTER 4: THE USE OF A PERFUSED, WHOLE-BODY PREPARATION TO MEASURE THE BRANCHIAL AND INTESTINAL INFLUX OF 137-CAESIUM IN THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM).

INTRODUCTION

The uptake of ions in freshwater teleosts.

The body fluids of fresh-water teleosts are osmotically more concentrated than the medium (hyperosmotic) and therefore they face a net influx of water and a net loss of salts. In order to balance these losses, ions must be taken up from the environment and water must be excreted.

The fluxes of sodium and chloride have been studied most extensively (Keys, 1931b; Krogh, 1939; Maetz and Garcia-Romeu, 1964; Kerstetter *et al.*, 1970; Maetz, 1971, 1973; Kerstetter and Kirschner, 1972; De Renzis and Maetz, 1973; De Renzis, 1975; Evans, 1975; Payan *et al.*, 1975; Evans and Cooper, 1976; Clairborne *et al.*, 1982) but a considerable number of cations have been shown to be taken up in fresh-water fish eg. cadmium (Pärt and Svanberg, 1982); calcium (Perry and Wood, 1985); potassium (Kerstetter and Kirschner, 1972; Eddy, 1985; Gardaire *et al.*, 1991) and zinc (Spry and Wood, 1988).

Caesium uptake.

A number of experimental studies have demonstrated that caesium may also be taken up from the environment by freshwater fish (Williams and Pickering, 1961; King, 1964; Morgan, 1964; Hewett and Jefferies, 1971, 1978). There are two principal routes by which this uptake may occur; directly from water across the gills and indirectly across the gut via contaminated food and any contaminated water ingested simultaneously. The relative importance of these two pathways has been evaluated by a number of authors. The uptake from water was measured by rearing fish in the laboratory in water to which radiocaesium was added, whilst any food given was initially free from radioactivity (Williams and Pickering, 1961; King, 1964; Morgan, 1964; Jefferies and Hewett, 1971; Pentreath and Jefferies, 1971; Hewett and Jefferies, 1976). Baptist and Price (1962), King (1964), Hewett and Jefferies (1978) and Kimura (1984) fed food containing radiocaesium to fish and measured the subsequent whole-body and tissue activity, whereas Pentreath and Jefferies (1971) and Hewett and Jefferies (1976) calculated the uptake from food based on models using data from environmental surveys. All studies cited food as a significant source of radiocaesium, with estimations (when made) of the proportion of the total body burden due to food varying from 42% (plaice, *Pleuronectes platessa*, Hewett and Jefferies, 1978) to 96% (common goby, *Acanthogobius flavimanus*, Kimura, 1984).

The above estimates however, are based on measurements of radiocaesium accumulation, ie. uptake - excretion, over relatively long time-courses. No attempts have previously been made to define the individual fluxes of radiocaesium across either the gill or gut epithelium. Therefore, it was decided to measure these, and determine

more accurately the relative importance of the two pathways of caesium uptake.

The measurement of branchial ionic fluxes

Two experimental approaches have been used in the study of branchial ion transport; the use of intact animals or the utilisation of some type of perfused gill preparation. In the basic intact animal experiment, the fish is simply placed in the medium, to which a radioisotope of the ion studied is added, and the depletion of counts in the water is monitored over an appropriate time course (Potts *et al.*, 1970; Eddy and Bath, 1979; McWilliams, 1980; Talbot *et al.*, 1982; 1983; Eddy, 1985; McDonald and Milligan, 1988; McWilliams and Shephard, 1989). In more sophisticated preparations the fish is surgically fitted with indwelling catheters such that continuous sampling of blood and/or urine can be performed (Kirschner *et al.*, 1973; McDonald and Wood, 1981; McDonald *et al.*, 1982; McDonald, 1983; Wood *et al.*, 1984). This ensures that only ions taken up across the gills are measured; in the "depletion" methods, losses of counts to mucus and skin, and even to the walls of the apparatus may produce significant errors.

The great advantage of these methods is the lack of disturbance to the normal physiology of the animal. The gills retain correct ventilation and perfusion, and neural and hormonal inputs remain. There are however, a number of disadvantages as noted by Perry *et al.* (1984). These include "...the inability to modify with precision the chemical composition of blood entering the gills, the difficulty in separating primary from secondary effects (eg. cardiovascular), and the impossibility of determining whether transport is across the basolateral or apical membranes." Moreover, the

problem of the physiological effects of handling stress arises. This has been shown to have significant effects on branchial ion fluxes (Maetz, 1974; Cameron, 1976) and although efforts may be made to minimise stress by confining the fish in darkened chambers well in advance of the experiment, uncertainties remain. Similar problems with intact animal preparations for the study of branchial ion transport were cited by Evans *et al.* (1982).

These problems have led many investigators to employ perfused gill preparations to examine branchial ionic transport. The principle behind perfusion techniques is that they allow close control of a number of factors which influence gill function, such as the composition, flow and pressure of the perfusate. In addition, the accuracy of flux measurements is increased. The disadvantages include the effects of anaesthesia if used, the introduction of unnatural stresses on the circulatory system and the slow but continuous deterioration of tissue function. A number of types of perfused preparation have been developed; these have been the subject of extensive reviews by Evans *et al.* (1982) and Perry *et al.* (1984).

Keys and co-workers were the first to describe a fish gill preparation where the fluids on both sides of the gill epithelium could be studied - the "heart-gill preparation" (Keys, 1931a, b; Bateman and Keys, 1932; Keys and Bateman, 1932; Keys and Willmer, 1932). Other preparations include the isolated, perfused branchial arch preparation (Richards and Fromm, 1970; Rankin and Maetz, 1971; Shuttleworth, 1972; Potts and Eddy, 1973; Kirschner *et al* , 1974; Shuttleworth and Freeman, 1974; Jackson and Fromm, 1980; Stagg and Shuttleworth, 1982) and the similar, isolated branchial

basket (Ostlund and Fange, 1962; Reite, 1969). Unfortunately, these are unable to acheive proper branchial irrigation. Even vigorous stirring of the bathing medium does not prevent the formation of boundary layers of fluid around the gill epithelium and these inhibit ionic transport (Shuttleworth and Freeman, 1974; Farmer and Evans, 1981). A further problem is that significant leakage of perfusate into the external medium is extremely difficult to prevent (Farrell *et al.*, 1979; Perry, 1981).

The most widely-used technique however, is the isolated, perfused head preparation (IPHP) used first by Payan and Matty (1975) and subsequently in a large number of studies (Payan *et al.*, 1975; Girard and Payan, 1976, 1977a, b; Payan, 1978; Clairborne and Evans, 1980; Pärt and Svanberg, 1981; Perry *et al.*, 1981; Perry *et al.*, 1984; Perry *et al.*, 1985; Spry and Wood, 1988). The perfused head is prepared by decapitating heparinised fish just posterior to the opercular openings. The gills are irrigated with water and perfused with an appropriate saline introduced via an input catheter inserted into either the bulbus arteriosus or the ventral aorta. The preparation allows close control over a number of important parameters such as external irrigation of the gills and the chemical composition and flow rate of the perfusate. It allows the separation of the efferent flow of perfusate from the gills such that it is possible to determine the ionic fluxes across respiratory cells and chloride cells in the gill epithelium of trout independently (Girard and Payan, 1976, 1977a, b; Clairborne and Evans, 1980).

There are however, a number of problems with this preparation. The most important is that the dorsal aortic pressure is normally at or around zero, attempts to

impose *in vivo* pressures artificially resulted in significant leakage of perfusate into the external medium (Pärt *et al*., 1984). Although this lack of back pressure will inevitably influence the flow of perfusate through the gills (Wood, 1974) very few studies have used physiological values of dorsal aortic pressure (Wood, 1974; Daxboeck and Davie, 1982). A further problem is the deterioration of the preparation, which is often countered by the inclusion of adrenaline or nor-adrenaline in the perfusate (Girard and Payan, 1977a; Payan, 1978; Girard and Payan, 1980; Perry *et al*., 1983; Perry *et al*., 1984). It is difficult however, to determine whether the stimulatory effects of adrenaline on ionic transport are specific, or due to accompanying haemodynamic alterations. One must also try to relate the preparation under these conditions to the *in vivo* situation.

When deciding on the most appropriate method to study the branchial influx of 137-caesium in the rainbow trout (*O. mykiss*) some further factors were considered. The simple depletion of counts from water by a non-catheterised intact animal could not be used as the proportion of counts that crossed the gill epithelium was very small. Small errors in sampling could therefore account for significant proportions of the actual flux and lead to large errors in calculation. It was decided to use a perfusion technique rather than a "flux chamber" intact animal preparation (McDonald and Wood, 1981; McDonald, 1983; McDonald *et al*., 1983) as the former allowed greater control over various physiological parameters (as explained above) and avoided the requirement to implant indwelling catheters.

The method used was a perfused whole-body preparation (see Materials and

Methods). This solved the main problem of the IPIP in that a dorsal aortic back pressure was provided by the post-branchial circulation. The surgery required was also simpler and less disruptive. Although it was not possible to separate the arterial and venous efferent flow from the gills, this was not necessary in this study.

The measurement of intestinal ion fluxes

The other major route for the uptake of ions in the aquatic environment apart from the gills is via the intestine. Marine teleosts are hyposmotic, and in order to compensate for the osmotic loss of water they drink the surrounding sea water. Large amounts of unwanted salts are simultaneously ingested and absorbed from the intestine and these must then be eliminated from the body. In hyperosmotic, fresh-water teleosts the main problem is one of osmotic water inflow, therefore drinking is minimal (Shehadeh and Gordon, 1969; Hirano, 1974). Ions may be taken in with food and the small volumes of water simultaneously ingested however, and these ions may be taken up across the intestinal epithelium. Spry *et al.* (1988) found that dietary sources of zinc were more important when the concentrations in water were relatively low.

A major advantage of studying ionic transport across the fish intestine is its functional stability at room temperature over relatively long periods (several hours) (Lahlou, 1983) in contrast to the fish gill which shows rapid *in vitro* deterioration of physiological function. Hence a number of *in vitro* techniques have been successfully developed to investigate physiological and cellular mechanisms in the fish intestine. The most common methods used to study ionic absorption are variations on the everted sac technique (Wilson and Wiseman, 1951; House and Green, 1963; Smith, 1964;

Ando, 1975; Ando and Kobayashi, 1978) and Ussing-type chambers (Ussing and Zerahn, 1951; Huang and Chen, 1973; Field *et al.*, 1978; Bell *et al.*, 1983; Colin *et al.*, 1985) although *in vivo* perfusion techniques have also been used (Shehadeh and Gordon, 1969; Skadhauge, 1969, 1974; Kirsch and Mayer-Gostan, 1973; Bogé *et al.*, 1979, Kirsch and Meister, 1982). In the particular case of sodium, the transepithelial electrical potential difference (P.D.) is determined by sodium movements (Field *et al.*, 1978) and hence, P.D. measurements can be made as an indicator of sodium flux (Huang and Chen, 1971; Ando, 1975; Ando and Kobayashi, 1978).

The principles of the sac and Ussing-chamber techniques are similar and straightforward. In both cases the intestine is carefully dissected out from the freshly-killed fish and small cylindrical sections of the area of the gut to be studied are isolated. If a sac method is to be used, one end of the section is tied off, the sac is filled with mucosal Ringer, the other end is tied off and the sac is suspended in a bath of serosal Ringer. If an Ussing-type chamber is to be used the intestine is opened out and a small section is clamped between the mucosal and serosal chambers. A radiotracer of the ion to be studied is introduced to one compartment and its appearance in the bathing medium on the other side of the intestine is monitored over an appropriate time-course. Both influx (mucosal-serosal) and efflux (serosal-mucosal) can be measured in this way. To measure the latter, the intestinal sac may be everted such that the serosal surface is on the inside (Wilson and Wiseman, 1951; Ando, 1975).

Although the intestine remains relatively stable during such *in vitro* techniques, the fluxes measured do not represent the *in vivo* situation. This is because the flux

measurements have been made across the entire intestinal wall including various layers of muscle and connective tissue. In the *in vivo* situation, ions crossing the intestinal epithelium would be transported away in blood vessels located mostly within the connective tissue. The fluxes measured are therefore likely to underestimate the real values. Ando and Kobayashi (1978) found that when the outer layers of intestine of the Japanese eel (*Anguilla japonica*) were stripped off the electrical P.D. increased four-fold. Unfortunately, it is not possible to strip the trout intestine in a similar manner (Lahlou, 1983).

In order to simulate *in vivo* conditions as closely as possible it was decided to use an *in situ* perfused gut preparation that allowed the influx of 137-caesium to be measured simultaneously across the entire intestine (see Materials and Methods). This chapter presents the results obtained using a perfused, whole-body preparations to study the branchial and intestinal influxes of 137-caesium in the rainbow trout (*O. mykiss*). In the previous chapter it was shown that the accumulation of 137-caesium from water in juvenile salmonids was significantly reduced at low pH. Therefore, the branchial influx of 137-caesium was also studied under acid conditions.

MATERIALS AND METHODS

Radioisotope and chemicals

137-Caesium (as CsCl in 1M HCl; 0.25μg Cs MBq^{-1} ^{137}Cs) was purchased from Amersham International, Amersham, U.K. Heparin (sodium salt), MS 222 (ethyl 3-

aminobenzoate methanesulphonate) and PVP-40 (polyvinylpyrrolidone, average molecular weight 40,000) were purchased from the Sigma Chemical Company Ltd., Poole, U.K.. All other chemicals were of AnalaR grade where available, from BDH Chemicals, Poole, U.K. or FSA Laboratory Supplies, Loughborough, U.K..

Animals

Rainbow trout (*Oncorhynchus mykiss*, Walbaum) weighing 200-450g were obtained from Swanswater Trout Farm, Sauchieburn, Stirling and College Mill Trout Farm, Almondbank, Perthshire. These were maintained indoors in large, circular, fibre-glass tanks (576l capacity) supplied with flowing, aerated, dechlorinated Stirling tap water. The water was moderately soft, typical major cation concentrations being Ca, 8mg l^{-1} (200 μM); Na, 2.7mg l^{-1} (120 μM) and K, 0.25mg l^{-1} (6.4 μM). Temperature was ambient and varied between 4 and 14°C and photoperiod was maintained at 10h light : 14h dark. The fish were fed on a diet of commercial trout pellets on an *ad lib* basis and starved for 4-5 days before use, to ensure that the intestine of the fish was fully evacuated.

Perfusion medium

The fish were perfused with a physiological saline based on Cortland saline (Wolf, 1963) but with some modifications (Perry *et al.*, 1984). This comprised (mM): NaCl, 124.1; KCl, 5.1; Na_2HPO_4, 2.9; $MgSO_4$, 1.9; $CaCl_2$, 1.4; $NaHCO_3$, 11.9; glucose, 5.6. Also included were 4% (w/v) PVP and 10,000 USP units l^{-1} heparin. Adrenaline/nor-adrenaline was not included in the saline but the solution was passed through a 0.22 μm Millipore filter before use, which has been shown to maintain high

branchial flow rates in the absence of catecholamines (Rankin and Maetz, 1971).

Surgical preparation

The initial stages of the preparation were the same for both the branchial and the intestinal flux measurements. The experiments were carried out at ambient temperature in a controlled temperature (C.T.) room at 10°C (±1.5°C). Each fish was anaesthetised to stage II plane 2 (after McFarland, 1959), ie. not responding to any external stimuli, with 0.05g l^{-1} MS 222 buffered to approximately pH 7.5 with sodium bicarbonate. The fish was injected intraperitoneally with 1ml heparin solution (1500 USP units ml^{-1} saline) which was allowed to circulate for 20 min before the fish was pithed using a metal seeker inserted via a small hole cut in the skull. This hole was plugged with "Blu-Tac" to prevent blood loss.

The fish was placed ventral-side-uppermost in a fibre glass holder (Fig. 4.1). The gills were ventilated using a continuous-circulation centrifugal pump (Eheim) at a rate of 0.5-0.75l min^{-1} via a moulded "Blu-Tac" mouthpiece. A minimal volume of water (0.5l) was used to reduce the amount of radioisotope required in the branchial influx experiments. The water was aerated as it drained through small holes in the base of the holder into the reservoir below. The head of the fish was surrounded by a circular well such that the gills were bathed continuously.

An incision was made in the ventral body surface posterior to the head along the *linea alba* and through the pericardium to expose the heart, the incision being held open using surgical retractors. Any minor blood vessels severed were cauterised with a hand-

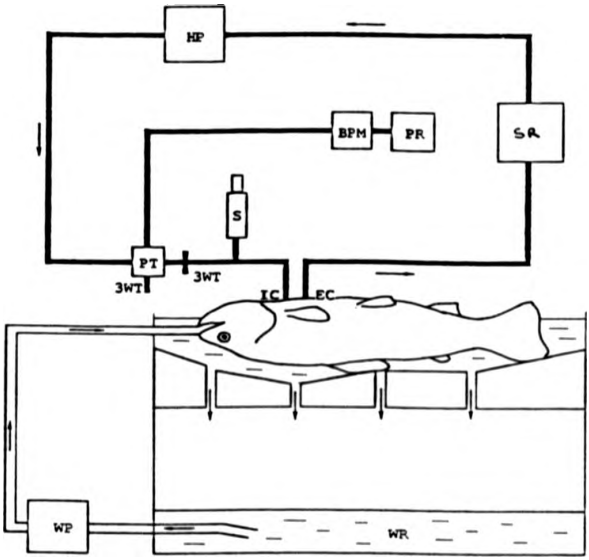

Fig. 4.1. The perfused whole-body preparation of the rainbow trout (*O. mykiss*) for the study of branchial and intestinal influx of 137-caesium. BPM, blood pressure monitor; EC, exit catheter; HP, heart pump; IC, input catheter; PR, pen recorder; PT, pressure transducer; S, syringe (windkessel); SR, saline reservoir; WP, water pump; WR, water reservoir; 3WT, three-way tap.

held, battery-powered cauteriser, and excess blood was removed using a syringe with a blunt, wide-bore needle.

The catheterisation used was the same as that in the spontaneously ventilating, blood-perfused trout preparation (SVBPTP) as described by Davie *et al.* (1982). Two silk ligatures were positioned under the bulbus arteriosus and a transverse incision was made close to the ventricle into which two heat-flared, PVC catheters were inserted and tied-off. The input catheter (Portex, 200μm outside diameter) was inserted anteriorly into the bulbus whilst the output (drain) catheter (Portex, 150μm outside diameter) was directed posteriorly through the ventricular/bulbous valve into the ventricle (Fig. 4.2).

The preparation was perfused via a small rodent heart pump (Harvard Apparatus model 1407) which provided a constant, pulsatile flow. This has been shown to be preferable to non-pulsatile perfusion (Bergman *et al*., 1974; Daxboeck and Davie, 1982). The stroke rate and stroke volume were set within physiological limits at 40min^{-1} and 0.07-0.15ml respectively to produce a flow rate of approximately 13ml min^{-1} kg^{-1}. A pressure transducer was fitted into the input via a T-junction to measure pulse pressures in the branchial vasculature and these were continuously monitored on a pressure box (CIB Blood Pressure Monitor, no.8138). Any preparation which developed pressures outwith the physiological range (>70mm Hg) was rejected. Once the circulatory system had been cleared of blood (20 min) the output catheter was directed into the saline reservoir to create a closed, re-circulating system.

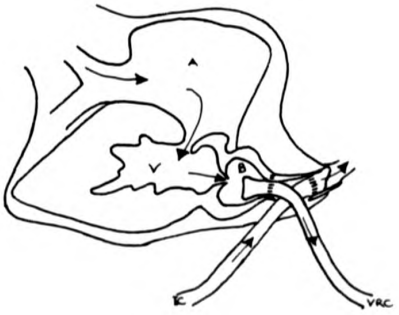

Fig. 4.2. Schematic sagittal section through the heart of the trout, to illustrate catheter positions used for perfusion experiments. Top of the page is dorsal, right is cranial. A, atrium; B, bulbus arteriosus; IC, input catheter into bulbus arteriosus/ventral aorta leading to gills; V, ventricle; VRC, venous return catheter, flow aided by ventricular contractions. Arrows within lumina of tubes and heart chambers indicate direction of blood flow. (From Davie *et al.*, 1982).

Measurement of the branchial influx of 137-caesium

Branchial 137-caesium influx was measured at four external caesium concentrations: 23.4nM (carrier-free 137-caesium only), 1mM, 3mM and 10mM. Background samples of perfusate and water were taken and carrier-free 137-caesium was added to the ventilating water at a concentration of 3.7MBq l^{-1} (100μCi l^{-1}). The additional caesium was added to the ventilating water as CsCl before the surgery took place.

Three 2ml samples were taken at five minute intervals from the reservoir of perfusate and at ten minute intervals from the water bathing the gills over a period of 50 min - one hour. These were counted for 137-caesium activity for ten minutes each in a gamma counter (Canberra-Packard A500C).

At the end of each experiment Evans blue dye was added to the ventilating water and allowed to circulate for a few minutes. The fish was then removed from the holder and the body cavity was opened to ensure that no radioactive water had entered the stomach and intestine. In early preparations the 2nd and 4th gill arches on the right-hand side of the head were excised and fixed in glutaraldehyde/cacodylate buffer to examine for any evidence of oedema in the secondary lamellae using scanning electron microscopy.

In order to determine the effect of low pH on 137-caesium influx, the apparatus and sampling procedure were somewhat modified: a pH probe (Radiometer, pHM 71b) was introduced into the well surrounding the head of the fish such that the pH of the

water passing over the gills could be monitored continuously. Sampling was delayed until 10min after the addition of 137-caesium and was carried out every 2.5min. After a further 20 minutes (t=30min), the pH of the water was reduced to approximately 5 by addition of 0.1M HCl. Further acid was added as necessary for a further 20min (ie. the total time of the experiment was reduced to 50min) to maintain an approximately constant pH of 5.0 (± 0.3).

For each preparation, a graph of total 137-caesium concentration in the perfusate (corrected for changes in total volume during the experiment) against time was plotted. In the first set of experiments, a straight line was fitted to all of the data, the slope of which was a measure of the rate of 137-caesium influx in cpm $^{137}Cs\ min^{-1}\ 100g^{-1}$ fish. This value was then converted to moles from the specific activity of caesium used in the preparation and expressed as J_{in} in standard units (moles $Cs\ kg^{-1}\ h^{-1}$. In the second set of experiments, two straight lines were fitted to the data, one either side of t=30 min to determine whether any change in slope could be observed on reduction of pH. The fluxes measured before and after the addition of acid from six individual preparations were compared statistically using a paired t-test (Minitab).

Measurement of the intestinal influx of 137-caesium.

The intestinal influx of 137-caesium was measured at three caesium concentrations; 0.6μM (^{137}Cs only), 100μM and 5mM. Samples of perfusate and water were taken before the start of the experiment to establish background levels of gamma-activity. 5.0μCi of carrier-free 137-caesium was added to 2ml trout saline made up to the required caesium concentration. The dye phenol red was also included in the saline

to check for leaks both during and after the experiment .

A purse suture was stitched around the anus using a surgical silk suture (Ethicon W200). The saline was introduced to the intact intestine *in situ* from a 5ml syringe via a 20cm length of PVC catheter tubing (Portex, 200μm outside diameter). The catheter was initially inserted as far as the stomach and then slowly withdrawn as the saline was discharged. Once the catheter was totally withdrawn the purse suture was tightened to seal off the posterior end of the intestine. At the end of each preparation the body cavity was opened up and if dye was found in either the peritoneum or the stomach on internal inspection the preparation was rejected. The pyloric sphincter of the stomach was found to provide an effective barrier to the forward movement of saline.

The entire intestine posterior to the stomach was removed from the fish and any fat deposits were dissected away. The excised intestine was cut longitudinally along its entire length, opened out and the gut contents and excess mucus gently scraped off. Any of the pyloric caeca that visibly had not been penetrated by the dye were also removed. The intestine was rinsed thoroughly with saline, dabbed dry on tissue paper and weighed. The total area of the intestine was also estimated by tracing the outline on a transparent sheet and placing this over a sheet of graph paper to count the squares enclosed.

The accumulation of 137-caesium in the perfusate, and any loss of 137-caesium via the gills to the ventilating water was measured by sampling, counting and graphical analysis as in the branchial influx experiments. It was observed that the rate of

intestinal caesium influx decreased with time during the individual preparations. The initial influx (cpm $^{137}Cs\ g^{-1}$ gut min^{-1}) was therefore calculated by two methods

1) A straight line was fitted to the initial part of the curve (STATGRAPHICS) and the influx was calculated from the slope, as was done for the branchial perfusions (above).

2) First-order rate equations were fitted to the data (Enzfitter, Elsevier Biosoft; STATGRAPHICS) of the form:

$$Q = Q_{eq}(1-e^{-Kt})$$

Where Q is the activity (cpm) of 137-caesium in the saline at time t (min), Q_{eq} is the equilibrium activity and K is the rate constant or "turnover" of 137-caesium (d^{-1}) between the gut and the circulation. Such equations have previously been fitted to radiotracer influx data by Motais and Isaia (1972) and Tytler and Bell (1989) and were used in Chapter three to describe the accumulation of 137-caesium in juvenile salmonids.

This equation was based on a linear differential equation:

$$dQ/dt = I-K.Q$$

Where I is influx of 137-caesium (a constant) and Q and K are as above. At equilibrium:

$$dQ/dt = 0$$

$$\Rightarrow \quad I = K.Q_{eq}$$

The intestinal caesium influx was therefore calculated from the parameters of the fitted first-order rate curves. Of the two methods, the second was preferred due to the

difficulty in accurately fitting straight lines to curvilinear data. The results were expressed in terms of moles Cs g^{-1} gut h^{-1} rather than in terms of area due to the difficulty in measuring accurately the area of the entire intestine, including the pyloric caeca. The estimated area showed a reasonable correlation with the weight of the intestine ($r = 0.851$) and therefore the units were converted wherever necessary for comparison with data from other studies.

RESULTS

Branchial influx

The perfused whole-body preparation used in these experiments proved to be successful; 137-caesium was easily measured in the internal perfusate. The gill epithelium remained fully intact (Plates 4.1 and 4.2), although an increased amount of mucus was observed on the gill surface at the end of the experiments.

Typical branchial perfusion plots of 137-caesium activity in the saline (ie. that which had crossed the gills) versus time for the four external caesium concentrations studied under "normal" conditions from individual preparations are shown if Figs. 4.3a-d. An initial "lag" period of 5-15min was seen in the majority of preparations. The reason for this was uncertain but it may have represented the time for caesium to equilibrate either in the external interlamellar spaces or on the mucus layer covering the gill surface. Similar "lags" were observed by Pärt and Svanberg (1981) and Spry and Wood (1988) for the branchial influx of cadmium and zinc respectively. This "lag" period was however, omitted from the influx analyses in the present experiments.

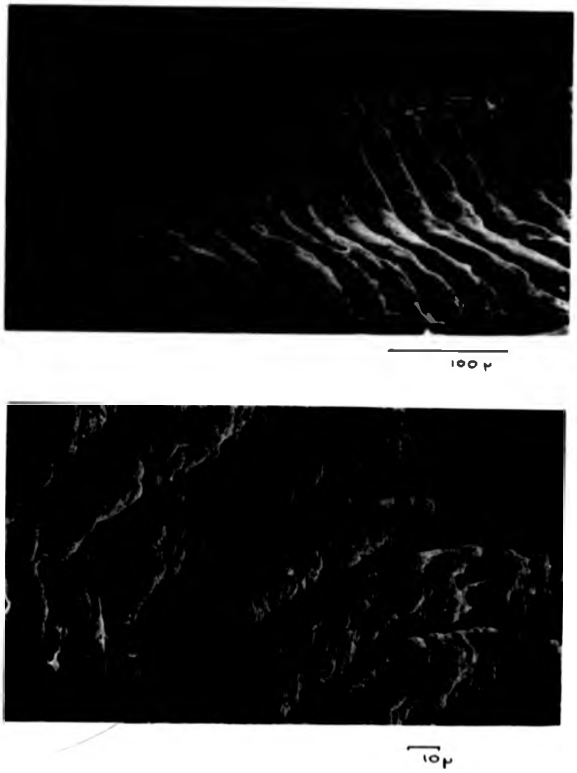

Plate 4.1. Scanning electron micrographs of secondary lamellae of unperfused (control) gills of rainbow trout (*O. mykiss*). Magnification: x200 (top), x490 (bottom).

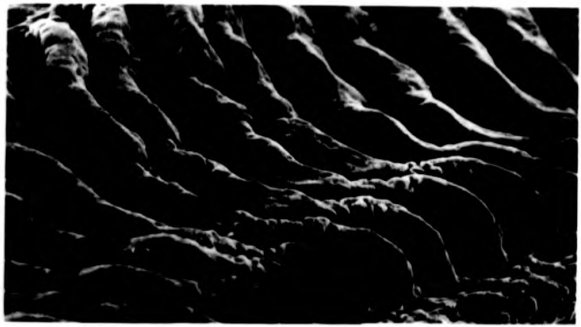

Plate 4.2. Scanning electron micrographs of secondary lamellae of gills from a perfused, whole-body preparation of rainbow trout (*O. mykiss*). Gills were saline perfused for 90 mins. No oedema of the secondary lamellae was apparent. Magnification; x194 (top), x430 (bottom).

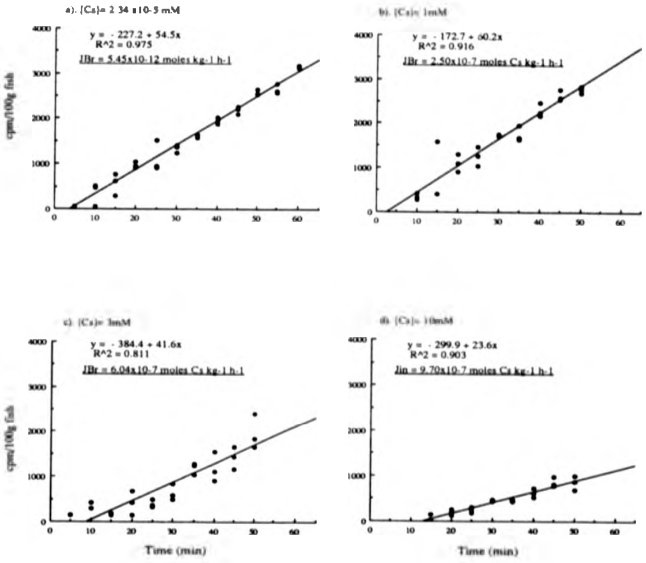

Fig.s 4.3a-d. Individual branchial caesium influx determinations: Typical plots of 137-caesium activity in the saline (cpm $100g^{-1}$ fish) versus time (min) at four external caesium concentrations in rainbow trout (*O. mykiss*). Caesium influx (J_{Br}) is calculated from the slope of the line.

Straight lines fitted the data well (R^2 = 0.811-0.975) and were highly significant ($p<0.001$; ANOVAR) in all cases (STATGRAPHICS). The resulting branchial caesium influx (J_{in}) is shown in each case. At the lowest external concentration of caesium, where only carrier-free 137-caesium was added to the water (3.7 MBq l^{-1} 137-caesium; [Cs]= 2.34 x 10^{-5} mM) the mean influx of caesium was 5.45 x 10^{-12} moles kg^{-1} h^{-1} (n=8). Considerable variation in individual influx determinations was recorded; the standard deviation was equal to 62% of the mean (± 3.38 x10^{-12}). (Table 4.1).

When Michaelis-Menten kinetics were fitted to the data (R^2 = 0.734, $p<0.001$; ANOVAR) the values of J_{max} and K_m were 1.05 x10^{-6} moles Cs kg^{-1} h^{-1} and 1.92 mM respectively (Fig. 4.4). Significant effects of reduced pH were seen in three of the six preparations carried out. These were inconsistent however, and overall there was no significant difference in the mean fluxes under "normal" and "acid" conditions (paired t-test, $p=0.25$, table 4.1).

Intestinal influx

The 137-caesium activity in the saline (ie. that which had crossed the intestine) versus time for typical intestinal perfusions at the three mucosal caesium concentrations studied are shown in Fig. 4.5a-c. The rate of 137-caesium accumulation in the saline gradually decreased throughout the preparations. First-order rate equations fitted the data well in all of the preparations (R^2 = 0.568-0.998, $p<0.001$; ANOVAR). In contrast to the branchial influx, the intestinal influx of caesium (J_{int}) did not display saturation

Table 4.1. Branchial influx (J_{Br}) of 137-caesium in rainbow trout (*O. mykiss*) at two acidities. J_{Br} in 10^{-12} moles Cs kg^{-1} h^{-1} ± 1 S.D. [Cs] = 0.58 μM. t = duration of experimental treatment (min).

pH	n	t	mean J_{Br}
6.4-6.8	8	60	5.45 ± 3.38
6.4-6.8	6*	20*	3.36 ± 1.53
4.6-5.2	6*	30*	4.25 ± 1.41

*Paired data, t = 1.31, p = 0.25 (paired t-test)

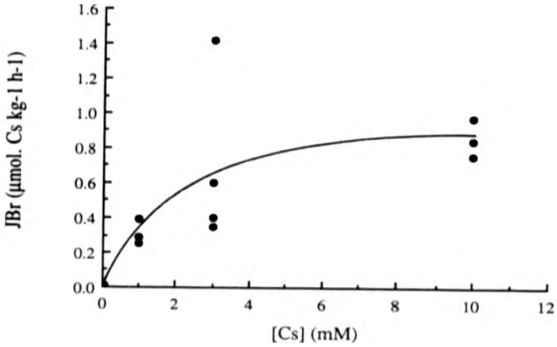

Fig. 4.4. Branchial caesium influx (JBr) as a function of external caesium concentration. Each point is an individual determination. The line is based on Michaelis-Menten kinetics ($p<0.001$, ANOVAR) and was fitted by computer.

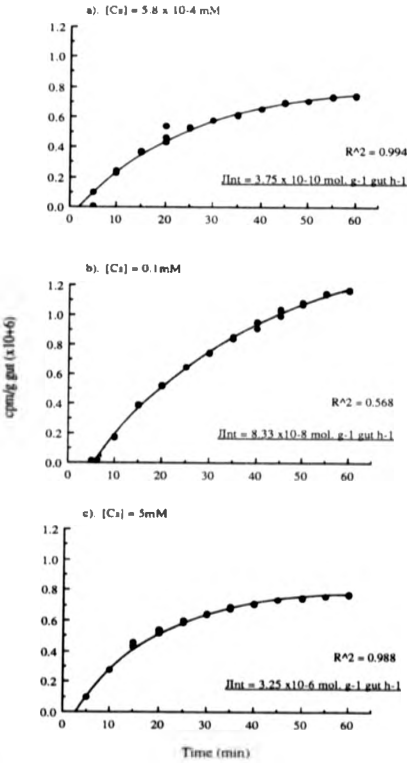

Fig.s 4.5a-c. Intestinal caesium influx determinations: Typical plots of 137-caesium activity in the saline (cpm g^{-1} gut) versus time (min) at three mucosal caesium concentrations in rainbow trout (*O. mykiss*). Caesium influx (J_{in}) is calculated from the initial slope of the curve.

kinetics over the range of caesium concentrations used. A plot of $\log_{10}$ caesium influx versus $\log_{10}$ mucosal caesium concentration was linear over the concentration range studied; $p<0.001$, ANOVAR (Fig. 4.6). The slope of the line was 1.0, indicating that the rate of influx was directly proportional to the mucosal concentration of caesium. In contrast, a log-log plot of branchial caesium influx was not linear (not shown).

A comparison of branchial and intestinal influxes at four caesium concentrations showed that the intestinal influx (moles Cs g^{-1} gut h^{-1}) was consistently greater than the branchial influx (moles Cs kg^{-1} fish h^{-1}), and that the difference increased with caesium concentration as the branchial influx approached saturation (Table 4.2). The intestinal influx could not be expressed accurately in terms of moles kg^{-1} h^{-1} as the mucosal saline did not consistently permeate the entire intestine. Only those areas of the intestine dyed by the phenol red included in the saline were considered to have been involved in the caesium influx. The measured intestinal caesium influx did not therefore correlate with the weight of the fish. When the difference in units of influx was taken into account (a 1kg fish would normally have an intestine of approximately 5-8g; I. Morgan, personal observation) the relative magnitude of the intestinal influx increased further, such that the intestinal influx was approximately 10-40 times the branchial influx.

DISCUSSION

The branchial caesium influx measured in the presence of 137-caesium only ($[Cs]$ = 23.4nM) was very small; 5.45×10^{-11} moles Cs kg^{-1} h^{-1}. Indeed, the rate of caesium uptake in the influx experiments was smaller than that recorded for the

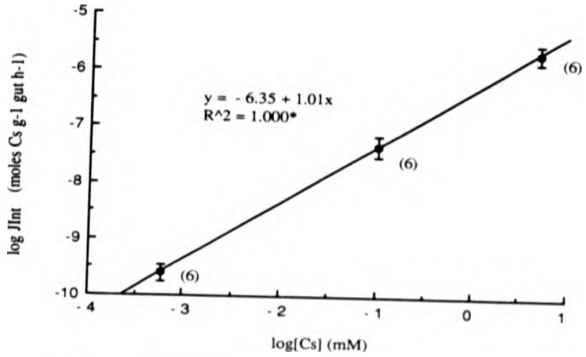

Fig. 4.6. Intestinal caesium influx (JInt) as a function of mucosal caesium concentration in the rainbow trout (Q. mykiss). Each point is the mean ± S.D. of the number of determinations in brackets. *($p<0.001$, ANOVAR; STATGRAPHICS).

Table 4.2. A comparison of recorded branchial influx (J_{Br}) with predicted intestinal influx (J_{Int}) at four external caesium concentrations in rainbow trout (*O. mykiss*). (* 1kg fish ≈ 5-8 g gut).

[Cs] (mM)	J_{Br} (mol Cs kg^{-1} h^{-1})	J_{Int} (mol Cs g^{-1}gut h^{-1})	J_{Int}/J_{Br}
2.4×10^{-5}	5.45×10^{-12}	1.25×10^{-11}	2.29
1.0	3.08×10^{-7}	4.78×10^{-7}	1.55
3.0	6.91×10^{-7}	1.42×10^{-6}	2.05
10.0	8.58×10^{-7}	4.67×10^{-6}	5.44

accumulation of 137-caesium in juvenile brown trout (Chapter 3), despite the much greater 137-caesium concentration and the fact that accumulation is a measure of net flux ie. influx - efflux (Table 4.3). The increased mucus layers observed on the gills may also have decreased the influx by increasing the diffusion distance for caesium ions (Spry and Wood, 1988). Previous work has shown that the rate of accumulation of caesium varies inversely with the size of fish (Morgan, 1964; Hewett and Jefferies, 1976) and hence the caesium influx recorded here is in accordance with these studies.

The 137-caesium concentrations used in the three experiments which are compared in Table 4.3 were, however, many orders of magnitude greater than those found in the environment. The maximum 137-caesium concentrations recorded in British fresh waters post-Chernobyl did not exceed 0.5Bq l^{-1} (1.88×10^{-11}M) (Camplin *et al.*, 1986). A 137-caesium concentration of 140 Bq l^{-1} (5.3×10^{-10}M) was however recorded on 28th April, 1986 in Lake Atnsjøen, Norway although this declined to less than 10Bq l^{-1} within a week (Brittain *et al*, 1991). At such caesium concentrations the curve fitted in Fig 4.4 predicted extremely small branchial caesium influxes of approximately 10^{-16} - 10^{-17} moles Cs kg^{-1} h^{-1}.

The saturable nature of the branchial caesium influx in rainbow trout suggests that caesium uptake is a mediated process dependent on a finite number of carriers or active sites. The branchial influx of sodium in freshwater fish both *in vivo* and *in vitro* has also been shown to display saturation kinetics (Kerstetter *et al.*, 1970; Kerstetter and Kirschner, 1972; Maetz, 1971, 1972, de Renzis and Maetz, 1973, Perry *et al*, 1985). The maximal branchial influx (J_{max}) of sodium however, is approximately 2-3 orders of

magnitude greater than that of caesium, and the external ion concentration at which $0.5J_{max}$ is acheived (K_m) is approximately 10 times less for sodium than for caesium (Table 4.4). The behaviour of caesium is however, considered to resemble more closely that of potassium (Chapter 2). Potassium has recently been shown to also display saturation uptake kinetics across the gills of rainbow trout (Gardaire *et al.*, 1991). The K_m and J_{max} values for potassium were 1.0mM and 75μmoles kg^{-1} h^{-1} respectively (Gardaire *et al.*, 1991). These values are much closer to those recorded for caesium in the present experiments than are the values in the literature for sodium (Table 4.4). The K_m of potassium influx is only approximately half of that measured for caesium, whilst the J_{max} of potassium is approximately seven times that of caesium.

Eddy (1985) recorded a potassium influx in rainbow trout (*O. mykiss*) of 70μmoles K kg^{-1} h^{-1} at an external potassium concentration of 0.05mM. This value is approximately three orders of magnitude greater than the corresponding caesium influx (this chapter) and is almost equal to the J_{max} for potassium recorded by Gardaire *et al.* (1991). The fish used by Eddy (1985) were however, ten times smaller (in terms of mass) than those used in the present experiments and by Gardaire *et al.* (1991), and the results of Chapter three suggested that caesium uptake per unit weight decreased as the size of the fish increased. Gardaire *et al.* (1991) concluded that branchial potassium influx in the rainbow trout (*O. mykiss*) occurred via specific potassium channels located on the secondary lamellae. The kinetic similarities between potassium and caesium influx suggest that branchial caesium influx also occurs via such channels.

Table 4.3. A comparison of rates of 137-caesium uptake (in 10^{-12} moles Cs kg^{-1} h^{-1}) recorded during measurement of branchial influx in rainbow trout (*O. mykiss*) with those recorded during the accumulation in brown trout (*S. trutta*) from two separate studies.

species	mean weight (g)	[^{137}Cs] (kBq l^{-1})	^{137}Cs uptake	source
(*O. mykiss*)	283	3700	5.45	^{137}Cs influx (This chap.)
(*S. trutta*)	0.9	130	8.01	^{137}Cs accum^{n}. (Chap. 3)
(*S. trutta*)	38.1	37	0.38	Hewett and Jefferies (1976)

Table 4.4. A comparison of branchial caesium influx with the influxes of other group-I metals in the rainbow trout (*O. mykiss*). Fluxes recorded as maximal value (J_{max}) and the external ion concentration at which $0.5J_{max}$ is achieved (K_m), or as value (J_{Br}) at a given ion concentration, [cation]. J_{max}, J_{Br} in moles Cs kg^{-1} h^{-1}, K_m and [cation] in mM.

cation	[cation] (mM)	J_{Br}	J_{max}	K_m	source
Na^+	0.1 - 6	-	4.54×10^{-4}	0.34	Perry *et al.* (1985)
K^+	0.1 - 10	-	7.50×10^{-6}	1.0	Gardaire *et al.* (1991)
Cs^+	5.8×10^{-4} - 10	-	1.05×10^{-6}	1.92	This chap.
K^+	0.05	7.00×10^{-5}	-	-	Eddy (1985)
Cs^+	0.05	2.14×10^{-8}	-	-	This chap.

In contrast to the results of Chapter three, reduced pH had no significant effect on the branchial influx of 137-caesium. This may have been due to the short time-course of the experiments. McDonald and Wood (1981) found that positive fluxes of sodium and chloride were maintained for the first twelve hours of acid exposure but after that period the fluxes decreased dramatically. Similar delays were observed in changes of plasma potassium concentrations in rainbow trout, *O. mykiss*, (Booth *et al.*, 1982) and plasma sodium and potassium concentrations in brown trout, *S. salar* (Stuart and Morris, 1985), although a delay in the effects of altered fluxes on plasma ion concentrations might be expected. Other studies have noted immediate effects of acute acid stress on sodium influx (McWilliams and Potts, 1978; McWilliams, 1980a, b; 1982a, b; Booth *et al*., 1987; Wood *et al*., 1987; Wood, 1988). The acute acidities used in these experiments (pH 4.4 - 4.8) were however consistently lower than that used in the present experiment (pH=5.0). Moreover, the dissolved ion concentrations used in previous studies, especially of calcium and aluminium, were not always comparable to those used in the present experiments. McDonald and Milligan (1987) found that under comparable concentrations of calcium (400μM) and sodium (145μM) to the present experiments, sodium influx was immediately affected only when the acute pH ≤ 4.8.

The conflicting nature of the data prevents a firm conclusion being reached on the absence of an effect of reduced pH on branchial caesium influx. It is also possible that the increased mucus layers observed during these experiments may have delayed the effects of increased acidity as was suggested earlier to explain the "lag" period seen at the start of the majority of the branchial influx preparations.

The intestinal caesium influx decreased rapidly in less than one hour following a single dose of caesium (Figs. 4.3a-c). Previous studies have measured only the accumulation of 137-caesium from fish intestine after either a single dose or daily doses, over time-courses measured in days (Baptist and Price, 1962; Hewett and Jefferies, 1976; Kimura, 1984). The initial influxes measured here cannot be compared accurately with such accumulation data. Intestinal influxes of sodium have however been studied in a number of freshwater or freshwater-adapted species of fish. The predicted intestinal caesium influxes are very similar to the recorded sodium fluxes in the freshwater-adapted trout (*O. mykiss*, Colin *et al.*, 1985) and goldfish (*Carassius auratus*, Ellory *et al.*, 1972), (Table 4.5).

In contrast to the branchial caesium influx, Fig. 4.6. indicates that intestinal caesium influx does not display saturation kinetics but is directly proportional to the mucosal caesium concentration. This type of relationship has previously been established for the uptake of sodium, chloride and calcium in the trout (*O. mykiss*, Lahlou, 1975), for sodium in the goldfish (*C. auratus*, Ellory *et al.*, 1972) and for a large number of anions in the intestine from eel (*Anguilla anguilla*, Bell *et al.*, 1983). These results are consistent with P.D. measurements *in vitro* which indicate that fish intestine is a "leaky" epithelium (Lahlou, 1983). It is likely therefore, that the majority of the caesium influx occurs by simple diffusion via leak pathways. The precise mechanisms of ionic transport across the fish intestine are however, uncertain. Ellory *et al.* (1972, 1973) concluded that sodium crossed the intestinal epithelium via mediated routes whilst Bell *et al.* (1983) suggested that the fluxes of oxyanions occurred across intercellular spaces and was not regulated. The present results fit the latter hypothesis;

the rapid decrease in intestinal caesium influx during the individual preparations suggested that the influx decreased as the chemical gradient across the intestinal epithelium became smaller.

Table 4.5. A comparison of intestinal caesium influx ($J_{int}Cs^+$) in rainbow trout (*O. mykiss*) with intestinal sodium influxes ($J_{int}Na^+$) in other freshwater fish. $J_{int}Na^+$ and $J_{int}Cs^+$ in μmoles Cs g^{-1} gut h^{-1}.

mucosal ion conc^n (mM)	$J_{int}Na^+$	$J_{int}Cs^+$	$J_{int}Na^+/J_{int}Cs^+$	source	species
156	64.0	71.1	0.90	Colin *et al* (1985)	Rainbow trout (*O. mykiss*)
140	44.0	65.7	0.67	Ellory *et al* (1972)	Goldfish (*Carassius auratus*)

CHAPTER 5. THE INTRACELLULAR BINDING OF 137-CAESIUM IN MUSCLE TISSUE OF THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*, WALBAUM)

INTRODUCTION

In the general introduction to this thesis (Chapter 1) it was seen that a considerable amount of data exists to show that radiocaesium accumulates in fish, especially in freshwater species, to concentrations many times greater than that in the surrounding water. This was confirmed for juvenile brown trout (*S. trutta*) and Atlantic salmon (*S. salar*) in the experiments of Chapter three. These experiments demonstrated that the greatest accumulation of 137-caesium occurred in muscle tissue and that this could account for some 80% of the total body-burden at equilibrium. Muscle also had the slowest rate of 137-caesium elimination; biological half-lives in excess of 400d were recorded at "normal" pH. Under environmental conditions, where the uptake of radiocaesium may be continuous, whole-body biological half-lives of the order of months or even years have been recorded (Gustafson, 1967; Häsänen *et al.*, 1967; Brittain, 1991).

A number of metal ions may bind to various ligands on organic molecules, including a number of amino acids, both free and within protein side chains (Martell and Smith, 1974). For example, carp muscle contains a calcium-binding protein

"parvalbumin" which has two calcium binding sites per molecule (Kretsinger and Nockolds, 1973). The requirement for calcium in muscle action is well-known; no such biochemical function has been determined for caesium. Caillé and Hinke (1973) produced evidence for the binding of potassium inside muscle fibers of the giant barnacle (*Balanus nubilus*) however, and given the similarity between caesium and potassium (Chapter 3), caesium could compete for any intracellular potassium binding sites, or indeed simply bind to any negatively-charged organic molecules in the tissue.

The experiments in the present chapter were therefore designed to determine the extent of the intracellular binding of caesium (particularly to proteins) in muscle tissue of rainbow trout (*O. mykiss*). This was done by measuring the binding of 137-caesium to two muscle-derived suspensions: a microsomal suspension, and a coarse muscle suspension prepared by removing only the largest cell fragments from a muscle homogenate. Microsomes are membrane-bound vesicles of 50-150nm diameter derived primarily from the endoplasmic reticulum but also from the cell plasma membrane. They are formed when cells are homogenised and then subjected to differential centrifugation. At slower speeds cell debris and larger organelles eg. nuclei and mitochondria sediment. At very high speeds (>100,000g) the microsomes, which are closed membrane fragments, often with ribosomes still attached to the outside, settle out. These provide a convenient membrane-bound protein preparation with which to examine intracellular caesium binding.

MATERIALS AND METHODS

Radioisotopes and chemicals

137-Caesium (as CsCl in 1M HCl; 0.25μg Cs MBq^{-1} ^{137}Cs) was purchased from Amersham International Plc., Amersham, U.K. Bovine serum albumin (BSA), Folin-Ciocalteu phenol reagent and HEPES (N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulphonic acid]), were purchased from the Sigma Chemical Company Ltd., Poole, U.K. All other chemicals were of AnalaR grade where available, from BDH Chemicals, Poole, U.K. or FSA Laboratory Supplies, Loughborough, U.K.

Animals

Rainbow trout (*Oncorhynchus mykiss*, Walbaum) weighing 200-450g were obtained from Swanswater Trout Farm, Sauchieburn, Stirling and College Mill Trout Farm, Almondbank, Perthshire. These were maintained in large (576l capacity), circular, fibre-glass tanks supplied with flowing, aerated, dechlorinated Stirling tapwater. The water was moderately soft, typical major cation concentrations being Ca, 8mg l^{-1} (200μM); Na, 2.7mg l^{-1} (120μM) and K, 0.25mg l^{-1} (6.4μM). The temperature was ambient and varied between 4 and 10°C and photoperiod was maintained at 10h light : 14h dark. The fish were fed *ad lib* on a diet of commercial trout pellets.

Preparation of "coarse" and microsomal suspensions from muscle.

Rainbow trout (*O. mykiss*) were killed by a sharp blow to the head. The fish were decapitated and gutted and a number of transverse sections ("steaks") were taken anteriorly to the dorsal fin and put on ice. The skin, sub-cutaneous fat and bones were

removed from 10-12g of white muscle. The isolated muscle was placed in a 50ml beaker in ice, together with 30ml ice-cold buffer solution. This buffer, used throughout the experiments, comprised 8% (w:v) sucrose in 10mM HEPES pH = 7.45 at 20°C. The muscle tissue was minced for 60s (2x30s) using a Polytron (Kinematica 10/35) and homogenised (12 strokes) with a teflon-in-glass barrel homogeniser at 500rpm (Tri-R Stir-R, model K43).

In order to produce the coarser muscle suspension, the homogenate was centrifuged once only; for 5min at 200g in a bench centrifuge, which removed only the larger cellular debris. The pellets were discarded and the supernatant was equilibrated to 10°C ready for use. In order to produce a microsomal suspension the initial homogenate was diluted to 190ml with ice-cold saline, stirred vigorously and centrifuged for 10min at 650g and 10°C to remove the larger debris. The pellets were discarded and the supernatant was centrifuged for 20min at 15,000g and 10°C in a Sorvall RC-5B Refrigerated Superspeed Centrifuge. The resulting pellets, consisting mainly of mitochondria with some microsomes, were discarded and the supernatant was spun at 120,000g for 1h in a MSE Superspeed 65 Mk.2 Ultracentrifuge (10°C). The final pellets, consisting primarily of microsomes, were removed from the centrifuge tubes with a clean spatula and were re-homogenised with 6 strokes at 700rpm in 6ml buffer. The microsomal suspension was diluted to 25ml with ice-cold buffer and equilibrated to 10°C ready for use. Samples of both the coarse and microsomal suspensions used were frozen for later protein determination (see below). The binding experiments were repeated three times for each of the muscle-derived suspensions.

Measurement of caesium binding

The binding of caesium to the rainbow trout (*O. mykiss*) muscle-derived microsomal suspension was measured in duplicate at five external caesium concentrations; 0.11μM (137-caesium only), 1mM, 3mM, 5mM and 10mM. The 137-caesium stock was diluted with buffer to produce a final activity of 50μCi ml^{-1}. 20μl of this solution were added to 2.5ml polycarbonate centrifuge tubes with the correct volume of 1M CsCl to achieve the required total caesium concentrations. The centrifuge tubes were equilibrated to 10°C (± 0.5°C) in a water bath. The binding reaction was started by the addition of 1.96ml of the test suspension. The tubes were shaken vigourously at regular intervals during the incubation, which lasted 1h, to achieve equilibrium binding. Caesium binding to the coarse trout muscle-derived suspension was measured in duplicate at 1, 5, 10, 25, and 50mM caesium. The procedure was similar to that outlined above except only 0.5ml of the test suspension was used per tube, the balance (1.46ml) being made up with buffer.

At the end of the incubations, the suspensions were spun down at 140,000g for 1h at 10°C (MSE). Two, 100μl samples of the supernatant were taken from each tube and the remaining supernatant was discarded. Each pellet was washed twice with 1ml clean, ice-cold buffer. The pellets and supernatant samples were analysed for 137-caesium activity in a gamma-counter (Canberra-Packard A500C). The bound and free concentrations of caesium were calculated from the 137-caesium activity of the two fractions and the specific activity of the isotope. No estimations of unbound caesium within the pellets was made, this was considered to be insignificant.

The amount of protein per tube was assayed by the method of Lowry *et al.* (1951) with Folin-Ciocalteu phenol reagent. Four solutions were prepared:

A) 10% Na_2CO_3 in 0.5M NaOH.

B) 0.5% $CuSO_4.5H_2O$ in 1% sodium-potassium tartarate.

C) 10ml of solution B was mixed with 100ml solution A. (Prepared daily).

D) Folin-Ciocalteu phenol reagent; the commercial reagent (Sigma) was diluted 1:11 with distilled water immediately prior to use. The method is suitable to measure protein concentrations of 0-500μg ml^{-1}. 1ml of solution C was added to 1ml of the microsomal suspension or 0.1ml of the coarse suspension (made up to 1ml with buffer) in a test tube. This was mixed and allowed to stand for exactly 10min. Three ml of diluted Folin-Ciocalteu reagent was added as rapidly and uniformly as possible to each tube and the contents were mixed thoroughly and allowed to stand for a further 10min. The optical density (O.D.) was measured in a Unicam SP1700 U.V. spectrophotometer at 740nm.

The protein concentrations of the test suspensions were determined by reading off the measured optical density against a calibration curve of O.D. for 0-100μg ml^{-1} standard protein (bovine serum albumin) solution. It was discovered that the buffer solution in which the trout muscle was homogenised produced a colour in the above assay and therefore an appropriate blank was subtracted from the measured O.D. prior to comparison with the calibration curve.

The equilibrium binding data were used to plot Scatchard plots of $[Cs]_b/[Cs]_f$ versus $[Cs]_b$, where $[Cs]_b$ = concentration of bound caesium and $[Cs]_f$ = concentration

of unbound or free caesium. This plot is based on saturation kinetics and is linear with a slope of $-1/K_d$ and a y-intercept of $n[P]t/K_d$. K_d = dissociation constant of the caesium-bound protein and indicates the external caesium concentration required for 50% of the binding sites to be occupied, and n[P]t = total concentration of caesium binding sites ([P]t = total protein concentration; n = number of identical and independent binding sites per molecule of protein), (Segel, 1976). In order to calculate the saturation binding capacity of caesium per mg protein (B_{max}), n[P]t was converted from mM to µmoles (from the experimental volume) and this was divided by the total mass of protein (mg) used in each experiment. Straight lines were fitted to the data from the individual experiments by computer (STATGRAPHICS) and the values of K_d and B_{max} were calculated from the slope and intercept(s) of the lines (as above).

RESULTS

Scatchard plots of caesium binding to coarse and microsomal suspensions of rainbow trout (*O. mykiss*) muscle were approximately linear over the ranges of external caesium concentrations used; R^2 = 0.64-0.97, $p<0.01$ in all cases, ANOVAR (Figs. 5.1 and 5.2). There was a possibility that the data were in fact curvilinear, indicating the presence of more than one type or site of binding with different binding affinities. In the present experiments simple linear analysis provided the required information. Moreover, more sophisticated analysis would require data at higher external caesium concentrations to be obtained and therefore such analysis was not undertaken.

The mean dissociation constant K_d of caesium binding to trout muscle-derived

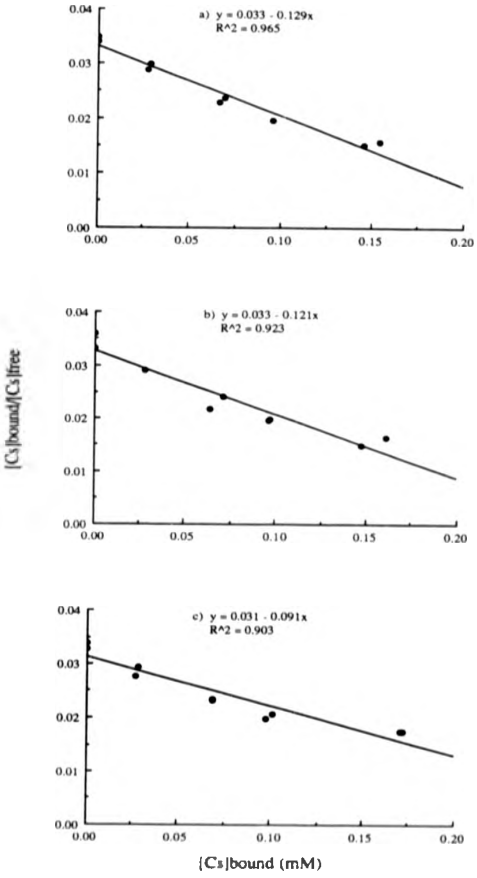

Fig. 5.1. Scatchard plots of the binding of 137-caesium to a supension of microsomes derived from muscle of rainbow trout (O. mykiss).

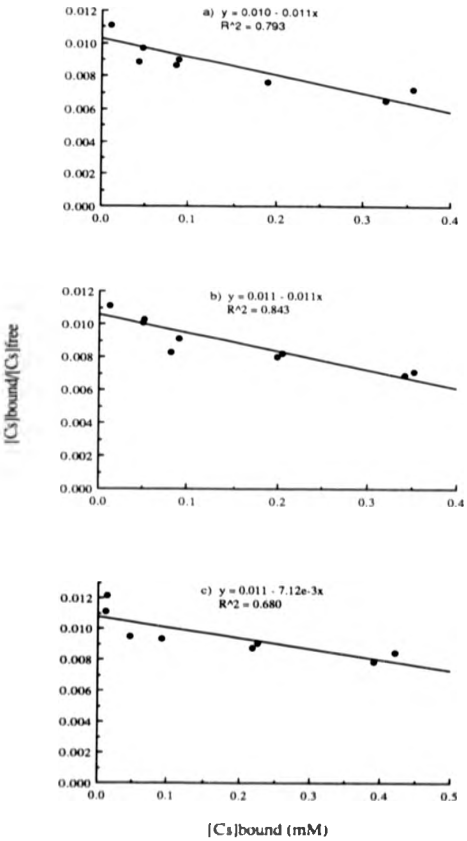

Fig. 5.2. Scatchard plot of the binding of 137-caesium to a coarse suspension of muscle of rainbow trout (O. mykiss).

microsomes was much smaller than that of the coarse trout muscle suspension; 9.0mM (± 1.75 S.D.) and 107.4mM (± 28.6 S.D.) respectively (Table 5.1). This indicated that the caesium binding affinity was significantly greater for the microsomal protein. The saturation binding capacity of the microsomal protein was, however, less than that of the coarse suspension protein, 0.43 (± 0.06 S.D.) and 0.82 (±0.03 S.D.) μmoles Cs mg^{-1} protein respectively.

DISCUSSION

The dissociation constant of caesium binding to trout-muscle derived microsomes was approximately 9mM. Few data of caesium binding to protein are available for comparison as caesium has no known biochemical function. The enzyme sodium plus potassium-dependent adenosine triphosphatase (Na^++K^+-ATPase) prepared from peripheral nerves of the shore crab (*Carcinus maenas* L.) may however, be activated by a number of univalent cations (Skou, 1957, 1960). The dissociation constant of membrane-bound Na^++K^+-ATPase, derived from the gills of the seawater-adapted eel (*Anguilla anguilla*), was 13mM for caesium and approximately 2mM for potassium (Bell *et al.*, 1977). The order of affinity of Na^++K^+-ATPase for group-I metal ions was $Rb^+ \geq K^+ > Cs^+ > Li^+$ (Skou, 1957, 1960; Bell *et al.*, 1977). Matsui and Homareda (1988) reported similar results for Na^++K^+-ATPase prepared from kidney tissue. The dissociation constants of caesium and potassium binding to K^+-dependent acyl phosphatase (as a part of the Na^++K^+-ATPase of cell membranes) were 3.0 and 0.9mM, respectively (Bader and Sen, 1966). It should be noted however, that muscle tissue is relatively poor in Na^++K^+-ATPase and although membrane-bound Na^++K^+-ATPase may

Table 5.1. Dissociation constants (K_d) and saturation binding capacities (B_{max}) of caesium binding to a microsomal and a "coarse" suspension of muscle tissue of rainbow trout (*O. mykiss*). K_d in mM, B_{max} in µmol Cs mg^{-1} protein.

	Microsomal suspension		"Coarse" suspension	
	K_d	B_{max}	K_d	B_{max}
a)	7.77	0.42	114.8	0.76
b)	8.26	0.38	90.9	0.79
c)	11.00	0.50	90.9	0.82
mean	9.01	0.43	107.4	0.82
(S.D.)	1.74	0.06	28.6	0.04

be partially purified in a similar manner to the method used in this chapter to produce microsomes (Bell *et al.*, 1977) the binding of caesium to the latter is likely to involve a number of proteins.

It might be thought that the similarity of the dissociation constant of caesium binding to trout muscle-derived microsomes to that of caesium binding to Na^++K^+-ATPase indicates relatively strong binding of the former. A number of studies have found however, that the binding of potassium to Na^++K^+-ATPase can be divided into two types. A specific, high-affinity (ouabain-sensitive) potassium binding with a K_d value of only a few μM under favourable conditions, and a low-affinity, non-specific (ouabain-insensitive) potassium binding with a K_d of greater than 10mM (Kanike *et al.*, 1976; Matsui *et al.*, 1977; Jorgensen and Petersen, 1982; Matsui and Homareda, 1988). In comparison to the former therefore, the affinity of the trout muscle-derived microsomes for caesium was comparatively low; the majority of the accumulated caesium remains "free" in the tissue water.

The much lower binding affinity of the coarse muscle suspension (K_d = 107.4 mM) was probably due to the heterogeneous nature of the preparation and a large amount of non-specific binding. Although the larger tissue and cell fragments were removed by centrifugation a large proportion of the cellular organelles were likely to have remained.

Whilst the binding affinity for caesium of the two trout muscle-derived suspensions was relatively low compared to specific potassium binding by Na^++K^+-

ATPase, the saturation binding capacity of the former was relatively large. Matsui *et al.* (1977) recorded a maximum specific binding of 6.2nmol K^+ mg^{-1} Na^++K^+-ATPase. Matsui and Homareda (1988) measured specific and non-specific saturation binding capacities of 3.5 and 182nmol K^+ mg^{-1} Na^++K^+-ATPase, respectively. The maximum binding capacity of the trout muscle-derived microsomes recorded in this experiment was 430nmol mg^{-1} protein and that of the coarse suspension was greater still at 820nmol mg^{-1} protein. Given the relatively high K_d values of intracellular caesium binding recorded in the present experiments, it is likely that the majority of the caesium binding is non-specific to negatively-charged protein side chains. The frequency of such glutamate (Glu) or aspartate (Asp) side chains in proteins (ie. the proportion of the total number of amino acids) can be obtained from protein sequence data bases such as SWISS-PROT (Bairoch and Boeckman, 1991). The frequency of Glu and Asp groups is approximately 11.5% and the mean residue molecular weight is approximately 110. An "average" protein of molecular weight = 50,000 would therefore contain:

50,000/110 x 0.115 = 52.3 negatively-charged side chains.

$\Rightarrow$ 1000g (0.02moles) protein contains 1.05moles Glu/Asp

$\Rightarrow$ 1mg protein contains 1.05μmoles or 1050nmoles Glu/Asp

This value of the number of negatively-charged side chains shows good agreement with the maximum caesium binding capacity of the two trout muscle-derived suspensions. This suggests that the majority of the intracellular caesium binding to muscle protein in rainbow trout (*O. mykiss*) is non-specific, and validates the values of B_{max} recorded in the present experiments.

The maximum caesium binding capacities would only be acheived however,

when the external cation concentration >K_d. Intracellular potassium concentrations are relatively high; 140mmol kg^{-1} tissue water in rainbow trout, *Salmo gairdneri*, (Holmes and Donaldson, 1969), such that maximum binding of potassium to the proteins mentioned above (even for non-specific binding) is likely to occur *in vivo*. Caesium however, is only encountered *in vivo* in trace amounts, much lower than the K_d values measured in this experiment. For example, the equilibrium concentration factor of 137-caesium in muscle tissue of juvenile brown trout (*S. trutta*) recorded in Chapter three was 10.03. This was equivalent to 10.8nmol Cs kg^{-1} muscle tissue. This concentration is approximately six and eight orders of magnitude smaller than the K_d values for the binding of caesium to trout muscle-derived microsomes and the course muscle suspension, respectively. Moreover, the present experiments were carried out in a potassium-free buffer. Chapter two suggested that caesium inhibited potassium uptake and *vice versa*, by the erythrocytes of rainbow trout (*O. mykiss*), by competition for binding and/or carrier sites of potassium channels. If the high intracellular potassium concentrations inhibited caesium binding to muscle protein in a similar way, intracellular caesium binding *in vivo* would be even less than that recorded in the present experiments. It is unlikely therefore, that significant intracellular caesium binding occurs at *in vivo* caesium concentrations.

CHAPTER 6: GENERAL DISCUSSION

The results of each series of experiments carried out in this thesis have already been discussed in detail in the relevant chapters. The aims of this general discussion are to summarise the main results and conclusions of the experimental chapters and fit these into an overall picture of the radioecology of 137-caesium in freshwater ecosystems. Whilst these aims may result in the repetition of some previous statements, this is limited to the main points of the thesis. The current chapter will hopefully provide an overview of the subject without the need to constantly refer back to individual chapters.

The Chernobyl disaster led to widespread contamination with 137-Cs over much of Europe. Previous studies of freshwater ecosystems had demonstrated that 137-caesium accumulated in freshwater animals and that the levels of accumulation increased with trophic level up the food chain. Concentration factors (C.F.s) of 137-caesium in the order of several thousands had been recorded in the top carnivores of such ecosystems eg. pike, perch and trout. There was, therefore, much radiological interest in the fate of 137-caesium contamination from Chernobyl, primarily with regard to potential human health hazards from eating contaminated food, but also concerning effects on the natural environment itself.

In order to understand fully the fate of 137-caesium in the environment, it is necessary to have an understanding of the chemical and biological behaviour of caesium. Unfortunately, caesium has no known biochemical function in its own right

and therefore there is little relevant physiological/biochemical data available. Similarities in the behaviour of caesium to other group-I metals, especially potassium however, have been observed since the work of Ringer (1882). Sjodin (1959) noted that at room temperature, caesium behaved like potassium with respect to its effect on the resting potential of the frog sartorius muscle. Bryan (1961) and Bryan and Ward (1962) treated caesium as potassium, but found that the former was taken up and lost more slowly, and was concentrated to a greater degree in decapod crustaceans.

A number of environmental studies also noted an inverse relationship between 137-caesium levels in fish and potassium concentrations in water (Gustafson *et al.*, 1966; Kolehmainen *et al*., 1966b; Preston *et al.*, 1967; Kevern and Spigarelli, 1971; Kolehmainen *et al.*, 1972). Much of the early work relating caesium to potassium was reviewed by Davis (1963). One of the main conclusions of this review was that caesium ions can replace potassium ions in a number of tissues but the extent to which this replacement occurred was very variable.

The experiments described in Chapter two were therefore devised to study the biochemical similarity of caesium to potassium through uptake by erythrocytes of the rainbow trout (*Oncorhynchus mykiss*). The total caesium influx was much smaller than that of potassium; 14.4 and 756.0 nmoles min^{-1} ml^{-1} packed cells respectively at an external concentration of 3mM. The most important results from this chapter were however, that the presence of caesium inhibited the uptake of potassium and vice versa by erythrocytes of the rainbow trout (*O. mykiss*). This inhibition was found to be either competitive or non-competitive (as opposed to uncompetitive). Two of the three routes

of potassium uptake into trout erythrocytes described by Bourne and Cossins (1984) involved active transport; the ouabain-sensitive Na^+-K^+ pump and the specific furosemide-sensitive route, comprising 50 and 46% of the total potassium influx respectively. Other studies have suggested that caesium interacts with potassium channels (Sjodin, 1961; Bezanilla and Armstrong, 1972; Beaugé *et al*, 1973). Indeed, caesium is a well-known as a blocking agent of potassium movement in neurophysiological studies (Adelman and Senft, 1966; Adelman *et al*.,1971; Blatz and Magleby, 1984; Matteson and Swenson, 1986). It is likely therefore, that the inhibition of potassium uptake by caesium and vice versa was due to competition for binding and/or carrier sites of the potassium channels. Chapter two concluded that caesium behaved as potassium in a qualitative but not quantitive manner. This conclusion, together with evidence from the literature was used to justify discussions on the behaviour of caesium in terms of that of potassium in later chapters, whenever knowledge of caesium itself was lacking.

Chapter three examined the accumulation and excretion of 137-caesium from water in the early stages of development of salmonids and compared these to results from previously published studies concerning adult fish. The accumulation of 137-caesium in the eggs of brown trout (*S. trutta*) was relatively small, even when the eggs were placed in radioactive water immediately post-fertilisation. Over an incubation period greater than 30d, the maximum C.F. recorded was only approximately 0.6. During the process of water-hardening and perivitelline fluid (pvf) formation, which is completed within 3-4h of fertilisation, the vitelline membrane surrounding the embryo becomes increasingly impermeable. The chorion is however, freely permeable to ions

(Eddy, 1974) and hence only the pvf is available for 137-caesium accumulation, resulting in the low C.F.s recorded for the whole egg but higher C.F.s (≈ 1.5) for the pvf itself.

In contrast, 137-caesium accumulated rapidly in newly-hatched alevins of Atlantic salmon (*S. salar*) and brown trout (*S. trutta*), achieving C.F.s in excess of 40 in less than 30 days. This change in susceptibility to radiocaesium accumulation from eggs to larvae could in fact be seen a few days prior to hatching in brown trout eggs, suggesting that changes in the permeability of the vitelline membrane were taking place.

The accumulation of 137-caesium in both alevins and juveniles of Atlantic salmon (*S. salar*) and brown trout (*S. trutta*) followed first-order rate kinetics ie. the rate of increase of accumulation decreased until a constant equilibrium C.F. was reached. This pattern of accumulation had previously beeen observed in a number of other studies of radiotracer accumulation (Jefferies and Hewett, 1971; Pentreath and Jefferies, 1971; Motais and Isaia, 1972; Pentreath, 1973; Hewett and Jefferies, 1976, 1978; Tytler and Bell, 1989). The C.F.s achieved at equilibrium in the alevins were however, much larger than those of the juvenile fish and the rate of accumulation was significantly lower in the latter. No evidence was found to suggest that this was due to different iono-regulatory mechanisms, although this remained a possibility. The difference was attributed to the ability to use food as a source of ions and/or differences in metabolic rate (MR). Alevins must initially obtain large proportions of their ions directly from water (Rombough and Garside, 1984). Alevins also have a greater MR per unit weight than juvenile and adult fish (Fry, 1967; Kamler, 1976; Brett and Groves, 1979;

Rombough, 1988), and although the evidence of a number of studies was conflicting, Edwards (1967) demonstrated that excretion of 65-zinc from juvenile plaice (*P. platessa*) was proportional to metabolic rate. The higher metabolic rate of the alevins could therefore have led to a greater turnover and accumulation of radiocaesium.

The accumulation of 137-caesium recorded in the juvenile salmonids at "normal" pH was of a similar magnitude to that recorded for juvenile fish of other species both in terms of C.F.s and rate constants (Williams and Pickering, 1961; Baptist and Price, 1962; King, 1964). Whilst the accumulation was less than that in the alevins (above) it was faster and achieved greater C.F.s when compared to larger fish in other studies (Morgan, 1964; Häsänen *et al.*, 1967; Hewett and Jefferies, 1978). Indeed, the results from the experiments in Chapter three showed good agreement with the inverse relationship between size and equilibrium C.F. in brown trout (*S. trutta*) observed by Hewett and Jefferies (1978).

Radiocaesium taken up via the gills was distributed throughout the body - it was recorded in all tissues analysed. The uptake of 137-caesium was greatest in kidney, liver, muscle and gills. The biological half-life ($t_{b\frac{1}{2}}$) of muscle was however, the longest of the tissues analysed and the greatest C.F.s at equilibrium were consistently recorded in muscle. This is also the most massive tissue in salmonids and up to 80% of the total body burden of 137-caesium at equilibrium may be located in muscle.

The importance of muscle as a sink for radiocaesium presents a potential health risk to humans as this is the part of the fish that is eaten. This problem was recognised

in earlier work regarding the accumulation of radiocaesium in fish (Häsänen and Miettinen, 1963; Gustafson *et al*., 1966; Preston *et al*, 1967). The MAFF survey of radioactivity in British waters post-Chernobyl (Camplin *et al*., 1986) concluded that consumption of freshwater fish provided the biggest potential dose of radiocaesium but that this was only 21% of the NRPB total annual dose (NRPB, 1986) at which a ban of foodstuffs may be considered following an accidental release of radioactivity. Fortunately, the relatively high radiocaesium concentrations in larval and juvenile fish do not present any additional problems as they are obviously too small for human consumption.

No experiments were carried out to determine whether the concentrations of radiocaesium used in these experiments were damaging to the health of the animals themselves. Developing fish are known to be particularly at risk; damage to eggs and larvae of marine fish has been recorded at radioisotope concentrations of 10^{-10} and 10^{-11} Ci l^{-1} (3.7 and 0.37Bq l^{-1}) (Fedorov *et al*., 1964; Polikarpov, 1966). Brown (1962) noted no deaths among eggs of sea trout (*S. trutta*) and Atlantic salmon (*S. salar*) in freshwater however, when exposed to 10^{-9} Ci l^{-1} (37Bq l^{-1}) $^{90}Sr/^{90}Y$. No evidence of radiation damage was observed in any of the current experiments using 137-caesium concentrations of approximately 10^{-6} Ci l^{-1} (37kBq l^{-1}).

The elimination of 137-caesium from Atlantic salmon was best described by a single exponential function. Most studies of whole-body radiotracer elimination in fish have described two components in which the "slow" component comprised the majority of the radioactivity at t=0 (Baptist and Price, 1962; Häsänen *et al*., 1967; Kolehmainen

and Miettinen, 1967; Nelson, 1967). The separate components do not necessarily represent depletion from individual tissues but it has been suggested that the "slow" component is strongly affected by the depletion from muscle tissue. The elimination of 65-zinc from plaice, *P. platessa*, (Pentreath, 1973) and inorganic 203-mercury from rays, *R. clavata*, (Pentreath, 1976) however, were also best described by a single exponential function. It should be noted however, that the rate constants and biological half-lives measured in Chapter three were obtained under laboratory conditions and are not therefore comparable to the *in vivo* situation. Under environmental conditions, radiocaesium uptake may be continuous, if erratic, and occurs both from water and from food. The half-lives of radiocaesium in natural populations of fish measured post-Chernobyl were therefore much greater than those observed in the present experiments (Brittain *et al.*, 1991).

Reduced environmental pH significantly reduced the rate of 137-caesium accumulation in Atlantic salmon (*S. salar*) and brown trout (*S. trutta*). Initially, this result was somewhat unexpected; the greatest 137-caesium concentrations recorded in fish in the MAFF post-Chernobyl monitoring programme (Camplin *et al.*, 1986) were from upland, nutrient-poor waters, which are often acidic. Further investigation revealed however, that these waters received much more radiocaesium owing to the pattern of fallout from Chernobyl via rainfall (Clark, 1986; Smith and Clark, 1986). These waters are also often oligotrophic with low dissolved potassium concentrations which has been shown to result in elevated radiocaesium C.F.s in fish (Hüsänen and Miettinen, 1963; Kolehmainen *et al.*, 1966b; Kevern and Spigarelli, 1971; Kolehmainen, 1972).

No data concerning caesium transport at different acidities were found in the literature. The effects of acid stress on fluxes of other group-I metal ions, especially sodium, are however, well documented. At low pH, branchial sodium influx decreases and sodium efflux increases (Packer and Dunson, 1970; McWilliams and Potts, 1978; McWilliams, 1980; Potts, 1980) resulting in net losses of plasma sodium (Lacroix and Townsend, 1987). Losses of potassium via the gills also occur (McDonald and Wood, 1981; McDonald, 1983) principally from the pool of intracellular potassium in muscle tissue (McDonald and Wood, 1981; Stuart and Morris, 1985).

The inhibition of branchial sodium influx has been ascribed to increased "... direct competition of H^+ with Na^+ for the transport sites and/or access channels to the carrier" (Wood, 1988). It seems likely that increased acidity reduces 137-caesium accumulation C.F.s in a similar manner as in this investigation low pH reduced the whole-body influx by approximately 50% in both Atlantic salmon and brown trout, whereas rate constants were much less affected. This requires that branchial caesium influx is saturable in the same way as are the influxes of sodium and potassium (below). This was indeed confirmed in Chapter four. The conclusion that it is the uptake of caesium that is most affected by low pH unfortunately means that increased acidity would be of little use in promoting the clearance of 137-caesium from contaminated organisms.

The branchial influx of 137-caesium measured using a perfused, whole-body preparation of the rainbow trout (*O. mykiss*) was very small. When factors such as the size of fish and the external caesium concentrations were taken into account however,

the results of Chapter four were in line with the influxes calculated from the accumulation studies of Chapter three. The external 137-caesium concentrations used in these experiments however, were much greater than those found *in vivo*. At concentrations recorded in the environment, the branchial influx of 137-caesium would be even smaller. It should be noted however, that apparently high concentrations of a radioisotope in terms of Bq l^{-1} or Bq kg^{-1} may be equivalent to very small concentrations of the element in terms of moles l^{-1} or moles kg^{-1} if the isotope has a high specific activity. For example, the carrier free 137-caesium used in these experiments, 1kBq $^{137}Cs\ kg^{-1}$ was equal to only 2.5×10^{-10} moles Cs kg^{-1}.

The saturable nature of the branchial caesium influx indicated that caesium uptake across the gills is a mediated process dependent upon a limited number of carriers or active sites. This had already been suggested in Chapter three where it was postulated that caesium accumulation was inhibited at low pH by an increased direct competition between protons and caesium ions for transport sites. Perversely, increased acidity had no significant effect on the branchial influx of 137-caesium in the perfused whole-body preparation of the rainbow trout (*O. mykiss*). This was thought to be a result of the short duration of the influx experiments. Some data is available to suggest that ionic influxes are initially unaffected by increased acidity (McDonald and Wood, 1981; McDonald and Milligan, 1987) but other studies suggest that the effects of acid exposure are immediate (McWilliams and Potts, 1978; McWilliams, 1980a, b; 1982a, b; Booth *et al*., 1987; Wood *et al*., 1987; Wood, 1988).

That the branchial influx of sodium in freshwater fish displays saturation kinetics

has been well-documented Kerstetter *et al.*, 1970; Maetz, 1971, 1972; Kerstetter and Kirschner, 1972; de Renzis and Maetz, 1973; Perry *et al.*, 1985; Avella *et al.*, 1987). More recently, Gardaire *et al.* (1991) demonstrated that branchial potassium influx is also saturable and suggested that these exchanges occur through potassium channels in the branchial epithelium of secondary lamellae. The K_m of branchial caesium influx measured in Chapter four was however, very large compared to *in vivo* external caesium concentrations indicating a poor efficiency of transport. Latorre and Miller (1983) classified four types of potassium channel depending upon their properties of conductance and selectivity. The potassium channels involved in branchial potassium influx were assigned to type A - "ion-specific channels" (Latorre and Miller, 1983), which had a high selectivity for monovalent cations. The V_{max} of branchial potassium influx (Gardaire *et al.*, 1991) and of potassium influx into erythrocytes (Chapter two; Bourne and Cossins, 1984) were both much greater in the rainbow trout (*O. mykiss*) than were the corresponding caesium fluxes. These results are incompatible with the other three types of potassium channel described by Latorre and Miller (1983) and it seems likely, therefore, that caesium influx also occurs via "ion-specific potassium channels" in these two systems; the mechanisms of transport are the same but the magnitude of the caesium flux is much smaller.

The kinetics of intestinal caesium influx in rainbow trout (*O. mykiss*) however, are very different to those across the gill epithelium and the erythrocyte plasma membrane. Intestinal caesium influx is not saturable but is directly related to the mucosal caesium concentration. This type of relationship has previously been observed for the intestinal uptake of a number of ions, including sodium and potassium, in

various freshwater fish (Ellory *et al.*, 1972, 1973; Lahlou, 1975; Bell *et al.* 1983) and also in the small intestine of a number of mammals (MacKnight, 1977). Indeed, the intestinal caesium influx in freshwater fish has been found to be greater than that of sodium under identical conditions (Ellory *et al.*, 1972; Colin *et al.*, 1985). These results suggest that the fish intestine is a "leaky" epithelium (Lahlou, 1985) and that ionic influx occurs via leak pathways, although these are not necessarily simple diffusional channels (Ellory *et al.*, 1972, 1973; Lahlou, 1975).

Some caution should be exercised in applying the absolute values of the branchial and intestinal influxes from the present experiments to the *in vivo* situation. Whilst the methods were chosen to simulate *in vivo* conditions as closely as possible, a number of differences exist between the *in vitro* and *in vivo* situation that could influence the recorded fluxes (see Introduction, Chapter four). The absolute values should therefore be used only as a pointer towards *in vivo* rates; more attention should be given to the relative fluxes and the information that these experiments have provided with respect to the mechanisms of radiocaesium influxes.

Environmental data have shown that radiocaesium is retained in fish for long periods. In Chapter three it was demonstrated that the majority of this radioisotope is deposited in muscle tissue and that muscle had the smallest rate of depletion of the tissues analysed. The binding experiments in Chapter five showed that caesium binding to intracellular muscle protein in rainbow trout (*O. mykiss*) was relatively weak, and that the majority of the caesium remained "free", presumably in the intracellular water. Although the saturation binding capacity of such proteins for caesium was quite high,

this only occurred at caesium concentrations much greater than those found *in vivo*. It was therefore concluded that intracellular binding was not a significant factor in the long-term retention of radiocaesium.

This conclusion begs the question, "What is causing the long retention of radiocaesium?" The most likely explanation is provided by the proposed structure and functional mechanism of the ion-specific potassium channels (Latorre and Miller, 1983). Bezanilla and Armstrong (1972) and Hille (1973) suggested that these channels select ions accurately by their dehydrated ionic radii. An internal, large, poorly-selective "mouth" may accept larger ions, eg. caesium and tetraethylammonium (TEA) ions. A similar acceptor site exists on the outside of the channel. Beyond the "mouth", the channel remains large enough to accept caesium, and long enough to allow the simultaneous occupancy of two or three ions. Farther into the channel, a ring of five negatively-charged oxygen atoms form a selective pore, through which dehydrated potassium ions fit, but larger ions do not (Fig. 6.1). Smaller monovalent ions, eg. sodium and lithium are prevented from penetrating the channel as the oxygen atoms cannot approach the ion sufficiently closely to replace the oxygen atoms from the water molecules of the hydration shell (Fig. 6.2). No indication was given as to how these channels could operate against the unfavourable electrochemical gradients that exist for ionic uptake from fresh water (Latorre and Miller, 1983).

This theory implies however, that the potassium channels are totally impermeable to caesium, and therefore it cannot accomodate the fluxes of caesium found in this thesis and in earlier work. Armstrong (1975) did however, point out that

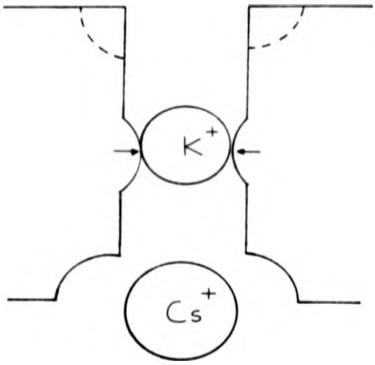

Fig. 6.1. Longitudinal section of a potassium channel. Caesium ions fit the internal binding site and also the first part of the channel but do not pass through the selective pore (arrows). Potassium ions however, pass through the entire channel. An external binding site (dashed line) is also shown. (After Lattorre and Miller, 1983).

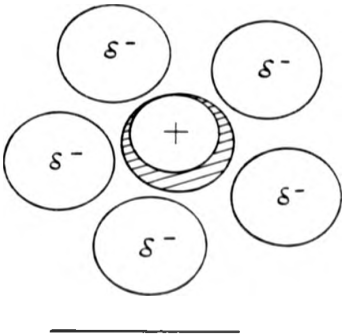

Fig. 6.2. Cross-section of the selective pore of a potassium channel. Five oxygen atoms with partial negative charge surround a space into which a dehydrated potassium ion (shaded circle) fits. Also shown (unshaded circle inside shaded circle) is the size of a dehydrated sodium ion. Scale bar is 0.5nm. (After Hille, 1973).

this model predicted a greater selectivity than was seen from the data of a number of investigations, and Latorre and Miller (1983) concluded that this problem could be alleviated by allowing greater deformability of the selectivity pore.

A further problem with this model is that the transport of caesium through the channels is bi-directional. The selectivity of the channel is greater to inward ion movement (Latorre and Miller, 1983), whereas the opposite might be expected given the rapid accumulation and slow elimination of radiocaesium. Unfortunately, potassium transport has been little studied in fish; none of the investigations included in extensive reviews of potassium transport (MacKnight, 1977, Latorre and Miller, 1983) were carried out on fish. Further studies on potassium transport mechanisms in teleosts will hopefully also provide more information on the behaviour of caesium.

The results and discussions of these experiments can be used to speculate on the effects of a hypothetical, single input of 137-caesium in a previously uncontaminated lake. This is essentially what occurred in many freshwaters in northern and western Europe following the Chernobyl disaster. Whilst 137-caesium may have been present in some waters via contamination from nuclear industries and from nuclear weapons fallout, the concentrations were small compared to those post-Chernobyl. For example, the 137-caesium concentration measured in a Swedish lake in 1971 was 0.03Bq l^{-1} (Carlsson and Liden, 1972), whilst 140Bq l^{-1} 137-caesium was recorded in a Norwegian lake on 28th April 1986, although this declined to <10Bq l^{-1} after one week (Brittain *et al.*, 1991).

The relative magnitudes of the branchial and intestinal caesium influxes suggest that food is likely to be the greater source of radiocaesium in freshwater fish. Under natural conditions however, a large number of physical, chemical and biological factors will influence the input of radiocaesium from these two sources. Over the short-term, the concentration of caesium in the water is likely to be relatively constant and therefore the branchial influx of caesium will also be constant. Initially, the majority of the radiocaesium available for uptake by fish would be that dissolved in the water column; little radiocaesium would be available for uptake into fish via food. Direct branchial uptake would therefore be the greater source of radiocaesium uptake. In this situation, the present experiments demonstrated that larval and juvenile fish are most susceptible to 137-caesium accumulation.

Over longer periods of time however, if the original input of radiocaesium into the environment was a single event eg. the input of Chernobyl fall-out, the concentration in the water would gradually decrease due to flushing and the loss of radiocaesium to sediments. Sediments have been shown to be an important sink for radiocaesium (Gustafson, 1967; Wrenn *et al.*, 1971; Lerman and Tunaguchi, 1972; Ritchie *et al.*, 1974; Eyman and Kevern, 1975; Alberts *et al.*, 1979; Stanners and Aston, 1982) especially on micaceous soils (Francis and Brinkley, 1976) and under acid conditions (Schindler *et al.*, 1980). At the same time, radiocaesium concentrations in food organisms would gradually increase.

Moreover, whilst the branchial uptake of caesium may be constant over short periods, the uptake of radiocaesium from food would be dependent on the diet of the

fish which may vary significantly over a short time. The opportunistic nature of feeding is such that it is intermittent and the amount of food eaten is very variable. Even under optimal conditions at sustained maximal feeding rates, the radiocaesium uptake will depend on the type of food ingested. Radiocaesium concentrations have been found to increase up the food chain (Pendleton *et al.*, 1965; Gustafson *et al.*, 1966; Kolehmainen *et al.*, 1967; Wrenn *et al.*, 1971; Carlsson and Liden, 1978) therefore larval and juvenile fish could themselves be important sources of radiocaesium for larger, piscivorous fish.

Hence it is very difficult to predict the relative importance of water versus food as sources of radiocaesium in freshwater fish unless a large number of parameters are known. In practice, most studies have cited food as the greater source of radiocaesium in fish (Baptist and Price, 1962; Hewett and Jefferies, 1976, 1978; Kimura, 1984). The data presented in Chapter four, show that intestinal caesium influx is significantly greater than the branchial influx and that except for the situation immediately after a pollution incident, food is indeed likely to be the principal source of radiocaesium in freshwater fish.

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Appendix A. Radiological units used in this thesis.

Quantity	Old unit and symbol	Definition	New SI unit and symbol	Definition
Radioactivity	curie (Ci)	3.7×10^{10} disintegrations s^{-1}	bequerel (Bq)	disintegration s^{-1}
Absorbed dose	rad (rad)	10^{-2} J kg^{-1}	gray (Gy)	J kg^{-1}
Dose equivalent	rem (rem)	10^{-2} J kg^{-1} x modifying factors	sievert (Sv)	J kg^{-1} x modifying factors