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TISSUE AND WHOLE-BODY EXTRACELLULAR, RED BLOOD CELL AND ALBUMIN SPACES IN THE RAINBOW TROUT AS A FUNCTION OF TIME: A REAPPRAISAL OF THE VOLUME OF THE SECONDARY CIRCULATION

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Summary

[^{58}Co]EDTA, [^{51}Cr]RBC and [^{125}I]albumin spaces in the whole body and 28 tissue samples were examined at timed intervals over 16 h in rainbow trout *Oncorhynchus mykiss*. [^{58}Co]EDTA space (which approximates extracellular fluid volume; ECF) in fins, skin, gallbladder and eye are reported for the first time. After a 16 h equilibration, ECF volume was large (376–726 μl g^{-1} wet tissue mass) in kidney, swimbladder, skin and fins, moderate (219–313 μl g^{-1} wet tissue mass) in stomach, skull, spleen, liver, intestine, gills, eye and cecum, and small (53–181 μl g^{-1} wet tissue mass) in red muscle, fat, brain, gallbladder and white muscle. Whole-body ECF was 387±10.6 μl g^{-1} (mean ± S.E.M.; *N*=11). [^{51}Cr]RBC space relative to [^{58}Co]EDTA space was large in spleen, liver, intestine and gill, and low in skin, fins, stomach and skull. Whole-body [^{51}Cr]RBC space was 9.9±0.6 μl g^{-1} body mass (*N*=17). Blood volume calculated from [^{51}Cr]RBC space at 16 h and a dorsal aortic hematocrit of 24.5 % was 40.4 μl g^{-1} body mass. Whole-body [^{125}I]albumin space at 16 h was 118.0±7.4 μl g^{-1} body mass (*N*=6), which resulted in an estimated blood volume of 156.6 μl g^{-1} body mass, nearly four times that estimated from the [^{51}Cr]RBC space. Tissue hematocrits, calculated from [^{125}I]albumin and [^{51}Cr]RBC spaces, were significantly lower than dorsal aortic hematocrit in all tissues except spleen, kidney and liver. [^{58}Co]EDTA and [^{51}Cr]RBC spaces reached equilibrium in nearly all tissues within 1 h, whereas [^{125}I]albumin continued to accumulate in many tissues up 24 h. The disparity between [^{125}I]albumin distribution kinetics compared with the kinetics of [^{58}Co]EDTA and [^{51}Cr]RBC distribution, as well as the accumulation of [^{125}I]albumin in tissues not known to have a secondary circulation, indicates that [^{125}I]albumin is a poor marker of plasma volume in trout and that previous studies based on [^{125}I]albumin clearance from the plasma have overestimated both the volume and the turnover rate of the secondary system. Revised estimates of secondary circulation volume, based on [^{58}Co]EDTA distribution rate, indicate that it is no more than 10–20 % of the volume of the primary circulation.

Key words: fish, *Oncorhynchus mykiss*, blood volume, extracellular fluid volume.

Introduction

Information about extra- and intracellular fluid compartment size, and the rate of fluid flux between them, is critical to an understanding of osmoregulatory processes and cardiovascular homeostasis. An appreciation of the size of these compartments and their dynamic properties in fish has been hampered both by technical difficulties associated with indicator dilution methods and by the lack of studies that have attempted to measure compartment volumes in all tissues from a single species (Olson, 1992).

One fluid compartment in fish that is particularly enigmatic is the so-called ‘secondary circulation’. This arterial–capillary–venous system is formed from narrow-bore arterioles that arise from larger gill and systemic arteries and subsequently reanastomoses to form a second vascular network. The secondary circulation is believed to parallel the original (primary) circulation in tissues such as gill and in the epithelium of the skin, fins and the body cavity (Steffensen and Lomholt, 1992). On the basis of anatomical evidence that red blood cells are nearly completely excluded from the secondary circulation (reviewed in Steffensen and Lomholt, 1992) and on the assumption that a plasma marker such as radiolabelled albumin is distributed to both primary and secondary

compartments, J. F. Steffensen, M. Heies, D. J. Randall and G. Iwama (unpublished data quoted by Steffensen and Lomholt, 1992) indirectly determined the size of the secondary system of rainbow trout by monitoring the disappearance of albumin from the primary system. Their analysis led to the conclusions that the secondary system was 50 % larger than the primary system and that the low flux rate between the two systems indicated that the secondary system fluid had a turnover rate of approximately 7 h. Olson (1992, 1996), however, suggested that a significant portion of this [^{125}I]albumin disappearance is due to extravasation across tissue capillaries of the primary system, and that this results in a significant overestimation of secondary system volume and an inaccurate turnover rate.

The present study was designed to provide a more complete picture of fluid compartments in fish tissues by measuring the distribution spaces of indicators that are restricted to specific compartments. To accomplish this, we measured the distribution volume of ^{51}Cr-tagged red blood cells (RBCs) as an indicator of the primary circulation, [^{58}Co]EDTA as an indicator of the extracellular compartment and [^{125}I]albumin which, as mentioned above, is a purported, but controversial, indicator of the primary and secondary circulation (additional details of the criteria and utility of volume indicators in fish are provided by Olson, 1992). Indicator distribution volumes were measured in whole rainbow trout and in 28 tissues, including those that possess a secondary circulation, at timed intervals over 16–24 h. These studies were designed to provide comprehensive baseline information on tissue fluid compartments in fish and to address the applicability of [^{125}I]albumin in measuring the volume of the secondary circulation. The rate of appearance of these volume indicators in tissues was also compared with the rate of disappearance of indicators from the plasma to provide further insight into the nature of the secondary circulation.

Materials and methods

Animals

Rainbow trout *Oncorhynchus mykiss* (Walbaum) (250–350 g) were purchased locally and maintained on a 12 h:12 h light:dark photoperiod at 14 °C in a 2000 l fiberglass tank provided with continuously flowing water. Trout were fed a maintenance diet of commercial trout pellets and fasted for 24 h prior to experimentation.

Experimental procedure

The surgical procedure and experimental protocol for measuring the size of fluid spaces in the various tissues have been described in detail previously (Duff and Olson, 1989). In brief, trout were anesthetized in benzocaine (0.4 g l^{-1}) and the dorsal aorta was cannulated with heat-tapered polyethylene tubing (PE 60). Fish were placed in individual black plastic tubes and allowed to recover for at least 24 h before experimentation.

Radiolabelled red blood cells ([^{51}Cr]RBCs) were prepared by incubating saline-washed RBCs, collected from donor fish, for 4 h in phosphate-buffered Ringer's solution (PBR; Duff *et al.* 1987) containing 74.0×10^4 Bq ml^{-1} (20 μCi ml^{-1}) RBC of ^{51}Cr as sodium chromate. After incubation, the RBCs were washed five times in PBR and resuspended in PBR to a final hematocrit of 40 %. Depending on the experiment, either 0.5 ml of iodinated bovine serum albumin (BSA) (29.6×10^4 Bq ml^{-1}; 8 μCi ml^{-1} of [^{125}I]BSA, New England Nuclear) or 0.5 ml of [^{58}Co]EDTA, prepared as described by Bridge *et al.* (1982), was then added to 0.5 ml of RBCs and injected into the fish (t=0 h).

Determination of marker distribution was made by sampling blood and tissues at timed intervals ([^{58}Co]EDTA, t=2, 4, 8 and 16 h; [^{51}Cr]RBC and [^{125}I]albumin t=0.5, 1, 2, 4, 8 and 16 h). It became evident that [^{125}I]albumin distribution to a number of tissues had not reached equilibrium by 16 h, and an additional set of experiments was performed to examine [^{125}I]albumin and [^{51}Cr]RBC distribution at 24 h. Limited availability of ^{58}Co precluded additional studies with that nuclide. A 1 ml blood sample was withdrawn from the dorsal aortic cannula at the end of the mixing interval, and the fish was then anesthetized by injecting 1 ml of Nembutal (50 mg ml^{-1}) into the dorsal aorta. The isthmus was immediately clamped to prevent further circulation through the ventral aorta. The carcass was then quick-frozen in an ethanol:water:dry-ice slurry, and stored at −60 °C for subsequent tissue analysis.

Samples (approximately 0.5–1.5 g) of internal organs, skin, fins, muscle and skull were removed from each fish, weighed and counted for radioactivity on a Beckman 4000 gamma counter. Hematocrit from dorsal aortic blood samples (dorsal aortic Hct) and radioactivity in the terminal blood samples were measured in duplicate.

Calculations and statistical analyses

[^{58}Co]EDTA space, [^{51}Cr]RBC space, [^{125}I]albumin space and blood volume were determined using standard indicator dilution relationships. Whole-body [^{58}Co]EDTA space was corrected for urine loss on the basis of a [^{58}Co]EDTA clearance rate of 5.7 μl g^{-1} h^{-1} (Duff *et al.* 1997). Statistical differences between tissue spaces determined at different time intervals within an individual tissue were tested using analysis of variance (ANOVA) and the Student–Newman–Keuls multiple-range test when indicated by significance ($P\leqslant0.05$) in the ANOVA. Apparent tissue hematocrit (Hct) was calculated from the following relationship:

$$\text{Hct} = [^{51}\text{Cr}]\text{RBC space} / ([^{125}\text{I}]\text{albumin space} + [^{51}\text{Cr}]\text{RBC space}).$$

Statistical differences between the calculated tissue hematocrits and measured dorsal aortic Hct were determined with a Student's t-test. Results are expressed as mean ± standard error of the mean.

Results

Indicator distribution volume and kinetics were virtually

Table 1. *Summary data for [^{58}Co]EDTA, [^{51}Cr]RBC and [^{125}I]albumin spaces in 28 tissues and the whole body of trout 16 h post-injection*

	Tissue space					
	[^{58}Co]EDTA, *N*=11		[^{51}Cr]RBC, *N*=17		[^{125}I]albumin, *N*=6	
Organ	(μl g^{-1})	Rank	(μl g^{-1})	Rank	(μl g^{-1})	Rank
Kidney (posterior)	725.8±81.3	1	104.9±12.9	3	292.1±36.0	4
Kidney (anterior)	618.2±33.0	2	132.5±14.9	2	354.5±73.7	1
Swimbladder	586.9±25.4	3	10.4±1.6	13	219.4±24.6	9
Skin	529.2±53.5	4	2.4±0.3	25	251.2±18.3	6
Dorsal fin	519.1±28.1	5	2.6±0.3	24	392.7±17.8	3
Caudal fin	478.5±13.0	6	2.0±0.3	26	232.0±12.1	8
Pelvic fin	438.3±13.8	7	3.3±0.5	20	297.9±27.5	2
Pectoral fin	375.7±9.9	8	3.2±0.5	21	246.5±17.8	7
Esophagus	342.9±23.9	9	5.4±0.6	17	64.5±9.5	24
Operculum	333.5±7.6	10	9.1±5.2	14	215.3±8.0	10
Stomach	312.6±16.1	11	4.2±0.5	19	58.3±7.7	25
Skull	303.6±15.5	12	3.1±0.4	23	104.9±13.9	21
Spleen	293.0±39.7	13	548.7±36.7	1	253.4±41.3	5
Intestine (posterior)	287.6±24.2	14	20.8±2.6	9	169.7±16.0	11
Liver	282.6±14.2	15	62.5±6.7	4	165.0±32.7	12
Intestine (anterior)	240.6±29.7	16	1.3±2.0	10	146.9±12.4	15
Gill I	239.4±12.4	17	44.8±4.0	5	153.5±5.2	14
Gill IV	235.6±11.3	18	41.0±4.0	8	140.7±7.6	19
Gill III	229.4±9.9	19	42.8±3.9	7	145.0±6.6	16
Gill II	228.8±10.3	20	44.9±4.1	6	144.4±7.5	17
Eye	227.8±10.3	21	7.7±0.6	16	65.9±5.4	23
Cecum	219.0±26.1	22	14.1±1.9	12	117.4±10.4	20
Red muscle	180.5±9.9	23	4.4±0.4	18	143.3±11.1	18
Fat	174.2±48.0	24	14.2±2.9	11	82.9±20.3	22
Gallbladder	127.3±13.5	25	8.0±1.8	15	155.8±35.6	13
Brain	115.4±7.7	26	3.2±0.3	22	37.6±7.6	27
White muscle (mid ventral)	54.3±3.8	27	0.8±0.1	28	31.6±4.3	28
White muscle (anterior dorsal)	53.1±6.7	28	0.9±0.2	27	55.0±19.6	26
Whole body	387.0±10.6		9.9±0.6		118.0±7.4	

Values are mean ± S.E.M.

Tissues are listed in the table in order of decreasing [^{58}Co]EDTA space.

Rank indicates the relative ranking (1 being the largest) of tissues when they are sorted in descending order of size of space calculated from the associated marker (e.g. the spleen has the largest [^{51}Cr]RBC space).

identical for several tissue subsamples, and in Figs 1–4 results for representative tissues only are presented. Tissues shown (or omitted) include: the first pair of gill arches (arches 2, 3 and 4), anterior kidney (posterior kidney), anterior intestine (posterior intestine), ventral white muscle (dorsal white muscle) and dorsal and caudal fins (pectoral and pelvic fins). In addition, indicator spaces in the ventricle, atrium and bulbus were not included as there was some difficulty in completely removing indicators trapped within the lumen.

[^{58}Co]EDTA, [^{51}Cr]RBC and [^{125}I]albumin spaces at 16 h

[^{58}Co]EDTA space 16 h after injection (Table 1; Fig. 1) was large (>450 μl g^{-1} wet tissue mass) in kidney, swimbladder, skin and some fins, moderate (approximately 200–450 μl g^{-1} wet tissue mass) in esophagus, operculum, stomach, skull, spleen, liver, intestine, gills, eye and cecum, and small (<200 μl g^{-1} wet tissue mass) in red muscle, fat, brain, gallbladder and white muscle. Whole-body [^{58}Co]EDTA space was 387±10.6 μl g^{-1} body mass.

There were no statistical differences in tissue [^{51}Cr]RBC spaces when this label was injected with either [^{58}Co]EDTA or [^{125}I]albumin, and these results were pooled (Table 1; Fig. 1). The largest [^{51}Cr]RBC spaces were found in spleen (549 μl g^{-1} wet tissue mass) and kidney (105–133 μl g^{-1} wet tissue mass). Liver and gill [^{51}Cr]RBC spaces were moderate (approximately 40–63 μl g^{-1} wet tissue mass), while [^{51}Cr]RBC spaces were smaller in all other tissues (0.8–21 μl g^{-1} wet tissue mass). Whole-body [^{51}Cr]RBC space was 9.9±0.6 μl g^{-1} body mass, and the estimated blood volume, based on a mean dorsal aortic hematocrit of 24.5±0.75 % (Table 2), was 40.4 μl g^{-1} body mass.

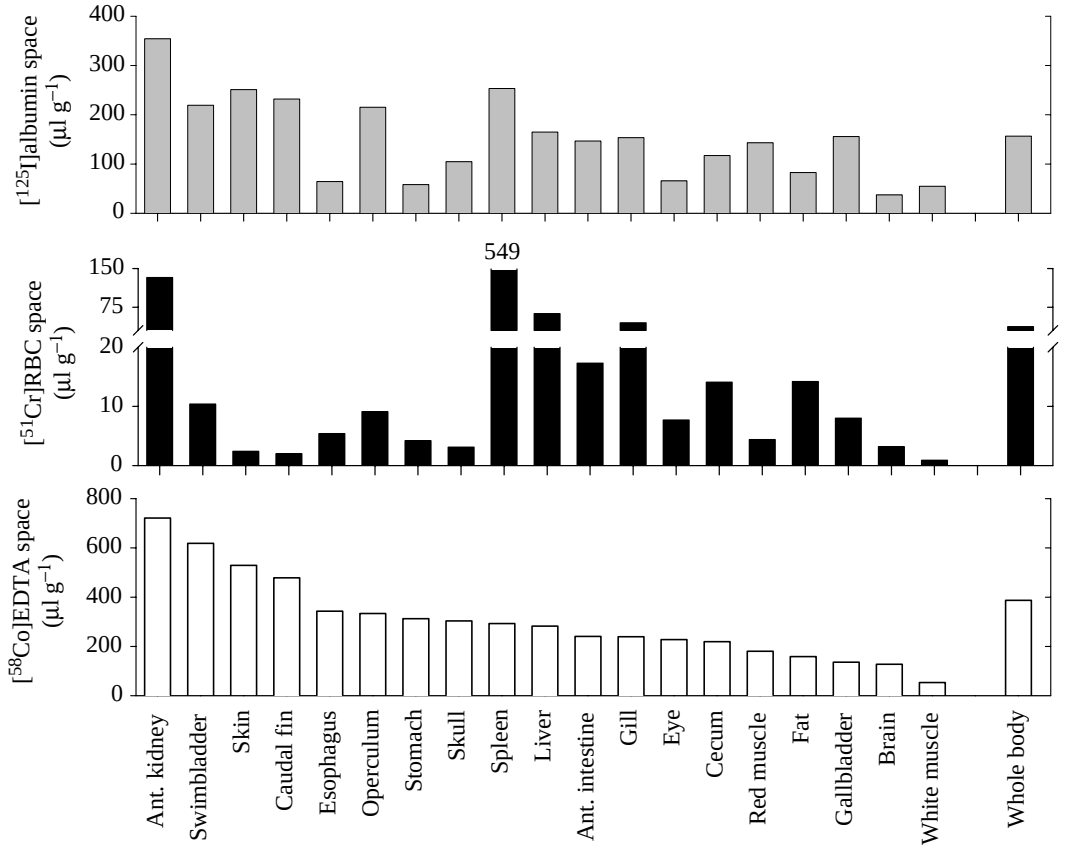

Fig. 1. [^{58}Co]EDTA, [^{51}Cr]RBC and [^{125}I]albumin space, at 16 h, in tissues of the rainbow trout. Tissues are arranged from left to right in order of largest to smallest [^{58}Co]EDTA space.

Large [^{125}I]albumin spaces (253–355 µl g^{-1} wet tissue mass) were correlated with large [^{51}Cr]RBC spaces in kidney and spleen, whereas in others tissues this was not the case. Fins and skin, for example, had very large [^{125}I]albumin spaces (232–393 µl g^{-1} wet tissue mass), but were among the tissues with the smallest [^{51}Cr]RBC spaces (<3.5 µl g^{-1} wet tissue mass). Intestine, gallbladder, gills and red muscle had moderate [^{125}I]albumin spaces (approximately 140–170 µl g^{-1} wet tissue mass), while the smallest spaces (approximately 30–80 µl g^{-1} wet tissue mass) were found in fat, eye, esophagus, stomach, brain and white muscle. Whole-body [^{125}I]albumin space was 118±7.4 µl g^{-1} body mass. Blood volume, calculated on the assumption that [^{125}I]albumin was retained in the plasma, was 156.6±10.6 µl g^{-1} body mass.

The relative sizes of the [^{51}Cr]RBC and [^{125}I]albumin spaces in different tissues were reflected in the estimated tissue hematocrits (Table 2). Splenic hematocrit (71.1±4.9 %) was significantly higher than dorsal aortic Hct. Only liver and kidney had calculated tissue hematocrits similar to dorsal aortic Hct. Tissue hematocrits in all other organs were significantly lower than dorsal aortic Hct and ranged from approximately 20 % in the gills to approximately 1 % in the fins and skin. Whole-body hematocrit calculated from blood values of [^{51}Cr]RBC space and [^{125}I]albumin spaces was 9.5±0.6 %.

[^{58}Co]EDTA, [^{51}Cr]RBC and [^{125}I]albumin distribution as a function of time

Of the three markers used in this study, [^{58}Co]EDTA distribution to the tissues was the most rapid (Figs 2–4). With the exception of the liver, kidney, eye and brain (Figs 3E,H, 4A,B), [^{58}Co]EDTA appeared to reach equilibrium in the tissues by the time the first sample was taken (t=1 h). Once equilibration had been achieved, the size of the [^{58}Co]EDTA space remained relatively constant for the duration of the experiment (16 h). Whole-body [^{58}Co]EDTA distribution was also rapid and remained stable (Fig. 5).

In general, the pattern of equilibration for [^{51}Cr]RBC was similar to that observed for [^{58}Co]EDTA. [^{51}Cr]RBC reached equilibrium in most tissue spaces within 0.5 h and remained stable throughout the course of the experiment. The only exceptions to this pattern were found in stomach, cecum and liver (Fig. 3B,C,E), where the [^{51}Cr]RBC volumes at 0.5 h were significantly higher than the [^{51}Cr]RBC volumes measured over the remaining 15.5 h. Whole-body [^{51}Cr]RBC space (Fig. 5) reached equilibrium in the first 0.5 h and did not change significantly thereafter.

While [^{58}Co]EDTA and [^{51}Cr]RBC could be described as markers that equilibrated quickly and remained stable in most tissues, [^{125}I]albumin distribution followed three general patterns; rapid, two-tiered or non-equilibration. In the rapid

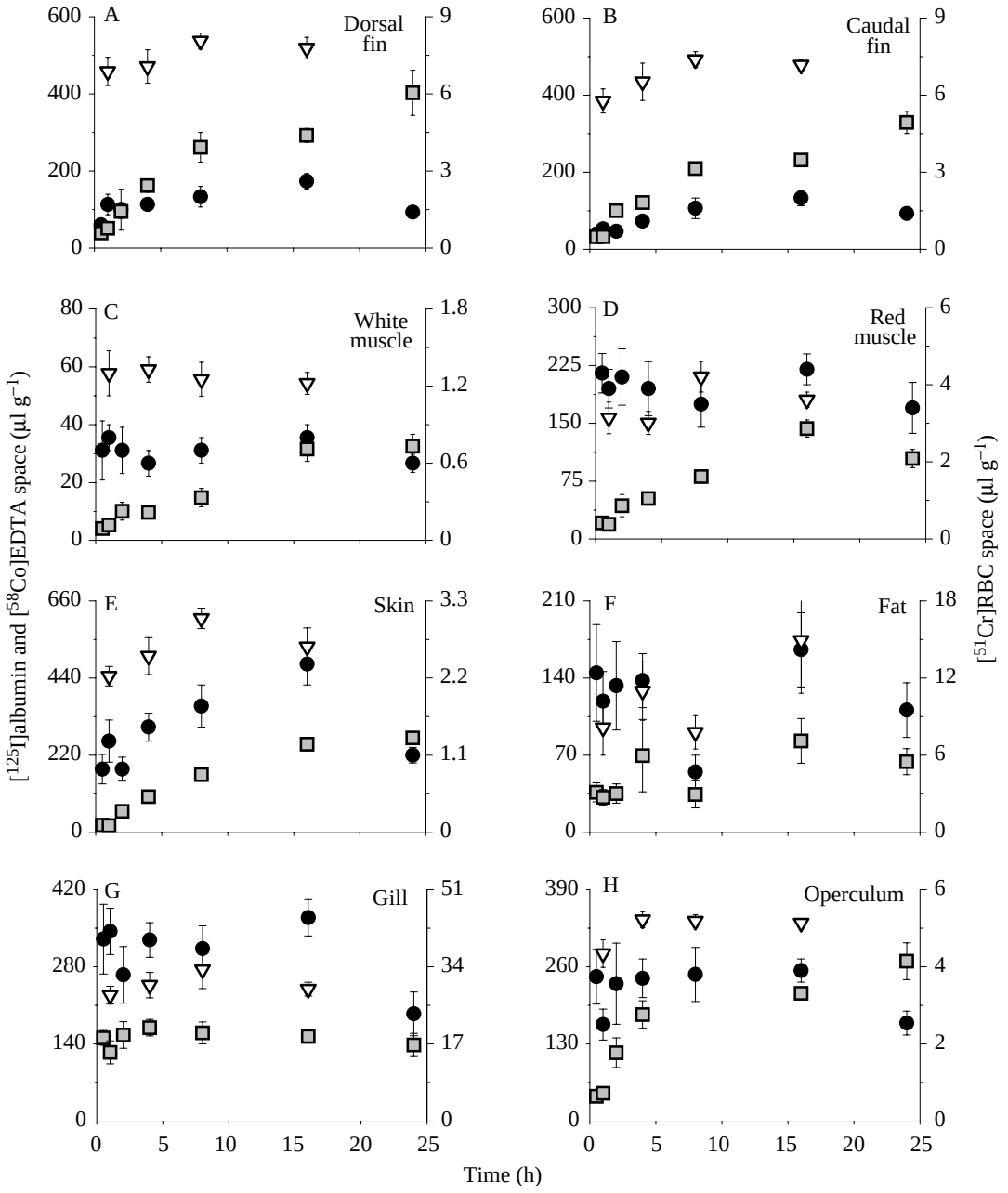

Fig. 2. [^{58}Co]EDTA (open triangles), [^{51}Cr]RBC (filled circles) and [^{125}I]albumin (shaded squares) spaces as a function of time in fins (A,B), white muscle (C), red muscle (D), skin (E), fat (F), gill (G) and operculum (H). Values are mean ± S.E.M., *N*=6–17.

equilibration pattern, [^{125}I]albumin equilibrium was reached in the first 0.5 h and remained constant ([^{125}I]albumin spaces determined at *t*=0.5 h were not statistically different from those measured over the next 23.5 h). In these tissues (fat, gills, liver, spleen and kidney; Figs 2F,G, 3E,G,H), the rate and stability of [^{125}I]albumin equilibration were similar to those of [^{58}Co]EDTA and [^{51}Cr]RBC. In tissues with a two-tiered equilibration pattern, [^{125}I]albumin initially appeared to equilibrate quickly: there were no significant differences between samples taken from the first four or five intervals (*t*=0.5–6–8 h). However, subsequent samples (*t*=8, 16 and 24 h) indicated that a new equilibrium had been established, as they were significantly higher than previous samples but not different among themselves. White muscle, cecum, intestine, gallbladder and brain (Figs 2C, 3C,D,F, 4B) exhibited this two-tiered equilibration pattern. [^{125}I]albumin did not reach equilibrium in fins, red muscle, skin and eye (Figs 2A,B,D,E, 4A) as continuous, and statistically significant, increases in [^{125}I]albumin spaces occurred from 0.5 to 24 h. Whole-body [^{125}I]albumin space (Fig. 5) did not appear to reach equilibrium during the 24 h experimental period.

Table 2. *Summary data for calculated tissue hematocrits at 16 h post-injection*

	Tissue hematocrit (%)	Tissue hematocrit/dorsal aortic hematocrit	Level of significance
Spleen	71.1±4.9	2.93±0.26	***
Liver	23.8±2.5	0.96±0.08	NS
Kidney (posterior)	21.3±4.1	0.86±0.15	NS
Gill III	20.3±1.0	0.83±0.03	**
Gill II	20.2±1.1	0.82±0.03	*
Kidney (anterior)	20.1±3.0	0.81±0.11	NS
Gill I	20.0±1.0	0.82±0.02	**
Gill IV	19.2±1.0	0.79±0.05	**
Fat	16.3±2.2	0.66±0.09	**
Cecum	12.5±1.8	0.50±0.07	***
Intestine (posterior)	11.7±1.8	0.47±0.07	***
Intestine (anterior)	10.0±1.7	0.41±0.07	***
Eye	8.4±0.6	0.34±0.02	***
Brain	8.1±1.0	0.33±0.04	***
Esophagus	7.4±0.8	0.30±0.03	***
Stomach	7.3±1.0	0.29±0.04	***
Operculum	6.5±4.7	0.28±0.21	**
Gallbladder	6.2±1.6	0.25±0.06	***
Swimbladder	5.2±1.0	0.21±0.04	***
Red muscle	3.5±0.5	0.14±0.02	***
Skull	3.2±0.8	0.13±0.04	***
White muscle (ventral)	2.6±0.3	0.11±0.01	***
White muscle (dorsal)	2.4±0.5	0.10±0.02	***
Pectoral fin	1.5±0.4	0.06±0.02	***
Pelvic fin	1.3±0.4	0.05±0.01	***
Caudal fin	1.2±0.3	0.05±0.01	***
Dorsal fin	1.1±0.3	0.04±0.01	***
Skin	1.0±0.3	0.04±0.01	***
Whole body	9.5±0.6	0.39±0.03	***

Tissue hematocrit = [^{51}Cr]RBC space/([^{51}Cr]RBC space + [^{125}I]albumin space).

Tissues are listed in the table in order of decreasing tissue hematocrit.

Values are mean ± S.E.M. (N=6).

Statistical differences from the measured dorsal aortic hematocrit (24.5±0.75 %, N=6) are noted in the final column. NS, not significantly different; *P<0.05; **P<0.01; ***P<0.001.

Discussion

[^{58}Co]EDTA, [^{51}Cr]RBC and [^{125}I]albumin spaces at 16 h

In the past 20 years, ECF measurements in fish tissues have been made using a number of different markers (reviewed by Olson, 1992). This probably accounts for as much, if not more, of the variability in tissue space values than actual interspecific differences.

The tissue distribution volume of [^{58}Co]EDTA (Table 1) agrees closely with tissue spaces measured previously using [^{58}Co]EDTA and an 8 h equilibration period (Duff *et al.* 1997). In other studies (Olson, 1992), tissue spaces (bone, brain, gill, gut, kidney, liver, skin, red and white muscle) measured in rainbow trout using inulin or polyethylene glycol (PEG) tended to be the same, or significantly smaller, than spaces measured using [^{58}Co]EDTA, while mannitol spaces were equal to or significantly larger than [^{58}Co]EDTA tissue spaces. Whole-body ECF volume measured in rainbow trout with either inulin (Munger *et al.* 1991; Nichols, 1987; Eddy and Bath, 1979; Beyenback and Kirschner, 1976) or mannitol (Munger *et al.* 1991; Milligan and Wood, 1982, 1986) yielded a significantly smaller space (127–287 μl g^{-1}) than those determined using [^{58}Co]EDTA (380±10.6 μl g^{-1}).

The present study provides, to our knowledge, the first estimate of ECF volume of swimbladder, fins, skin, gallbladder and eye. The ECF volume in the swimbladder (587 μl g^{-1} wet tissue mass) was second only to that of the kidney in size. The two main components of the swimbladder are vascular gas-secreting and resorption capillary beds, and the bladder itself. Anatomical studies by Wagner *et al.* (1987) indicate that the arterial and venous vessels of the gas-secreting rete mirabile are in such close apposition that there is very little interstitial space. A large ECF volume could reside in the connective tissues of the mucosal and submucosal layers underlying the inner epithelium of the bladder (Morris and Albright, 1975) or may result from fluid accumulation inside the bladder itself (Kobayashi *et al.* 1989). The large [^{58}Co]EDTA space observed in this study suggests the latter to be the case. It is not known whether fluid movement into the lumen of the gland has any physiological significance.

ECF volumes in fins (376–519 μl g^{-1} wet tissue mass) and skin (529 μl g^{-1} wet tissue mass) were the third and fourth largest, respectively, among the tissues measured. In view of the loose connective tissue matrix in the hypodermis of skin and fins (Whitear *et al.* 1980), a large ECF volume in these two tissues is not surprising. The ECF volume of fish skin is only slightly larger than the value of 350–490 μl g^{-1} wet mass reported for back and hindleg skin in rats (Renkin and Tucker, 1995), and thus a large ECF volume may be a common feature of the vertebrate epidermis. In addition, the secondary circulation of the epithelium may contribute to the increased [^{58}Co]EDTA distribution volume, although it is doubtful if this is a major factor (see below).

As has been reported previously (Duff *et al.* 1997), the largest ECF volume was found in the kidney (approximately 675 μl g^{-1} wet tissue mass). Even after subtracting the plasma volume (approximately 325 μl g^{-1} wet tissue mass), the interstitial fluid volume (350 μl g^{-1} wet tissue mass) was higher than the value of 130–170 μl g^{-1} wet tissue mass reported for mammalian kidneys (Aukland and Tenstad, 1995). Since fish kidneys are known to clear EDTA (Duff *et al.* 1997), glomerular filtration may concentrate EDTA in the tubules and collecting ducts, as it does in mammals, thus leading to an overestimate of ECF size in this tissue. It is also possible that

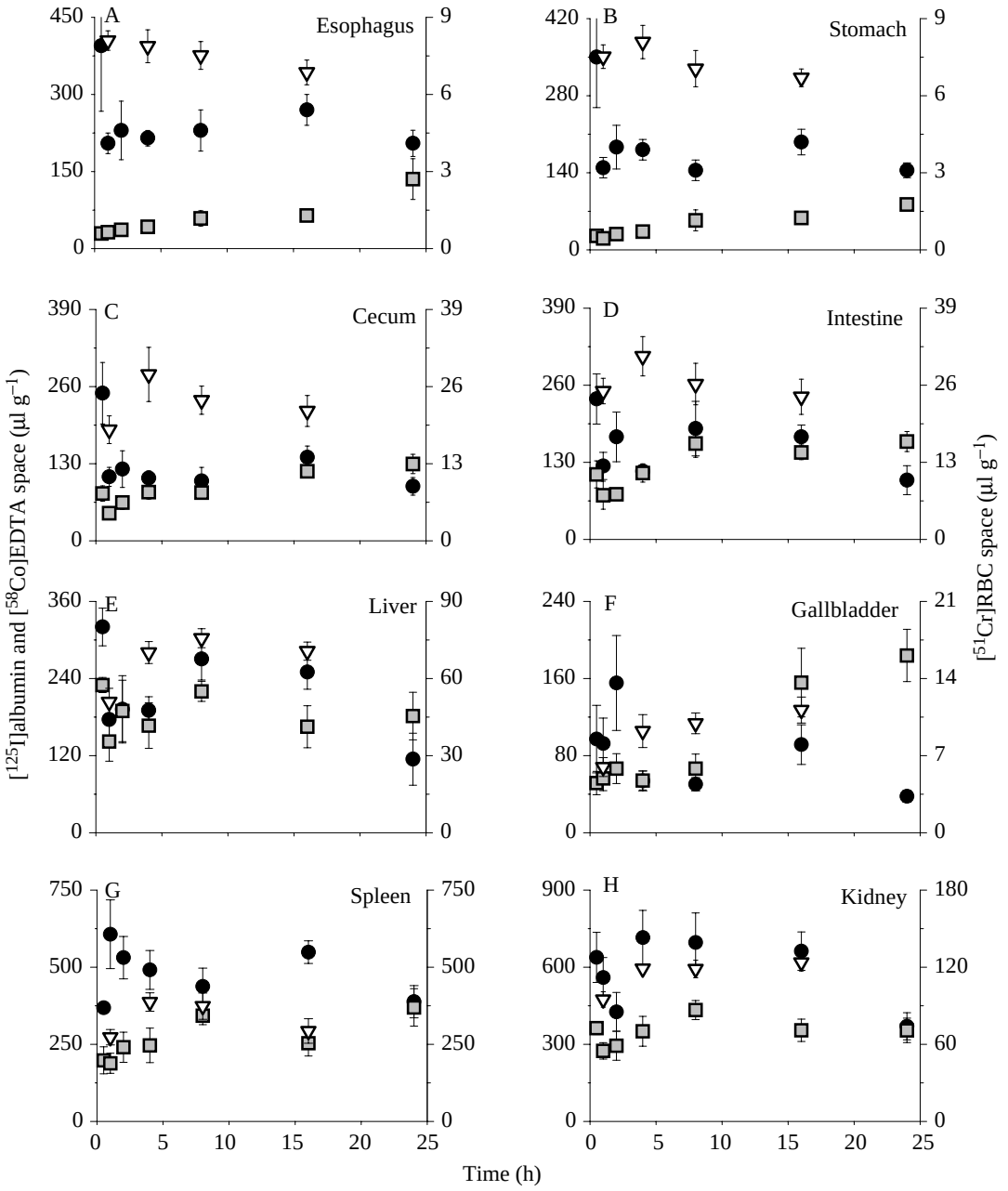

Fig. 3. [^{58}Co]EDTA (open triangles), [^{51}Cr]RBC (filled circles) and [^{125}I]albumin (shaded squares) spaces as a function of time in the esophagus (A), stomach (B), cecum (C), intestine (D), liver (E), gallbladder (F), spleen (G) and kidney (H). Values are mean ± S.E.M., *N*=6–17.

some renal binding of ionic ^{58}Co or [^{58}Co]EDTA occurred, although non-ionic polyethylene glycol spaces may be as high in the fish kidney (Olson, 1992) as [^{58}Co]EDTA spaces measured in the present study.

[^{51}Cr]RBC tissue spaces (Table 1) are in good agreement with other [^{51}Cr]RBC spaces reported in trout (Duff and Olson, 1989; Gingerich and Pityer, 1989; Gingerich *et al.* 1987, 1990). As noted previously, highly perfused, metabolically active organs (kidney, liver, gills) had the largest [^{51}Cr]RBC spaces. However, there is a discrepancy in brain [^{51}Cr]RBC spaces. Gingerich and Pityer (1989) and Gingerich *et al.* (1987, 1990) found relatively large [^{51}Cr]RBC spaces in brain (29–34 µl g^{-1} wet tissue mass), even though this tissue is not as well perfused as the kidney or the liver. Brain [^{51}Cr]RBC spaces reported by Gingerich's group are nearly an order of magnitude larger than the 3.4–4.2 µl g^{-1} wet tissue mass found in the present study and by Duff and Olson (1989). Whether this is due to differences in the technique used to kill the fish (a blow to the head in the former *versus* an overdose with Nembutal in the latter), tissue extraction procedures or strain of trout used is unclear.

Skin [^{51}Cr]RBC space, reported in the present study for the

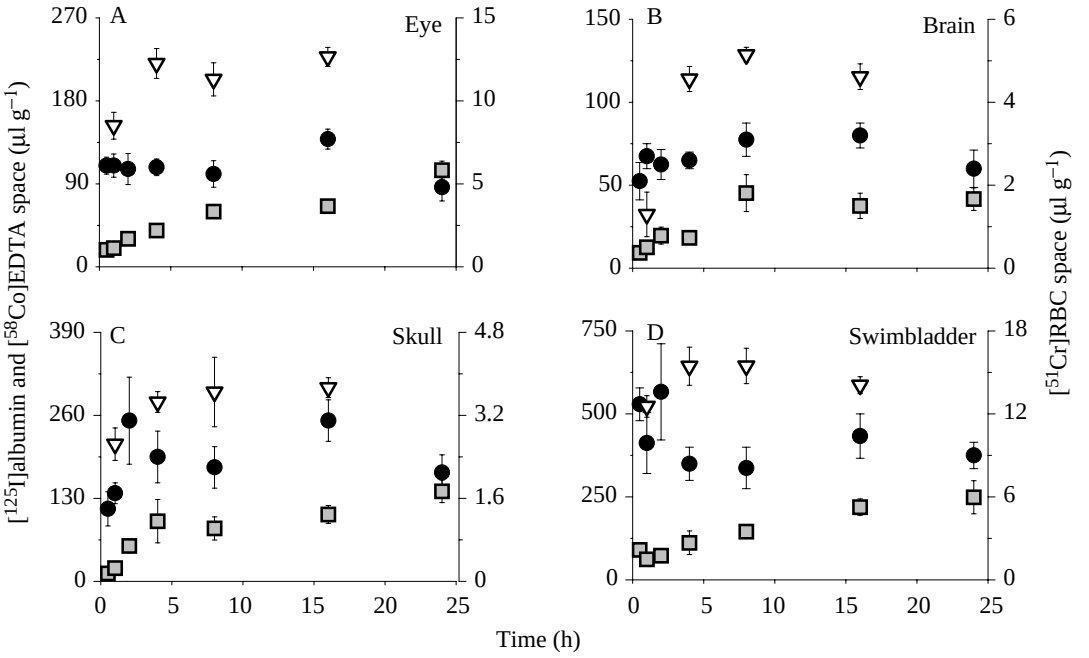

Fig. 4. [^{58}Co]EDTA (open triangles), [^{51}Cr]RBC (filled circles) and [^{125}I]albumin (shaded squares) spaces as a function of time in the eye (A), brain (B), skull (C) and swimbladder (D). Values are mean ± S.E.M., *N*=6–17.

first time, was quite small (2.4 μl g^{-1} wet tissue mass). On the basis of Wardle's (1971) measurement of [^{125}I]albumin space in the skin of the flatfish *Pleuronectes platessa* (607 μl g^{-1} wet tissue mass), the [^{51}Cr]RBC space would be predicted to be 100–200 μl g^{-1} wet tissue mass if skin hematocrit were similar to dorsal aortic Hct. Skin [^{51}Cr]RBC space is also two orders of magnitude smaller than predicted from [^{125}I]albumin space measured in the present study.

In general, [^{125}I]albumin tissue spaces measured at 16 h (Table 1) are somewhat larger than spaces measured in trout after mixing periods of either 0.5 h (Duff and Olson, 1989) or 4 h (Gingerich and Pityer, 1989; Gingerich *et al.* 1990).

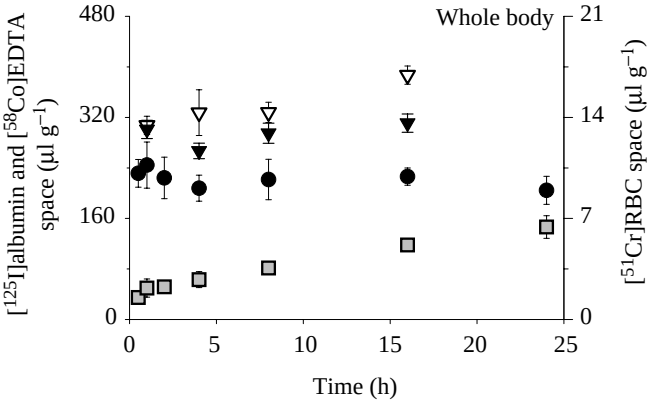

Fig. 5. Whole-body [^{58}Co]EDTA (open triangles), [^{58}Co]EDTA spaces corrected for urine loss (filled triangles), [^{51}Cr]RBC (filled circles) and [^{125}I]albumin (shaded squares) spaces as a function of time. Values are mean ± S.E.M., *N*=6–17.

[^{125}I]albumin space in the skin of the flatfish measured after an even longer (50 h) circulation period (607 μl g^{-1} wet tissue mass; Wardle, 1971) was considerably larger than the value for trout at 16 h (251 μl g^{-1} wet tissue mass). Tissue hematocrits estimated from [^{51}Cr]RBC and [^{125}I]albumin spaces at 16 h are lower than dorsal aortic Hct in all tissues except spleen, kidney and liver (Table 2). Similarly, whole-body plasma volume, i.e. [^{125}I]albumin space (118 μl g^{-1} body mass), was nearly four times larger than would have been predicted from plasma volume calculated from the [^{51}Cr]RBC space at 16 h and a dorsal aortic Hct of 24.5 % (30.5 μl g^{-1} body mass).

The F_{cell} ratio, which is the whole-body hematocrit (calculated from the RBC and plasma indicator spaces) divided by the large vessel hematocrit (hematocrit of blood in a central vessel) is approximately 0.91 in mammals (Albert, 1971). This less than unitary F_{cell} ratio is attributable, to a large extent, to a low (⩽10) hematocrit in tissue capillaries (Johnson, 1971). The F_{cell} ratio in trout at 16 h is 0.39 (Table 2). This is considerably less than the F_{cell} ratio reported for mammals and is also less than the ratio of 0.63 or 0.78 obtained in trout after a 4 h circulation time (present study; Gingerich and Pityer, 1989, respectively). In order for an F_{cell} ratio to be as low as 0.5, a trout would need to have half of its blood volume in the capillaries, and this blood would have to be devoid of red cells. Because this is an improbable situation, alternative explanations, such as extravasation of [^{125}I]albumin and/or equilibration with a secondary circulation, must be considered. Furthermore, the observation that calculated tissue hematocrits are, with very few exceptions, smaller than those predicted from dorsal aortic Hct (Table 2) indicates that this is probably

not a tissue-specific phenomenon. In fact, like the F_{cell} ratio, tissue hematocrits calculated after 16 h of indicator equilibration tend to be much smaller than those reported in previous studies that employed shorter sampling times (0.5–4 h; Gingerich and Pityer, 1989; Gingerich *et al.* 1990; Duff and Olson, 1989). Aberrant properties of [^{125}I]albumin as a plasma indicator become even more evident when the distribution dynamics of this indicator are examined in detail (see below).

Dynamics of [^{58}Co]EDTA, [^{51}Cr]RBC and [^{125}I]albumin equilibration

The distribution of vascular compartment indicators is dependent on the degree of tissue vascularization and on convective mixing of blood and its flow through the tissues. A number of studies have tracked the disappearance of markers from the vascular system over time (for a review, see Olson, 1992), but only one has documented the temporal nature of their appearance in the tissues, and in that study only ^{51}Cr-labelled RBCs were monitored for 4 h (Duff *et al.* 1987). The temporal distribution of [^{51}Cr]RBC in the present study (Figs 2–4) was similar to that reported previously by Duff *et al.* (1987), in that an apparent equilibrium was reached within 1 h in nearly all the tissues examined. A similar rapid time course was evident for [^{51}Cr]RBC dilution in the plasma, as reflected by the relatively constant value for whole-body [^{51}Cr]RBC space (Fig. 5; Duff *et al.* 1987). Thus, convective mixing of RBCs and their distribution to the tissues appear to be relatively rapid processes.

The distribution of extravascular indicators depends on the factors affecting the dispersal of intravascular indicators, as described above, together with (1) the rate of indicator escape across the capillaries, (2) the rate of convective and diffusive permeation into the interstitial space and (3) the volume of the interstitial compartment. The rate of [^{58}Co]EDTA accumulation in the tissues (Figs 2–4) and its disappearance from the central circulation (as reflected in whole-body [^{58}Co]EDTA space; Fig. 5) are, within the temporal discrimination of the present study, as rapid as those observed for [^{51}Cr]RBC. Thus, it can be concluded that permeant extravascular indicators are also quickly distributed throughout the ECF volume and are at, or near, equilibrium in most, if not all, tissues within 1 h.

The kinetics of [^{125}I]albumin distribution to tissues (Figs 2–4) or plasma volume calculated from the disappearance of label from the plasma (Fig. 5) are different from those observed for either [^{51}Cr]RBC or [^{58}Co]EDTA and indicate that other factors are involved. These other factors could include albumin extravasation from the vasculature or distribution into another fluid compartment, i.e. the secondary circulation. For reasons discussed below, extravasation seems more probable.

Implications of this study for the volume of the secondary circulation

J. F. Steffensen, M. Heies, D. J. Randall and G. Iwama (unpublished data cited by Steffensen and Lomholt, 1992) reported that the volume of the secondary circulation in trout is 48.4 μl g^{-1} body mass. This is 1.5 times the volume of the primary circulation, and its magnitude becomes even more impressive because secondary vessels are found only in gill, skin, fins, peritoneal lining, oral mucosa and in the heat exchanger rete in tuna (reviewed in Steffensen and Lomholt, 1992; Olson, 1996). However, in the study cited by Steffensen and Lomholt (1992), the secondary circulation volume was estimated from the disappearance of [^{125}I]albumin from the primary circulation (dorsal aorta), and it was assumed that extravasation of [^{125}I]albumin during the 24 h sampling period was negligible. Fig. 6 compares the fractional recovery of [^{125}I]albumin calculated from the study cited by Steffensen and Lomholt (1992) with fractional [^{125}I]albumin recovery from the present study. Despite the fact that serial samples were collected from the same fish in the earlier study, whereas in the present study only a single blood sample was obtained from an individual fish, the results from the two protocols are virtually identical. It can therefore be assumed that there are no significant effects of methodological differences between the two studies and that the dynamics of indicator distribution to the tissues were the same in both. Thus, the volume of the secondary circulation can now be re-evaluated on the basis of the appearance of indicators in the tissues.

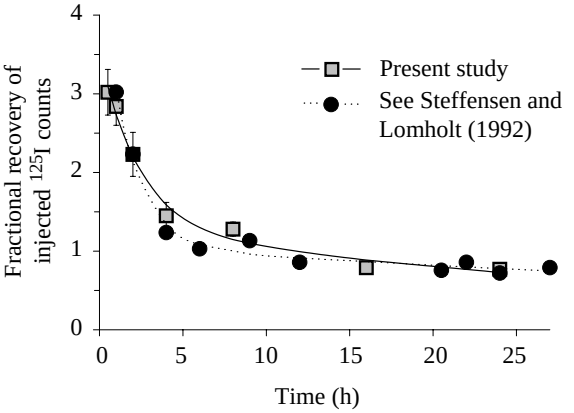

Fig. 6. Fractional recovery of a dose of [^{125}I]albumin injected into the dorsal aorta of rainbow trout as a function of time. Shaded squares, data from the present study, in which a single blood sample was withdrawn from each fish. Filled circles, data from a study cited in Steffensen and Lomholt (1992), in which serial samples were withdrawn from individual fish at timed intervals. The solid and broken lines are double-exponential curves fitted to the respective data by computer. Values for the present study are mean ± S.E.M., N=6–17.

The present study shows that there are two inconsistencies in using [^{125}I]albumin disappearance from the primary circulation as an index of indicator distribution to the secondary system. First, the distributions of different indicators in tissues are not temporally correlated and, second, there is no tissue-specific correlation between albumin space and the presence or absence of a secondary system.

If [^{125}I]albumin is retained within the capillaries of the

secondary circulation, then its distribution in these tissues should reach equilibrium before that of the extracellular volume indicator [^{58}Co]EDTA. Presumably both indicators are distributed to the tissues through the same convective (perfusion) processes; however, final [^{58}Co]EDTA equilibrium must also entail transfer across the capillary wall and diffusion into the interstitial matrix, as described above. Figs 2–4 clearly show that, in virtually all tissues, [^{58}Co]EDTA reaches equilibrium as fast as, or even faster than, [^{125}I]albumin. Thus, albumin distribution to many tissues is not limited solely by convection. This discrepancy is especially apparent in tissues with a known secondary system (fins, skin and operculum), where it is quite evident that [^{58}Co]EDTA reaches equilibrium well before the first sample at 1 h, whereas [^{125}I]albumin concentration continues to increase for the full experimental period of 24 h (Fig. 2A,B,E,H). It could be argued that there is sufficient primary circulation in fins and skin to permit rapid [^{58}Co]EDTA delivery to the entire extracellular compartment; however, it seems more plausible to attribute the delay in [^{125}I]albumin equilibration to a slower rate of [^{125}I]albumin transfer across the capillaries and/or decreased diffusivity in the extracellular matrix. Dissimilar [^{58}Co]EDTA and [^{125}I]albumin equilibration kinetics are observed in tissues where a secondary system is absent, i.e. muscle and gut (Figs 2C,D, 3A–D), providing further evidence that [^{125}I]albumin extravasation is the predominant factor. The high permeability of fish capillaries to plasma protein had also been noted (Hargens *et al.* 1974). Slow equilibration of [^{51}Cr]RBC in fins and skin does, however, appear to be due to the unique narrow-bore origins of the secondary system, which physically delays the entry of RBCs (Steffensen and Lomholt, 1992).

The tissue distribution volume of indicators at 16 h is also inconsistent with the presence or absence of a secondary circulation. While [^{125}I]albumin spaces are large in skin and fins, they are also larger than predicted in most other tissues (Table 2), even though the latter lack a secondary system (Steffensen and Lomholt, 1992; Olson, 1996). Conversely, although gills have a well-documented secondary system (Steffensen and Lomholt, 1992; Olson, 1996), their [^{125}I]albumin space is only slightly greater than predicted (Table 2). In fact, it appears that the gills are relatively impermeable to [^{125}I]albumin, a fact previously noted and attributed to the necessity of countering the high intravascular hydraulic pressure unique to the gill (Olson, 1992, 1996).

If it can be assumed that convective delivery of [^{125}I]albumin to the tissues is as fast as, or faster than, that of [^{58}Co]EDTA and that [^{58}Co]EDTA reaches equilibrium within 1 h, then the volumes of the primary and secondary circulations can be approximated from [^{125}I]albumin spaces obtained within the first hour of indicator circulation. Whole-body [^{125}I]albumin spaces at 0.5 and 1 h are 34.8±2.0 and 49.7±14.4 μl g^{-1} body mass, respectively, and [^{51}CR]RBC spaces at the same intervals are 10.1±1.0 and 10.7±1.2 μl g^{-1} body mass, respectively. At 0.5 and 1 h, the total intravascular volumes, calculated as the sum of [^{125}I]albumin and [^{51}Cr]RBC spaces, are 44.9 and 60.4 μl g^{-1} body mass, respectively, and the volumes in the primary circulation, calculated from [^{51}Cr]RBC space and 24.5 % dorsal aortic Hct, are 41.2 and 43.7 μl g^{-1} body mass, respectively. Subtracting the volume of the primary circulation from the total volume yields an estimated secondary circulation volume of 3.7 μl g^{-1} body mass at 0.5 h and 16.7 μl g^{-1} body mass at 1 h. Clearly, these values will underestimate the actual volume of the secondary circulation if convective delivery of plasma to this compartment is delayed, whereas secondary volume will be overestimated as [^{125}I]albumin enters the interstitium (which may be rapid in tissues with a discontinuous or very permeable endothelium, such as liver and spleen; Fig. 3E,G). Nevertheless, it is doubtful whether the volume of the secondary circulation is greater than 10–20 % of the volume of the primary circulation. Clearly, this issue cannot be resolved until a better plasma indicator is identified.

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