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Fate of ^{125}I-IGF-I Administered into the Amniotic Fluid of Late-Gestation Fetal Sheep

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ABSTRACT

Large amounts of amniotic fluid (AF) are swallowed in late gestation. AF is the most accessible fetal compartment and provides a possible paraplacental route for the therapeutic administration of hormones and nutrients to the fetus. We therefore wished to investigate the fate of the predominant fetal growth factor, IGF-I, administered into AF of late-gestation ovine fetuses. Seven chronically catheterized fetuses at 124 d gestation had approximately 800×10^6 dpm of ^{125}I-IGF-I injected into the AF. AF and blood samples were withdrawn for up to 6 d. At 131 d gestation a postmortem examination was performed. All AF, blood, and tissue samples were counted. Selected samples of AF, blood, and gut contents underwent size-separation chromatography. ^{125}I-IGF-I was rapidly mixed in AF, with a significant difference in counts from different regions of the cavity persisting for only 3 h ($p < 0.05$). *In vivo* binding of ^{125}I-IGF-I in AF correlated highly with AF IGF binding protein 3 concentrations ($r^2 = 0.93$, $p < 0.0001$). In some animals, free ^{125}I-IGF-I persisted in AF and in plasma for the duration of the experiments. Chromatography of plasma samples demonstrated that intact ^{125}I-IGF-I was taken up from the fetal gut. Only fetal gut and thyroid contained appreciable counts at postmortem examination. Gut contents had more counts than gut wall, and the number of counts in gut contents increased distally ($p < 0.05$ for colon contents *versus* other regions). We conclude that there is sustained delivery of ^{125}I-IGF-I from the AF to the gut and systemic circulation of the ovine fetus after a single intraamniotic injection. **(*Pediatr Res* 51: 361–369, 2002)**

Abbreviations

AF, amniotic fluid
EGF, epidermal growth factor
IGFBP, IGF binding protein
^{125}I-IGF-I, ^{125}iodine-labeled IGF-I

In late gestation, fetuses swallow large amounts of AF (1, 2) and turnover of AF volume has been estimated to occur every 24 h (3). AF contains growth factors, such as EGF and IGF-I (4, 5), and nutrients, which can be taken up by the fetal gastrointestinal tract (4, 6, 7). The amniotic route of administration for growth factors or nutrients has been proposed as a potential therapeutic approach to intrauterine growth restriction in which the placenta is the cause of a limited nutrient supply to the fetus (8).

IGF-I is probably the predominant fetal growth factor in late gestation (9). We have recently demonstrated that IGF-I administered once daily for 10 d into AF of intrauterine growth-restricted ovine fetuses increased gut growth, but reduced circulating fetal IGF-I levels and growth of the spleen, liver, and thymus (10). We also demonstrated that IGF-I levels in AF remained elevated for at least 24 h after injection.

Gastrointestinal uptake of IGF-I administered directly into the gut has been demonstrated in several species in postnatal life (11–13), and uptake of EGF has been demonstrated in the fetal rat (4). However, gut uptake has not been studied in the fetus after administration of a hormone into AF, rather than directly into the gastrointestinal tract.

We therefore designed this study to investigate the fate of IGF-I administered into AF. We wished to determine the clearance of ^{125}I-IGF-I from AF, its uptake by the fetal gut, and its binding characteristics in AF and fetal plasma *in vivo*. The occurrence of a gut stenosis at the site of portal venous catheter insertion in two animals has also allowed us to compare the gut uptake of ^{125}I-IGF-I in animals with normal and stenotic guts.

METHODS

Animal procedures. Approval for the experiment was obtained from the institutional animal ethics committee. Seven ewes carrying singleton pregnancies underwent surgery at 110–112 d (term = 145 d). Catheters were inserted into the fetal tarsal arteries and veins, the portal vein, the umbilical vein, the uteroovarian vein, and maternal carotid artery and jugular vein. Two AF catheters were attached to the fetal neck

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(the upper catheters) and two to the fetal hindlimbs (the lower catheters). On day 124 of gestation, baseline samples of blood and AF were withdrawn from each catheter for estimation of IGF-I levels by RIA (14, 15) and the characterization of IGFBPs by ligand blot analysis. Freshly iodinated ^{125}I-IGF-I was then injected as a bolus into one of the upper amniotic catheters, and the catheter was flushed with 2 mL of AF. All upper amniotic samples were subsequently withdrawn from the other upper catheter. Samples of blood and AF were withdrawn simultaneously from all catheters at increasing intervals of time for up to 144 h. The first animal received 81×10^6 dpm, but there were insufficient counts in fetal plasma for chromatography. Subsequent animals therefore received approximately 10 times as many counts (Table 1). Postmortem examinations were performed on six animals as quickly as possible after the last samples were taken, usually within 1–2 h.

Size-separation gel chromatography. The radioactivity of all samples was counted at the end of the experiment, together with tissues obtained at postmortem examination, and selected blood and amniotic samples underwent size-separation gel chromatography. Gut contents were collected for chromatography in two animals. Blood and AF samples for size-separation chromatography were collected onto ice and centrifuged at 4°C for 15 min. They were transported to the laboratory on ice as soon as possible. Samples were then filtered through a 0.22-μm filter (Millipore Corp., Bedford, MA, U.S.A.). Two HPLC systems were used to enable all the samples to be processed in an acceptable time interval from collection. These were an Äkta Explorer 10 using Unicorn 2.3 software (Pharmacia Biotech AB, Uppsala, Sweden) and a BioCAD Sprint using BioCAD 2.7 software (PerSeptive Biosystems Inc., Framingham, MA, U.S.A.). Two hundred fifty microliters of filtered sample was injected onto a 30-cm $\times$ 10-mm Superose 12 column (Pharmacia) and eluted with 0.01 M PBS, pH 7.4, running at 0.5 mL/min. Fractions of 0.3 mL each were counted on a Cobra gamma counter (Packard Instruments Co., Meriden, CT, U.S.A.) for 1 min. Both columns were calibrated with dextran blue, BSA, IGF-I, and phenol red as molecular weight markers. These peaks eluted over the same fractions on both columns. The columns were also calibrated with ^{125}I-IGF-I (eluting in fraction 48) and ^{125}I. Again, the elution profiles were identical between the two columns, with ^{125}I-IGF-I eluting beyond the column volume, presumably because of ionic interactions. The fraction at which ^{125}I eluted off both columns remained consistent throughout the experiments.

Gut contents were collected on ice at postmortem examination. Samples were diluted with PBS until a consistency suitable for homogenization was achieved. Homogenates were centrifuged at 100,000 $\times$ *g* for 30 min, and the supernatant was filtered through a 0.22-μm filter (Millipore Corp.) before injection onto the column.

To analyze the degree of *in vivo* binding of ^{125}I-IGF-I to IGFBPs in AF and plasma, the total number of counts in the fractions of the peak eluting around fraction number 48 was regarded as free ^{125}I-IGF-I. Counts in fractions eluting earlier than this were regarded as representing ^{125}I-IGF-I bound to various IGFBPs. The sum of all counts in fractions containing free and bound ^{125}I-IGF-I was taken to represent intact ^{125}I-IGF-I. The percent of intact ^{125}I-IGF-I that was bound was calculated by dividing the bound ^{125}I-IGF-I counts by the total counts representing intact ^{125}I-IGF-I, and expressing this as a percentage.

Ligand blot. Samples of plasma and AF were diluted to 20 μL in 0.01 M PBS and then mixed with 20 μL of nonreducing buffer and 3 μL of bromophenol blue. A sample of maternal plasma was included as a control. Samples were boiled for 3 min and loaded onto the gel. After electrophoresis the binding proteins were transferred to nitrocellulose. As IGFBPs bound to nitrocellulose are able to bind IGFs owing to their stable tertiary structure derived from the formation of multiple cystine cross-linkages (16), ^{125}I-IGF-II is used as the ligand to detect the presence of IGFBPs rather than a specific antibody as in Western blotting. Labeled IGF-II is used in preference to ^{125}I-IGF-I as most IGFBPs preferentially bind IGF-II. Validation of this technique in our laboratory has confirmed that the use of ^{125}I-IGF-II gives more consistent results (17).

After electrotransfer the membrane was incubated with a saline buffer containing 1% BSA and 0.1% Tween and 125,000 cpm/mL ^{125}I-IGF-II (1 mL per sample lane) at 4°C for 16 h.

The membrane was then placed next to a phosphoimaging cassette overnight. The cassette was imaged using a Molecular Dynamics Storm 860 phosphoimager (Amersham Pharmacia Biotech, Uppsala, Sweden), and the density of the bands was

Table 1. *Data for individual animals, showing some morphometric data, baseline IGF-I levels, counts of ^{125}I-IGF-I injected, and whether the gut was normal or stenotic*

	Animal number						
	99019	99054	99079	99124	99150	99158	99165
Gut stenosis	No	No	No	Yes	Yes	No	No
Counts injected (dpm $\times$ 10^6)	81	1025	1210	990	754	620	886
Sampling period (h)	72	72	144	144	144	144	36
Baseline IGF-I concentrations (ng/mL)							
Maternal artery		87.1	146.3	158.1	171.8	220.6	211.4
Fetal artery		98.3	114.8	97.9	71.6	72.3	93.4
Amniotic fluid		2.47	1.89	1.57	0.67	1.16	3.54
Fetal weight (g)	3608	3995	3816	5035	5573	4168	
AF volume (mL)	2334	3090	1277	2726	1327	1661	
Uteroplacental wt (g)	1355	2475	1297	1557	1665	2138	
Gut weight (g)	146	152	165	206	163	143	

AF volume, amniotic fluid volume at postmortem examination.

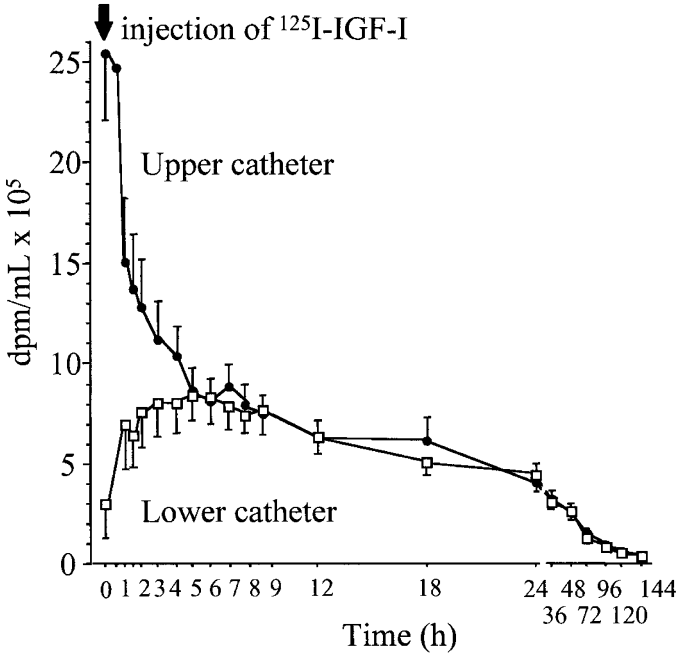

Figure 1. Counts in samples taken from upper and lower amniotic catheters ($n = 7$ until 36 h, then $n = 6$ until 72 h, and then $n = 5$ until 144 h). Values are mean ± SEM. Counts in upper and lower catheters are significantly different until 3 h after injection ($p < 0.05$).

then assessed using a Molecular Dynamics densitometer and ImageQuaNT software (Version 4.2a, Build 13). To obtain an image for reproduction, the nitrocellulose was exposed to x-ray film at −80°C for 7 d, and the film was then developed. The IGFBP-1, -2, -3, and -4 bands were identified with reference to previously validated Western ligand blots and Western immunoblots of ovine IGFBPs obtained in an identical manner in this laboratory (17–19).

Data analysis. Data are presented graphically either for each individual animal or as mean ± SEM as appropriate. Amniotic counts from upper and lower catheters were compared by the Wilcoxon signed rank test. Portal venous counts were compared between animals with normal and stenotic guts for the first 12 h after injection by repeated measures ANOVA with the Games-Howell *post hoc* test. Counts in tissues were compared between animals with normal and stenotic guts by the Mann-Whitney *U* test and among different gut regions in the same animal by the Wilcoxon signed rank test.

RESULTS

All fetuses were well grown, and baseline plasma IGF-I levels were similar in all animals (Table 1). Of the seven fetuses, two had a gut stenosis or atresia at the site of portal vein insertion. The stenoses were 150 cm (no. 99124) and 176 cm (no. 99150) distal to the beginning of the duodenum. These were unplanned stenoses, and a complication of portal vein catheter insertion. However, the occurrence of these stenoses has allowed investigation of the gastrointestinal uptake of ^{125}I-IGF-I in animals with abnormal guts.

Turnover of ^{125}I-IGF-I in AF. Mixing of ^{125}I-IGF-I in AF was rapid, with a statistically significant difference between counts from the upper and lower catheters persisting for only 3 h ($p < 0.05$; Fig. 1). The half-life of ^{125}I-IGF-I in AF was approximately 24 h (Fig. 1). The presence of gut stenoses did not affect this half-life (data not shown).

In vivo *binding of ^{125}I-IGF-I in AF and plasma.* The proportion of ^{125}I-IGF-I in AF that was bound to binding proteins 10 min after injection varied substantially among animals (Table 2 and Fig. 2). Serial chromatograms from samples taken during the course of the experiment demonstrated that, in most animals, the peak representing bound ^{125}I-IGF-I became progressively more prominent, although the total counts in all peaks decreased with time (Fig. 2). Nevertheless, 144 h after injection there were still an appreciable number of counts in the bound fractions (Fig. 2). The major bound peak eluted several fractions before the 66 kD M_r marker (Fig. 2). Of note is that in two animals (nos. 99054 and 99150) the majority of ^{125}I-IGF-I was present as free ^{125}I-IGF-I 10 min after injection (Fig. 2*A*) and remained so until the end of the experiment (Fig. 2*C*).

Table 2. *Densities of each IGFBP band on ligand blot expressed as a percent of the control lane, the percent of intact ^{125}I-IGF-I bound to binding proteins, and the correlation coefficient from regression of the two, in AF and fetal arterial plasma*

Sheep No.	% intact ^{125}I-IGF-I bound	Density of IGFBP bands (% control lane)			
		IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4
Amniotic fluid samples					
99054	12.2	12.6	16.8	7.5	12.3
99079	84.3	9.7	26.0	28.6	18.8
99124	32	8.6	21.2	18.2	10.9
99150	3.3	6.0	7.4	1.8	8.5
99165	80.5	10.4	34.4	36.2	24.5
	r^2	0.07	0.81*	0.93**	0.82*
Fetal arterial plasma samples					
99054	27.0	108.5	212.1	34.1	90.0
99079	71.3	111.2	192.0	57.8	107.7
99124	24.6	106.2	255.2	35.6	92.2
99150	0.0	116.6	235.4	41.9	110.6
99165	82.3	98.1	221.7	32.5	106.7
	r^2	0.46	0.33	0.05	0.07

* $p < 0.05$, ** $p < 0.01$.

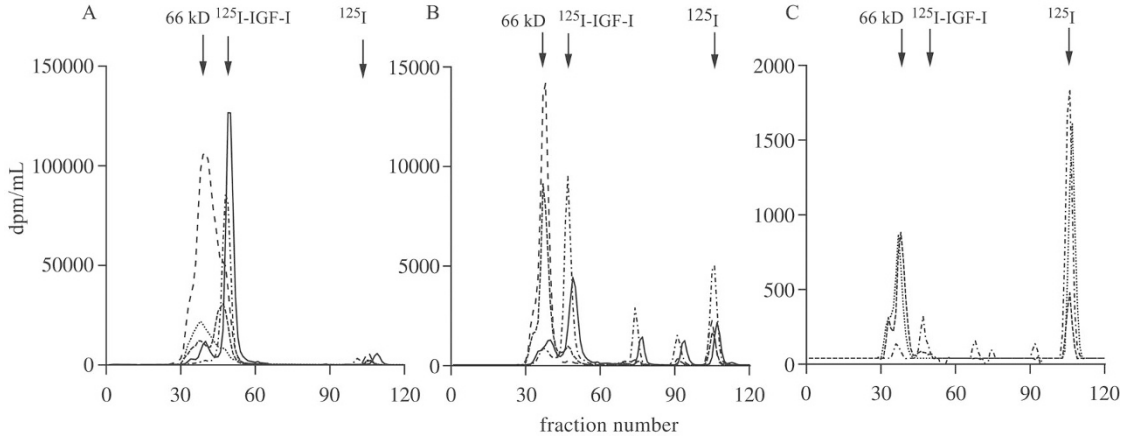

Figure 2. Counts in different fractions after size-separation chromatography of AF samples. *A*, 10 min; *B*, 36 h; and *C*, 144 h after injection of ^{125}I-IGF-I into AF. Lines represent individual animals (— no. 99054, ····· no. 99079, ----- no. 99124, -·-·-·- no. 99150, ____ no. 99165).

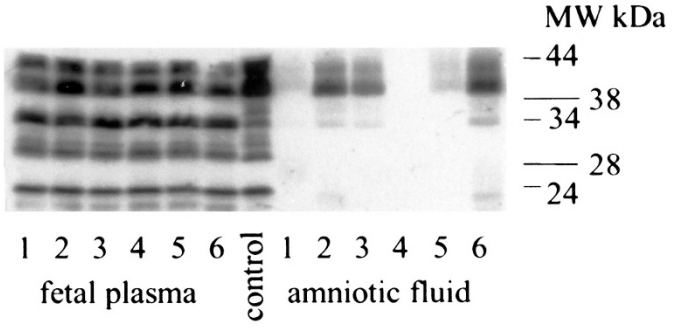

Figure 3. Ligand blot of IGFBPs in baseline samples of fetal plasma and AF taken immediately before injection of ^{125}I-IGF-I into AF. *Lanes 1–6*, animal nos. 99054, 99079, 99124, 99150, 99158, and 99165, respectively. *Control lane*, maternal plasma.

By 6 h after injection several smaller molecular weight peaks, presumably representing degradation products, were apparent (data not shown).

A ligand blot of samples of AF and fetal plasma taken immediately before injection of ^{125}I-IGF-I showed that the predominant binding protein in both AF and plasma had an M_r of 38–44 kD and ran in the position of IGFBP-3 (Fig. 3). The bands with M_r of approximately 34 kD, 28–30 kD, and 24 kD were identified as running in the positions of IGFBPs-2, -1, and -4, respectively. Levels of all IGFBPs were higher in plasma than in AF (Fig. 3). The ligand blot suggests that there is little intact IGFBP-1 in AF. Regression analysis of band density against percent intact ^{125}I-IGF-I bound (calculated from the first chromatograms obtained from each animal: 10 min after injection for AF, and up to 6 h for plasma) showed a very high correlation between the percent ^{125}I-IGF-I bound and the density of the IGFBP-3 band in AF ($r^2 = 0.93$, $p < 0.01$; Table 2). Hence the animals with predominantly unbound ^{125}I-IGF-I on chromatography had only faint bands on ligand blot in the molecular weight region for IGFBP-3. Correlations against individual IGFBPs in plasma were less good (Table 2). Thus we conclude that IGFBP-3 accounts for most of ^{125}I-IGF-I binding in ovine AF *in vivo*.

The chromatographic profiles of plasma samples taken from fetal portal vein and fetal artery were not different, except that counts were higher in the portal vein samples for the first 48 h. Therefore, chromatograms are only shown from fetal arterial samples (Fig. 4). As in AF, the proportion of intact ^{125}I-IGF-I in fetal plasma that was bound was variable among animals with a significant proportion remaining unbound in some animals (Table 2). The percent of ^{125}I-IGF-I bound in plasma correlated best with the relative density of the IGFBP-1 band ($r^2 = 0.46$; Table 2). The chromatograms of plasma samples from fetal artery and portal vein demonstrated the presence of free and bound ^{125}I-IGF-I, with the appearance of degradation fragments as the experiment proceeded (Fig. 4). Degradation products were present earlier and in greater quantities than in AF (Fig. 4 *versus* Fig. 2).

The relative densities of the IGFBP-3 bands in AF and plasma did not correlate with baseline fetal arterial glucose concentrations or oxygen content, or with fetal size at postmortem examination (data not shown). However, the densities of the IGFBP-3 bands in AF did correlate reasonably well with baseline AF IGF-I levels (Table 1; $r^2 = 0.56$, $p = 0.08$). Baseline plasma IGF-I levels did not correlate well with either plasma IGFBP-3 ($r^2 = 0.04$, $p > 0.1$) or IGFBP-1 ($r^2 = 0.01$, $p > 0.1$) band densities.

Appearance and disappearance of counts in fetal plasma. Counts increased in portal vein blood samples before fetal artery blood samples, and remained higher for 48 h (Fig. 5*A*), but with the small number of animals this was not statistically significant. However, counts in portal vein samples were significantly higher for the first 12 h in samples from animals with normal guts than in those from animals with stenotic guts ($p < 0.05$; Fig. 5*B*). In the latter animals the increase in counts in portal vein samples was delayed and peaked later.

Counts appeared in maternal plasma from 12 h after injection (Fig. 5*B*), demonstrating loss of label across the placenta. However, the counts in maternal plasma samples did not reach levels high enough for size-separation chromatography to be performed, so we were unable to determine whether these counts represented labeled hormone or free ^{125}I.

Distribution of counts in tissues at postmortem examination. Very few counts were detected in most fetal tissues at postmortem examination 72–144 h after injection of ^{125}I-

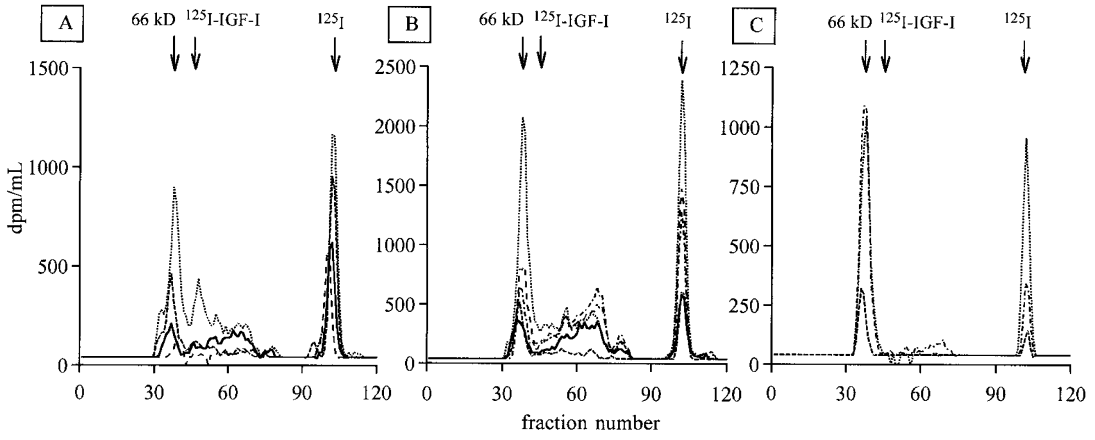

Figure 4. Counts in different fractions after size-separation chromatography of fetal arterial plasma samples. *A*, 6 h; *B*, 36 h; and *C*, 144 h after injection of ^{125}I-IGF-I into AF. Lines represent individual animals (— no. 99054, ····· no. 99079, ----- no. 99124, -·-·-·-· no. 99150, ____ no. 99165).

IGF-I, with the exception of fetal gut and maternal and fetal thyroid (Fig. 6). Gut contents consistently contained more counts than gut wall ($p < 0.05$ for all regions of gut; Fig. 6*B*). Counts in gut contents were higher in distal regions of gut, with the counts in colon contents being approximately 5-fold higher than in other parts of the gut ($p < 0.05$ for contents of colon *versus* all other regions of gut; Fig. 6*B*). Counts in gut contents were higher in normal guts than in stenotic guts from the jejunum distally ($p < 0.05$; Fig. 6*B*). The chromatographic profile of gut contents in the animal with a normal gut showed that the profile of stomach contents was identical to that in AF just before postmortem examination (Fig. 7*A*). In contrast, in the animal with a stenotic gut, the profile of stomach contents resembled that seen from contents of more distal bowel with very little ^{125}I-IGF-I bound to IGFBPs (Fig. 7*B*). This suggests that no degradation had occurred in the stomach of the animal with a normal gut, whereas in the animal with gut stenosis degradation had occurred. The proportion of counts that were associated with degradation products increased from proximal to distal bowel in both animals (Fig. 7). Colon contents from the animal with a normal gut contained some bound and some free ^{125}I-IGF-I, but mostly smaller molecular weight degradation products and free ^{125}I (Fig. 7). Counts in colon contents obtained from the animal with a stenotic gut were substantially

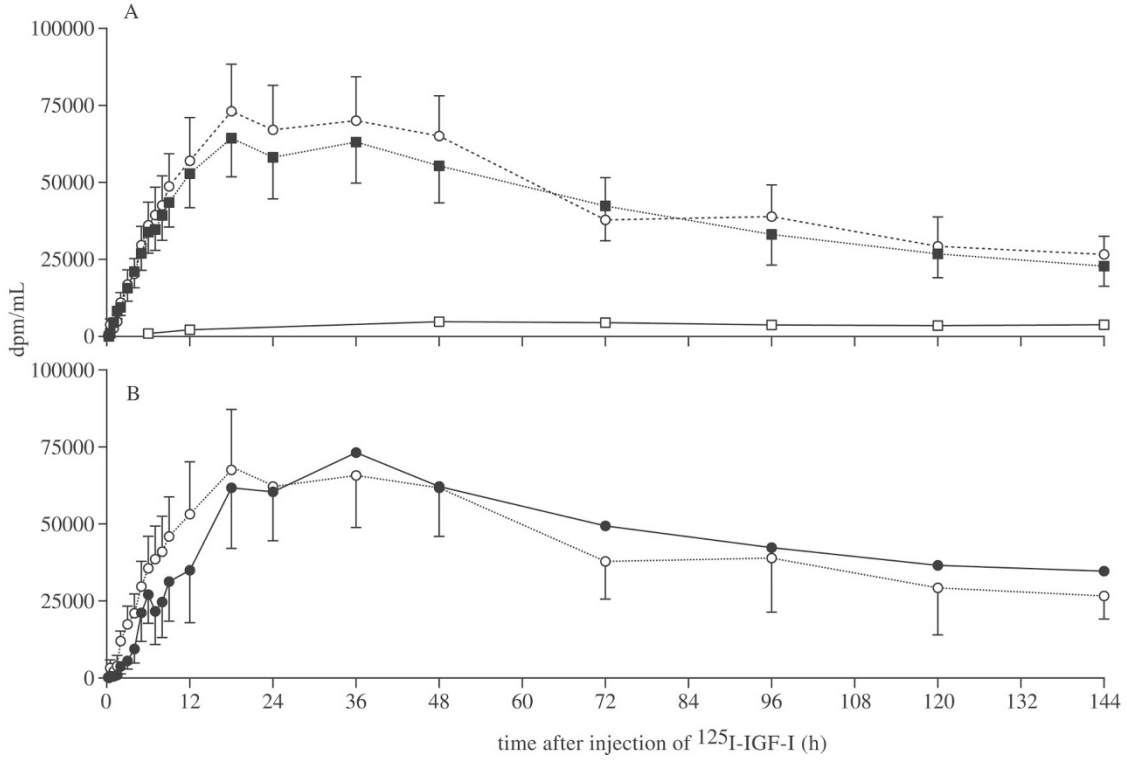

Figure 5. Counts in fetal and maternal blood samples during the course of the experiments. *A*, fetal portal vein (○), fetal artery (■), and maternal artery (□); $n = 6$. *B*, fetal portal vein from animals with normal ($n = 4$, ○) and stenotic ($n = 2$, ●) guts. Counts are lower in animals with stenotic guts for the first 12 h after injection ($p < 0.05$).

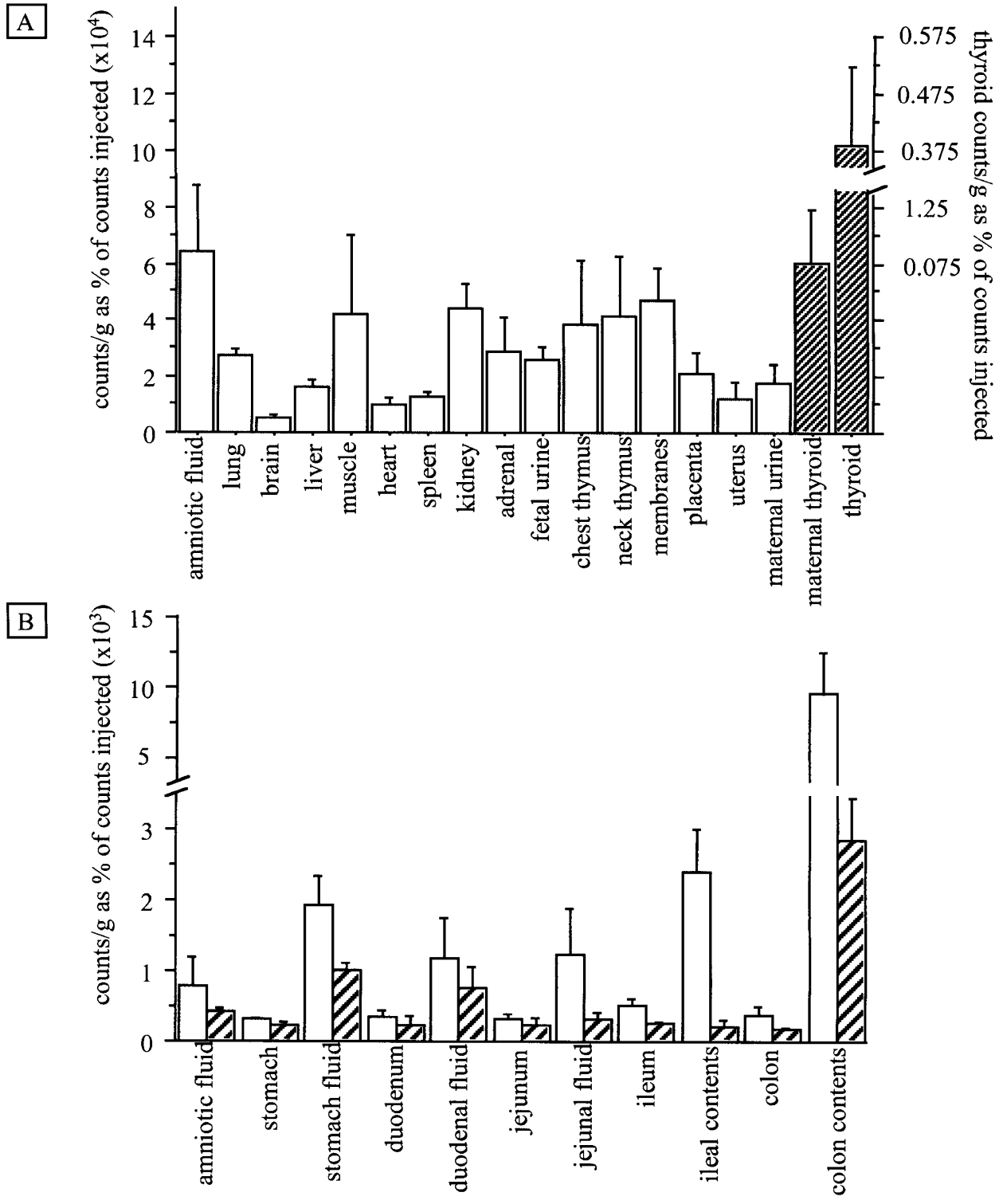

Figure 6. Counts in fetal and maternal tissue at postmortem examination, expressed as a percent of counts injected ($n = 6$). *A*, tissues other than gut. *Shaded bars* are counts in thyroid. *B*, gut tissues. *Clear bars* indicate animals with normal guts ($n = 4$). *Shaded bars* indicate animals with stenotic guts ($n = 2$). All tissues are fetal unless otherwise specified. Values are mean ± SEM. Counts in gut contents are higher than in gut wall for all regions ($p < 0.05$), and counts in colon contents are higher than in contents from other regions ($p < 0.05$). Counts in contents from normal gut are higher than from stenotic guts from the jejunum distally ($p < 0.05$).

lower than those from the animal with a normal gut, and there was very little intact ^{125}I-IGF-I detectable (Fig. 7).

Overall, approximately 13.5% of injected counts were accounted for at postmortem examination: 7.2 ± 1.3% in AF, 1.3 ± 0.5% in fetal blood (assuming a fetal blood volume of 100 mL/kg), 0.3 ± 0.1% in fetal gut, 0.55 ± 0.08% in fetal thyroid, 2.6 ± 0.9% in other fetal tissues counted, and 1.3 ± 0.4% in maternal thyroid.

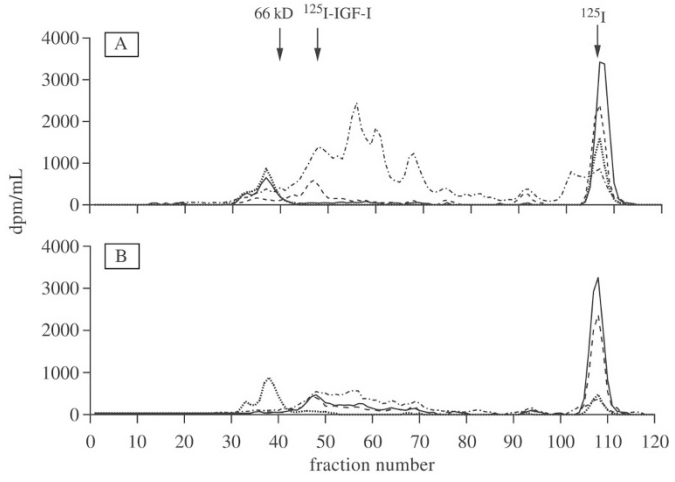

Figure 7. Counts in different fractions after size-separation chromatography of gut contents from an animal with a normal gut (*A*; no. 99079) and an animal with a small intestinal stenosis (*B*; no. 99124). (···· AF taken at 144 h, — stomach contents, ---- small intestine contents, -·-·-· colon contents.) Note the similarity of profiles from AF and stomach contents in no. 99079, and the difference in these profiles in no. 99124.

DISCUSSION

These data demonstrate that ^{125}I-IGF-I injected into AF of the late-gestation ovine fetus is rapidly distributed throughout the AF volume, is swallowed by the fetus, and is taken up across the gut into the portal venous circulation. Furthermore, detectable ^{125}I-IGF-I persists in AF for at least 6 d in both bound and free forms. These data therefore provide evidence for sustained delivery of ^{125}I-IGF-I from the AF to the fetal systemic circulation after a single intraamniotic injection.

The estimated half-life of 24 h for IGF-I in AF is similar to that of various radiolabeled proteins injected into human fetuses in the experiments of Gitlin *et al.* (3). In addition, our finding of good mixing of ^{125}I-IGF-I throughout the AF within 3 to 4 h of injection agrees with the results of Tomoda *et al.* (2) in which ^{125}I-labeled BSA, ^{51}Cr-labeled red blood cells, and ^{103}Ru-labeled microspheres were injected into the AF of late-gestation fetal sheep.

In vivo *binding of ^{125}I-IGF-I in AF.* The chromatography profiles demonstrate that a significant amount of injected ^{125}I-IGF-I remained unbound in both the AF and fetal plasma of some animals for the duration of the experiment. Most of the bound ^{125}I-IGF-I eluted in fractions before the 66-kD marker. These fractions may represent aggregates of binding proteins with ^{125}I-IGF-I or aggregates of ^{125}I-IGF-I alone, but are unlikely to represent the ternary IGFBP-3 acid-labile subunit–^{125}I-IGF-I complex, as acid-labile subunit expression is reportedly present only in very low amounts in ovine tissue before birth (20). The high correlation coefficient between the density of the IGFBP-3 band in the ligand blot from AF and the percent *in vivo* binding of ^{125}I-IGF-I suggests that these higher molecular weight fractions do contain IGFBP-^{125}I-IGF-I complexes, and also suggests that IGFBP-3 is the most important IGFBP in ovine AF. This contrasts with human AF, in which IGFBP-1 is predominant (21). The explanation for the variability in AF IGFBP-3 levels, and thus the degree of ^{125}I-IGF-I binding *in vivo*, among animals is not clear, but may relate to protease activity in AF. The presence in human AF of a cation-dependent serine protease, which degrades IGFBP-3, -4, and -5, is well known (22). To our knowledge, there are no publications reporting protease activity in ovine AF.

The Western ligand blot that we used detects IGFBPs on the basis of molecular weight and their ability to bind ^{125}I-IGF-II. We therefore cannot exclude the possibility that some of the bands contain fragments or aggregates of IGFBPs as well as intact proteins. This may lead to a discrepancy between levels measured by Western ligand blot and actual levels present in AF. However, the bands obtained in this study are quite well defined and migrate in positions identical with previously validated pure IGFBPs (17–19). Furthermore, as ^{125}I-IGF-II is used as the ligand, only proteins binding IGF-II are detected, thus eliminating nonspecific bands unrelated to IGFBPs and also immunogenic fragments of IGFBPs that may not be able to bind IGFs.

In contrast to postnatal life, in which IGFBP-3 plasma concentrations are predominantly regulated by GH, fetal plasma levels of IGFBP-3 are regulated by nutritional supply and other hormones such as insulin and IGF-I (23). The regulation of amniotic IGFBP-3 levels is not known. In this study there were no significant correlations between amniotic IGFBP-3 band densities and fetal size, arterial glucose levels, or arterial oxygen content. The correlation with baseline amniotic IGF-I levels was reasonably good, although it did not reach statistical significance. Levels of IGF-I in AF may be regulated by binding protein levels, or, perhaps less likely, IGF-I levels may regulate binding protein levels. However, the marked difference in IGFBP band density among different animals, and the correlation of these densities with the *in vivo* binding of IGF-I, raises the possibility that IGFBP-3 has a role in modulating the action of IGF-I in AF. If so, the role of IGFBP proteolysis would also be very important and remains to be explored.

Gut uptake of ^{125}I-IGF-I. Gut uptake of ^{125}I-IGF-I administered directly into the gastrointestinal tract has been reported before in neonatal animals (11, 24–27). The bioavailability of ^{125}I-IGF-I in one such study was estimated to be as high as 9% of the administered dose (11). Two studies, one in suckling rats (24) and one in neonatal pigs (25), demonstrated higher radioactivity levels in portal than systemic blood, strongly suggesting uptake across the gut lumen. The data we report showing a portosystemic difference in radioactivity levels for the first 12 h after intraamniotic injection suggests a similar process in fetal life. The delayed appearance of radioactivity in the portal vein of animals with a gut stenosis confirms gastrointestinal uptake, and suggests that any contribution from uptake across other epithelial surfaces, such as skin, lung (28), or umbilical cord (29), must be very small. Although we did not measure counts in fetal skin, the ovine skin at this stage of gestation is very well keratinized with a well-developed wool covering and is thus unlikely to be a major route of IGF-I uptake.

By 6 d after injection we could account for only 13.5% of the injected tracer at postmortem examination. The remainder of the injected tracer is assumed to be distributed to other maternal or fetal tissues or to have been excreted by the

mother. Further description of the distribution of injected IGF-1 into fetal and maternal tissues in future studies would require dynamic measurements of AF volume, fetal gut blood flow, and blood volumes and blood flows on both sides of the placenta.

The chromatograms in fetal plasma confirm that intact labeled hormone is being taken up into the fetal circulation. It has been reported that a substantial proportion of ^{125}I-diiodotyrosine given into the stomach of the suckling rat is deiodinated during intestinal transmission (30), and that 60% of ^{125}I-IGF-I may be degraded after 10 h incubation in the contents from rat stomach or intestines (11). The chromatograms from portal venous and fetal arterial blood samples taken after 36 h in our study still demonstrate significant quantities of intact ^{125}I-IGF-I. Nevertheless, increasing amounts of ^{125}I are also present as the experiment progresses, and if deiodination has occurred in the gut then free ^{125}I may be taken up by the gut and appear in the portal vein. Similarly, deiodinated IGF-I may also be taken up by the gut. Therefore, the data from this experiment may underestimate the actual amount of IGF-I that was taken up across the gut.

The mechanism of gut uptake of hormones is not yet clear. After 3 d of intraluminal IGF-I treatment in late-gestation fetal sheep, the presence of IGF-I within enterocytic vesicles was demonstrated by immunocytochemistry (31). Using similar techniques, EGF has been demonstrated within the enterocytic vesicles of the fetal rat after an acute, direct intraluminal infusion (4). Thus endocytosis seems the most likely mechanism for transport of the ^{125}I-IGF-I from the lumen to the portal circulation. However, the precise mechanism of IGF-I uptake across the gut has not been definitively determined, and may involve more than one mechanism.

The chromatograms of gut contents from the animal with a normal gut demonstrated increasing quantities of degradation products from proximal to distal bowel. These samples were processed 145–150 h after injection into the AF, yet small quantities of bound and free ^{125}I-IGF-I were still present. In the animal with a gut stenosis the chromatographic profile of gut contents was different. Counts were still present beyond the obstruction, perhaps reflecting progressive obstruction after interruption of the blood supply. However, compared with the animals with normal guts, counts were much lower in the distal bowel, the proportion of counts that were accounted for by degraded fragments or free ^{125}I were much greater, there was almost no intact ^{125}I-IGF-I present, and the profile in stomach contents was different from that in AF. Swallowed ^{125}I-IGF-I might be cleared by the gut less quickly in animals with a gut stenosis and therefore be exposed to proteolytic enzymes for a longer period of time, allowing more extensive degradation. The delay in the increase in counts in portal venous samples would support this interpretation. The fact that portal venous counts reached a similar level to those in normal animals, although with some delay, suggests that differences in swallowing rate or volume are not present.

More extensive enzymatic degradation of IGF-I in the small bowel of the animal with a gut stenosis may also explain the differences in chromatographic profiles of colon contents. Nevertheless, the finding of intact ^{125}I-IGF-I in the colon of the animal with a normal gut was surprising and raises the intriguing possibility of the secretion of previously absorbed ^{125}I-IGF-I back into the gut lumen. After i.v. injection of ^{125}I-IGF-I into suckling rats, 2% of the injected radioactivity was collected in bile during a 2-h period (32). Furthermore, 30% of this radioactivity coeluted on gel chromatography with native IGF-I. The concentration of IGF-I in the bile of rats is greatest in the neonatal period, declining as the rat matures (33). It is therefore possible that enterohepatic circulation of ^{125}I-IGF-I could account for the relatively high proportion of intact ^{125}I-IGF-I still present in the bowel in our experiment after 6 d.

Previous animal studies of AF turnover (2) and postnatal enteral administration of ^{125}I-IGF-I (11, 24–27) have followed the disappearance of labeled protein from AF or the gut uptake of ^{125}I-IGF-I for at most 12 h. By extending the experiments we describe in this paper to 144 h, we have been able to show that ^{125}I-IGF-I persists in AF for this length of time, and that gut uptake continues for up to 18 h in animals with normal guts, and for up to 36 h in those with a gut stenosis.

The results of the experiments described in this paper confirm that IGF-I given into AF as a bolus reaches the fetal gut, and is absorbed from the gut into the portal circulation. The half-life of 24 h, a peak portal venous level at 18 h, and persisting levels of radioactivity in AF for several days suggest that the amniotic route of administration of IGF-I is feasible, with a dosing interval of more than 24 h being a possibility. However, the large variability in the degree of binding of the ^{125}I-IGF-I may have implications for its bioavailability, and the role of proteases in ovine AF in generating this variability requires further investigation. Further studies should investigate the explanation behind this variability and could also investigate the precise mechanism of uptake, the possibility of enterohepatic circulation of transported hormone, the receptor-binding capability of the transported IGF-I, and the effects of different dosing intervals.

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