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DISTRIBUTION AND ELIMINATION OF THE SOMATOSTATIN ANALOGUE (^{111}In-DTPA-D-Phe^1)-OCTREOTIDE (OCTREOSCAN111)

HENRIK ARNBERG, JAN-ERIK WESTLIN, STIG HUSIN and STEN NILSSON

The distribution and elimination characteristics of the ^{111}In-labelled somatostatin analogue OctreoScan111 were studied in 23 patients with malignant tumours. The substance exhibited a rapid blood elimination following a bi-phasic pattern. The initial part of the elimination curves showed a $t_{\frac{1}{2}a}$ of between 0.27 and 3.6 h. The patients investigated had creatinine clearance rates ranging from 33 to 124 ml/min. However, within this range, no apparent correlation was found between the OctreoScan111 elimination rate and kidney function. Also no correlation was observed between the amount of administered activity and the elimination rate of OctreoScan111. The serum radioactivity of 6 patients was analyzed with respect to molecular size. These experiments showed that OctreoScan111 circulated unbound in serum. About 3% of the radioactivity, most probably representing ^{111}In-chloride of DTPA-^{111}In-chloride, circulated protein-bound. The elimination of OctreoScan111 radioactivity in urine displayed a bi-phasic pattern. Size separation of the radioactivity appearing in the urine after 24 h showed a higher molecular weight when compared with OctreoScan111, indicating the existence of a metabolite of the injected substance. The results obtained are discussed in the light of a potential role for the substance in systemic radiotherapy.

During the last decade, long-acting somatostatin analogues have been used in the therapy of patients with malignant endocrine tumours. The treatment has, in most cases, a good effect on the patients' tumour-related symtoms (1, 2). In vitro data using autoradiographic techniques have shown that approximately 90% of this tumour type express somatostatin cell-surface receptors (3, 4). The ^{123}I-labelled somatostatin analogue, octreotide, has been developed for the scintigraphic detection of endocrine tumours (5, 6). This analogue, however, is secreted via the biliary system, thus making the diagnostics of intra-abdominal somatostatin receptor-positive tumours difficult. Further development has led to the synthesis of DTPA-D-Phe1-octreotide (7, 8) which can be easily labelled with ^{111}In. This substance (OctreoScan111), which is excreted via the kidneys, results in a lower intra-abdominal background activity when compared with the ^{123}I-labelled somatostatin analogue. This facilitates the diagnosis of somatostatin receptor-positive tumours located in the abdomen (7). In a recently performed study we have shown that OctreoScan111 is a useful tool in the detection and localization of carcinoids, endocrine-active pancreatic tumors, and MEN1 (multiple endocrine neoplasia 1) tumours (9). An analysis of tumour uptake of the radio-pharmaceutical has shown tumor/blood ratios of up to 100 (Öhrvall et al., unpublished study). The substance is therefore of interest with respect to a possible use in targeting with beta-emitting radionuclides, such as ^{90}Y for systemic radiotherapy. The present study was performed to investigate the elimination characteristics of OctreoScan111 in serum and urine in patients with differing kidney functions and also to study the gross molecular weight distribution of the serum and urine radioactivity over time.

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From the Section of Nuclear Medicine and Hospital Physics, Department of Oncology, University Hospital, Uppsala, Sweden (all authors).
Correspondence to: Dr Henrik Arnberg, Section of Nuclear Medicine, Department of Oncology, University Hospital, S-751 85 Uppsala, Sweden.
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Material and Methods

Patients. Twenty-three patients were included in the study (9 with carcinoid tumors, 5 with endocrine pancreatic tumors, 5 with elevated hormonal levels indicating the presence of a neuro-endocrine tumour, 2 with medullary thyroid carcinoma, and 2 with multiple endocrine neoplasia, the MEN1-syndrome). The blood and urine ^{111}In activities were monitored in 15 and 12 patients respectively. Size separation of serum and urine samples on Sephadex was performed on 6 and 4 patients respectively. The calculation of plasma creatinine clearance values was based on serum creatinine concentration and body weight according to the equation of Cockroft & Gault (10).

Labelling and administration of OctreoScan111. Lyophilized DTPA-D-Phe^1-octreotide and ^{111}In-chloride were obtained in a separate vials from the manufacturer (Mallinckrodt Medical, Petten, Netherlands). The ^{111}In-chloride (240 MBq) was added to the lyophilized DTPA-D-Phe^1-octreotide (20 ug) and incubated for 30 min at room temperature. The labelling yield was controlled with reversed phase chromatography using SEP-PAK (11). In short, a small aliquot of the ^{111}In-labelled pentratreotide was applied on top of a 1.0 × 1.5 cm column. Elution was then performed with 5 ml of water (Fraction A), followed by 5 ml of methanol (Fraction B). The activities in the separate fractions and the remaining activity in the column (Column) were analyzed using a scintigraphic well counter (Capintec). The labelling yield, which was calculated using the formula Fraction B/(Fraction A + Fraction B + Column), always exceeded 97%. After labelling, 2 ml of a sterile isotonic sodium chloride solution was added to the vial to make the injection volume easier to handle. The patients received 132–260 MBq of the OctreoScan111 solution (3.5–4.5 ml) as an intravenous bolus injection.

Scintigraphy. Static anterio-posterior whole-body images, with the exception of the extremities, were collected 4

Table

Summary of patient and OctreoScan111 data from 23 patients. The biological half-life refers to the first slope in the elimination curve ($t_{\frac{1}{2}a}$)

Patient No.	Sex	Age	Diagnosis	Positive/Negative OctreoScan111 investigation	Urine activity	Serum G.C.	Urine G.C.	Injected Activity MBq	Creatinine clearance ml/min	$t_{\frac{1}{2}a}$
1	F	66	carc.	+	+			242	64	0.87
2	M	59	carc.	+	+			166	75	1.6
3	M	73	elv. hormones	−	+			250	60	0.89
4	F	74	med. thyr. ca.	+				190	N.D.	2.4
5	M	53	end. pancr.	+	+			168	86	1.2
6	M	21	end. pancr.	+	+			260	124	0.59
7	F	69	carc.	+				260	39	0.54
8	M	53	carc.	+	+			239	96	0.67
9	M	46	carc.	+	+	+		240	60	N.D.
10	F	44	elev. hormones	−	+			240	N.D.	N.D.
11	F	69	elev. hormones	−				207	33	3.6
12	F	39	end. pancr.	+	+	+		182	104	0.48
13	F	51	carc.	−	+			200	108	0.27
14	F	79	carc.	+		+		222	33	N.D.
15	M	42	end. pancr.	−	+			182	96	0.32
16	F	60	MEN1	−	+			196	68	0.87
17	F	64	elev. hormones	−		+		240	64	N.D.
18	M	59	carc.	+				239	59	0.63
19	M	46	MEN1	−			+	240	107	N.D.
20	M	60	carc.	−		+	+	235	62	N.D.
21	F	31	elev. hormones	−			+	238	95	N.D.
22	M	21	end. pancr.	+			+	139	85	0.41
23	M	43	med. thyr. ca.	−		+		182	104	N.D.

carc. = carcinoid tumor
end. pancr = endocrine pancreatic tumor
med. thyr. ca. = medullary thyroid carcinoma
MEN1 = MEN1 syndrome
elev. hormones = elevated neuro-endocrine hormonal levels
G.C. = gel chromatography
OctreoScan111 investigation: (+) = positive; (−) = negative
Urine activity: (+) = urine activity measured
Serum G.C.: (+) = serum samples analysed on Sephadex column
Urine G.C.: (+) = urine samples analysed on Sephadex column
N.D. = not done

and 24 h after injection. Twenty-four hours after injection SPECT (Single Photon Emission Tomography) was performed over the abdomen. A gamma scintillation SPECT-camera, delivered by Nuclear Diagnostics, Hägersten, Sweden and London, was used.

Sampling. Blood samples from 15 patients were drawn repeatedly, from 5 min to 48 h after injection of the OctreoScan111, and analyzed for radioactivity. The blood samples which were collected in glass vacuum tubes were analyzed for radioactivity and thereafter allowed to coagulate in order to obtain serum. The total urine volumes from 12 patients were collected during the following intervals: 0–10, 10–22, 22–34 and 34–46 h respectively after the injection of OctreoScan111. Samples from the collected urine volumes were analyzed for radioactivity.

Gel chromatography. Serum samples obtained 4 and 22 h after OctreoScan111 injection were subjected to gel chromatography on Sephadex G-25 (1 × 15 cm column). The elution fractions were analyzed with respect to radioactivity. In 2 cases serum samples were also analyzed on Sephadex G-100 (1 × 60 cm column) at different time intervals. Urine samples from 4 patients were subjected to Sephadex G-25 chromatography and the elution fractions were analyzed for radioactivity. Phosphate buffered saline (PBS), pH 7.4, was used as the elution buffer in all gel chromatographies. The radioactivity was analyzed in a scintillation well counter (Capintec). Corrections were made for the natural decay radioactivity.

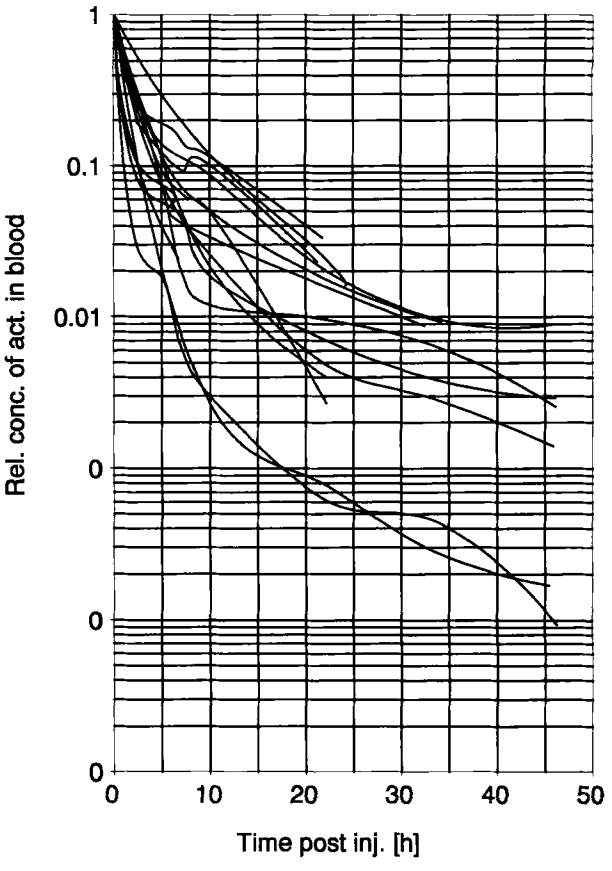

Fig. 1. Elimination of blood radioactivity after administration of ^{111}In labelled DTPA-D-Phe^1-octreotide (OctreoScan111). The activities in the initial individual blood samples, which were drawn 5 min after injection, were normalized to 1.0.

Results

OctreoScan111 scintigraphy was performed in 23 patients. The clinical characteristics and the outcome of the scintigraphic investigations have previously been described (9) and are summarized in the Table. Fifteen of these patients were analyzed with respect to the half-life of blood radioactivity. As can be seen from Fig. 1, the radioactivity in blood decreased rapidly with time. The median percentage of injected activity remaining in blood after 1 h was 50%. The variation was, however, considerable and ranged from 20 to 80%. The elimination of the radioactivity followed a biphasic curve in the semi-logarithmic diagram, with the initial steep part of the curve corresponding to a $t_{\frac{1}{2}a}$ of 0.21–3.6 h. As can be seen in Fig. 1, the elimination rates of blood radioactivity varied markedly between the patients. The activity remaining in blood after 10 h ranged from 1 to 25%. The results from these analyses are summarized in the Table.

The serum radioactivity from 6 of the patients was analyzed with respect to molecular size. The result from a representative gel chromatography on Sephadex G-25 is depicted in Fig. 2. The majority of the radioactive material chromatographed with the total volume of the gel (peak 2). In control experiments performed prior to the injection, OctreoScan111 was eluted at this position (data not shown). About 3% of the radioactivity eluted with the void volume of the gel (peak 1). In control experiments, ^{111}In-chloride and OctreoScan111 were incubated with patients' sera drawn prior to the study and thereafter analyzed on Sephadex G-25 gel chromatography under conditions identical to those described above. In these experiments, the ^{111}In-chloride behaved as peak 1 and OctreoScan111 as peak 2 (data not shown). In a separate set of experiments, serum samples drawn at different time intervals (4–24 h post OctreoScan111 administration) were analyzed with Sephadex G-100 chromatography. Representative data from these experiments are depicted in Fig. 3. The bulk of the radioactivity was eluted with the total gel volume. A high molecular weight component was seen corresponding to the elution volume of human serum albumin. In control experiments, ^{111}In-chloride added to serum migrates to this position as well as ^{111}In-chloride added to human serum albumin (data not shown). OctreoScan111 is eluted with the total volume of both the G-100 gel and the G-25 gel (see above). These data indicate that OctreoScan111 does not bind to carrier proteins in serum and that the radioactive component with a high molecular weight (peak 1),

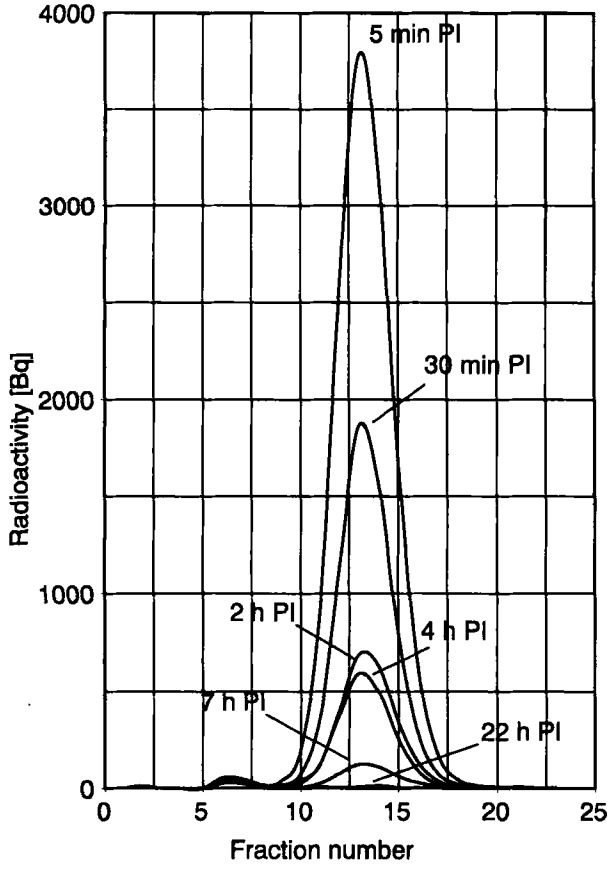

Fig. 2. Sephadex G-25 gel chromatographies on sequential serum samples obtained from a patient 5 min.-22 h after injection with 220 MBq OctreoScan111. The buffer used was PBS, pH 7.4. The volume per fraction was 1.0 ml.

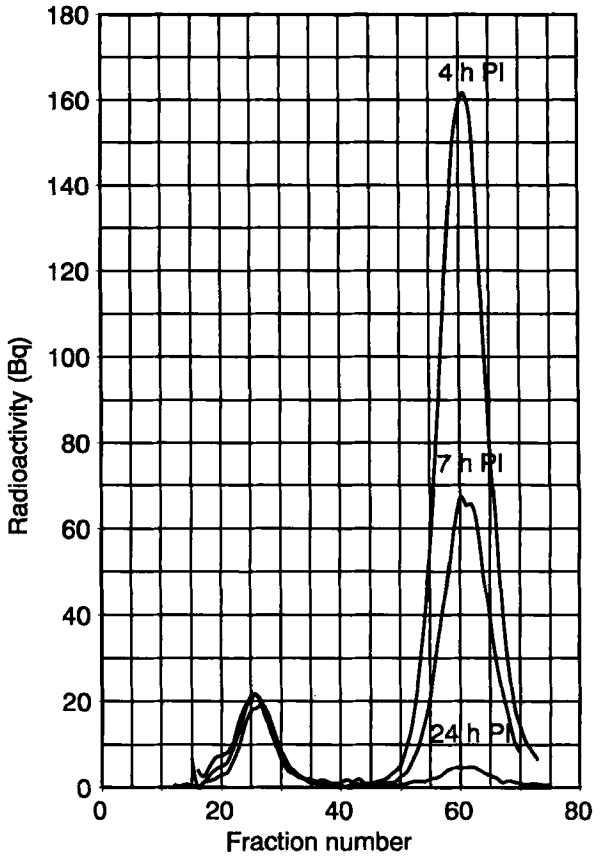

Fig. 3. Sephadex G-100 gel chromatographies on sequential serum samples obtained from a patient 5 min.-22 h after injection with 220 MBq OctreoScan111. The buffer used was PBS, pH 7.4. The volume per fraction was 1.0 ml.

seen on Sephadex G-25 and Sephadex G-100 chromatography, most probably represents protein-bound ^{111}In-chloride or ^{111}In-chloride-DTPA. In an attempt to explain the marked differences between patients with respect to the half-life in serum of OctreoScan111, a comparison was made with respect to their renal function, as defined by creatinine clearance. The $t_{\frac{1}{2}a}$ values plotted against creatinine clearance show, however, that no apparent correlation existed between the two parameters (Fig. 4). Thus, a prolonged elimination time does not seem to depend on impaired kidney function. Nor was any apparent correlation observed between the administered activity of the radio-pharmaceutical and the $t_{\frac{1}{2}a}$ values (Fig. 5).

No apparent correlation was seen between tumour burden, assessed as gross nuclide uptake in tumours/normal tissues, and the half-life, measured as $t_{\frac{1}{2}a}$ of the radiopharmaceutical (data not shown). Urine radioactivity was followed in 12 patients. As can be seen from the log-linear diagram presented in Fig. 6, the elimination followed a bi-phasic pattern. We decided to further investigate the nature of the radioactive component(s) at two different time intervals after the injection of OctreoScan111. Urine

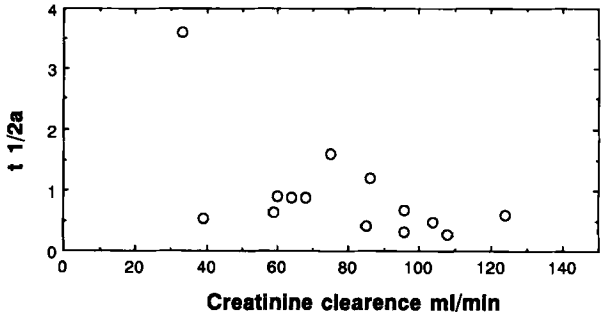

Fig. 4. The initial part ($t_{\frac{1}{2}a}$) of the radioactivity elimination curves from Fig. 1 were plotted against the creatinine clearance values from the individual patients.

samples from 4 patients were therefore analyzed on Sephadex G-25 chromatography after 4 and 24 h respectively. Representative results from these experiments are shown in Fig. 7. As can be seen from the figure, all radioactivity from the 4-h urine samples eluted with the total gel volume, indicating that the radioactive substance is not protein-bound under the experimental conditions studied. However, the elution profiles from the 24-h samples were

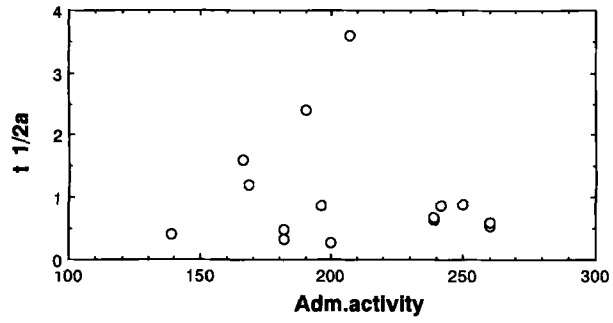

Fig. 5. The initial part ($t_{\frac{1}{2}a}$) of the radioactivity elimination curves from Fig. 1 were plotted against the OctreoScan111 activity administered to the individual patients.

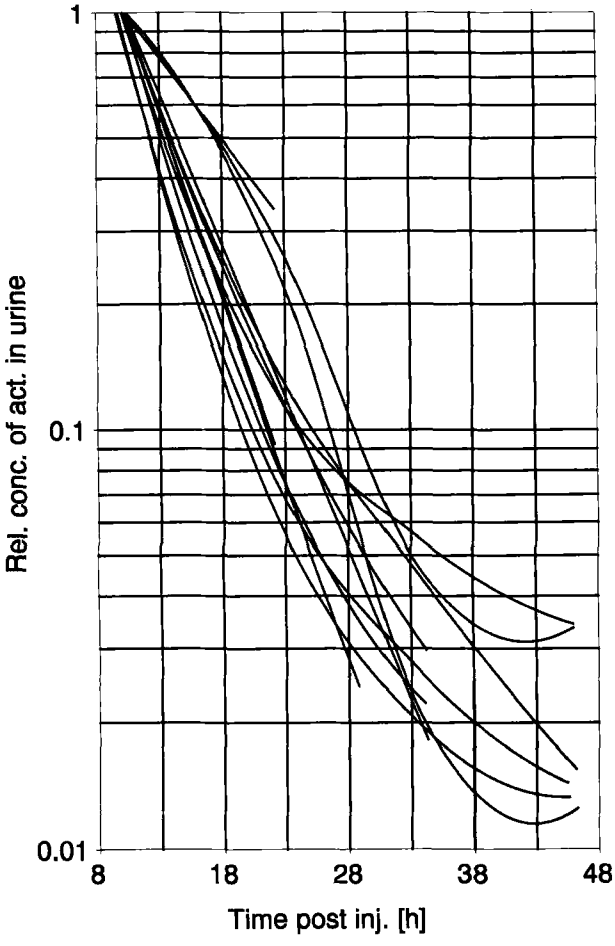

Fig. 6. Elimination of urine radioactivity after administration of OctreoScan111. The radioactivity was measured from samples of collected total volumes of the urine. The activities in the initial individual urine samples, which were obtained from collected 15-h urine volumes, were normalized to 1.0.

different from those of the 4-h samples, with a major radioactivity peak indicating the presence of a new component with a slightly higher molecular weight, as assessed from the elution position (Fig. 7). The radioactivity peak with a low molecular weight corresponding to the total gel

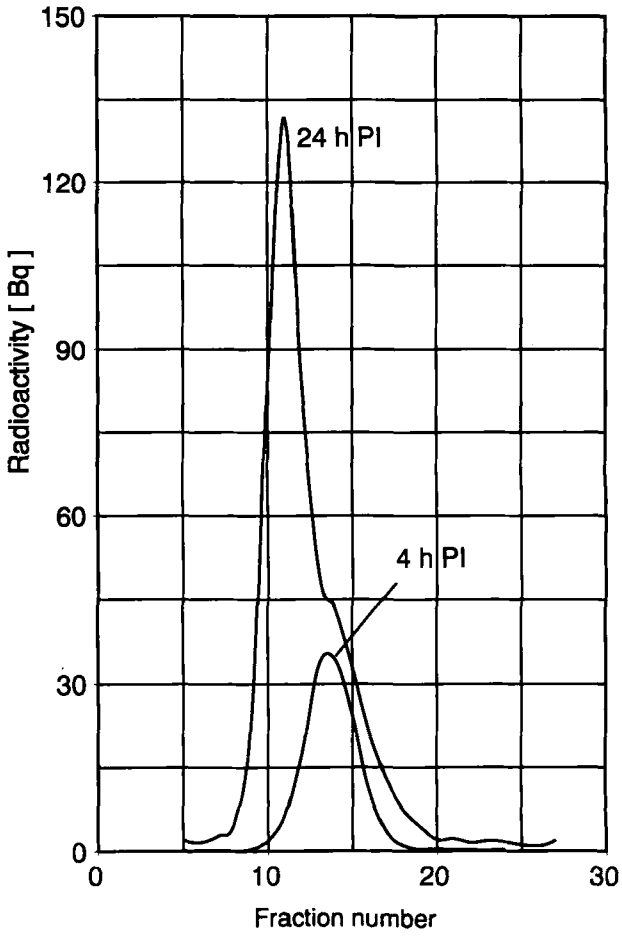

Fig. 7. Sephadex G-25 gel chromatographies of samples from sequentially collected urine total volumes. The buffer used was PBS, pH 7.4. The volume per fraction was 1.0 ml.

volume could still be seen in the 24-h urine samples, although in lower quantities.

Discussion

OctreoScan111 is useful in the in vivo detection of endocrine tumours (7–9). In contrast to the iodinated somatostatin analogue which is metabolized mainly in the liver, OctreoScan111 is excreted by the kidneys. Among the patients included in the present study, no apparent correlation existed between the blood half-life of OctreoScan111 and creatinine clearance. Nor does the creatinine clearance difference noticeably affect the scintigraphic imaging. The radio-nuclide was not bound to serum proteins under physiological conditions. A small proportion of the total radioactivity eluted with the void volume on Sephadex G-25 (peak 1). We interpret this activity as either representing free ^{111}In-chloride, which never bound to the DTPA-chelated somatostatin analogue or ^{111}In-chloride-DTPA which might have been released from the ^{111}In-chloride-DTPA-Phe^{1}-octreotide molecule.

The results obtained from the chromatographies performed on ^{111}In-chloride after incubation with patient sera and with purified human serum albumin indicate that peak 1 represents albumin-bound activity.

Radioactivity appears rapidly in the urine after the intravenous administration of OctreoScan111. The bulk of the injected activity, (approximately 80%) is excreted via the kidneys within 10 min (7). The radioactivity in the urine is represented mainly by the active substance, OctreoScan111, since it is still able to specifically bind to somatostatin receptor-expressing rat cortex cell membranes (7). The urine samples analyzed in present study show, in analogy with the situation in serum, that OctreoScan111 is not protein-bound. Interestingly, the radioactive substance seems to become metabolized or conjugated with time. This finding is evident from the gel chromatographic analyses performed on urine samples obtained from one and the same patient after 4 and 24 h. In the 4-h specimen, all radioactivity is eluted with the total volume, whereas a portion of the radioactivity in the 24-h specimen is eluted slightly before the total volume, most probably indicating that OctreoScan111 has been conjugated with an additional chemical group or that released ^{111}In-chloride or ^{111}In-chloride-DTPA have associated with another low-molecular weight compound. This finding is in agreement with results presented previously, showing that most of the biological activity of the radioactivity remaining in urine has been lost afer 24 h (7). It is of interest to perform further studies to discover whether the supposedly modified molecule represents a substance released from the tumour cells or from a normal organ, such as the kidney or the spleen. The $t_{\frac{1}{2}a}$ in the elimination curve from blood ranged from 0.21 to 3.6 h but the follow-up time of up to 48 h after injection does not allow for a reasonable estimate of $t_{\frac{1}{2}b}$. OctreoScan111 is of interest as a potential tumour-seeking agent for future radionuclide therapy. The substance exhibits a high tumour/blood uptake. Experimental data obtained from our laboratory have shown that a tumour/blood ratio of >100 can be obtained in endocrine tumours (Öhrvall et al., unpublished study). The major part of the substance is rapidly cleared from the circulation, which speaks in favour of developing the therapeutic concept. However, the kidneys are the main organs at risk, since most of the administered radioactivity is excreted through them. In addition, a substantial fraction of the radioactivity can be expected to be taken up by the bone marrow if ^{90}Y is to be exploited in the therapeutic situation. Thus, further experimental studies are warranted before entering clinical trials in a therapeutic setting.

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