# Reduction of Renal Uptake of Monoclonal Antibody Fragments by Amino Acid Infusion

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The renal uptake of radiolabeled antibody fragments and peptides presents a problem in radioimmunodetection and therapy, compromising lesion sensitivity, especially with intracellularly-retained isotopes. Previously, we showed that cationic amino acids and their derivatives are capable of significantly reducing kidney uptake in animals (*1–4*). We report our initial clinical results of successful renal uptake reduction in five patients who underwent cancer radioimmunodetection with $^{99m}$Tc-anti-CEA Fab′ fragments. **Methods:** The patients were infused with two liters of a commercially-available nutritive amino acid solution (containing approximately 2.25 g/liter lysine-glutamate and 2.50 g/liter arginine), whereas 75 control patients received the same volume of saline (quantification of organ and tumor kinetics from conjugate whole-body views by ROI technique). **Results:** The renal uptake in the amino acid group was significantly lower ($p < 0.05$) than in the control group (11.1 ± 2.0% injected dose versus 17.7 ± 7.0% injected dose at 24 hr postinjection), whereas the uptake of all other organs remained unaffected. Gel filtration chromatography of the urine taken from amino-acid-treated patients showed that a significantly higher amount of excreted activity was bound to intact Fab′ (53% of excreted activity) in contrast to only less than 10% in the control group. **Conclusion:** The renal uptake of monoclonal antibody fragments in patients can be reduced significantly by amino acid infusion, even at considerably lower doses than those that were safe and effective in animals. As was found in animals, the mechanism seems to rely on an inhibition of the re-absorption of tubularly-filtered proteins by the proximal tubule cells. These results encourage further clinical trials to lower the renal uptake experienced in radioimmunodetection, as well as in therapeutic trials with antibody fragments and peptides.

**Key Words:** monoclonal antibodies; Fab′ fragments; renal uptake reduction; cationic amino acids

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Renal uptake of monoclonal antibody fragments and peptides is a problem in radioimmunodetection or receptor scintigraphy (*5,6*). It compromises reliable diagnostic accuracy in the retroperitoneal, periaortic and epigastric regions (Fig. 1), especially with intracellularly-retained isotopes (e.g., $^{111}$In, $^{99m}$Tc) (*7*). With $^{99m}$Tc-labeled Fab′ fragments, for example, renal uptake values of up to 25% of the injected dose, within 24 hr, have been reported (*8*). The kidney is well known as a major site of catabolism of low-molecular-weight proteins (*8,9*).

Three previous studies have suggested basic amino acids to be effective in decreasing the renal uptake of radiolabeled peptides. Hammond et al. reported the effect of administration of an amino acid solution on the renal uptake of the $^{111}$In-labeled somatostatin analogue, octreotide, in patients (*10*). Only a semiquantitative analysis, however, was performed by these authors. Pimm et al. showed a preliminary report on the effect of repeated intraperitoneal injections of high doses of L-lysine on the renal uptake of $^{111}$In-labeled Fab′ fragments in BALB/c mice (*11*), and was able to show highly significant effects in these animals. Recently, we demonstrated that cationic amino acids, amino sugars, as well as their polymers, are capable of reducing the renal uptake of monoclonal antibody fragments (Fab′, F(ab′)$_2$) with various labels by as much as 85% (*1–4*). The inhibition of the tubular reabsorption of the antibody fragments, presumably due to neutralization of negative charges on the cell surface of the kidney tubule cells, was identified as a possible mechanism (*1–4*).

In this study, we report our initial clinical results on the reduction of renal uptake of monoclonal antibody Fab′ fragments by infusion of a commercially-available, low-dose, nutritive amino acid solution. Additionally, we have analyzed the possible physiological mechanism of this effect.

## MATERIALS AND METHODS

### Monoclonal Antibodies

The murine anti-carcinoembryonic antigen (CEA) monoclonal antibody clones NP-4 and F023C5 were used. Both belong to the IgG$_1$ subclass and react with a related class-III peptide antigenic determinant of the CEA molecule (*8*). The directly $^{99m}$Tc-labeled Fab′ fragments of both have been described in detail previously (*6,8,12*). Also, their identical biodistribution patterns in patients, as well as their metabolic fates, have been decribed (*8*).

### Patients and Pharmaceuticals Used for Kidney Uptake Reduction

Five patients (4 men, 1 woman; aged 45–72 yr) with recurrent or metastatic CEA-expressing tumors (four recurrent colorectal cancers, one gastric primary after curative surgery of a colonic tumor) were examined in this study. Two of these patients were examined with the $^{99m}$Tc-Fab′ fragment of NP-4, and three with the $^{99m}$Tc-Fab′ fragment of the clone F023C5.

Approximately 30 min prior to the antibody injection, an intravenous infusion of the parenteral amino acid nutritive solution was started. Contents of this solution are listed in Table 1. The infusion proceeded for approximately 2 hr after antibody injection (total volume 2 liter; i.e., total amount of basic amino acids administered: 4.50 g lysine-glutamate and 5.00 g arginine). The organ kinetic data of a total of 75 patients who received the same amount of 0.9% saline, and whose data were reported earlier (*8*), were taken as controls (17 were examined with $^{99m}$Tc-NP-4 Fab′, 58 with $^{99m}$Tc-F023C5 Fab′).

Serum creatinine, BUN and serum electrolytes were determined in all patients before the antibody administration, and in the amino-acid-treated patients also at 1, 7 and 28 days after the examination.

### Antibody Administration and Scintigraphic Protocol

A dose of 740–1295 MBq (0.3–0.5 mg protein) was injected as a slow bolus (2–3 min) through the intravenous line within 30 min

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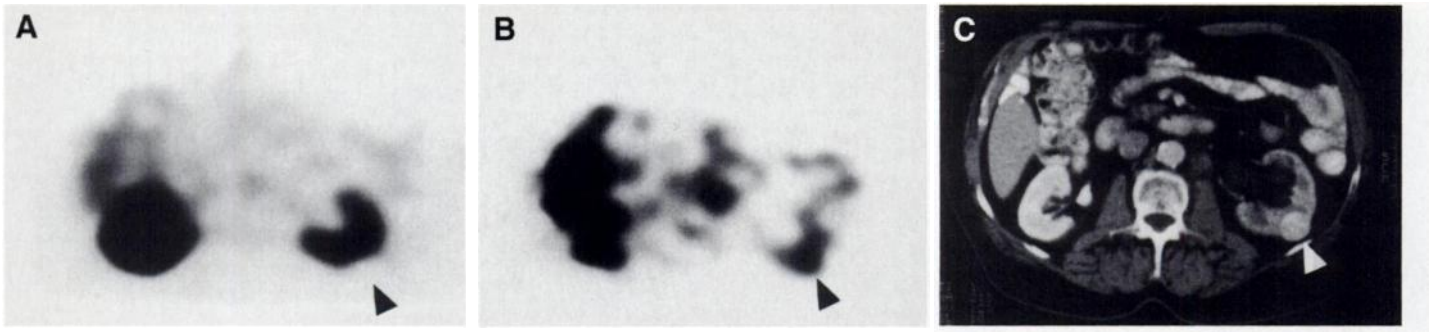


**FIGURE 1.** A 65-yr-old patient with a metastasis of colorectal cancer in the left kidney. (A) Radioimmunodetection study with F023C5 $^{99m}$Tc-Fab'; the lesion appears as a cold defect (arrowhead) in the kidney parenchyma in the abdominal SPECT at 4 hr postinjection. (B) Complete IgG [for methodological details, (*6*)] given 24 hr later is able to localize the metastasis as hot (arrowhead) in comparison to the normal kidney parenchyma, due to its lower physiological kidney uptake. (C) Corresponding CT scan depicts hydronephrotic changes of the left kidney that are reflected in both antibody scans by a thinner parenchymal margin.

of labeling. Whole-body scans were acquired at 10 min, 1, 4 and 24 hr postinjection, and planar imaging of the pelvis, abdomen, thorax and head was performed 4 to 6 hr and 18 to 24 hr postinjection (anterior-posterior and lateral projections). SPECT of the pelvis and abdomen was performed 4 to 6 hr postinjection; SPECT of the thorax and a second abdominal SPECT were completed 18 to 24 hr postinjection.

### Camera Equipment and Imaging Technique

The cameras used were a double-headed rotating gamma camera Rota-II and a Siemens Bodyscan™, interfaced with a Microdelta-Plus Computer (Siemens Gammasonics, Erlangen, Germany). Images were acquired in an analog manner and digitally in a 128 × 128 (spot views) and 512 × 2048 (whole-body scans) matrix (energy window 140 keV ± 10%; planar scans at 1 and 4 hr after antibody administration with 500,000 counts/view, at 24 hr with 200,000 counts/view). For SPECT, 60 planar projections in a 360° step-and-shoot technique (4–6 hr postinjection: 30 sec/view; 24 hr postinjection: 40 sec/view) were acquired in a 64 × 64 matrix. The data were processed by filtered back projection (Butterworth filter, Nyquist frequency 0.4–0.5) and reconstructed in three planes (transaxial, coronal and sagittal).

### Whole-Body and Organ Kinetics

For determination of the whole-body clearance and organ uptake kinetics, the ROI technique was applied to whole-body scans obtained at 10 min, 1, 4 and 24 hr after antibody administration (conjugate view technique; calculation of the geometric mean, physical half-life correction, expression in percent of injected dose by referring to whole-body counts at 10 min after MAb injection without previous voiding of the urinary bladder: by definition 100% of injected dose), as described earlier (*6,8*). Statistical analysis of the biodistribution data was performed using the Wilcoxon rank sum test (*13*).

**TABLE 1**
Amino Acid Solution Contents

| | | | |
|---|---|---|---|
| L-arginine | 2.50 g | | |
| L-lysine-glutamate (1:1) | 2.25 g | | |
| L-histidine | 0.75 g | | |
| L-isoleucine | 0.77 g | L-alanine | 3.00 g |
| L-leucine | 1.10 g | L-proline | 3.50 g |
| L-methionine | 0.93 g | L-serine | 1.50 g |
| L-phenylalanine | 0.75 g | N-acetyl-tyrosine | 0.48 g |
| L-threonine | 0.50 g | K-hydrogenglutamate | 3.80 g |
| L-tryptophane | 0.23 g | NaCl | 1.46 g |
| L-valine | 0.75 g | $MgCl_2 \times 6\ H_2O$ | 1.01 g |
| acetylcysteine | 0.10 g | L-malate | 0.92 g |
| glycine | 3.50 g | K-citrate | 0.68 g |
| $CaCl_2 \times 2\ H_2O$ | 0.44 g | | |
| $ZnCl_2$ | 0.004 g | | |
| Xylitol | 50.0 g | | |
| ad 1000 ml with $H_2O$ | | | |

### Serum Clearance Determination: Molecular Analysis of Serum and Urine Components

We determined serum clearance by drawing 10-ml blood samples at 10 min, 1, 4 and 24 hr after antibody injection. They were collected, centrifuged, and 1 ml of the supernatant serum was measured in a well counter. The 10-min value was taken as 100% (time-point of complete homogenous distribution of injected activity in the whole blood before significant extravasation or excretion has begun). Urine was quantitatively collected over 4 hr, starting immediately after the MAb injection.

The molecular size composition was analyzed by size exclusion gel filtration chromatography, essentially as described earlier (*8*). Briefly, the native pre-injection antibody solutions, 1 ml of serum at the different time-points postinjection and of the 4-hr urine, respectively, were applied to a column of Sephadex G-100 Superfine (Pharmacia, Freiburg, Germany; column size 0.5 × 120 cm). The gel bed was pre-equilibrated with phosphate-buffered saline (pH 7.4). Elution was performed with the same buffer as the eluant (elution speed 5 ml/hr). Fractions (4 fractions per hour) were collected and analyzed for their activity in a well counter. The column was calibrated with defined molecular weight protein standards. L-cysteine standard (Sigma Chemie, Deisenhofen, Germany) was labeled with $^{99m}$Tc according to procedures described earlier (*8,14*).

## RESULTS

### Reduction of Renal Uptake of Technetium-99m-Fab' Fragments

The clinical characteristics of the five patients infused with the amino acid solution are summarized in Table 2. Four of them suffered from recurrent colorectal cancer, and the fifth had a primary gastric tumor metastatic to the left hepatic lobe (Fig. 2) 5 yr after resection of a colon carcinoma. All patients included in this study had normal kidney function parameters (creatinine ≤ 1.2 mg/dl, BUN ≤ 23 mg/dl). In none of the treated patients could any changes of these parameters, including the serum electrolytes and acid-base status, be noticed over a period of 4 wk. The amino acid infusion was well tolerated by all patients without any subjective or objective side effects.

The quantitative analysis of the whole-body and organ uptake kinetics under amino acid challenge, in comparison to control patients, is shown in Figure 3. A statistically significant effect on renal uptake was observed at 4 hr as well as after 24 hr postinjection (10.9 ± 2.0% versus 14.1 ± 5.2% of injected activity in the treated versus the control group at 4 hr [$p <$

**TABLE 2**
Characteristics of Five Patients Examined with Technetium-99m-Labeled Fab' Fragments*

| Patient no. | Patient Age (yr) | Patient Sex | Site of primary lesion | Lesions known at time of scanning | Antibody used | Scan results |
|---|---|---|---|---|---|---|
| 1 | 72 | M | Rectum | Gastric ca. (second primary lesion in the antrum)<br>(Liver metastasis left hepatic lobe) | F023C5 $^{99m}$Tc-Fab' | Strongly positive 4 and 24 hr p.i. (planar)<br>Large cold lesion at 4 hr, filling in at 24 hr (planar) |
| 2 | 67 | M | Rectum | Local recurrence | F023C5 $^{99m}$Tc-Fab' | Positive on 4-hr (SPECT) |
| 3 | 63 | M | Rectum | Local recurrence | F023C5 $^{99m}$Tc-Fab' | Positive on 24-hr (SPECT) |
| 4 | 48 | M | Sigma | Recurrence at site of anastomosis | NP-4 $^{99m}$Tc-Fab' | Positive at 1 and 4 hr (SPECT) |
| 5 | 45 | F | Rectum | Two liver metastases (rt. lobe 1 cm)<br>(lt. lobe 10 cm) | NP-4 $^{99m}$Tc-Fab' | Positive at 4 hr (SPECT)<br>planar and SPECT at 4 hr |

*Labeled Fab' fragments of the anti-CEA antibodies were the clones NP-4 and F023C5. These patients underwent amino acid infusion.
ca. = carcinoma; p.i. = postinjection.

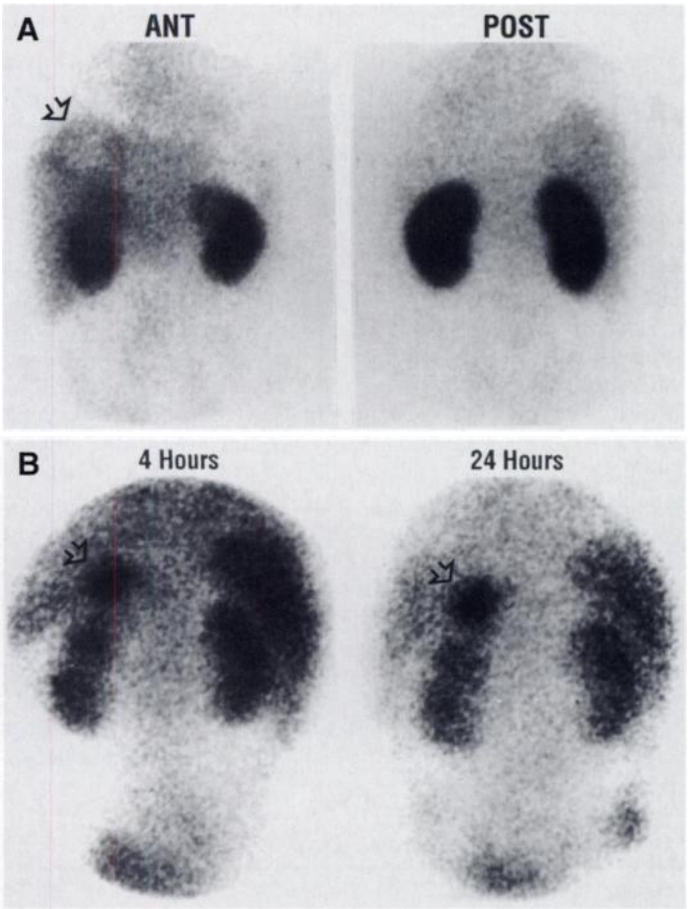


**FIGURE 2.** (A) Abdominal scan of a 53-yr-old patient with a 5-cm liver metastasis in the dome of the liver (NP-4 $^{99m}$Tc-Fab', 4 hr postinjection). The lesion (hot rim in the anterior view; arrow) is barely visualized in the posterior view, since the hot kidneys deliver most of the counts. (B) Posterior abdominal scan of a patient with a gastric carcinoma in the antral region (F023C5 $^{99m}$Tc-Fab') who was treated with 2 liters of the amino acid infusion (Patient 1, Table 2). The kidneys appear to be much less intense than usual with fragments (Fig. 2A) and the tumor is well visualized (arrow).

0.10], 11.1 ± 2.0% versus 17.7 ± 7.0% at 24 hr [$p < 0.05$]), despite the relatively small number of patients in the treated group. There was no statistically significant difference at 1 hr postinjection, and no effect could be observed on the uptake in any other organ or in the blood clearance at any time. The effect (53.6 ± 6.1 versus 63.5 ± 14.3, $p < 0.10$) on the whole-body retention at 24 hr is easily explained by the difference in the retention in the kidneys. Also, the lesion sensitivity, as far as it can be judged reliably from only five patients, was in the expected range, when compared to the previously reported results in control patients (*6,8*). All known tumor sites were targeted with the exception of a 10-cm liver metastasis in patient KJ (Table 2), which appeared at 4 hr postinjection scintigraphically as a cold lesion, when compared to the normal liver parenchyma, with subsequent filling-in by 24 hr postinjection. This is a usual finding for liver lesions of this size (*6,8*).

### Mechanism of Action

For assessment of the physiological mechanism of this renal uptake reduction, analysis of the molecular composition of the patients' urine was carried out. Whereas no fundamental differences were found in the molecular composition of the activity in the serum between the control group and amino-acid-treated patients (Fig. 4, upper panel), molecular species analysis of the urine excreted by the patients revealed significant differences between control and treated patients (Table 3, Fig. 4, lower panel).

For identification of these compounds, protein standards of defined molecular weight, the intact $^{99m}$Tc-labeled Fab' fragment, as well as an L-cysteine standard, labeled with $^{99m}$Tc, were run as molecular weight standards on gel filtration chromatography under the same conditions as used for serum and urine samples. The first peak in the serum (fractions 15–19) coeluted with a 100-kD protein standard, consistent with a $F(ab')_2$ fragment. The second peak (fractions 19–24) present in serum in control and treated patients, but in the urine in larger amounts (53% of total urine activity) only in the amino-acid treated group, coeluted with intact Fab' (50 kD); the group of small-molecular-weight peaks was found in a similar range as the $^{99m}$Tc-cystine standard (fractions 40–50). Thus, the

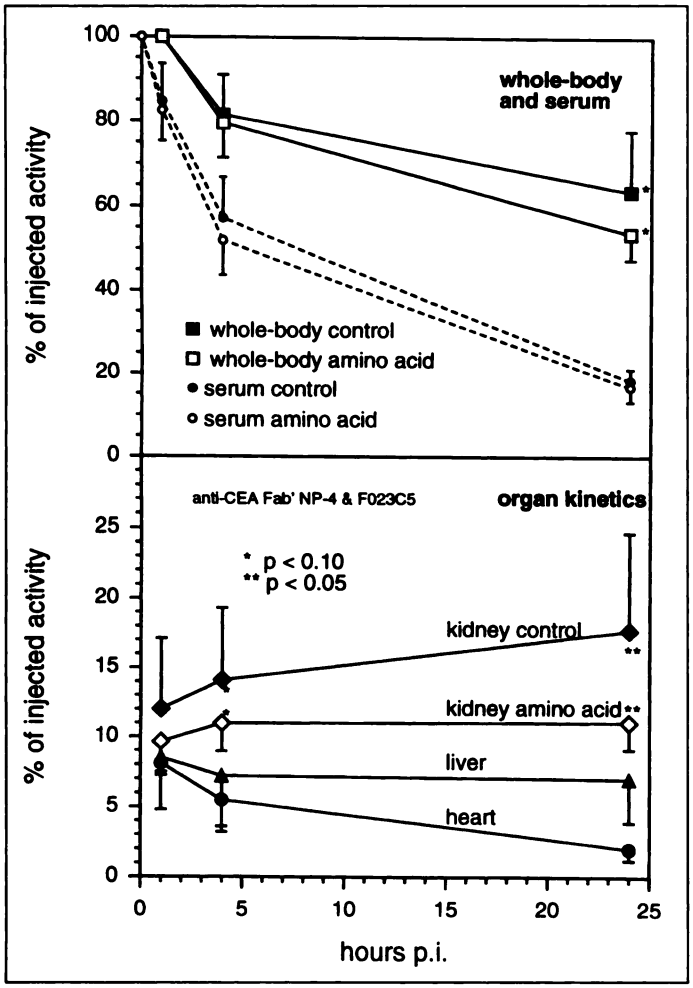


**FIGURE 3.** Summary of the reduction of the renal uptake in the five patients who underwent radioimmunodetection studies with $^{99m}$Tc-Fab' fragments of the anti-CEA MAbs F023C5 and NP-4. The effect on renal uptake is most pronounced at 24 hr postinjection ($p < 0.05$). No difference was found in the serum clearance as well as in the uptake kinetics of all other organs.

serum of the patients contained essentially Fab' and $F(ab')_2$ fragments (ratio at 3 hr postinjection, 2.2:1.0; the relatively high amount of $F(ab')_2$ is due to a more rapid excretion of Fab' when compared to $F(ab')_2$ (*6,8*)).

Additionally, the serum contained smaller amounts of low-molecular-weight metabolites (approximately 12% of total serum activity), which most likely represent $^{99m}$Tc-cystine and other sulfhydryl-containing peptides, as was demonstrated earlier (*8,14*). In the urine of the control group, these metabolites of low molecular weight were predominant (over 90% of the total activity excreted). Only traces of a labeled compound in the molecular weight range (50 kD) of the original intact Fab' (approximately 7.5% of total urine activity) could be detected in these control patients. In the amino-acid-treated group, however, the amount of this substance in the 50-kD range, with 53% of the total urine activity, was much more prominent. The mechanism of the reduction of the renal uptake of radiolabeled fragments seems, therefore, to rely on an inhibition of the tubular reabsorption of glomerularly-filtered proteins, so that they appear directly in the urine without prior lysosomal degradation to low-molecular-weight compounds in the proximal tubule cells.

## DISCUSSION

High renal uptake is a major problem in radioimmunodetection with monoclonal antibody fragments, as well as in scintigraphy with radiolabeled peptides (*5–7*). The hot kidneys can

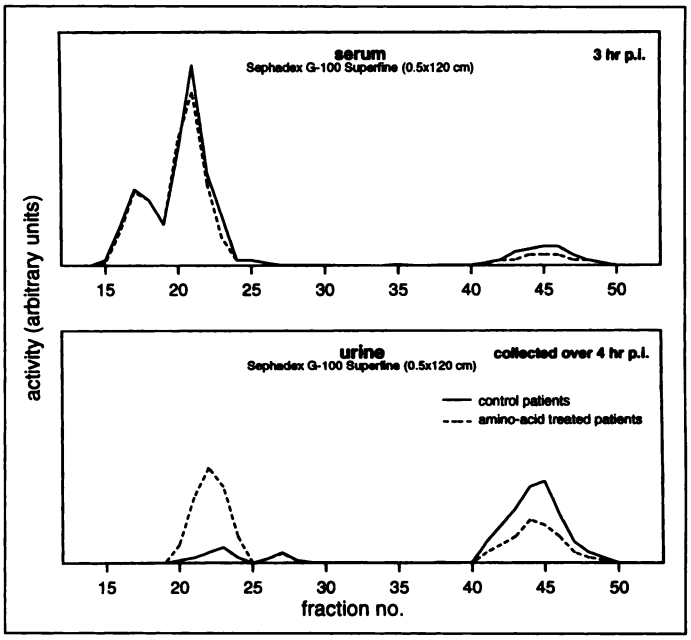


**FIGURE 4.** Gel filtration chromatographic profile of a patient who was amino-acid-treated, in comparison to a control patient. Upper panel: No significant differences become apparent in the molecular composition of the labeled compounds in the serum at 4 hr postinjection. Lower panel: In the urine, significantly higher amounts of unmetabolized proteins appear in the molecular weight range of native Fab', whereas the amount of low-molecular-weight metabolites seems decreased (Table 3).

compromise lesion sensitivity in their surroundings, even when SPECT techniques are used (*6,15*) (Figs. 1, 2A). Furthermore, even with pure gamma emitters, such as $^{99m}$Tc-Fab', the dose to the kidney is, at 0.11 ± 0.04 mGy/MBq, relatively high (*16*).

Attempts to modify the antibody itself, e.g., by neutralizing positive charges via N-acetylation, have been shown to be of limited success (*17*), and have compromised the immunoreactivity and tumor uptake of the antibody (*17*). In this study, no effect on lesion sensitivity was seen, as far as this can be concluded from the data of only five patients. This is in accordance to our previous animal experimental findings (*1–4*), where no apparent effects on tumor uptake were observed.

It is known in renal physiology that high doses of basic amino acids can induce proteinuria (*9,18*), presumably by blocking the tubular reabsorption of glomerularly-filtered peptides (*9*), although several contradictory theories have been published (*9–11,18*). Preliminary animal experimental data by Pimm et al. (*11*), and semiquantitative human data from Hammond and coworkers (*10*), suggested that L-lysine can reduce the renal uptake of $^{111}$In-labeled Fab' fragments and $^{111}$In-labeled somatostatin analogues. Our previous animal studies showed that the effect of lysine is not restricted to indium-labeled compounds,

**TABLE 3**
Molecular Composition of 3-Hour Serum Sample and Urine*

| | Serum | | Urine | |
|---|---|---|---|---|
| Total activity (%) | Control | Amino-acid | Control | Amino-acid |
| $F(ab')_2$ | 27.3% | 29.5% | — | — |
| Fab' | 59.7% | 57.5% | 7.5% | 53.4% |
| Low-molecular-weight metabolites† | 12.2% | 8.1% | 90.4% | 38.2% |

*Samples from a control subject an amino-acid-treated patient were collected over a 4-hr period (Fig. 4).
†$^{99m}$Tc-cystine and other metabolites (*8,14*).

but seems to be a general principle, since it extends to all isotopes tested (*1–4*). Furthermore, it could be shown that the molecular characteristics that enable a compound to inhibit protein uptake seem to be very variable, provided the substance carries a positive charge through an amino group.

With respect to the mechanism by which these basic compounds are capable of reducing the renal uptake of proteins and peptides, we found, similar to our animal studies (*1–4*), that significantly higher amounts of unchanged Fab′ are excreted in the amino-acid-treated patients, in contrast to low-molecular-weight products in the controls. This supports the view of Morgenson et al. (*9*) that the major principle involved is inhibition of tubular reabsorption of primarily glomerularly-filtered peptides.

Our data are still preliminary, especially when comparing the moderate reduction of the renal uptake in our patients to an 85% reduction obtained in animals (*1–4*). This is certainly due to the very low doses of the amino acids used in patients to animals; in mice, the optimal effectiveness was found with a total of 8 mg of lysine per gram of body weight (*1–4*). We believe, however, that further clinical trials, also with other isotopes and peptides as well as larger fragments (e.g., $F(ab')_2$), are warranted. A major concern that needs to be considered before starting clinical trials on the effect of renal uptake reduction in patients on a larger scale, however, is the potential toxicity of high doses of these compounds. Hammond et al. (*10*) gave approximately 40 g of basic amino acids (lysine and arginine) over a 4-hr time period, which is approximately fourfold higher than the amount used in this study, obviously without major side-effects. Since we did not want to give a too hyperosmotic solution via a peripheral vein, we chose the lower amount. Eventual differences in renal uptake reduction may be due this difference in the amino-acid dose.

Indeed, there are contradictory opinions in the literature on the toxicity of amino acids given in high dose (*19–22*). Especially the toxicity of L-lysine seems to be species-dependent. The study of Abel et al. (*20*) showed that the administration of amino acids in acute renal failure in humans can improve the survival rate from 44 to 75%, whereas high lysine doses in rats can cause an acute renal failure-like histological pattern, characterized predominantly by brush border loss, dilatation of the tubule up to tubular necrosis (*21*). Evidence exists that the toxic effect of high doses of amino acids is related to the alpha-amino and carboxyl groups, whereas the inhibitory effect on protein reabsorption relies on a basic side chain, so that the desired effect and the side effect may be separable (*21*). In humans, genetically-elevated serum lysine levels (familial hyperlysinemia) seem to have no adverse effects (*23*). Since we could demonstrate in animals an equal effectiveness of both isomers of lysine (*1–4*), and since the toxicity of lysine seems to be restricted to its L-isomer (*18*), D-lysine should be metabolically inert and applicable without endangering the metabolic balance between the different amino acids and their metabolites, especially since no transmembrane transporters are known in humans to be capable of accreting D-lysine (*18*).

Further studies are therefore necessary to determine a clinically practical setting to reduce the renal uptake found in antibody and peptide scintigraphy with certain agents, to evaluate the pharmacology and toxicity of potential substances used for this purpose, and to assess the role of renal uptake in therapies with radiometal-labeled antibody fragments and receptor-binding peptides.

## CONCLUSION

The renal uptake of monoclonal antibody fragments in patients can be reduced significantly by amino acid infusion, even at considerably lower doses than those that were safe and effective in animals (*1–4*). As was found in animals, the mechanism seems to rely on an inhibition of the reabsorption of tubularly-filtered proteins by the proximal tubule cells (*4*). These results encourage further clinical trials to lower the renal uptake experienced in radioimmunodetection, as well as in therapeutic trials with antibody fragments and peptides (Behr et al., unpublished data). Before application of higher amounts of basic amino acids in patients, formal toxicological studies are indicated.


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