Plasma clearance and endocytosis of mitochondrial malate dehydrogenase in the rat

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1. Pig mitochondrial malate dehydrogenase was labelled with ^{125}I and intravenously injected into rats. Enzyme activity and radioactivity were cleared from plasma identically, with first-order kinetics, with a half-life of only 7 min. 2. Radioactivity accumulated in liver, spleen, bone (marrow) and kidneys, reaching maxima of 31, 4, 6 and 9% of the injected dose respectively, at 10 min after injection. 3. Our data allow us to calculate that in the long run 59, 5, 11 and 13% of the injected dose is taken up and subsequently broken down by liver, spleen, bone and kidneys respectively. 4. Differential fractionation of liver showed that the acid-precipitable radioactivity was mainly present in the lysosomal and microsomal fractions, suggesting that the endocytosed protein is transported via endosomes to lysosomes, where it is degraded. 5. Radioautography of liver and spleen suggested that the labelled protein was taken up by macrophages of the reticuloendothelial system. 6. Mitochondrial malate dehydrogenase is probably internalized in liver, spleen and bone marrow by adsorptive endocytosis, since uptake of the enzyme by these tissues is saturable.

Plasma contains a large number of enzymes which probably originate from damaged cells or from the intestinal tract (for a review see Fishman & Doellgast, 1975). The (generally low) concentration of these circulating enzymes, like that of other plasma proteins, is determined by the rates of deliverance to, and removal from, the vascular compartment. Plasma enzymes are cleared from the blood at widely different rates (Posen, 1970). Well-documented mechanisms for rapid and irreversible removal of enzymes from the circulation are glomerular filtration of low-molecular-weight enzymes (Kooistra *et al.*, 1979a, 1980; Maack *et al.*, 1979) and endocytosis of carbohydrate-containing enzymes by liver cells (Schlesinger *et al.*, 1980; Scholtens, 1980).

Friedel *et al.* (1976) have determined half-lives in plasma of a number of enzymes after intravenous injection into rats. One of these enzymes, mitochondrial malate dehydrogenase (EC 1.1.1.37), was cleared very rapidly from plasma, with a half-life of about 5 min. Since its mol.wt. (67 000) is nearly the same as that of serum albumin, extensive glomerular filtration seemed unlikely. Furthermore, there is no evidence that mitochondrial malate dehydrogenase is a glycoprotein. It seemed there-

fore of interest to study the cause of the exceptionally rapid clearance of the enzyme.

Since the reticuloendothelial system appears to be involved in the removal of several tissue-derived plasma enzymes (Fishman & Doellgast, 1975; Sinke *et al.*, 1979), we determined the uptake of the enzyme by liver, spleen and bone marrow.

Experimental

Materials

Pig heart mitochondrial malate dehydrogenase [suspension in $(\text{NH}_4)_2\text{SO}_4$] was obtained from Boehringer, Mannheim, Germany; Na^{125}I (carrier-free) was from The Radiochemical Centre, Amersham, Bucks., U.K.; Iodogen (1,3,4,6-tetrachloro-3a,6a-diphenylglycouril) was from Pierce, Rockford, IL, U.S.A.; 5'AMP-Sepharose 4B and Sephadex G-150 were obtained from Pharmacia, Uppsala, Sweden; NAD^+ (grade III), NADH (grade III) and bovine serum albumin were from Sigma, St. Louis, Mo., U.S.A.; glutaraldehyde was from O.C.S., Soesterberg, The Netherlands; Fluothane was from I.C.I., Macclesfield, Cheshire, U.K. All other chemicals used were analytical grade.

Protein labelling

Mitochondrial malate dehydrogenase was dissolved in 0.2 M-sodium borate buffer, pH 9.2; insoluble material was removed by centrifugation for 30 min at 15 000 g. To 3 ml of the clear supernatant, containing 12 mg of the enzyme, were added 47 nmol of Na^{127}I and 0.75 mCi of Na^{125}I . The mixture was transferred to a 50 ml Erlenmeyer flask that had been coated with a thin film, containing 2.4 mg of the water-insoluble oxidizing agent Iodogen as described by Fraker & Speck (1978). The reaction was allowed to proceed under gentle shaking for 45 min at 0°C.

Labelled enzyme was separated from free iodide and labelled non-enzymic material by affinity chromatography on 5'AMP-Sephadex 4B. After application of the solution, the column (0.9 cm × 17 cm) was washed with 3 vol. of 20 mM sodium phosphate buffer, pH 7.0. Subsequently, malate dehydrogenase was eluted with a linear gradient (total volume 44 ml) of 0–0.2 mM-NADH in 20 mM-sodium phosphate buffer, pH 7.0.

The ^{125}I -labelled enzyme had a specific radioactivity of 8–10 $\mu\text{Ci}/\text{mg}$ of protein. Highly labelled enzyme (35 $\mu\text{Ci}/\text{mg}$ of protein) was prepared in the same way as described above by adding 4 times the usual amount of Na^{125}I to the enzyme. Enzyme activity was not changed by the iodination procedure. Only one band of radioactive material could be detected after electrophoresis in sodium dodecyl sulphate-containing polyacrylamide gels as described by Laemmli (1970). Less than 1% of the labelled material was trichloroacetic acid-soluble.

Handling of animals and liver fractionations

Male rats of an inbred Wistar strain, weighing between 160 and 250 g, were used. All experiments were done with anaesthetized animals. Anaesthesia was induced and maintained with Fluothane in a mixture of N_2O and O_2 .

Rats were injected, via the penile vein, with 0.20 ml of phosphate-buffered saline (6 mM-sodium phosphate buffer, pH 7.35, containing 0.15 M-NaCl) per 100 g body wt., usually containing 0.1 mg of the enzyme. Solutions used for injection were always centrifuged for 30 min at 15 000 g immediately before injection, in order to remove any traces of insoluble material. Blood samples (about 0.15 ml) were obtained from the orbital plexus, and centrifuged in heparinized tubes. Duplicate samples (20 μl) of plasma were used for radioactivity measurements and/or enzyme-activity determinations. Plasma volumes were calculated by using the equation: plasma volume (ml) = $[0.0291 \times \text{body wt. (g)}] + 2.54$ (Bijsterbosch *et al.*, 1981).

After suitable intervals, rats were killed. Liver, spleen and kidneys were excised, dropped im-

mediately into ice-cold 0.25 M-sucrose and homogenized. Livers were perfused with ice-cold phosphate-buffered saline until they became yellowish. Spleen and kidneys were not perfused. Bone marrow (red) was obtained from the femora and tibiae and suspended in 0.25 M-sucrose. To determine uptake in bone (including bone marrow), the skeleton was dissected free from other tissues and subsequently dissolved in 300 ml of 60% (w/v) NaOH at 90°C for 2 h, and samples of the resulting solution were counted for radioactivity. Uptake in the tissues was corrected for the amount of radioactivity in residual plasma, assumed to be present in the tissue at the time of sampling. Values for spleen (84 μl of plasma/g fresh wt.) and kidneys (100 μl of plasma/g fresh wt.) were taken from Everett *et al.* (1956). For the skeleton a value of 210 μl of plasma/100 g body wt. (Everett *et al.*, 1956; Lippman & Finch, 1972) was used. The amount of plasma left behind after perfusion of liver with phosphate-buffered saline (35 μl of plasma/g fresh wt.) was taken from Munniksmas *et al.* (1980). The amount of plasma in bone marrow (84 μl of plasma/g fresh wt.) was determined by measuring radioactivity in this tissue shortly after injection of ^{125}I -labelled poly(vinylpyrrolidone) (see Munniksmas *et al.*, 1980).

In one type of experiment, the circulation through liver, spleen and kidneys was interrupted by ligating the hepatic artery, the portal vein and the blood vessels of spleen and kidneys.

Differential fractionations of livers were done as described by Bouma & Gruber (1966).

Assay of radioactivity

Radioactivity in plasma was determined after precipitation of protein as follows: to 20 μl of plasma was added 0.1 ml of a carrier solution containing 10% (w/v) bovine serum albumin and 0.22% (w/v) NaI, followed by the addition of 0.1 ml of 22% (w/v) trichloroacetic acid. Radioactivity in homogenates and subcellular fractions was measured after adding 0.5 ml of 20% (w/v) trichloroacetic acid to an equal volume of the homogenate or fraction. Supernatants and precipitates were counted for radioactivity.

Radioactivity measurements were made in a Nuclear Chicago mark I scintillation counter. Samples were counted as gamma emitters in a heterogeneous system by using tetrabutyltin-loaded scintillator (Gamma-vial C, Koch-Light). Counting efficiency was about 50%.

Determination of protein and enzymes

The concentration of mitochondrial malate dehydrogenase was determined spectrophotometrically by using $A_{280,1\text{ cm}}^{1\%}$ 2.88 (Frieden *et al.*, 1978). Malate dehydrogenase activity was assayed by measuring the rate of change of A_{340} in 2.5 ml of

0.1 M-diethanolamine/HCl buffer, pH 9.0, containing 3.2 mM-NAD⁺, 20 mM-malate, 1 mM-EDTA and 4 mM-2-mercaptoethanol, in a thermostatically controlled cell kept at 25°C. Acid phosphatase activity was measured as described by Gianetto & de Duve (1955). Glucose 6-phosphatase was assayed as described by de Duve *et al.* (1955), with minor modifications. Protein concentrations in homogenates and subcellular fractions were measured by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

Radioautography

For radioautography, highly labelled mitochondrial malate dehydrogenase preparations (35 μ Ci of ¹²⁵I/mg of protein) were used. Radioautograms were prepared as described by Sinke *et al.* (1979).

Results

Plasma clearance of enzyme activity

After intravenous injection of pig heart mitochondrial malate dehydrogenase, enzyme activity in plasma was rapidly cleared with first-order kinetics (Fig. 1). Clearance remained monophasic until at least 90% of the dose had disappeared from plasma. Amelung (1960) obtained similar results with rabbits, but Friedel *et al.* (1976) found biphasic clearance of enzyme activity after injection

of the enzyme into rats. However, these authors injected an amount of enzyme activity exceeding the activity naturally present in plasma by only one order of magnitude; moreover they followed the clearance up to 180 min or longer. The amount of enzyme activity that we injected exceeded the activity naturally present in plasma by more than two orders of magnitude.

In a first-order clearance process, plasma concentration P (% of dose/ml) at any time t (min) is given by: $P = P_0 e^{-kt}$, where P_0 is the initial plasma concentration (% of dose/ml) and k is the rate constant of plasma clearance (min^{-1}). The rate constant of mitochondrial malate dehydrogenase clearance, as determined by measuring enzyme activity, was $0.103 \pm 0.008 \text{ min}^{-1}$ (mean $\pm$ S.E.M.; five rats). This value corresponds to a half-life of only 6.7 min. When the clearance curve is extrapolated to the time of injection, a concentration close to the dose divided by the plasma volume is found. An initial phase of very rapid clearance, for instance of aggregated material, is clearly absent.

Inactivation of mitochondrial malate dehydrogenase in plasma

Mitochondrial malate dehydrogenase is known to be thermolabile (Dodd, 1973). On incubation *in vitro* in rat plasma at 38°C (25 μ g of enzyme/ml of plasma) enzyme activity is lost with first-order kinetics. However, the rate constant of inactivation, $0.0057 \pm 0.0002 \text{ min}^{-1}$ (mean $\pm$ S.E.M.; four experiments), corresponding to a half-life of more than 2 h, is far too small to account for the rapid decline of plasma enzyme activity after injection of the enzyme.

Plasma clearance of ¹²⁵I-labelled enzyme

We labelled mitochondrial malate dehydrogenase with ¹²⁵I to determine which tissues are responsible for the plasma clearance of the enzyme. Iodination of the enzyme did not affect its clearance. In animals injected with labelled enzyme, acid-precipitable radioactivity and enzyme activity were cleared with rate constants of 0.104 ± 0.004 and $0.109 \pm 0.005 \text{ min}^{-1}$ (means $\pm$ S.E.M.; five rats) respectively. As described in more detail below (Table 1), unlabelled mitochondrial malate dehydrogenase competes with the labelled enzyme for uptake by several tissues, indicating that they are taken up via the same receptors.

Uptake and breakdown of ¹²⁵I-labelled enzyme by liver and spleen

Trichloroacetic acid-precipitable and -soluble radioactivities in liver and spleen were determined at various times after injection of ¹²⁵I-labelled mitochondrial malate dehydrogenase (Figs. 2a and 2b).

Fig. 1. Clearance of enzyme activity from plasma after intravenous injection of mitochondrial malate dehydrogenase

Rats were injected with 1.0 mg of enzyme/kg body wt. Blood samples were taken at 2, 5, 10 and 15 min after injection. Each point represents the mean value $\pm$ S.E.M. for five animals.

In these tissues, acid-precipitable radioactivity increased up to 10 min after injection and subsequently decreased. The decline of acid-precipitable radioactivity in these tissues was accompanied by an increase of acid-soluble radioactivity. These changes can be ascribed to endocytosis of the

Fig. 2. Uptake and breakdown of ^{125}I -labelled mitochondrial malate dehydrogenase by liver (a) and spleen (b).

Rats were injected with 1.0 mg of ^{125}I -labelled enzyme (sp. radioactivity 8–10 $\mu\text{Ci}/\text{mg}$ of protein)/kg body wt. At various times after injection, trichloroacetic acid-precipitable (○) and -soluble (●) radioactivities were determined in liver (a) and spleen (b). Each point represents the mean value for at least three animals. Vertical bars give the range of the individual values. Note the different scales on the ordinates of the Figures.

enzyme followed by intracellular proteolysis to acid-soluble products that rapidly diffuse from the tissues into the blood. Up to 10 min after injection, the amount of acid-soluble radioactivity in the organs is negligible. This lag period probably reflects the time required for transport of the endocytosed enzyme from the cell membrane to the site of breakdown (probably lysosomes) and subsequent proteolysis.

Evidence for involvement of the lysosomal apparatus in digestion of the endocytosed protein was obtained by differential fractionation of liver homogenates 15 min after injection of the labelled enzyme (Figs. 3a–3d). The sedimentation pattern of acid-precipitable radioactivity (Fig. 3c) was intermediate between those of the lysosomal marker acid phosphatase (Fig. 3a) and the microsomal marker glucose 6-phosphatase (Fig. 3b). We interpret this localization of the acid-precipitable radioactive material as both lysosomal and endo-

Fig. 3. Distribution patterns of radioactivity and enzymes after differential fractionation of liver.

Liver homogenates were fractionated by differential centrifugation 15 min after injection of ^{125}I -labelled mitochondrial malate dehydrogenase (1.0 mg of enzyme/kg body wt.). Blocks from left to right represent fractions in the order in which they were isolated: nuclear, mitochondrial, lysosomal, microsomal and supernatant (cytosol) fractions. The percentage of the recovered protein is given on the abscissa. The ordinate represents the relative specific activity (percentage of recovered activity divided by percentage of recovered protein). Thus the area of each block is proportional to the percentage of activity per fraction. Panels represent the distribution of acid phosphatase, glucose 6-phosphatase, acid-precipitable radioactivity and acid-soluble radioactivity. Results given are means for two experiments. Average recoveries from the homogenates were: acid phosphatase, 93%; glucose 6-phosphatase, 89%; acid-precipitable radioactivity, 93%; acid-soluble radioactivity, 93%; protein, 103%.

EXPLANATION OF PLATE 1

Cellular localization of endocytosed ^{125}I -labelled mitochondrial malate dehydrogenase in liver (a) and spleen (b)

A rat was injected with 1.0mg of radioiodinated enzyme (specific radioactivity $35\mu\text{Ci}/\text{mg}$ of protein) per kg body wt. The liver was perfused first with phosphate-buffered saline and subsequently with 2% (w/v) glutaraldehyde in 0.1 M-sodium phosphate buffer (pH 7.4) 15 min after injection of the protein. The spleen was not perfused. Small blocks of both tissues were fixed in 2% (w/v) glutaraldehyde, and radioautograms were prepared as described by Sinke *et al.* (1979). Scale markers represent $25\mu\text{m}$.

EXPLANATION OF PLATE 2

Cellular localization of endocytosed ¹²⁵I-labelled mitochondrial malate dehydrogenase in kidneys 15 min after injection
A rat was injected and radioautograms were prepared as described in the Explanation of Plate 1. The arrow points to grains located above the origin of a proximal tubule close to the corresponding glomerulus. The scale marker represents 100 μ m.

somal. Acid-soluble radioactivity (Fig. 3d) was mainly present in the supernatant fraction and, to lesser extent, in the lysosomal fraction. The latter distribution pattern probably reflects rapid diffusion of acid-soluble breakdown products out of the lysosomes into the cytosol.

The cellular localization of endocytosed ^{125}I -labelled enzyme in liver and spleen was studied by radioautography of tissue sections 15 min after injection. In liver, radioactivity was present in both hepatocytes and sinusoidal cells, but grains were much more concentrated above the latter cell type (Plate 1a). In spleen, grains were located predominantly above the red pulp (Plate 1b), probably owing to the presence of many macrophages in this part of the tissue. Obviously, endocytosis of mitochondrial malate dehydrogenase by cells of the reticuloendothelial system plays an important part in clearance of the enzyme from blood.

Uptake of ^{125}I -labelled enzyme by bone

Uptake of ^{125}I -labelled mitochondrial malate dehydrogenase by bone was determined 10 min after injection of the enzyme. At this time, $7.4 \pm 0.4\%$ of the dose (mean $\pm$ S.E.M.; four rats) had accumulated in bone. Probably accumulation of radioactivity in bone occurs mainly in bone marrow. At 10 min after injection, bone marrow had taken up $5.5 \pm 0.4\%$ of the dose per g of tissue (mean $\pm$ S.E.M.; four rats). Bone marrow contains many cell types, including macrophages. We have not determined the cellular localization of the endocytosed enzyme, but very

likely the enzyme is internalized in bone marrow by macrophages belonging to the reticuloendothelial system, as it is in liver and spleen.

Glomerular filtration and uptake by kidneys

Patterns of acid-precipitable and acid-soluble radioactivities in kidneys resembled those from liver and spleen (Fig. 4). No acid-precipitable radioactivity could be detected in urine. Radioautograms of kidney sections, made 15 min after injection of labelled enzyme, showed grains exclusively in the cortex, above convoluted tubules (Plate 2). Clearly, mitochondrial malate dehydrogenase is filtered through the glomeruli and subsequently endocytosed by proximal convoluted tubules (see the arrow in Plate 2).

Adsorptive endocytosis of mitochondrial malate dehydrogenase

The rapidity of the uptake of injected mitochondrial malate dehydrogenase by the tissues investigated points to adsorptive endocytosis rather than to the slow and non-specific process of fluid endocytosis (see Sinke *et al.*, 1979). Adsorptive endocytosis entails binding of the protein to receptors on the plasma membrane of the endocytosing cells and should in principle be saturable.

In the experiments described in Table 1, rats were injected with 0.2, 1.0 or 4.0 mg of the enzyme per kg body wt. The uptake by liver, spleen, bone marrow and kidneys was determined 10 min after injection. At this time, the amount of protein taken up by the tissues gives an estimate of the rate of endocytosis that is not, or at least not much, masked by breakdown. The results do indeed show (partial) saturation of receptors in liver, spleen and bone marrow, at a dose of 4.0 mg of enzyme/kg body wt. No saturability was observed in kidneys. Clearly, here glomerular filtration is rate-limiting.

The factors governing adsorptive endocytosis of mitochondrial malate dehydrogenase are not known. Uptake of the enzyme cannot be attributed to irreversible binding of the enzyme to plasma proteins or to aggregation. We checked this by gel filtration on a calibrated Sephadex G-150 column of enzyme that had been preincubated with rat plasma (25 μg of enzyme/ml of plasma) for 5 min at 38°C. The enzyme was eluted as one symmetrical peak at a position corresponding to a mol.wt. of 67000 (recovery about 90%). This molecular-weight estimate is in close agreement with values reported by others (see Banaszak & Bradshaw, 1975). Nor can endocytosis of the enzyme be attributed to the presence of carbohydrate chains on the protein. We did not find any evidence for the presence of hexoses in our preparations by the anthrone reaction (Spiro, 1966), nor for that of hexosamines by a slightly modified Elson-Morgan reaction

Fig. 4. Uptake and breakdown of ^{125}I -labelled mitochondrial malate dehydrogenase by kidneys

Rats were injected and data are expressed as described in the legend to Fig. 2.

Table 1. *Effect of dose on uptake of 125 I-labelled mitochondrial malate dehydrogenase*

Rats were injected with 0.2, 1.0 or 4.0 mg of enzyme (containing about $8\mu\text{Ci}$ of label) per kg body wt. Radioactivities in liver, spleen, bone marrow and kidneys were determined 10 min after injection. Values are means $\pm$ S.E.M. for the numbers of rats given in parentheses. Differences in respect to the lowest dose were tested for significance by Wilcoxon's two-sample test (one-sided; Wilcoxon, 1945).

Tissue	Dose ...	Uptake (% of dose/g of tissue protein)		
		0.2 mg/kg	1.0 mg/kg	4.0 mg/kg
Liver		30.4 ± 4.2 (5)	25.8 ± 2.2 ns (8)	$11.2 \pm 0.9^*$ (9)
Spleen		57.6 ± 2.6 (5)	52.5 ± 6.3 ns (8)	$16.7 \pm 1.6^*$ (9)
Bone marrow		31.4 ± 2.9 (5)	39.0 ± 2.6 ns (7)	$18.3 \pm 0.9^*$ (4)
Kidneys		36.4 ± 2.3 (5)	42.6 ± 5.3 ns (8)	37.6 ± 3.0 ns (9)

* $P < 0.025$; ns, not significant.

(Davidson, 1966). In addition, the occurrence of a carbohydrate moiety has not been reported by the groups that have determined the amino acid composition of the protein or that have elucidated parts of the amino acid sequence (for a review see Banaszak & Bradshaw, 1975).

Calculation of uptake of the enzyme by the tissues

To what extent can plasma clearance of mitochondrial malate dehydrogenase be accounted for by uptake in liver, spleen, bone and kidneys? Owing to breakdown of the protein and consequent loss of label, this problem cannot simply be solved by measuring the radioactivity in the tissues when (nearly) all radioactivity has been cleared from plasma. However, the lower curves in Figs. 2 and 4 show that breakdown of endocytosed enzyme is negligible up to about 10 min after injection. By extrapolation from measurements of tissue radioactivity made within 10 min after injection, the amount of enzyme taken up in the long run can be calculated, provided that plasma clearance is a first-order process and that the rate of uptake by the tissues is proportional to the concentration in plasma (see Sinke *et al.*, 1979). The data in Table 1 show that, up to the usual dose of 1.0 mg of enzyme/kg body wt., uptake is proportional to the dose, and thus to plasma concentration. At this dose, plasma clearance of the radioiodinated enzyme is first-order and remains monophasic up to at least 30 min after injection (at this time only 4.5% of the dose is left in plasma).

Using values for total radioactivity in the tissues up to 10 min after injection and a rate constant for plasma clearance of 0.104 min^{-1} , we have calculated the amounts of enzyme that in the long run will be taken up (and broken down) by liver, spleen, bone and kidneys (see Table 2). The validity of this calculation has been demonstrated by de Jong *et al.* (1981), who compared the calculated uptake of ^{125}I -labelled lactate dehydrogenase M_4 with the observed uptake of the enzyme tagged with a label

Table 2. *Calculated values of 125 I-labelled mitochondrial malate dehydrogenase taken up in the long run*

The values were calculated by extrapolation from the uptake in the tissues within 10 min after injection of the enzyme (1.0 mg/kg body wt.), assuming a rate constant of plasma clearance of 0.104 min^{-1} , as described by Sinke *et al.* (1979). Values are means $\pm$ S.E.M. for the numbers of rats given in parentheses.

Tissue	Percentage of dose taken up by the tissue
Liver	59.1 ± 4.9 (14)
Spleen	4.5 ± 0.5 (14)
Bone	11.4 ± 0.6 (4)
Kidneys	13.4 ± 0.9 (14)

that is retained in the lysosomes. The data in Table 2 suggest that plasma clearance of mitochondrial malate dehydrogenase is largely, if not completely, brought about by uptake of the enzyme in liver, spleen, bone and kidneys. Major uptake by other tissues seems unlikely.

The view that liver, spleen and kidneys play important parts in plasma clearance of the enzyme has been corroborated in the following experiment. Rats in which the circulation through liver, spleen and kidneys had been interrupted were injected with the enzyme. Enzyme activity in plasma decreased to $59.3 \pm 3.0\%$ (mean $\pm$ S.E.M.; four rats) of the initial value in 30 min, whereas in controls only about 4% was left. The 40.7% decrease of plasma activity in treated rats can probably be attributed to uptake by bone, enzyme inactivation (15% of the enzyme activity is lost during incubation in rat plasma *in vitro* for 30 min at 38°C), distribution over extravascular spaces and, perhaps, some uptake by other tissues.

Discussion

Our findings on mitochondrial malate dehydrogenase are very similar to our previous results on

lactate dehydrogenase M_4 (Sinke *et al.*, 1979) and point to a similar mechanism, except for the uptake of mitochondrial malate dehydrogenase in kidneys. Indeed, in some preliminary experiments uptake of lactate dehydrogenase M_4 was retarded by the simultaneous injection of mitochondrial malate dehydrogenase. This phenomenon suggests competition for a common receptor.

It is not yet clear what the chemical basis of the affinity of these proteins for the receptor is. Mitochondrial malate dehydrogenase (pI9.3; Noyes *et al.*, 1974) and lactate dehydrogenase M_4 (Sinke *et al.*, 1979) are both basic proteins. Wachsmuth & Klingmüller (1978) have given evidence suggesting that the positive charge of lactate dehydrogenase M_4 is responsible for its endocytosis by cells of the reticuloendothelial system. We have obtained similar evidence for derivatives of pancreatic ribonuclease A and egg-white lysozyme (Kooistra *et al.*, 1979b, 1980). Thus the basic character of mitochondrial malate dehydrogenase may contribute to its rapid uptake, but it is quite possible that in addition other features of the molecule are involved.

Probably the positive charge of the enzyme is also responsible for its glomerular filtration. Mitochondrial malate dehydrogenase has about the same molecular weight as serum albumin, but its pI is much higher (9.3 versus about 4.7 for serum albumin). Several studies have shown that glomerular filtration of macromolecules is accelerated by positive charge. Rennke *et al.* (1978), for instance, demonstrated a marked increase of filtration rate of 'cationized' horseradish peroxidase preparations (cationized by substitution of free carboxyl groups with hexanediamine) concomitant with a rise in pI. Endocytosis of mitochondrial malate dehydrogenase by cells of the proximal convoluted tubules is possibly also enhanced by the positive charge of the enzyme. Christensen *et al.* (1980) found that cationized ferritin was taken up much better by proximal tubules than was unmodified ferritin. Dissociation of the dimeric enzyme followed by glomerular filtration of subunits is unlikely. As stated above, gel filtration of enzyme that had been preincubated with plasma gave no indication of the presence of monomers. Neither has dissociation of the enzyme been found by Jaenicke *et al.* (1979), even at a concentration as low as $0.2 \mu\text{g/ml}$.

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