24 h after injection, while it can be calculated (from Eqn. 5, ref. 1) that about 90% of the injected ^{14}C-labelled LDH 5 had disappeared from the circulating body fluids during that period. Since dialysing the urine removed all ^{14}C radioactivity, it is apparent that intact ^{14}C-labelled LDH was not excreted in urine.

In all three experiments samples of plasma containing 100 mU of injected LDH 5 were incubated with rabbit antiserum. There was no decrease in the percentage immune inhibition of lactate dehydrogenase activity during the course of an experiment. If present with its antigenic sites intact, inactive circulating LDH 5 would have caused a fall in percentage immune inhibition. A 1:1 ratio of inactive to active LDH 5 would be expected to reduce inhibition from 65 to 55% since that is the effect of decreasing antiserum concentration by half.

It can be concluded that the rapid loss of LDH 5 activity from plasma following its intravenous injection does represent the actual disappearance of the isoenzyme from plasma. If LDH 5 is inactivated in the circulation then the inactive form must be removed very rapidly indeed.

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Relative levels of the disulfide-interchange enzyme in the microsomes of the bovine tissues

It has been shown that rat and beef liver microsomes[1,3] and pig, pigeon and chicken pancreas tissue[2] contain an enzyme that catalyzes the reactivation of the inactive fully reduced or of randomly cross-linked forms of bovine pancreatic ribonuclease, lysozyme[3], soy bean trypsin inhibitor[4] and pepsinogen[5]. The enzyme isolated from beef-liver microsomes has been purified and characterized[6,7] and it has been shown to catalyze sulfhydryl–disulfide interchange in proteins[8].

The enzyme may be assayed by measuring the reactivation of fully reduced proteins in the presence of an added oxidizing agent[1,2] or of the inactive forms of ribonuclease and soy bean trypsin inhibitor (in which incorrect disulfide bonds have been introduced) in the presence of low levels of a reducing agent[8].

Since the disulfide interchange enzyme catalyzes the reactivation of several animal and plant proteins and since the enzyme is localized at the site of protein

synthesis, it has been suggested that the enzyme may catalyze, *in vivo*, the correct pairing of half-cystine residues in newly synthesized polypeptide chains[6].

We have investigated the enzymatic reactivation of fully reduced ribonuclease in the presence of the microsomes of the bovine tissues.

The tissues were removed as soon as possible after the animals were slaughtered and rapidly processed to prepare microsomes as previously described[1]. Bovine pancreatic ribonuclease (Type II-A, Sigma) was fully reduced with β-mercaptoethanol in 8 M urea, and the reduced protein was separated from the reagents by gel filtration as previously described[9]. Titration of aliquots of the effluent with *p*-mercuribenzoate as described by BOYER[10] showed the presence of eight half-cystine residues per mole of ribonuclease. Stock solutions of reduced ribonuclease in 0.1 M acetic acid were kept for no longer than 1 day at 0° to minimize the possibility of spontaneous reactivation. The supernatant fraction of liver microsomes was used as the oxidizing system[1]. The concentration of proteins in the microsomal suspensions were deter-

TABLE I

COMPARISON OF SPECIFIC ACTIVITIES OF THE DISULFIDE INTERCHANGE ENZYME FROM VARIOUS BOVINE TISSUES

For experimental procedures see Fig. 1.

Microsomes	*Specific activity**	*Microsomes*	*Specific activity**
Lymphatic gland	163	Parotid gland	23
Testis	83	Kidney	21
Liver	70	Brain	20
Ovary	55	Heart	20
Thyroid	35	Spleen	13
Lung	30		

* Calculated at protein levels below 1 mg/ml: in this range an essentially linear relationship between ribonuclease reactivation and enzyme concentration was found.

mined by the method of LOWRY *et al.*[11]. Assays for reactivation of reduced ribonuclease were performed in duplicate; aliquots were removed from the incubation mixture and assayed for ribonuclease activity by measurement of the rate of digestion of yeast RNA (Type II, Sigma) at pH 5.0 (ref. 12). Blank determinations were carried out to permit correction for endogenous ribonuclease activity in the microsomal preparations. Table I summarizes the results obtained with the microsomal fraction of 11 beef tissues. It can be seen that the highest amounts of the enzyme were present in the microsomes of lymphatic gland, testis, liver, ovary and thyroid; as noted earlier, inhibition was observed when higher levels of microsomal proteins were used for the reactivation process (Fig. 1). It is noteworthy that the level of the disulfide interchange enzyme is highest in the tissues which secrete large amounts of proteins containing disulfide bonds (γ-globulins, serum albumin, thyroglobulin *etc.*).

The general occurrence of the disulfide interchange enzyme in the beef tissues, and the elevated levels of this enzyme in the microsomes of secretory beef tissues, supports the suggestion for the *in vivo* role of the enzyme in the terminal stages of biosynthesis of proteins containing S–S bonds.

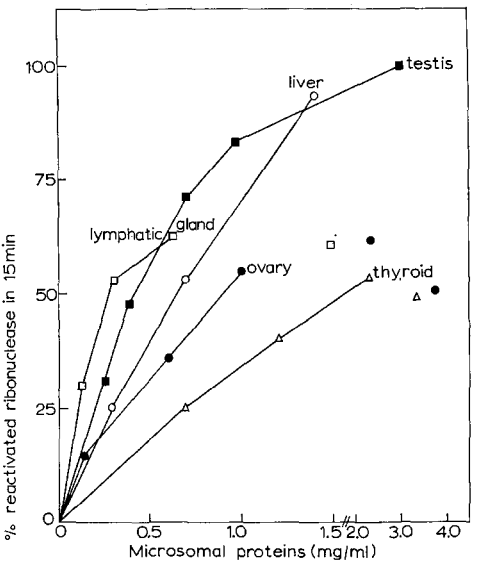

Fig. 1. Effect of increasing amounts of the microsomal fraction from beef lymphatic gland, testis, liver, ovary and thyroid on the reactivation of reduced ribonuclease. All incubations were carried out in a Dubnoff shaking bath at 37° for 15 min. Reactivation mixtures contained (total volume of 1 ml) 0.1 M Tris–HCl (pH 7.4), 25 μg of reduced ribonuclease, 25 μl of the dialyzable beef-liver supernatant fraction, and washed microsomes. The percentage of the reduced ribonuclease reactivated of that theoretically obtainable from the amount of reduced ribonuclease added, is shown as a function of the disulfide interchange enzyme present in the mixtures.

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