# LOCALIZATION OF ENZYMES RESPONSIBLE FOR THE HYDROLYSIS OF GLUTATHIONE

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It has been recognized for some time in this laboratory that the enzymes of renal tissue responsible for the hydrolysis of glutathione are found in the microsomal fraction. Glutathionase was described as a lipoprotein (Binkley, 1951) and the relationship of the cysteinylglycinase to microsomal nucleoprotein has been emphasized (Binkley, 1952). However, others (Semenza, 1957) have attempted to compare easily extractable activities with this cysteinylglycinase and there have been rather contradictory reports (Connell and Hanes, 1956; Cliffe and Waley, 1959) as to the distribution of the glutathionase in various tissues. It would seem worthwhile, therefore, to present a concise report of the distribution of the glutathionase in renal tissue of the pig, a major source for the preparation of soluble as well as insoluble peptidases and various gamma-glutamyl transferases.

The tissue fractions were prepared from fresh pig kidney essentially by the methods summarized by Hogeboom (1955) with the exception that the particles were finally suspended in 0.1 M Tris buffer, pH 8.0. "Lipoprotein" and "ribonucleoprotein" particles of the microsomes were prepared by the procedure utilizing deoxycholate (Littlefield et al, 1955); this procedure worked quite well with microsomes of renal tissue and the residue was found to contain most if not all the orcinol reactive material. A tabulation of the activities found in the various fractions is given in Table 1; for comparison, alkaline phosphatase was included. There seems to be universal agreement that alkaline phospha-

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tase of renal tissue is found in the microsomes (Hers et al, 1951) and this was confirmed; the assay was according to the procedure of Morton (1954). Glutathionase was assayed with 0.003 M glutathione, 0.006 M glycylglycine and 0.001 M magnesium chloride and the results are expressed as micromoles glutathione hydrolyzed per minute. It is obvious that glutathionase was concentrated almost quantitatively in the microsomes and, in turn, in the lipoprotein fraction. The bulk of the cysteinylglycinase activity, assayed as previously described (Binkley et al, 1957), was found to be concentrated in the microsomes and, like the alkaline phosphatase of the microsomes, was found with the ribonucleoprotein particles.

TABLE I

Distribution of enzymatic activities in fractions of renal tissue

| Fraction | Glutathionase | Cysteinylglycinase | Phosphatase |
|---|---|---|---|
| Homogenate | 144 | 11 | 63 |
| Nuclei | 28 | 3 | 15 |
| Mitochondria | 24 | 2 | 21 |
| Microsomes | 192 | 26 | 94 |
| "Lipoprotein" | 216 | 3 | 10 |
| "Ribonucleoprotein" | 32 | 26 | 91 |
| Supernatant | 0 | 6 | -- |

Results are expressed in units per ml. homogenate or resuspended particles.

The alkaline phosphatase and peptidase of swine kidney as isolated in this laboratory (Binkley et al, 1957; Binkley, 1959a) are of low molecular weight and are released from the insoluble material of kidney tissue by digestion with proteolytic enzymes; the activities released by proteolysis are resistant to proteolytic activity. The alkaline phosphatase and cysteinylglycinase activities of the ribonucleoprotein particles are also resistant to proteolysis under conditions not permitting a decrease in pH below 7 (where loss of activity is observed with or without proteolytic enzymes). The resistance to proteolysis has been observed over extended digestion with crystalline trypsin, chymotrypsin,

ficin, papain or carboxypeptidase. The glutathionase is unstable at 37° and is difficult to test as to resistance to proteolysis; it has been observed, however, that trypsin and chymotrypsin, like inert protein, will delay the loss of activity at 37°. Various factors in the enzymatic release of the peptidases and alkaline phosphatases from insoluble material of swine kidney have been described (Binkley, 1959b) and further studies will be detailed in the future. It is to be recalled that the activities are not destroyed by ribonuclease or by snake venom.

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