the enzyme activity of the supernatant was raised threefold more than that of the pellet. The further addition of deoxycholate decreased the phosphate activation of glutaminase. This decrease was greater for the pellet enzyme than for the supernatant enzyme.

The supernatant and pellet enzymes were then preincubated at elevated temperatures for various times. Preincubation at 60°C for 10min almost completely destroyed the glutaminase activity of the pellet, but only slightly decreased the enzyme activity of the supernatant. In the presence of phosphate similar heat treatment almost completely destroyed the enzyme activity of both supernatant and pellet. These results indicate that the enzyme which is released in the supernatant is different from that which remains in the pellet.

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Mechanisms of Ammoniagenesis in Human Kidney

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It has been demonstrated that glutamine is the major and direct source of renal ammonia production in man (Owen & Robinson, 1963). Two distinct metabolic pathways of glutamine metabolism have been demonstrated in the kidney of the rat, guinea pig and dog. The predominantly intramitochondrial glutaminase I isoenzymes that hydrolyse glutamine to ammonia and glutamate and the subsequent deamidation of glutamate to ammonia and 2-oxoglutarate constitute the major metabolic route in the rat (Goldstein, 1967). However, the extramitochondrial glutamine aminotransferase–ω-amidase pathway (glutaminase II) has been shown to play an important role in the dog (Bourke, Frindt, Rubio-Paez & Schreiner, 1971). In human kidney glutaminase I activity has been demonstrated (Mattenheimer & De Bruin, 1964), but values obtained have ranged widely owing to enzyme instability in autopsy specimens. We obtained healthy portions of renal cortex from patients undergoing nephrectomy. Washed kidney mitochondria were incubated with glutamine (70mM) in tris buffer, pH 8. Significant deamidation of glutamine occurred. Ammonia production increased by 75% in the presence of maleate and fivefold in the presence of phosphate. This suggests that, as has been demonstrated in the rat, there are two isoenzymes of glutaminase I in man.

Previously there has been no direct evidence for a glutaminase II pathway in human kidney. We investigated this pathway using substituted glutamine, ω-N[^{14}C]-methyl-L-glutamine. In contrast with glutamine, this compound on deamidation yields methylamine. Initial studies indicated that this compound was not metabolized by human kidney glutaminase I. The demonstration by Meister (1954) that it was deamidated by rat liver glutaminase II suggested its application for investigating the glutaminase II pathway in human kidney slices. Slices were incubated in Krebs–Ringer phosphate medium, pH 7.4, at 37°C under continuous bubbling with O_2+CO_2 (95:5) with added glutamine or ω-N[^{14}C]-methylglutamine (70mM). Significant quantities of either ammonia or methylamine production were demonstrated. In the presence of pyruvate (39mM), a keto acid utilized in the glutaminase II pathway, methylamine production increased by over 50%, as did ammonia production. Under these circumstances methylamine production amounted to 10% of ammonia production, indicating a functionally important glutaminase II pathway in human kidney.

Malonate, which leads to accumulation of tissue 2-oxoglutarate, decreased ammoniagenesis from glutamine. High concentrations of fluorocitrate and pyruvate, which also inhibit the rate of utilization of 2-oxoglutarate, similarly depressed ammoniagenesis, supporting the postulate that 2-oxoglutarate utilization affects the activity of glutaminase I. Inhibition of methylamine production was also demonstrated under these circumstances, suggesting a similar control by 2-oxoglutarate on glutaminase II activity.

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The Permeability of Mitochondria to Carnitine and Acetylcarnitine

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Three distinct spaces may be operationally defined in mitochondria that have been sedimented into a pellet. Polymers such as dextran (mol.wt.