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 10 min 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 Ammoniogenesis 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 (70 mM) 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 O₂+CO₂ (95:5) with added glutamine or ω -N[14 C]-methylglutamine (70 mM). Significant quantities of either ammonia or methylamine production were demonstrated. In the presence of pyruvate (39 mM), 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 ammoniogenesis from glutamine. High concentrations of fluorocitrate and pyruvate, which also inhibit the rate of utilization of 2-oxoglutarate, similarly depressed ammoniogenesis, 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.

75000) are totally excluded from the mitochondrion, certain smaller molecules such as sucrose penetrate only as far as the inner membrane and the total water space may be measured with tritiated water. Using these spaces as markers, we have investigated the distribution volumes for (-)-carnitine and acetyl-(-)-carnitine in rat liver mitochondria incubated at 0°C for 3 min at pH 7.2 in a medium consisting of tris-HCl buffer (20 mM), potassium chloride (80 mM), EDTA (1 mM) and sucrose (20 mM). After incubation mitochondria were obtained by centrifugation at 12000g for 10 min. In these experiments the total pellet water averaged 2.22 µl/mg of protein and the [¹⁴C]sucrose and [¹⁴C]dextran spaces were 68% and 38% respectively of the total pellet water. The penetration of tritiated (-)-carnitine was identical with that of sucrose. However, values for the acetyl-(-)-carnitine space, measured enzymically, were quite erratic and occasionally less even than the dextran space. This anomaly was traced to a breakdown of acetyl-(-)-carnitine during the incubation and subsequent manipulations. Experiments were therefore repeated, but the technique of high-speed centrifugation of mitochondria through a silicone layer into perchloric acid was employed to give a near-instantaneous deproteinization of the mitochondria after separation from the incubation medium. In this system the acetyl-(-)-carnitine space was also found to be identical with the sucrose space. In parallel experiments the addition of acetyl-(-)-carnitine was observed to elevate the intramitochondrial acetyl-CoA concentration from 0.88 to 1.4 nmol/mg of protein. This suggests that acetyl groups enter the mitochondrial matrix under conditions in which acetyl-(-)-carnitine does not penetrate the inner membrane.

Results reported suggest the concept of an anisotropic mechanism for carnitine facilitation of fatty acyl group translocation (Haddock, Yates & Garland, 1970), provided that two assumptions are valid. First, acetyl-(-)-carnitine must not have been rapidly removed as soon as it entered the matrix. Secondly, it is assumed that discrete subcompartments of the mitochondrial matrix do not exist. If there were compartmentation within mitochondria, small changes in accumulation of acetyl-(-)-carnitine might well be non-detectable. The validity of these assumptions will be discussed.

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Lipase Activity of Bovine Mammary Gland

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The lipase activity of the mammary-gland extracts (0.4–1.5 µequiv. of acid/min per g of tissue; substrate tributyrin emulsion) is associated with macromolecules. These are not sedimented by centrifugation at 38000g for 2 h and are eluted at the void volume of Sepharose 4B columns, indicating an apparent molecular weight greater than 20×10^6 . On mixing pancreatic lipase with the extracts the enzyme binds to the macromolecules, which thus appear to be complexes composed of mammary-gland lipase(s) in association with other proteins. Lipid or other hydrophobic interactions are apparently involved in maintaining the mammary-gland lipase complexes; the addition of dimethylformamide (final concn. 1.3 M) to the extract followed by gel filtration on Sepharose 4B columns equilibrated with eluent containing dimethylformamide dissociates the complexes, and the lipase activity is eluted (recovery 60%) in a position corresponding to a protein of molecular weight 0.63×10^6 . Similarly the complexes are also completely dissociated by gel filtration on Sepharose 2B in the absence of dimethylformamide and, since the eluted lipase again has an apparent molecular weight of approximately half a million, it may represent the free enzyme.

Milk lipases may be derived from the mammary gland and the casein-lipase complexes of milk (mol.wt. $> 100 \times 10^6$) exhibit a number of properties (Downey & Murphy, 1970) similar to those of the mammary-gland activity.

In the absence of dimethylformamide the lipase activity is quantitatively eluted from Sepharose 4B columns, which had been calibrated for molecular-weight determination with spherical plant viruses and macroproteins, as two overlapping peaks at or near the void volume (mol.wt. $> 20 \times 10^6$) followed by a third lipase peak (mol.wt. approx. 1.5×10^6). The first peak appears to represent the intact lipase-protein complexes and exhibits little esterase activity. The other two are attributed to intermediate dissociation products, and the esterase activity of the extracts (0.4–0.8 µequiv. of acid/min per g of tissue; substrate *p*-nitrophenyl acetate) is quantitatively eluted with the latter of these two peaks. After dimethylformamide treatment the esterase is eluted (recovery 60%) coincidentally with the free lipase in a single peak of molecular weight 0.63×10^6 . These results may indicate an alteration in the substrate specificity of the lipase resulting from dissociation of the lipase-protein complexes, similar