The amino acid composition of rat bile[1]

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Summary. The pattern of amino acids in the bile of rats differs from the pattern in the serum of these animals, since bile contains significantly greater amounts of acidic and sulphur-containing amino acids and glycine than serum, while the serum contained more basic amino acids than bile, indicating that secretion of amino acids into bile may involve specific transport processes.

As part of a study of the incorporation of amino acids into pancreatic enzymes and the secretion of these enzymes[4], we studied the appearance of amino acids in the bile of rats. Our findings indicate that the secretion of amino acids into bile is selective and specific.

Materials and methods. Male Wistar rats, weighing 300 g (± 30 g) were studied. 2 experimental schedules were employed.

1. 9 rats were fasted for 24 h and then anaesthetized with urethane (0.6 ml/100 g b.wt of a solution containing 250 g/l) by i.p. injection. During each study, the body temperature of the rats was maintained at 37 °C. The bile duct was catheterized near the liver and the duct distal to the cannula was ligated. A catheter was placed in the jugular vein and 0.15 moles/l sodium chloride solution was infused at 0.9 ml/h. Bile was collected in 30-min batches in plastic tubes and then pooled. Blood was obtained by aortic puncture at the end of each study.

2. In order to study the effect of fasting and feeding on the biliary excretion of amino acids, a further 12 rats were divided into 2 subgroups, half of which were fasted for 24 h and studied as above, while the remaining 6 rats were fed until the time of study and then had bile collected, as above.

Samples (100 µl) of bile or serum were treated with trifluoroacetic acid (5 µl) at 10 °C. After centrifugation, the supernatant and 4 washings of the precipitate (each washing 50 µl of triple-distilled water) were combined and evaporated in a vacuum desiccator. The amino-acid composition of the resulting residues was determined with a LoCarte Amino-acid Analyser (single 22 cm column; buffer changes – pH 3.25 for 65 min, 4.25 for 60 min, 6.65 for 45 min; buffer temperature – 52 °C for first 45 min, then 60 °C thereafter) and Autolab Computing Integrator System AA.

Results. Bile contained significantly greater amounts of the acidic amino acids aspartic and glutamic (table) ($p < 0.01$) while the amounts of these amino acids in serum were small. On the other hand, serum contained more of the basic amino acids lysine and ornithine than did bile ($p \leqslant 0.001$). While bile contained appreciable amounts of the sulphur-containing amino acids cystine and methionine, cystine was absent from serum and only traces of methionine could be detected (table) ($p < 0.001$). Furthermore, there were significantly greater amounts of glycine in bile compared with serum ($p < 0.001$). The levels of histidine, valine, tyrosine, isoleucine, leucine and phenylalanine in bile and serum did not differ signifi-

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Amino acid composition of serum and bile

	Group I Fasted Serum Median	Range	Bile Median	Range	Group II Serum Fasted Median	Fed Median	Bile Fasted Median	Fed Median
Histidine	0.050	0.025–0.148	0.076	0.023–0.100	0.056	0.057	0.062	0.045
Lysine	0.237	0.158–0.325	0.089	0.052–0.181	0.381	0.328	0.103	0.070
Arginine	0.027	0 –0.078	0	0 –0.017	–	–	–	–
Ornithine	0.134	0.096–0.273	0.066	0.042–0.078	0.261	0.282	0.067	0.049
Aspartic acid	0	0 –0.041	0.770	0.371–1.118	0.016	0	> 2.500	> 2.500
Threonine + Gln + Asn + Serine	0.548	0.426–0.606	0.659	0.128–0.902	0.890	0.637	0.357	0.236
Glutamic acid	0.147	0.063–0.206	1.356	0.788–2.536	0.197	0.143	2.049	1.742
Glycine	0.235	0.190–0.309	1.074	0.611–2.649	0.378	0.408	1.008	0.937
Alanine	0.264	0.181–0.428	0.233	0.175–0.376	0.573	0.590	0.143	0.152
Half cystine	0	0 –0.024	0.472	0.373–0.929	0	0	0.540	0.130
Valine	0.131	0.098–0.162	0.187	0.106–0.247	0.299	0.154	0.231	0.179
Methionine	0.012	0 –0.020	0.086	0.051–0.408	0.027	0.031	0.382	0.383
Isoleucine	0.078	0.058–0.107	0.145	0.108–0.247	0.134	0.062	0.327	0.153
Leucine	0.164	0.115–0.219	0.362	0.124–0.666	0.285	0.206	0.446	0.347
Tyrosine	0.055	0.038–0.090	0.091	0.077–0.118	0.133	0.152	0.150	0.091
Phenylalanine	0.082	0.058–0.122	0.132	0.085–0.219	0.134	0.162	0.210	0.132

All values in mmoles/l.

cantly. An unidentified peak, travelling between leucine and tyrosine in bile, was not found in any serum sample.

Discussion. While there have been many studies of the protein content of bile[5], very little information is available about the amino acid composition of bile. Miller[6] analysed cadaveric bile and found free lysine, tyrosine and glycine which he considered represented the excretion of waste products. Dassi and Gianni[7] examined human bile obtained during laparotomy and detected water-soluble substances reacting with ninhydrin, which they considered compatible with the presence of free amino acids or polypeptides. The present study is therefore the first to quantify the amino acids in bile and to compare the pattern of amino acids in bile with those found in serum.

The high concentrations of acidic and sulphur-containing amino acids in bile and the relative lack of basic amino acids has not been explained. In part, the concentrations in bile may reflect the concentrations of the amino acids inside the hepatic cells, since it has been shown that there are significantly greater amounts of some amino acids (glutamate, aspartate and glycine) in the liver cells than in serum[8]. However, other amino acids are also found to be concentrated in the hepatocytes (e.g. alanine) but are not found concentrated in bile, while amino acids which are not concentrated by the liver cells (e.g. cystine) may be found in greater amounts in bile than in serum. Lysine has been shown to be specifically transported by the mucosa of the gall bladder, but so are methionine and glycine[9], so that the relative lack of lysine in the bile is probably not attributable to selective transport of the lysine out of the lumen of the biliary tract.

Unlike proteins, which appear in bile by processes involving bulk transfer and molecular sieving of the plasma proteins[10,11], it appears that the transfer of amino acids into the bile involves specific transport processes. It has previously been shown that organic anions[12] and cations[13] can be secreted into bile by active transport processes but multiple excretory processes are probably involved[14] and the role of these processes in the transport of amino acids has not been studied. In the small intestine, the transport of the neutral, acidic and basic amino acids is highly specific[15] so that there may be similar processes involved in the hepatic secretion of these amino acids, in view of the significantly different handling of these 3 groups of amino acids by the liver cells.

The function of the biliary amino acids has not been defined, but amino acids are known to stimulate pancreatic exocrine secretion[16] and, perhaps in conjunction with bile salts (which also stimulate pancreatic secretion[17]) may have some functional role in the early stages of the digestive response to the entry of food into the alimentary tract.

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Nerve endings isolated from chick embryonic optic tectum. 2. Developmental aspects of synaptosomal membrane

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Summary. Fractions enriched in nerve endings (synaptosomes) have been isolated from chick embryonic optic tectum during development. After osmotic shock, these fractions appeared to be enriched in membranes which during development acquire typical features of mature synaptosomal membranes.

The development of improved fractionation techniques has permitted biochemical and morphological studies to be carried out into the nature of individual components of nerve endings (synaptosomes).

The most attention has been focused on synaptosomal membranes, both in analysis of gross composition and identification of specific components[1] both in ultrastructural organization of synaptic densities[2], in correlation with its critical role played in synaptic transmission. It is also generally accepted that synaptosomal membrane is to a certain extent involved in neuronal receptor recognition and specificity of cell adhesion during the development of neuronal circuits[3]. Consequently, isolation of embryonic synaptic membrane can be regarded as an essential step for biochemical and morphological studies on properties of maturing synaptic complexes.

	16th		18th		2nd	
	a)	b)	a)	b)	a)	b)
Homogenate	100	48.35	100	91.45	100	66.00
Crude mitochondrial fraction	19.86	9.60	17.32	15.84	20.72	13.68
Synaptosomes	4.34	2.10	6.90	6.31	5.21	3.44
Membranes	1.86	0.90	5.68	5.20	7.15	4.72

a) Percentage of protein content of each fraction based on the homogenate being 100%; b) total protein content of the fractions (mg).