FURTHER STUDIES ON RENAL TUBULAR CONSERVATION OF FREE AMINO ACIDS IN EARLY INFANCY

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The higher rates of excretion in the urine of the premature infant of both total free alpha amino nitrogen and of individual amino acids as compared to the full-term infant, the older child, and the adult have been repeatedly documented (Childs, 1952; Dustin, Moore and Bigwood, 1955; Fowler, Norton, Cheung and Pratt, 1957; Jagenburg, 1959; O'Brien, Bergstedt, Butterfield, Ibbott and Lubchenco, 1960; Sereni, McNamara, Shibuya, Kretchmer and Barnett, 1955; Woolf and Norman, 1957). In only one study, however, has there been an attempt to extend these observations to the measurement of the renal clearances of single amino acids. Using the semi-quantitative technique of paper chromatography, Sereni *et al.* (1955) demonstrated in the case of 13 individual amino acids that this aminoaciduria in the premature infant was due to diminished renal tubular reabsorption and that it was also limited by the small glomerular filtration rate in the first month of life. Additional and more precise data obtained by column chromatography in both normal adults and adults and children with a variety of metabolic defects has been reviewed in detail (Scriver, 1962).

The purpose of this study was to obtain comparable information for the premature and full-term infant at the time of maximal aminoaciduria and so

to extend the earlier observations based on paper chromatography as well as to provide a more accurate baseline for the appraisal of aminoacidurias in this age-group.

Clinical Material and Methods

Four healthy premature male infants and three full-term male infants (Table 1) were studied at an age between 30 and 60 days when previous observations (O'Brien *et al.*, 1960; Sereni *et al.*, 1955) had shown that the aminoaciduria would be most pronounced. The subjects were maintained for four days before the collection period on 120-150 ml./kg./24 hours of a conventional partially skimmed milk and carbohydrate mixture containing 67 calories and 2.7 g. protein per 100 ml. They were placed on a metabolic frame (Hepner and Lubchenco, 1960) from which all urine could be collected into a time flow fraction collector. After a fast of not less than four hours, the time of the next voiding was noted. The infant was then given 25 ml./kg. of 5% dextrose in water and all urine was subsequently collected for a timed period which ranged from 97-174 minutes; samples were frozen pending the estimation of amino acids. Similar studies were carried out on three fasting healthy male adults.

A scalp vein sample of approximately 5 ml. was taken for the assay of creatinine and free amino acids at the end of the collection period rather than in the middle because of evidence that such procedures might signi-

TABLE 1
COLLECTION DATA AND CREATININE CLEARANCES

	Prem. 1	Prem. 2	Prem. 3	Prem. 4	Full-term 1	Full-term 2	Full-term 3	Adult 1	Adult 2	Adult 3
Age (days)	36	50	57	53	31	39	46	37 yrs.	34 yrs.	35 yrs.
Birth weight (g.)	1,230	980	1,225	1,250	3,520	3,030	2,551	—	—	—
Current weight (g.)	2,100	1,920	1,970	1,930	3,990	3,720	3,120	88 kg.	70 kg.	79.5 kg.
Length (cm.)	44	42.5	49	44	57.5	53	53	184	180	183
Surface area (m. ²)	0.153	0.144	0.158	0.147	0.24	0.22	0.205	2.1	1.75	1.9
Collection time (min.)	174	108	109	107	97	132	163	207	120	122
Urine volume (ml.)	31.0	26.0	21.0	28.0	41	74	74	217	70	374
Plasma creatinine (mg./100 ml.)	0.45	0.56	0.88	0.66	0.55	0.56	0.58	1.20	1.11	1.04
Urine creatinine (mg./100 ml.)	7.5	5.4	13.6	8.5	14.6	6.3	9.1	137	209	44.3
Creatinine clearances (C _{cr}) (ml./min./1.73 m. ²)	33.6	27.9	32.6	39.7	80.8	51.3	60.4	98.6	108	121

TABLE 2
AMINO ACID CONCENTRATIONS IN PLASMA (P_{aa})

	Prem. 1		Prem. 2		Prem. 3		Prem. 4		P _{aa}
	P _{aa}	UV	P _{aa}	UV	P _{aa}	UV	P _{aa}	UV	
Taurine ..	0.077	0.047	0.058	0.045	0.062	0.078	0.050	0.029	0.049
Hydroxyproline ..	Tr	1.18	Tr	1.36	0.072	1.15	0.063	2.12	Tr
Aspartic acid ..	0.020	Tr	0.010	0.042	0.011	Tr	0.818	Tr	0.008
Threonine ..	0.197	1.07	0.240	1.98	0.330	0.982	0.155	0.860	0.204
Serine ..	0.161	1.21	0.102	1.32	0.171	0.792	0.112	1.04	0.197
Glut./Asp. ..	0.423	1.54	0.440	1.25	0.421	0.490	0.432	1.32	0.563
Proline ..	0.104	0.634	0.131	1.72	0.314	0.670	0.147	0.990	0.473
Glutamic acid ..	0.144	0.022	0.087	0.130	0.125	0.076	0.085	0.028	0.060
Citrulline ..	0.068	0.173	0.044	0.098	0.044	0.025	0.036	0.069	0.014
Glycine ..	0.217	4.11	0.124	2.93	0.204	2.50	0.162	4.22	0.191
Alanine ..	0.213	0.700	0.208	0.770	0.390	0.573	0.221	0.700	0.508
α-Am-n-butyric ..	0	Tr	0	Tr	Tr	Tr	Tr	Tr	0.019
α-Am-adipic ..	0	0.210	0	Tr	0	0.044	0	0.100	0
Valine ..	0.148	0.065	0.140	0.052	0.227	0.045	0.128	0.077	0.344
Half-cystine ..	0.070	0.252	0.017	0.149	Tr	0.037	Tr	0.172	Tr
Homocitrulline ..	Tr	0.136	Tr	0.289	Tr	0.302	Tr	0.373	0
Cystathionine ..	0.005	0.109	0.009	0.090	0.008	0.109	0.005	0.125	0
Methionine ..	0.043	0.097	0.025	0.079	0.039	0.100	0.024	0.140	0.046
Isoleucine ..	0.063	0.066	0.043	0.043	0.074	0.033	0.038	0.037	0.102
Leucine ..	0.100	0.044	0.508	0.042	0.111	0.051	0.066	0.085	0.180
Tyrosine ..	0.388	0.560	0.381	0.604	0.306	0.173	0.097	0.248	0.201
Phenylalanine ..	0.073	0.088	0.057	0.097	0.064	0.110	0.056	0.126	0.114
β-Alanine ..	0	0	0	0	0	0	0	0	0
βAIB ..	0	0.124	0	0.092	0	0.121	0	0.156	0
Hydroxylysine ..	0	0.131	0	0.159	0	0.272	0	0.190	0
γ-Am-n-butyric ..	0	0	0	Tr	0	Tr	0	0	0
Ornithine ..	0.100	0.085	0.090	0.091	0.112	0	0.079	0.068	0.094
1-Me-histidine ..	0	0	0	0	0	0	0	0	0
Histidine ..	0.048	0.827	0.131	0.595	0.048	0.340	0.050	0.619	0.061
3-Me-histidine ..	0	0	0	0	0	0	0	0	0
Arginine ..	0.060	0	Tr	0	Tr	0	0.068	0	0.049
Lysine ..	0.146	0.580	0.081	0.418	0.125	0.196	0.097	0.425	0.342
Total alpha amino acids*	2.9	14.0	2.4	14.4	3.3	9.2	2.2	14.3	3.8

P_{aa} = Plasma free amino acid concentration in μmol./ml.
Tr = Detectable but insufficient to measure accurately.

* Derived from sum of measured amino acids.

significantly alter glomerular filtration rate (McCance, Naylor and Widdowson, 1954). Creatinine in plasma and urine was measured specifically in triplicate within two hours of sample collection by the Jaffe reaction after adsorption on to and elution from Lloyd's reagent (Owen, Iggo, Scandrett and Stewart, 1954). The remainder of the plasma was at once deproteinized (Stein and Moore, 1954) and assayed for amino acids on sulphonated polystyrene resin columns (Spackman, Stein and Moore, 1958) using a Beckman/Spinco amino acid analyser. Urine samples were similarly assayed after acidifying to pH 2.0. Glutamine and asparagine are eluted simultaneously from the column and are expressed in terms of glutamine; these values must be treated with reserve because of the variable destruction of glutamine during deproteinization. Tryptophane is not recovered quantitatively from such a column (Stein and Moore, 1954), and in none of the runs on urine or plasma was there a measurable peak in the tryptophane position. Reproducibility for individual plasma amino acids for all stages of this assay as expressed by the coefficient of variation was $\pm 5.7\%$ (2.6-7.6%) except for half-cystine and histidine which gave values of $\pm 10\%$. However, as Jagenburg (1959) has pointed out, there are technical reasons for supposing that cystathionine, methionine, leucine and isoleucine in urine are overestimated; but the error is not sufficient to affect the

general conclusions on tubular reabsorption of these amino acids.

Results and Discussion

Values for creatinine concentration in plasma and urine and for endogenous creatinine clearance rates are given in Table 1. Plasma amino acid concentrations (P_{aa}) and urinary amino acid excretion rates (UV) are shown in Table 2, and amino acid clearances (C_{aa}) and percentage tubular reabsorption (TR) in Table 3. In calculating TR, the endogenous creatinine clearance has been accepted as the measure of glomerular filtration rate (Barnett and Vesterdal, 1953).

Two major factors must be considered in any evaluation of the higher urinary losses of amino acids in infants as compared to older children and adults. The first of these is the size of the filtered load presented to the proximal tubule. Doolan, Harper, Hutchin and Shreeve (1955) and Doolan, Harper, Hutchin and Alpen (1956) have shown, for example, that in adults the intravenous loading of a mixture of amino acids produces a striking reduction in the tubular conservation of threonine, serine,

AND URINARY EXCRETION RATES (UV)

Term 1	Full Term 2		Full Term 3		Adult 1		Adult 2		Adult 3	
UV	P _{aa}	UV	P _{aa}	UV	P _{aa}	UV	P _{aa}	UV	P _{aa}	UV
0.183	0.103	0.013	0.020	0.023	0.074	0.501	0.047	1.31	0.052	0.366
2.58	Tr	0.705	Tr	0.790	Tr	0	0	0	0	0
Tr	0.017	0.160	0.020	0.323	0.004	0.057	0.010	0.090	0.008	0.028
1.32	0.178	0.675	0.226	1.34	0.092	0.084	0.139	0.102	0.116	0.044
2.32	0.165	1.09	0.203	1.95	0.094	0.296	0.105	0.253	0.089	0.089
2.00	0.365	1.15	0.477	1.71	0.500	0.475	0.519	0.485	0.406	0.175
5.40	0.413	1.11	0.422	0.730	0.243	0	0.154	0	0.201	0
0.041	0.204	0.622	0.115	0.255	0.056	0.016	0.155	0.014	0.192	0.008
0.040	Tr	Tr	0.035	Tr	0.027	Tr	0.023	Tr	Tr	0
8.40	0.180	3.76	0.238	5.28	0.150	0.825	0.208	0.886	0.233	0.414
2.12	0.496	1.21	0.474	1.36	0.373	0.207	0.364	0.263	0.354	0.087
0	Tr	0	0	0	0.011	0.025	0.027	0.039	0.017	0.014
0.230	0	0.176	0	0.230	0	0.019	0	0.017	0	0
0.120	0.330	0.165	0.327	0.106	0.182	0.032	0.248	0.036	0.206	0.005
0.260	Tr	0.343	0.060	0.148	0.055	0.051	0.076	0.056	0.075	0.020
0.240	0	0.233	0	0.129	0	0.038	0	0.036	0	0.026
0.170	Tr	0.119	Tr	0.118	0	0.014	0	0.020	0	0.013
0.127	0.033	0.141	0.048	0.140	0.020	0.035	0.032	0.031	0.012	0.023
0.102	0.080	0.137	0.122	0.156	0.055	0.022	0.071	0.036	0.065	0.009
0.154	0.143	0.172	0.215	0.167	0.103	0.032	0.141	0.048	0.121	0.016
0.380	0.119	0.224	0.142	0.243	0.051	0.092	0.067	0.102	0.043	0.081
0.118	0.065	0.123	NA	0.137	0.047	0.049	0.066	0.063	0.045	0.043
0	0	0	0	0	0	0	0	0	0	0
0.250	0	0.176	0	0.425	0	0.036	0	0.093	0	0.014
0.110	0	0.110	0	0.057	0	0.019	0	Tr	0	0
Tr	0	0	0	0	0	Tr	0	Tr	0	Tr
0.082	0.077	0.084	0.090	0.057	0.059	Tr	0.083	Tr	0.072	Tr
0.029	0	0	0	0	0	0.578	0	0.196	0	0.528
1.80	0.052	0.855	0.072	1.49	0.064	0.533	0.069	0.294	0.067	0.147
0.065	0	0	0	Tr	0	0.229	0	0.280	0	0.082
0.045	0.096	0.097	0.095	Tr	0.038	0	0.057	0	0.041	0
1.37	0.215	1.36	0.316	0.724	0.169	0.209	0.178	0.114	0.170	0.022
30.1	3.3	15.0	3.7	18.1	2.5	4.5	2.8	4.8	2.6	2.3

UV = Urinary amino acid excretion in $\mu\text{mol./min./1.73 m}^2$.

NA = Technically unsatisfactory chromatogram.

glycine and histidine. Webber, Brown and Pitts (1961) and Webber (1962) have also demonstrated in dogs that the infusion of any single basic, acidic or neutral amino acid will primarily increase tubular rejection of other acids in the same group. No data on the effects of increased tubular loads are available for the small infant; but the fasting serum levels of alanine, valine, leucine, isoleucine and proline are higher in the infant than in the adult. Sereni *et al.* (1955) showed, however, that the impact of these increased plasma levels on the tubular load was counteracted in the least mature infants by their small glomerular filtration rate.

The second factor is that of transport across the luminal membrane, in turn a function of molecular charge and steric relations. Thus the neutral amino acids, alanine, α -aminobutyric, valine, leucine and isoleucine are reabsorbed as effectively in the infant as in the adult despite uniformly higher plasma levels in all cases except α -aminobutyric. It is of interest that glycine does not behave in this manner; its special position may relate not only to its being the shortest chain and therefore the least lipophilic of the neutral acids but to the other

factors that affect its absorption. Histidine with its cyclic side chain is also less well absorbed in the infant than the longer chain neutral acids.

The dicarboxylic acid glutamic is as well reabsorbed in the infant as in the adult although its monoamide glutamine shows some impairment in premature and full-term infants. Tubular rejection of aspartic acid on the other hand, although similar at all ages, is uniformly greater than of glutamic acid. These two acidic amino acids have been shown by Webber (1962) to inhibit each other competitively at the tubular membrane but not to interfere significantly with the reabsorption of the neutral amino acids.

The neutral aromatic acids, phenylalanine and tyrosine, are efficiently conserved at all stages of maturity. Phenylalanine has been shown by Webber (1962) to compete for reabsorption with other neutral acids, but whether it does so with tyrosine in the brain (Neame, 1961) is not known.

The basic amino acids ornithine, arginine, lysine and cystine have a known common reabsorptive pathway (Robson and Rose, 1957). Plasma levels are similar at the different ages except for the high

RENAL CLEARANCES (C_{aa}) AND RENAL TUBULAR

	Prem. 1		Prem. 2		Prem. 3		Prem. 4		C _{aa}
	C _{aa}	TR	C _{aa}	TR	C _{aa}	TR	C _{aa}	TR	
Taurine	0·60	98	0·77	97	1·3	96	0·58	98	3·8
Hydroxyproline	—	(1)	—	(1)	16	51	34	15	—
Aspartic acid	—	(2)	4·2	85	—	(2)	—	(2)	—
Threonine	5·4	84	8·2	71	3·0	91	5·5	86	6·5
Serine	7·5	78	13	54	4·6	86	9·3	77	12
Glut./Asp.	3·6	89	2·8	90	1·2	96	3·1	92	3·6
Proline	6·1	82	13	53	2·1	94	6·7	83	14
Glutamic acid	0·15	99	1·5	95	0·61	98	0·33	99	0·68
Citrulline	2·5	93	2·2	92	0·57	98	1·9	95	2·9
Glycine	19	44	24	15	12	63	26	34	44
Alanine	3·3	90	3·7	87	1·5	95	3·2	92	4·2
α-Am-n-butyric	—	(1)	—	(1)	—	(1)	—	(1)	—
α-Am-adipic	—	(1)	—	(1)	—	(1)	—	(1)	—
Valine	0·44	99	0·37	99	0·20	99	0·60	98	0·35
Half-cystine	3·6	89	8·8	68	—	(1)	—	(1)	—
Homocitrulline	—	(1)	—	(1)	—	(1)	—	(1)	—
Cystathionine	22	35	10	64	14	58	25	37	—
Methionine	2·3	93	3·2	88	2·6	92	5·8	85	2·7
Isoleucine	1·0	97	1·0	96	0·44	99	0·97	98	1·0
Leucine	0·44	99	0·72	97	0·46	99	1·3	97	0·86
Tyrosine	1·4	96	1·6	94	0·56	98	2·6	93	1·9
Phenylalanine	1·2	96	1·7	94	1·7	95	2·2	94	1·0
β-Alanine	—	—	—	—	—	—	—	—	—
βAIB	—	(1)	—	(1)	—	(1)	—	(1)	—
Hydroxylysine	—	(1)	—	(1)	—	(1)	—	(1)	—
γ-Am-n-butyric	—	—	—	—	—	—	—	—	—
Ornithine	0·85	97	1·0	96	—	—	0·86	98	0·87
1-Me-histidine	—	—	—	—	—	—	—	—	—
Histidine	17	49	19	31	7·1	78	12	69	29
3-Me-histidine	—	—	—	—	—	—	—	—	—
Arginine	—	(2)	—	(2)	—	(2)	—	(2)	0·91
Lysine	4·0	88	5·2	81	1·6	95	4·4	89	4·0

(1) = O-Tr in plasma but present in significant amounts in urine.

(2) = Detectable in plasma but not in urine except in traces.

lysine in the full-term infants, and there is a variable impairment of tubular reabsorption in the infant which is most marked in the premature. This pathway appears to be quite distinct from that for the acidic amino acids, although there may be some overlap with that for the neutral acids (Webber, 1962). The pattern appears to be shared by the two other basic amino acids citrulline and hydroxylysine.

The neutral hydroxy acids are the one group affected by pre-renal factors in that the high urinary losses are linked to high plasma levels. This is also the case for methionine, a neutral but structurally unrelated amino acid.

The imino acids and glycine have also been shown by Scriver, Schafer and Efron (1961) to have properties in common during tubular reabsorption. These three amino acids show significant tubular rejection as a group in infancy, and this is particularly demonstrated in the first full-term infant. Glycine, in addition, may compete with phosphorus for reabsorption (Evered, (1956) as well as being reabsorbed less effectively after a load of neutral amino acids (Doolan *et al.*, 1955, 1956; Webber, 1962).

The five amino acids hydroxylysine, homocitrulline, cystathionine, β-aminoisobutyric and

α-amino adipic have in common an unusually low renal threshold at all ages. Cusworth and Dent (1960) have pointed out that substances behaving in this way are not normally transported in the extracellular water in significant amounts.

The possibility that homocitrulline might be an artefact derived from lysine and cyanate within the bladder or on storage of the urine has been suggested (Gerritsen, Lipton, Strong and Waisman, 1961; Stark, Stein and Moore, 1960). However, incubation of a sterile urine sample at 37° C. for 96 hours showed no increase in homocitrulline or diminution in lysine, a finding that had been confirmed by T. Gerritsen (1962, personal communication) on sterile urine to which urea and lysine had been added.

1- and 3-methyl histidines were observed only in the adult urines except for small amounts in one of the full-term infants; these amino acids have not previously been reported in the urine under 5 months of age (Scriver, 1960). Taurine, which is not an amino acid is efficiently reabsorbed at all stages of maturity despite the higher plasma levels in the infants.

Summary

The plasma levels and urinary excretion rates of

REABSORPTION (TR) OF AMINO ACIDS

Term 1	Full Term 2		Full Term 3		Adult 1		Adult 2		Adult 3	
TR	C _{aa}	TR	C _{aa}	TR	C _{aa}	TR	C _{aa}	TR	C _{aa}	TR
95	0.12	99	1.2	98	6.8	93	28	72	7.0	94
(1)	—	(1)	—	(1)	—	—	—	—	—	—
(2)	9.4	82	16	73	14.2	86	9.0	87	3.5	97
92	3.8	93	5.9	90	0.91	99	0.73	99	0.38	99
85	6.6	87	9.6	84	3.1	97	2.4	98	1.0	99
96	3.1	94	3.6	94	0.95	99	0.93	99	0.43	99
86	2.7	95	1.7	97	(2)	(2)	(2)	(2)	(2)	(2)
99	3.0	94	2.2	96	0.28	99	0.09	99	0.04	99
96	—	—	—	(2)	(2)	(2)	(2)	(2)	(2)	(2)
45	21	59	22	64	5.5	94	4.3	96	1.8	98
95	2.4	95	2.9	95	0.55	99	0.72	99	0.25	99
(1)	—	(1)	—	(1)	2.3	98	1.4	99	0.82	99
(1)	—	(1)	—	(1)	(1)	(1)	(1)	(1)	(1)	(1)
99	0.50	99	0.32	99	0.18	99	0.15	99	0.02	99
(1)	—	(1)	—	96	0.93	99	0.74	99	0.27	99
(1)	—	(1)	—	(1)	(1)	(1)	(1)	(1)	(1)	(1)
(1)	—	(1)	—	(1)	(1)	(1)	(1)	(1)	(1)	(1)
97	4.3	91	2.9	95	1.7	98	1.0	99	1.9	99
99	1.7	97	1.3	98	0.40	99	0.51	99	0.14	99
99	1.2	98	0.78	99	0.31	99	0.34	99	0.13	99
98	1.9	96	1.7	97	1.8	98	1.5	99	1.9	98
99	1.9	96	—	—	1.0	99	1.0	99	1.0	99
(1)	—	(1)	—	(1)	—	(1)	—	(1)	—	(1)
(1)	—	(1)	—	(1)	—	(1)	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—
99	1.1	98	0.63	99	—	(2)	—	(2)	—	(2)
(1)	—	—	—	—	(1)	(1)	—	(1)	—	(1)
64	16	69	21	65	8.3	92	4.3	96	2.2	98
(1)	—	—	—	—	—	(1)	—	(1)	—	(1)
99	1.0	98	—	(2)	—	(2)	—	(2)	—	(2)
95	6.3	88	2.3	96	1.2	99	0.64	99	0.13	99

C_{aa} = renal amino acid clearance = UV/P_{aa} ml./min./1.73 m². (5).TR = per cent. renal tubular reabsorption of amino acids = 100 (1 - UV/P_{aa} · 1/C_{cr}).

32 amino acids and related compounds have been measured in four fasting premature infants and three full-term infants. The values for renal clearance and renal tubular reabsorption have been calculated for 21 of these and compared to the figures for three adults.

The main patterns of renal tubular conservation of amino acids have been discussed in relation to pre-renal factors, to known common reabsorptive pathways and to structural relations.

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