Renal Clearances of Amino Acids in Normal Adults and in Patients with Aminoaciduria

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The development of new methods of amino acid analysis during the past 15 years has resulted in a very large increase in our knowledge of the way in which these important constituents are handled by plant and animal tissues and organisms. Of these new methods, ion-exchange chromatography has many advantages when quantitative measurements are required, as it is capable of much greater precision than paper-chromatographic techniques and is more specific than microbiological methods. In addition, microbiological methods can only give results for the number of compounds for which assays are done; no information is obtained about the presence of, or changes in, unknown or unexpected amino acids, and this is a serious disadvantage, especially when pathological material is being examined. The main disadvantage of the ion-exchange method at present is that it is time-consuming and laborious. The advent of a fully automatic self-recording modification of the ion-exchange-column method (Spackman, Stein & Moore, 1958) should enable this objection to be overcome. The many discoveries which have been made with these new methods in the field of human amino acid excretion have been reviewed by Dent (1952*a*), Harris (1955) and Harrison & Harrison (1957), and will not be discussed in detail here.

The majority of measurements which have been made so far in man with the ion-exchange method have been based on 24 hr. urine collections (e.g. Stein, 1953) or isolated plasma samples (Stein & Moore, 1954). Evered (1956), however, has reported approximate renal amino acid clearances in normal humans and in patients with certain metabolic diseases which were calculated by comparing 24 hr. urine excretions with the concentrations of amino acids in a plasma sample taken on a different day. Sereni, McNamara, Shibuya, Kretchmer & Barnett (1955) calculated fasting renal clearances of individual amino acids in normal premature babies, infants, children and adults with estimates of plasma and urine amino acid concentrations obtained from paper chromatograms. Doolan, Harper, Hutchin & Shreeve (1955) and Doolan, Harper, Hutchin & Alpen (1956), using microbiological methods, have measured renal clearances of amino acids in normal human adults in acute experiments both in the fasting state and during an amino acid infusion. Arrow & Westall (1958), using techniques identical to those employed in the present study, have reported from this Department renal clearances in normal adults and in patients with cystinuria as well as in the heterozygous carriers of the disease.

The studies reported here were undertaken to confirm or otherwise previous theories of the mechanism of amino acid excretion (Dent, 1954) and to elucidate further the mode of amino acid excretion in certain metabolic diseases.

EXPERIMENTAL

Subjects studied. Physical data are given in Table 1, and further clinical details are in the Appendix.

Collection of samples. Subjects were fasted overnight and rested in bed for at least an hour before collections were commenced, until the end of the urine collection. Water was given to drink (about 250 ml. every 30 min.) from an hour before collections commenced and throughout the experimental period to maintain a reasonable urine flow and so minimize bladder-emptying errors. An accurately timed 3 hr. sample of spontaneously voided urine was collected; catheterization was not done. Blood samples of about equal volume were taken from an arm vein with a dry syringe at ½, 1½ and 2½ hr. after the start of the urine collection. Each was transferred to a heparinized tube, centrifuged immediately and plasma removed. Plasma and urine samples were stored at −10° until required for analysis. The collection was arranged to coincide with a time when blood was required for routine biochemical tests whenever possible, especially in the children. In the children also the number of venepunctures was reduced to two, at 1 and 2 hr.

Preparation of samples. Urine was applied to the analytical column without preliminary treatment. At the end of the collection period equal volumes of the two or three plasma specimens from the subject were mixed to give a single sample, which was then prepared by the method of Stein & Moore (1954) by deproteinizing with picric acid and removing the picric acid from the protein-free filtrate with a small column of Dowex 2 anion-exchange resin in chloride form.

Amino acid analysis. The method of Moore & Stein (1954*a*) was employed, a 0·9 cm. × 150 cm. column of Dowex 50X4 cation-exchange resin in the sodium form being used and gradient elution with sodium citrate buffers in the range pH 3·1–5·1. Fractions (2 ml.) were collected

Table 1. *Details of subjects studied*

Subject	Sex	Age (years)	Height (cm.)	Weight (kg.)	Surface area ($m.^2$)	Diagnosis
D.A.W.	M.	27	168	59·6	1·66	Normal
D.C.C.	M.	28	171	63·2	1·73	Normal
H.G.R.	F.	21	173	61·3	1·72	Normal
C.H.	F.	29	164	73·9	1·81	Normal
V.C.	F.	24	139	35·8	1·18	Osteomalacia with increased glycine excretion (Type I of Dent, 1952*b*)
E.G.	M.	37	167	56·8	1·54	
S.K.	M.	7	116	24·8	0·90	Renal-tubular rickets with glycosuria (Type II of Dent, 1952*b*)
L.R.	F.	46	147	47·0	1·35	Nutritional osteomalacia
M.P.	F.	40	147	44·3	1·35	Adult hypophosphatasia
E.Hu.	F.	46	148	60·3	1·58	Chronic renal failure with secondary hyperparathyroidism
M.Y.	F.	56	161	51·0	1·53	Adult Fanconi syndrome
J.J.	M.	7	91	13·2	0·61	Cystinosis
G.J.	F.	9	110	20·3	0·80	Cystinosis
E.Ha.	M.	17	153	46·0	1·39	Hartnup disease
K.R.	M.	7	118	21·0	0·88	Argininosuccinic aciduria

with a drop-counting collector and analysed by the photometric ninhydrin method of Moore & Stein (1954*b*).

Estimation of glomerular filtration rate. Creatinine concentrations of some samples of plasma and urine were estimated during the amino acid analysis by collecting 1 ml. fractions in the region in which creatinine emerges from the column and analysing alternate fractions with the ninhydrin reagent referred to above, and with alkaline picrate (Jaffe, 1886), which is much more sensitive to creatinine. It was possible to measure plasma-creatinine concentrations in this way in those subjects with renal damage with elevated plasma-creatinine concentrations, but the method was not sensitive enough to make accurate plasma measurements in those subjects with normal glomerular function. For these subjects, who had normal renal function as judged clinically and from biochemical evidence such as blood urea and urea clearances, an estimate of glomerular filtration rate was obtained from body-surface area, with reference values 127 ml./min./1·73 $m.^2$ and 118 ml./min./1·73 $m.^2$ respectively for males and females (Smith, 1951). For the subjects for whom a plasma-creatinine value was obtained, the endogenous creatinine clearance was calculated. Although it is fully appreciated that neither of these methods gives an accurate value for glomerular filtration rate, it was not felt that the more complicated procedure for inulin clearances was justified, as the values were required for approximate calculation and comparative purposes only. Moreover the presence of inulin in the samples might have interfered with the amino acid analysis. With these estimates, calculations were made of the load of amino acids filtered at the glomerulus and the fractions of this load excreted and reabsorbed. Owing to their approximate nature, they are not presented in detail but are discussed in the text. Body-surface area was estimated from height and weight with the chart given by Sendroy & Cecchini (1954).

RESULTS

Plasma concentration, urinary excretion and renal clearance of individual amino acids are given in Tables 2–4. Glutamine emerges as a mixed peak with asparagine and is also unstable on the column, recoveries being in the range 25–50% (Moore & Stein, 1954*a*), but a large deviation from values obtained in normal subjects presumably reflects a real difference in the sample. Tryptophan is also unstable on the column, but in none of the samples examined here has a definite peak been seen in this position, so that no values are reported for this amino acid. Throughout the analyses of urine samples, and to a much smaller extent in the plasma samples, small unidentified peaks were frequently encountered, but no attempt has been made to characterize them here. These probably represent some of the many unidentified amino acid-like compounds which are known to occur in human urine (Westall, 1955).

Normal subjects

The values reported here are in good agreement with previous work by ion-exchange chromatography (Evered, 1956) and microbiological assay (Doolan *et al.* 1955). Evered (1956) has compared the results obtained by ion-exchange analysis with those obtained by other methods and this will not be discussed further here. Approximate calculations of the filtered loads of the individual amino acids indicate that 99% or more of most of the amino acids are normally reabsorbed by the renal tubules, 95–98% of glycine is reabsorbed and 90–95% of histidine. The reabsorption of taurine appears to vary considerably, ranging between 88 and 99% in the four subjects studied here. Moderate amounts of 1- and 3-methylhistidines and of β-amino*iso*butyric acid may be excreted while the plasma concentrations of these compounds is very low. Although it is impossible to measure accurately such low plasma values by the present methods, the results suggest that relatively small proportions of these amino acids are reabsorbed by the renal tubules, leading to a high rate of renal clearance.

Table 2. *Plasma amino acid concentrations (mg./100 ml.)*

See text for additional values and details. In this and subsequent tables amino acid abbreviations are those of Brand & Edsall (1947) with the addition of: Tau, taurine; Gln, glutamine + asparagine; α-AB, α-amino-*n*-butyric acid; β-AB, β-amino*iso*butyric acid; 1mH, 1-methylhistidine; 3mH, 3-methylhistidine. A dash (—) indicates that a result was not obtained for technical reasons; a nought (0) indicates that a peak was not detected or was too small to be identified or measured with certainty; figures in parentheses indicate that the result is approximate.

	Normal adults				Severe Type I osteomalacia		Type II rickets	Nutritional osteomalacia
Subject ...	D.A.W.	D.C.C.	H.G.R.	C.H.	V.C.	E.G.	S.K.	L.R.
Tau	0·88	0·79	0·96	1·3	0·73	0·81	—	1·3
Asp	0·09	0	0	0	0	0	—	0
Thr	2·0	2·0	1·5	1·3	1·5	1·8	0·81	1·7
Ser	1·4	1·2	1·3	1·3	1·1	1·3	0·70	1·3
Gln	2·6	3·2	4·0	4·5	3·6	3·1	0·42	3·9
Pro	1·7	2·0	2·6	1·9	3·2	5·5	2·1	2·4
Glu	1·3	1·3	0·56	1·1	0·61	0·94	1·3	1·9
Gly	1·8	2·2	2·0	2·1	2·0	2·6	1·9	1·8
Ala	2·3	3·3	3·6	2·4	2·5	4·9	3·4	3·5
α-AB	0·29	0·22	0·18	0·24	0·24	0·27	0	0
Val	3·3	2·7	2·3	2·4	2·2	2·5	2·0	1·6
Cys	1·5	0·63	0·88	0·20	0·76	1·3	1·3	0·80
Met	—	—	0·27	—	0·42	0·30	0·22	0·21
iLeu	0·87	0·89	0·70	0·61	0·53	0·81	0·61	0·67
Leu	1·7	1·4	1·3	1·2	1·3	1·5	1·2	0·98
β-AB	0	0	—	0	(0·015)	0	(0·03)	0
Tyr	0·88	0·87	0·99	0·64	0·69	0·93	0·67	0·77
Phe	0·91	0·84	0·90	0·80	0·80	0·96	0·77	0·75
Orn	1·2	0·84	0·99	0·63	0·46	1·1	0·46	0·67
Lys	2·4	2·4	2·1	2·3	2·0	2·3	1·5	2·1
His	1·3	1·5	1·2	1·2	1·2	1·5	1·2	1·3
1mH	0·29	(0·09)	(0·05)	—	0	0·47	(0·1)	0·35
3mH	0	(0·06)	(0·05)	—	0	0	(0·1)	0·14
Arg	1·0	2·4	1·4	1·3	0·65	2·3	1·2	1·2

	Adult hypophosphatasia*	Hyperparathyroidism*	Adult Fanconi syndrome	Child Fanconi syndrome with cystinosis		Hartnup disease	Arginino-succinic aciduria*
Subject ...	M.P.	E.Hu.	M.Y.	J.J.	G.J.	E.Ha.	K.R.
Tau	1·1	0·97	0·77	0·78	2·3	0·72	—
Asp	0	0·17	—	0·26	0·12	0·12	—
Thr	0·58	0·92	1·4	0·91	1·2	0·84	1·7
Ser	1·6	0·92	0·98	0·84	0·87	1·1	1·5
Gln	2·5	1·8	4·5	2·5	2·0	1·7	4·1
Pro	4·9	3·1	5·3	3·1	3·2	3·7	2·7
Glu	0·77	1·0	2·3	1·0	1·1	1·2	1·1
Gly	1·5	1·3	2·8	1·5	1·4	1·4	3·6
Ala	3·9	3·6	3·7	3·0	3·0	2·5	2·8
α-AB	0·25	0	0·19	0·17	0·18	—	0·19
Val	3·0	1·6	2·0	1·7	1·9	2·2	1·7
Cys	1·5	2·6	1·1	1·4	1·4	1·0	0
Met	0·17	—	—	0·21	—	0·17	0·39
iLeu	0·84	—	—	0·62	—	0·82	0·66
Leu	1·5	1·0	1·2	1·0	1·3	1·6	1·3
β-AB	0	0·12	0	0·15	0·26	0	—
Tyr	1·1	—	1·0	0·78	0·60	0·66	2·2
Phe	0·98	—	1·0	0·90	0·96	0·97	1·0
Orn	0·82	0·90	0·70	0·47	0·48	0·79	0·44
Lys	2·1	1·5	1·9	1·6	1·6	3·0	1·9
His	1·3	1·0	1·3	0·74	0·93	1·5	1·4
1mH	0·39	1·0	0·46	0·34	1·2	0	—
3mH	(0·08)	0·40	0	0·18	0·29	0	—
Arg	2·0	0·98	1·7	1·1	1·0	1·7	0·61

* See text for additional values and details.

Table 3. *Amino acid excretions* (*μg./min./1·73 m.²*)

See Table 2 for explanation of abbreviations.

	Normal adults				Severe Type I osteomalacia		Type II rickets	Nutritional osteomalacia
Subject ...	D.A.W.	D.C.C.	H.G.R.	C.H.	V.C.	E.G.	S.K.	L.R.
Tau	90	51	130	22	140	120	27	115
Asp	2·2	—	0	0	8·1	—	6·3	0
Thr	17	19	22	10	18	19	15	62
Ser	27	38	37	24	10	49	29	96
Gln	18	58	24	30	6·8	25	16	86
Pro	0	0	0	0	0	0	0	1·5
Glu	4·6	4·2	3·4	8·0	11	11	6·0	13
Gly	49	91	116	89	340	260	180	300
Ala	10	30	26	6·9	21	42	28	64
α-AB	0	2·9	1·9	0	3·0	—	3·5	3·3
Val	4·4	8·9	1·4	0·9	2·6	3·0	3·2	7·8
Cys	10	4·5	10	5·7	6·2	—	10	0
Met	—	3·7	3·1	3·4	4·3	7·4	7·9	7·1
iLeu	1·8	8·0	3·9	6·1	1·1	6·5	3·1	5·3
Leu	4·0	3·5	12	3·4	5·5	4·3	1·9	3·4
β-AB	14	12	12	9·1	34	20	8·4	—
Tyr	15	15	10	8·6	8·6	14	14	24
Phe	6·5	8·1	12	11	12	10	18	9·4
Orn	0	0	0	0	0	0	0	0
Lys	45	5·2	28	11	15	7·3	12	56
His	120	115	79	58	120	120	150	255
1mH	51	32	41	26	36	38	59	180
3mH	—	44	27	18	35	45	17	32
Arg	8·0	7·5	4·3	2·2	5	13	0	3·7

	Adult hypophosphatasia*	Hyperparathyroidism*	Adult Fanconi syndrome	Child Fanconi syndrome with cystinosis		Hartnup disease	Arginino-succinic aciduria*
Subject ...	M.P.	E.Hu.	M.Y.	J.J.	G.J.	E.Ha.	K.R.
Tau	150	23	94	14	146	98	64
Asp	14	4·5	—	7·8	6·7	20	—
Thr	2·7	43	310	49	113	470	7·0
Ser	14	45	260	53	104	920	16
Gln	44	120	1030	91	210	1300	13
Pro	4·3	120	800	110	180	0	0
Glu	40	6·6	67	15	26	65	8·3
Gly	70	86	590	130	180	220	58
Ala	25	120	820	145	240	580	6·6
α-AB	3·3	2·0	30	3·2	7·2	19	—
Val	8·0	32	370	62	100	350	—
Cys	8·4	78	300	69	98	32	—
Met	9·7	3·2	24	2·8	—	38	—
iLeu	3·8	11	120	19	43	210	—
Leu	3·9	23	200	29	58	240	—
β-AB	15	7·4	—	28	47	—	—
Tyr	22	27	190	32	54	320	25
Phe	13	30	180	31	56	200	6·5
Orn	1·5	19	150	15	22	0	—
Lys	3·6	44	420	56	110	200	24
His	100	54	300	61	96	950	8·3
1mH	27	61	110	16	110	—	23
3mH	35	29	—	18	16	—	4·8
Arg	17	15	290	—	50	—	—

* See text for additional values and details.

Table 4. *Renal clearances of amino acids (ml./min./1·73 m.²)*

See Table 2 for explanation of abbreviations.

	Normal adults				Severe Type I osteomalacia		Type II rickets	Nutritional osteomalacia
Subject ...	D.A.W.	D.C.C.	H.G.R.	C.H.	V.C.	E.G.	S.K.	L.R.
GFR	127	127	118	118	118	127	127	118
Tau	10	6·4	14	1·7	19	15	—	9·2
Asp	2·4	—	—	—	—	—	—	—
Thr	0·9	1·0	1·5	0·8	1·2	1·1	1·8	3·6
Ser	2·0	3·1	3·0	1·9	1·0	3·7	4·2	7·4
Gln	0·7	1·8	0·6	0·7	0·2	0·8	3·8	2·2
Pro	0	0	0	0	0	0	0	0·06
Glu	0·4	0·3	0·6	0·7	1·8	1·2	0·5	0·7
Gly	2·7	4·1	5·8	4·3	17	10	9·8	16
Ala	0·4	0·9	0·7	0·3	0·8	0·8	0·8	1·8
α-AB	0	1·3	1·1	0	1·1	—	—	—
Val	0·1	0·3	0·06	0·04	0·1	0·1	0·2	0·5
Cys	0·7	0·7	1·2	2·9	0·8	—	0·7	0
Met	—	—	1·1	—	1·0	2·5	3·5	3·4
iLeu	0·2	0·9	0·6	1·0	0·2	0·8	0·5	0·8
Leu	0·2	0·2	0·9	0·3	0·4	0·3	0·2	0·3
β-AB	—	—	—	—	—	—	—	—
Tyr	1·7	1·7	1·0	1·4	1·2	1·5	2·1	3·2
Phe	0·7	1·0	1·3	1·4	1·5	1·1	2·4	1·3
Orn	0	0	0	0	0	—	0	0
Lys	1·9	0·2	1·3	0·5	0·7	0·3	0·8	2·7
His	9·7	7·9	6·6	4·7	9·9	7·9	13	19
1mH	17	—	—	—	—	8·1	—	50
3mH	—	—	—	—	—	—	—	23
Arg	0·8	0·3	0·3	0·2	0·7	0·6	0	0·3

	Adult hypophosphatasia*	Hyperparathyroidism*	Adult Fanconi syndrome	Child Fanconi syndrome with cystinosis		Hartnup disease	Arginino-succinic aciduria*
Subject ...	M.P.	E.Hu.	M.Y.	J.J.	G.J.	E.Ha.	K.R.
GFR	118	7·3	29	23	15	127	127
Tau	14	2·4	12	1·8	6·5	13	—
Asp	—	2·7	—	2·9	5·5	17	—
Thr	0·5	4·7	22	5·4	9·6	56	0·4
Ser	0·9	4·9	27	6·4	12	85	1·1
Gln	1·8	6·7	23	3·6	11	74	0·3
Pro	0·09	3·9	15	3·5	5·6	0	0
Glu	4·0	0·8	2·9	1·5	2·4	5·4	0·7
Gly	4·6	6·8	21	8·5	12	16	1·6
Ala	0·7	3·3	22	4·8	8·0	23	0·2
α-AB	1·3	—	16	2·0	4·1	—	—
Val	0·3	2·0	19	3·7	5·4	16	—
Cys	0·6	3·0	27	4·8	7·2	3·0	—
Met	6·0	—	—	1·4	—	22	—
iLeu	0·5	—	—	3·0	—	26	—
Leu	0·3	2·2	16	2·9	4·5	15	—
β-AB	—	6·4	—	18	18	—	—
Tyr	2·0	—	19	4·1	9·0	49	1·1
Phe	1·3	—	18	3·4	5·8	21	0·6
Orn	0·2	2·2	22	3·1	4·6	0	—
Lys	0·2	2·9	22	3·5	7·4	6·8	1·3
His	7·8	5·4	22	8·3	10	66	0·6
1mH	7·0	6·1	23	4·4	8·9	—	—
3mH	—	7·1	—	9·8	5·5	—	—
Arg	0·9	1·6	17	—	4·9	—	—

* See text for additional values and details.

Severe osteomalacia with increased glycine excretion

The urine results fully confirm previous findings of increased glycine excretion in these cases (Dent & Harris, 1956; Evered, 1956), and the plasma concentrations and clearance values suggest that this increased excretion is of renal origin. The plasma glycine of patient V.C. was within normal limits and the renal clearance was considerably elevated above the normal range. Patient E.G. also showed an elevated glycine clearance but the plasma glycine was higher than that of any of the normal subjects studied here and above the normal range found by others with the same method (Evered, 1956; Stein & Moore, 1954). The tubular reabsorption of glycine by V.C. and E.G. was estimated to be 90 and 92% respectively, both somewhat lower than the range of 95–98% found in the normal subjects. These results strongly suggest a renal origin of the increased glycine excretion, but owing to the raised plasma glycine found in E.G. further analyses are needed to confirm this view.

Renal-tubular rickets with glycosuria

Although the presence of renal glycosuria in addition to renal phosphaturia in this patient might suggest that the tubular lesion was more extensive than in the adult patients with osteomalacia discussed above, the amino acid results show that the only notable departure from normal was a slight increase of glycine excretion and clearance with a normal plasma level. These changes are not quite so marked as in the two adults, but the reabsorption of glycine was estimated to be 92%, lower than in the normal subjects. The excretion and clearance of histidine were higher than in the normals but this may be a dietary effect. Apart from this only minor changes were seen.

Nutritional osteomalacia

Jonxis & Huisman (1953) have reported an increased excretion of amino acids by children suffering from classical rickets. Rickets in children due to simple vitamin D deficiency is now extremely rare in this country, but adults are occasionally seen who develop minimal signs and symptoms of osteomalacia due to unusual dietary habits (Dent, 1957). In the case studied here all the plasma amino acids were in the normal range but there was a two- to three-fold increase in the excretion of threonine, serine, glycine, alanine and histidine; glutamine excretion was probably similarly increased. There were small increases in the excretions of lysine and tyrosine and a large excretion of 1-methylhistidine. There was a very small excretion of proline, which is not detected at all in normal urine, and no peak was seen for cystine, indicating that the excretion of this amino acid was very much reduced. The increased clearance values mirror exactly the increases in excretion, and the estimated tubular reabsorptions of the affected amino acids were lower than normal, including glycine 86%, histidine 84%, serine 94% and threonine 97%. The urinary findings in this adult are very similar to those reported for rachitic children by Jonxis & Huisman (1953), and the other measurements show that this aminoaciduria is also of renal origin.

Adult hypophosphatasia

The particular point of interest in studying this patient was to obtain more information about the excretion of phosphoethanolamine, originally reported in children with the disease (McCance, Morrison & Dent, 1955; Fraser, Yendt & Christie, 1955) and subsequently confirmed in child and adult cases (Cusworth & Dent, 1956; Cusworth, 1958). The estimation of phosphoethanolamine was technically difficult, especially in plasma as the separation obtained between phosphoethanolamine and taurine on the column was not as good as that reported by Moore & Stein (1954*a*). A reasonably satisfactory analysis was obtained on urine giving an excretion rate of phosphoethanolamine of 140 μg./min./1·73 m.^{2}, which corresponds to a 24 hr. excretion of about 200 mg. A very small peak was obtained in plasma and, owing to the uncertainty of its measurement due to the inadequate resolution and low colour yield with the ninhydrin reagent, the fractions containing the peak were combined and their total phosphorus content was estimated by the method of Berenblum & Chain (1938) after digestion with potassium sulphate and sulphuric acid. This procedure was carried out for two separate column analyses of the same plasma sample but the agreement of results was poor, the ninhydrin measurements giving 0·32 and 0·49 mg./100 ml. and the phosphorus estimations 0·28 and 0·17 mg./100 ml. as phosphoethanolamine. An accurate clearance cannot be calculated from such results, but they indicate that the clearance is high, probably greater than 30 ml./min. Apart from the phosphoethanolamine and a high taurine excretion, the excretion rates of most of the amino acids were within normal limits. Threonine and serine were lower than normal and there was a small excretion of proline. In the plasma, the threonine concentration was low, possibly accounting for the low excretion although the serine concentration was normal. The plasma-proline concentration was raised. The concentrations of other amino acids in plasma were normal and the renal clearances were normal. These results do not confirm a previous suggestion, based only on paper

chromatography, that the excretion of the common amino acids in this disease was a little lower than normal (Cusworth & Dent, 1956).

Chronic renal failure with secondary hyperparathyroidism and aminoaciduria

The plasma concentrations of several of the amino acids were lower than normal, possibly as a result of their continual heavy loss in the urine. The excretion rates of most of the amino acids were raised two to ten times above normal. The most striking relative increases were in proline and ornithine, but in absolute amounts glutamine/asparagine, alanine, proline and glycine were excreted in greatest quantity. The renal clearances of most of the amino acids were increased several times above normal, and when they are compared with the estimated glomerular filtration rate of less than 10 ml./min. they are relatively very high indeed, and show the renal origin of the increased amino acid excretion. Approximate calculations showed that although the amino acid-filtered loads were greatly below normal owing to the low glomerular filtration rate, the fractions reabsorbed were much reduced, ranging from 60 to 80% for valine, leucine, ornithine, lysine and arginine to 7% for glycine. There was a gross reduction of tubular reabsorption of practically all the amino acids, so that in spite of the very low filtered loads, considerable amounts of amino acids were excreted. The acquired tubular defect in this case is similar qualitatively to the inborn defect found in cases of adult Fanconi syndrome discussed below.

Adult Fanconi syndrome

The plasma concentrations of most of the amino acids were normal, but glycine was slightly raised and proline markedly raised above normal. The excretion of nearly all the amino acids was grossly raised, ranging from a tenfold increase of threonine, serine and glycine to a 100-fold increase of valine. The indicated increase in glutamine/asparagine was even greater but this value is approximate owing to decomposition of glutamine on the column. Earlier paper-chromatographic studies (Dent, 1947) suggest that this increase is largely due to glutamine. There were large excretions of proline and ornithine, neither of which is detected in normal urine. In absolute terms, glutamine/asparagine, alanine, proline and glycine were excreted in greatest amount. The renal clearances of all the amino acids were considerably raised and should be compared with the estimated glomerular filtration rate of about 30 ml./min. The clearances of serine and cystine approached the filtration rate. Calculation of tubule functions indicated that, in spite of the considerably reduced filtered loads, only taurine was reabsorbed by more than 50%, whereas the reabsorption of serine and cystine was very low at 8%. These results confirm the renal origin of the aminoaciduria in the adult Fanconi syndrome and show the gross and generalized nature of the defect.

Cystinosis

The plasma concentrations of most of the amino acids were slightly lower than normal in both children. The excretion of most of the amino acids was moderately raised, serine and glycine at two to three times normal showing a lesser increase than alanine, valine and cystine, which were five to ten times normal. The increases of proline and ornithine excretion were relatively large but not so marked as in the adult with Fanconi syndrome described above. In absolute amounts alanine, glycine, proline and glutamine/asparagine were excreted in greatest quantity. Although the amino acid-excretion pattern was very similar in both children to that of the adult patient, and to children in earlier stages of the disease (Dent & Harris, 1956), the increase was not so marked. The renal clearances of the amino acids in the two children were raised above normal but not to the same extent as in the adult patient, even allowing for their lower filtration rates. Estimates of tubule function showed that the filtered loads of all the amino acids were very low and that the tubules reabsorbed a relatively higher proportion of the load compared with the adult patient, although the absolute reabsorption of the individual amino acids (corrected for surface area) was of the same order as in the adult. These results confirm the suggestion of Dent & Fowler (quoted by Dent, 1954), based on total α-amino-nitrogen measurements, that the aminoaciduria in cystinosis is of renal origin. This is contrary to the claim of Bickel & Smellie (1952) and of Woolf (1951) that this aminoaciduria is due to an elevation of the plasma amino acids and is in accordance with the more guarded later report of Bickel (1955). Our results also indicate that in the later stages of the disease glomerular function declines more rapidly than tubular amino acid reabsorption. Filtered loads decrease whereas reabsorption probably undergoes little change, so that the total amount of amino acids excreted decreases. This decrease in amino acid excretion as uraemia increases, in later stages of the disease, has frequently been noted on paper chromatograms.

Hartnup disease

In the plasma, threonine and serine concentrations were less than normal and the glutamine/asparagine peak was smaller than seen in normal subjects. The low values may have resulted from the high urinary losses. The concentrations of

proline and lysine were slightly raised. The excretion of most of the amino acids was grossly raised but glycine was increased only twofold, cystine threefold and proline and ornithine were not detectable. Arginine excretion was probably raised only to a small extent. The largest peak in the urine analysis was that due to glutamine plus asparagine; paper chromatograms have shown that a large amount of asparagine is present in the urine in addition to glutamine. Histidine and serine excretions were also very high. The clearance values reflect these measurements and give very high values for serine, glutamine/asparagine, histidine and threonine. On the other hand the clearances of glycine and cystine were only two to three times normal, whereas those of proline and ornithine were normal. Tryptophan was not detected in the plasma; in the urine a small diffuse peak was seen in the tryptophan/arginine region, which it was not possible to identify by inspection. Indole chromatograms (Jepson, 1955) have shown that the excretion of tryptophan is increased in this disease and it is unfortunate that the instability of tryptophan under the conditions used here have prevented any measurements from being made. Estimates of tubule function underline the unusual nature of this gross renal aminoaciduria. Renal function as measured by all the usual tests is quite normal, but there is a gross defect of amino acid reabsorption of a highly specific nature. Although only 30–50 % of the filtered load of some amino acids is reabsorbed, the reabsorption of glycine, cystine, lysine and possibly arginine is affected to a very much smaller extent, whereas that of proline and ornithine is normal.

Argininosuccinic aciduria

These measurements were carried out before the amino acid-like substance excreted in large amounts by the two patients of Allan, Cusworth, Dent & Wilson (1958) had been identified as argininosuccinic acid (Westall, 1958). A preliminary column analysis was carried out on a random sample of urine and revealed a moderately large peak immediately after leucine, followed immediately by a second extremely large peak. Paper chromatography of alternate fractions from the region of these peaks showed that the first peak to emerge consisted almost entirely of a substance found in small amount on paper chromatograms of fresh urine from these cases and considered to be a decomposition product of the main spot (see fig. 3 of Allan *et al.* 1958). The second large peak consisted of roughly equal amounts of the main spot seen on paper chromatograms of whole urine from the affected children and another spot seen only in badly stored and somewhat decomposed urine samples. It is beyond the scope of this paper to discuss the detailed chemistry of these various degradation products, and to obtain an approximate figure for the amount of material being excreted the colour values of both peaks found in the column runs were added together. This was felt to be justified as paper chromatograms of fresh urine and plasma had shown the presence of traces only of the degradation products arising on the ion-exchange column. It was assumed by Allan *et al.* (1958), who gave approximate estimates of the quantity of the unknown 'amino acid' excreted, that the colour yield in the ninhydrin reaction was equivalent to that of leucine and that the molecular weight was 150. The molecular weight is now known to be 290. A similar picture of two unknown peaks was found in the timed urine sample, in the plasma and in a sample of cerebrospinal fluid. The results from these analyses were also treated as indicated above. In this paper we are calculating the excretions on the basis of the now-known molecular weight of the compound but have still to assume as a first approximation that the ninhydrin colour yield is the same as that with leucine. A small correction may be necessary later when pure samples of argininosuccinic acid are available. The results for the argininosuccinic acid calculated in this manner were: cerebrospinal fluid 10·0 mg./100 ml., plasma 3·3 mg./100 ml., excretion 3·5 mg./min./1·73 m.^{2}, clearance 105 ml./min./1·73 m.^{2}. Westall (1958) has now explained the phenomenon of double peaks on the elution curve and of the triple spots on the chromatograms as being due to the formation of two cyclic anhydrides from the argininosuccinic acid. It is a fair assumption that their ninhydrin values are similar to that of argininosuccinic acid and that our technique of quantitation is therefore probably correct. We emphasize that any errors introduced as above cancel out in determining the renal clearance.

The plasma concentrations of the other amino acids are now published for the first time. Many are normal but glycine was raised, 3·6 mg./100 ml., the highest value encountered in this series. Tyrosine was also elevated, being twice as large as that in any of the other subjects studied here. On the other hand, valine, lysine, arginine and ornithine were all slightly lower than normal and no peak at all was seen for cystine. The urine results confirm the paper-chromatographic observations of Allan *et al.* (1958) in that threonine, serine and possibly glutamine excretions were less than normal. The glycine excretion was at the low end of the normal range in spite of the elevated plasma concentration. Tyrosine excretion was slightly raised but histidine was very low indeed, only one-tenth of that seen in normal subjects. Excretion values for many of the neutral amino acids in the middle

of the chromatographic run were not obtained owing to the technical difficulties imposed by the presence of the very large abnormal peaks. All of the renal clearances for which values were obtained (except the argininosuccinic acid) were very low, notably glycine, serine and threonine; the histidine clearance was very low. These minor changes of other amino acids are difficult to explain but hardly alter the outstanding fact that the only gross abnormality concerns argininosuccinic acid.

DISCUSSION

The results confirm the renal nature of most of the conditions of aminoaciduria studied by our clearance techniques. The quantitative figures illustrate in addition the highly specific nature of some of the excretion patterns.

The abnormal amino acid excretion in argininosuccinic aciduria and in hypophosphatasia is of the 'overflow' type, but differs from that which occurs in phenylketonuria, liver disease and the recently described 'maple-syrup urine' disease (Westall, Dancis & Miller, 1957). In the last-named diseases there is an increased plasma concentration, and hence filtered load, of amino acids so that increased amounts are able to leak past the renal tubules into the urine, even though the tubules reabsorb a large part of the increased load. However, argininosuccinic acid, and probably phosphoethanolamine also, does not appear to be normally reabsorbed by the renal tubules. There is no renal threshold for them so that like inulin they are excreted in the urine whenever present in the plasma, even at low concentration.

The clearance of argininosuccinic acid is approximately equal to the filtration rate, indicating that little or no tubular reabsorption occurs. The calculated clearance of phosphoethanolamine in the patient with hypophosphatasia was not as high as the filtration rate, but owing to the low plasma concentration and the difficulty of its measurement, the true clearance may be somewhat higher than the value obtained here. If the excretion of phosphoethanolamine in hypophosphatasia were of renal origin it should be present in normal and hypophosphatasia plasmas at about the same concentration; it is not detected in normal urine, so if present in normal plasma it must normally have a very low clearance. This seems unlikely as intravenously administered phosphoethanolamine is very rapidly excreted by normal individuals (McCance, Fairweather, Barrett & Morrison, 1956), indicating that it has in fact a high clearance. The available evidence therefore suggests that phosphoethanolamine undergoes little or no tubular reabsorption in the normal state or in hypophosphatasia.

The present studies and those of Evered (1956) suggest that β-amino*iso*butyric acid may also be excreted in this manner. The excretion of large amounts of this amino acid by normal individuals (Crumpler, Dent, Harris & Westall, 1951) was initially considered to be due to defective renal-tubular reabsorption of the compound. Evered (1956) obtained an approximate clearance of β-amino*iso*butyric acid in one of these individuals which approached the filtration rate, but found that the plasma concentration in several subjects who excreted only small amounts of the amino acid was too small to be measured. This latter observation is confirmed in the present series of analyses; in the majority of subjects with normal renal function no peak was obtained in the β-amino*iso*butyric acid region of the plasma analysis. Consideration of the β-amino*iso*butyric acid excretion of these subjects shows that the clearance must have been high to hold the plasma concentration below the limit of detection. On the other hand, in three subjects (E.Hu., J.J. and G.J.) with severe renal damage small but measurable peaks were present in the plasma as if the β-amino*iso*butyric acid had been dammed back in a similar manner to creatinine and urea, and in each case the clearance of the amino acid was similar to the creatinine clearance. Feeding experiments have shown that β-amino*iso*butyric acid is rapidly excreted in the urine when ingested by normal subjects (Evered & Westall, quoted by Dent & Senior, 1955), again suggesting a high rate of renal clearance.

In addition to these examples, a similar mechanism appears to operate in the excretion of homogentisic acid (Neuberger, Rimington & Wilson, 1947) and porphobilinogen (Goldberg & Rimington, 1954). Many of these substances have something in common in that they appear to be normally exclusively intracellular, that is they are not transported about the body in the plasma, each cell being responsible for its own supply of the compound. Many substances such as glucose and most amino acids are transported in the plasma and if they were continually excreted in large amounts, owing to a low renal threshold, it would represent a serious loss to the body's metabolic economy. On the other hand a low or non-existent renal threshold is of no consequence with substances normally confined to the cell interior, and the arrangement only becomes conspicuous and possibly disadvantageous when for some reason they enter the plasma. If the unusual excretion of substances such as argininosuccinic acid and phosphoethanolamine is not due to a reduction of tubular reabsorption it is necessary to look for an extrarenal cause for the increased plasma concentration. β-Amino*iso*butyric acid is normally present in plasma at very low concentration; the increased

excretion shown by certain individuals is thus presumably also due to an increased plasma concentration. A possible cause is a metabolic block in which, due to an enzymic defect, the concentration of the non-metabolized substance increases in the cell and so leaks into the plasma. This may be so in hypophosphatasia, where there is a demonstrable deficiency of an enzyme or enzymes which can hydrolyse phosphoethanolamine. Another possibility is an alteration of the permeability of the cell membrane so that the substance enters the plasma even though the intracellular concentration is not increased. These two mechanisms are analogous to overflow and renal aminoaciduria, but operating at the level of the cell in question, which is not necessarily in the kidney.

Another point which arises from these studies is the not infrequent association of renal aminoaciduria and impaired vitamin D and phosphate metabolism. This has also been noted by Harrison & Harrison (1957). Examples studied here include renal-tubular oesteomalacia, simple vitamin D deficiency, chronic renal failure with secondary hyperparathyroidism and the Fanconi syndrome. A similar association occurs in the syndrome of Lowe, Terrey & MacLachlan (1952), and further evidence has been obtained from an extended study of a patient with this syndrome in which there was a marked reduction of amino acid excretion during treatment with vitamin D (J. P. Bound, D. C. Cusworth, C. E. Dent and J. M. Smellie, in preparation). A possible common factor in this group of diseases is the apparent absence of vitamin D action, due either to simple deficiency or to its failure to act when present in normal amounts. Another possibility is that parathyroid-gland hormone is involved, but in any case the mechanism does not seem to be a simple one such as a common reabsorption pathway for phosphate and amino acids. Further study of the renal handling of amino acids in various vitamin D states is needed before this association can be interpreted.

SUMMARY

1. Ion-exchange chromatography has been used to estimate the free amino acid content of timed urine and simultaneous plasma samples collected under fasting conditions from healthy adults and from patients with abnormal amino acid excretion.

2. Calculation of renal clearances confirms that 98% or more of the filtered load of most amino acids is normally reabsorbed by the renal tubules.

3. The increased glycine excretion by some cases of renal-tubule osteomalacia and the aminoaciduria of adult vitamin D deficiency are of renal origin. The depression of tubular amino acid reabsorption in these cases is small and limited to one or a few amino acids.

4. In the renal aminoaciduria of Hartnup disease, adult Fanconi syndrome, cystinosis and in a patient with acquired renal damage with a tubular lesion similar to that in the Fanconi syndrome, the reabsorption of many amino acids was very much reduced, being as low as 10% of the filtered load in some cases.

5. A form of overflow aminoaciduria is described which involves amino acids not normally detectable in plasma, and for which no renal reabsorptive mechanism exists. Argininosuccinic acid is excreted in this manner and the excretion of phosphoethanolamine in hypophosphatasia probably has a similar origin. β-Amino*iso*butyric acid, normally present in plasma in very low concentration, is also possibly excreted in this way.

APPENDIX

Clinical details of subjects studied

Normal adults. These were healthy laboratory workers with no history of renal disease.

Osteomalacia with increased glycine excretion. V.C. and E.G. were patients with Type I renal-tubular osteomalacia (Dent, 1952*b*) in which the tubular defect described is of diminished reabsorption of phosphate only. The increased glycine excretion in these patients, which might indicate a further tubular defect, was reported by Dent & Harris (1956) and their 24 hr. urine amino acid excretions were measured by Evered (1956). The increased glycine excretion has not been found in all Type I cases but only in those that present in adolescence or early adult life and develop severe bone disease. Both V.C. and E.G. previously had severe osteomalacia, which, however, responded well to treatment with large doses of vitamin D. E.G. had been off vitamin D for 1 month owing to a previous mild intoxication. The clinical investigations suggested a slight continuing vitamin D action at the time the clearance was done. V.C. at the time was responding fully to a large daily dose (5 mg.) of dihydrotachysterol given as AT 10.

Renal-tubular rickets with glycosuria. S.K., from the age of 18 months, had been noted to have rickets not cured by normal doses of vitamin D and only slowly responsive to very large doses. He otherwise had good general health with no evidence of renal or intestinal disease. On further study recently he was shown to have a mild renal glycosuria and high phosphate clearance (Type II of Dent, 1952*b*). There was no aminoaciduria detectable by paper chromatography. When the clearances were determined he had had no vitamin D treatment for some months previously.

Nutritional osteomalacia. L.R. had for many years chosen to live on an unusual diet from which milk, butter, cheese, eggs and fish had been rigidly excluded. Her vitamin D intake was estimated to be less than 20 units/day. She suffered from severe bone pains and although there was no radiological evidence of bone changes, biochemical findings were typical of osteomalacia with plasma calcium 8·8 mg., phosphorus 2·0 mg. and alkaline phosphatase 20 King-Armstrong (King & Armstrong, 1934)

units/100 ml. She responded well to treatment with dihydrotachysterol. The amino acid studies were carried out before treatment was commenced. Her clinical history was briefly summarized by Dent (1957).

Adult hypophosphatasia. The full clinical description of this patient, M.P., has been given by Bethune & Dent (1960). She had suffered from 'rickets' in childhood from which a slow recovery was made. She was well from 20 years of age until she was 34. During the next 6 years she suffered increasing pains in her feet, back and lately in both hips, so that she had become severely crippled. X-ray examination showed unhealing fractures in the middle of both femurs. All routine biochemical investigations were normal except for a constantly low plasma alkaline phosphatase level. Paper chromatography, however, showed a constant large excretion of phosphoethanolamine in the urine. The identity of the phosphoethanolamine has been confirmed by isolation and analysis from this patient's urine (Cusworth, 1958). No treatment was being given when the amino acid clearances were determined.

Chronic renal failure with secondary hyperparathyroidism. E.Hu. had long-standing renal damage which was first noted during her third pregnancy 17 years previously. During the last 2 years increasing bone pains and muscular weakness heralded the gradual development of secondary hyperparathyroidism. More recently the renal failure and radiological changes in her bones had become more severe. On admission to hospital blood urea was 168, plasma calcium 10·3 and plasma phosphorus 10·2 mg./100 ml., alkaline phosphatase was 23 King–Armstrong units, standard urea clearance was 11% of normal.

X-ray examination showed gross generalized arterial calcification as well as advanced hyperparathyroidism. Rather unexpectedly her urine paper chromatograms showed a definite aminoaciduria. Her amino acid clearances were determined soon after admission while no treatment was being given. She died 2 months later in uraemia. At post-mortem examination gross secondary enlargement of the four parathyroid glands was found as well as the other expected changes.

Adult Fanconi syndrome. The clinical history of this patient, M.Y., was described in full (case 1) by Milne, Stanbury & Thomson (1952). She had presented herself with typical osteomalacic symptoms developing 8 years previously with subsequent attacks of weakness due to 'low-potassium attacks'. She showed all the clinical and biochemical signs of the so-called 'adult Fanconi syndrome' (Dent & Harris, 1956) and had made an excellent clinical response to vitamin D and alkalis.

At the time the amino acid studies were carried out the patient had become seemingly 'resistant' to the continuing calciferol and alkali therapy and balance studies showed that she was in a virtually untreated state. She subsequently responded well to dihydrotachysterol and alkali. She has continued symptom-free to the time of writing (June 1959).

Cystinosis. J.J. and G.J. were brother and sister both showing the typical clinical and biochemical features of this disease. At the time of the amino acid studies both were in uraemia but with the rickets healed and in fair general health. They were each receiving 1·25 mg. of calciferol and 8 g. of sodium bicarbonate daily. Both children died 1 year and 6 months later respectively, with uraemia and hypertensive failure.

Hartnup disease. E.Ha. was case 1 of Baron, Dent, Harris, Hart & Jepson (1956). He had had the typical attacks with pellagra-like rash and cerebellar ataxia. In addition he showed always a gross aminoaciduria of unvarying pattern, an increased excretion of indole derivatives and other features which these authors have described. At the time of the clearance determinations he was in good general health.

Argininosuccinic aciduria. K.R. was case 2 of Allan *et al.* (1958). He was severely mentally retarded and was still manifesting the constant gross excretion of an amino acid-like compound that these authors described and which has now been identified by Westall (1958) as argininosuccinic acid. He was on a normal diet and having no treatment at the time of the clearance determinations.

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Studies on the Binding of ^{65}Zn by Equine Erythrocytes *in vitro*

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The administration of zinc to rabbits has been found to cause an increase in the zinc content of the erythrocytes unaccompanied by an increase in the content of carbonic anhydrase (Berfenstam, 1952). In humans also, the injection of ^{65}Zn was found to result in an uptake of about 10% of the injected element by the entire blood-cell mass in approximately 10 min. The specific activity of the erythrocytes continued to rise progressively until ultimately it exceeded by two- to three-fold that of the plasma (Gibson, 1953). Sheline, Chaikoff, Jones & Montgomery (1943) have shown that, in dogs, 10 hr. after the injection of ^{65}Zn all of the isotope is found in the erythrocyte and none in the plasma, even though initially the plasma had bound an appreciable amount of the isotope. The erythrocytes therefore have a marked capacity to take up ^{65}Zn even from a medium such as plasma, for instance, where many physiologically important metal-binding ligands are present. However, little is known about the process except that when the cells are incubated *in vitro* with ^{65}Zn they take up the isotope and fix it in a form that is stable to exhaustive washing with sodium chloride solution (Gibson, 1953; Tupper, Watts & Wormall, 1951, 1952), and that carbonic anhydrase is not involved in this binding (Tupper *et al.* 1952). In this paper, certain features of the binding of ^{65}Zn by washed erythrocytes *in vitro*, and the influence of other cations, metabolic inhibitors and some amino acids on this uptake, are presented.

EXPERIMENTAL

Erythrocyte suspension. Horse blood cells, freshly obtained, were used in all experiments. The cell suspension was prepared from citrated blood by separating the erythrocytes from the plasma and repeatedly washing the cells with large amounts of iso-osmotic NaCl soln. (0·9%) until free of citrate and plasma constituents. The suspension was then diluted with 0·9% NaCl so that the final suspension had a total solids content of 170 mg./ml. This suspension thus had approximately the same erythrocyte content as whole blood, since the latter contains 17 g. of haemoglobin/100 ml. The cells were invariably used with minimum delay after preparation of the above suspension. It was found that some differences existed between different samples of blood in zinc-binding capacity. Hence, in each set of experiments, the same sample of blood was employed.

Incubation procedure. Erythrocyte suspension (1 ml.) and 1 ml. of ^{65}Zn salt solution (preparation and activity given below) were incubated in a total volume of 4 ml. The rest of the volume was 0·9% NaCl when the erythrocytes were incubated with zinc alone, or of a suitable solution in 0·9% NaCl of any compound(s) whose influence on zinc uptake was to be tested. Under these conditions, the dilution of ^{65}Zn in the incubation mixture was such as to provide a final concentration of zinc of 0·25 mM.

When the effect of metal ions was studied the procedure was somewhat different. Erythrocyte suspension (1 ml.), 1 ml. of 4 mM (or where desired 40 mM) solution of the respective metal ion prepared in 0·9% NaCl and 1 ml. of 0·9% NaCl were first incubated for 2 hr. Thereafter, 1 ml. of ^{65}Zn salt solution was added and the incubation continued for another 2 hr. This procedure was adopted to eliminate, as far as possible, any differences in the final