above r values would have been close to zero.

The nature of the cellular acidity associated with K-deficiency remains unknown. But our findings that (i) diffusion of plasma CO_2 is not the major factor and (ii) that the buffer capacity of low-K skeletal muscle is significantly reduced are in harmony with the suggestion of Irvine *et al*(1) that H^+ may be less efficiently extruded during K-deficiency.

Summary. The plasma pCO_2 of control and dietary K-deficient rats was mechanically altered between 20 and 90 mm Hg. The object was to compare the intracellular pH and HCO_3 content of control and low-K skeletal muscle at identical pressures of plasma pCO_2. This made it possible to study the intracellular pH and HCO_3 changes associated with dietary K depletion without complications due to plasma pCO_2 changes. The data indicate that the intracellular acidity of low-K skeletal muscle from dietary depleted rats results mainly from the conditions imposed by the low-K regimen. The results also show that the *in vivo* buffer capacity of low-K skeletal muscle is significantly less ($P<.01$) than the buffer capacity of control skeletal muscle.

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Free Amino Acids in Serum, Cerebrospinal Fluid, and Urine in Renal Disease With and Without Uremia. (32562)

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The cause of uremia as the final stage of renal insufficiency is related to an increase of toxic substances of protein metabolism in the blood and to disturbances in the acid-base balance. In animal experiments, however, it is known that urea, uric acid, and creatinine do not produce symptoms of uremia. Amines and derivatives of phenol and indol have been suspected of being inducing factors of uremic coma(1,2,3,4,5). In this report, the concentration of free amino acids in the serum, cerebrospinal fluid (c.s.f.), and urine in renal diseases, with and without uremia, will be investigated. The relationship between the severity of renal insufficiency and the disturbances of amino acid metabolism have been examined by long-term observations and is reported here. In a previous paper, determinations of free and bound phenolic compounds, indican, and glucuronic acid have been reported(11).

Materials and methods. Two groups of patients with renal diseases and a control group of 50 healthy adults were investigated. The first group consisted of 51 renal patients with severe uremia, which was fatal in 47 cases. The non-protein nitrogen of these patients was between 155 and 412 mg %. The

distribution of the causes of renal insufficiency were: chronic glomerulonephritis (30), chronic pyelonephritis (13), subacute glomerulonephritis (2), malignant nephrosclerosis (2), renal amyloidosis (1), and acute renal failure (3). In the second group, consisting of 20 renal patients without uremia, the causes and distribution of the renal insufficiency were as follows: chronic glomerulonephritis (5), chronic pyelonephritis (3), diabetic nephropathy (3), acute renal failure (3), renal amyloidosis (2), nephrosclerosis (2), acute glomerulonephritis (1), and bilirenal syndrome (1).

The c.s.f. was taken by lumbar punctures. In the patients with renal disease, lumbar puncture was done either *in vivo*, as a therapeutical measure, or immediately post mortem. Serum and 24-hour-urine samples were investigated over periods of 2 to 7 days. In 12 renal patients 2 to 4 lumbar punctures were necessary in each case.

Comparative investigations were also made in the control group of 50 healthy adults.

The quantitative determination of free α-amino-nitrogen in the serum, c.s.f., and urine was made according to the method of Müting and Kaiser(6), which is a modification, for clinical purposes, of the ninhydrin method of Moore and Stein(7). This modification needs only 0.1 ml of serum, c.s.f., or urine. There is no interference by ammonia. Free amino acids were determined by quantitative paper chromatography according to the method of Fischer and Dörfel(8), for details see Müting(9).

Results. Analyses of free α-amino-nitrogen in body fluids from healthy adults and from patients with renal disease revealed the following in the c.s.f. (Table I).

(a) In the control group, the free α-amino-nitrogen of the c.s.f. averaged 0.76 ± 0.24 mg % (range, 0.40 to 1.15 mg %); (b) In 20 renal patients without uremia the free α-amino-nitrogen averaged 1.42 ± 0.24 mg % (range 0.90 to 1.45 mg %); (c) In 51 renal patients with uremia the free α-amino-nitrogen averaged 2.32 ± 0.49 mg % (range 1.52 to 3.40 mg %).

The differences in content of free α-amino-nitrogen in c.s.f. between the healthy adults on the one hand, and the 2 groups of renal patients on the other, are statistically significant ($p<0.001$). There is also a significant

TABLE I. Free Amino Acids in the Cerebrospinal Fluid of Renal Insufficiency Without and With Uremia. (Mean values in mg per cent.)

	A		B			C		
	Normal adults (50)*		Renal insufficiency without uremia (25)		Statistical significance, B compared with A	Renal insufficiency with uremia (51)		Statistical significance, C compared with B
	Mean	S.D.	Mean	S.D.	P	Mean	S.D.	P
Aspartic acid	.13	.03	.19	.07	<.0025	.28	.14	<.05
Glutamic acid	.27	.13	.34	.12	=.05	.47	.15	<.01
Lysine	.31	.15	.53	.14	<.0005	.51	.31	<.01
Arginine	.20	.06	.28	.14	=.0125	.36	.17	>.05
Histidine	.26	.08	.38	.09	<.0005	.53	.20	<.01
Tyrosine	.18	.06	.41	.16	<.0005	.66	.31	<.01
Tryptophane	.20	.06	.47	.22	=.05	.90	.53	<.01
Phenylalanine	.19	.06	.39	.13	<.0005	.74	.34	<.0025
Proline	.27	.08	.39	.14	<.0025	.59	.20	<.005
Cystine	.24	.07	.28	.13	>.15	.43	.21	<.025
Methionine	.13	.03	.19	.08	<.0125	.33	.13	<.0025
Taurine	.32	.12	.58	.22	<.0005	.85	.31	<.01
Leucine	.23	.05	.54	.20	<.0005	.79	.41	<.05
Isoleucine	.12	.03	.21	.08	<.0005	.33	.22	<.01
Valine	.26	.11	.53	.21	<.0025	.83	.31	<.01
Glycine	.39	.17	.90	.46	<.0005	1.99	.72	<.0005
α-Alanine	.34	.10	.79	.31	<.0005	1.43	.72	<.0025
Serine	.28	.11	.36	.18	>.05	.44	.17	>.05
Threonine	.18	.06	.45	.30	<.0005	.59	.27	>.05
Glutamine	.45	.18	1.16	.32	<.0005	1.89	.73	<.0025
α-Amino-N	.76	.24	1.42	.24	<.0005	2.32	.49	<.0005

* No. of patients.

TABLE II. Free Amino Acids in the Serum of Renal Insufficiency Without and With Uremia. (Mean values in mg per cent.)

	A Normal adults (50)*		B Renal insufficiency without uremia (25)		Statistical significance, B compared with A	C Renal insufficiency with uremia (51)		Statistical significance, C compared with B
	Mean	S.D.	Mean	S.D.	P	Mean	S.D.	P
Aspartic acid	1.01	.31	1.04	.34	>.35	1.34	.51	<.05
Glutamic acid	1.40	.30	1.11	.34	<.0005	1.51	.44	<.0025
Lysine	1.31	.28	1.79	.59	<.0005	2.84	1.40	<.0025
Arginine	1.06	.24	1.07	.27	>.45	1.49	.50	<.0025
Histidine	1.29	.31	1.18	.41	>.10	1.87	.83	<.0005
Tyrosine	1.22	.22	1.73	.56	<.0005	2.87	.93	<.0005
Tryptophane	1.30	.30	1.95	.95	<.0005	3.12	1.13	<.0005
Phenylalanine	1.00	.21	1.12	.45	>.05	1.93	.74	<.0005
Proline	1.41	.34	1.57	.49	>.05	2.30	1.03	<.005
Cystine	.58	.20	.45	.16	<.01	.91	.42	<.0005
Methionine	.34	.09	.44	.17	<.0025	.79	.48	<.0125
Taurine	1.53	.50	1.84	.44	<.01	2.54	.54	<.0005
Leucine	1.23	.23	1.93	.77	<.0005	3.06	1.00	<.0005
Isoleucine	.66	.15	.90	.44	<.0025	1.34	.48	<.005
Valine	2.28	.48	2.30	.53	>.45	2.98	.81	<.0025
Glycine	2.30	.50	3.03	.80	<.0005	4.33	1.45	<.0025
α-Alanine	2.72	.65	3.15	.54	<.01	4.72	1.74	<.0005
Serine	1.45	.29	1.41	.31	>.45	1.71	.44	<.0005
Threonine	1.08	.23	1.23	.60	>.05	1.89	.56	<.0005
Glutamine	2.26	.47	3.01	.80	<.0005	4.32	1.35	<.0005
α-Amino-N	4.06	.18	4.25	.37	<.005	6.50	1.33	<.0005

* No. of patients.

difference between renal insufficiency with and without uremia.

The increase of free α-amino-nitrogen in the serum is not so great (Table II). The control group had an average of 4.06 ± 0.18 mg % (range 3.7 to 4.3 mg %), in the renal patients without uremia 4.25 ± 0.37 mg % (range 3.8 to 4.9 mg %), and the renal patients with uremia 6.50 ± 1.33 mg % (range 4.7 to 8.5 mg %).

Evident in renal insufficiency without uremia is a significant increase of lysine, tyrosine, tryptophane, glycine, leucine, and glutamine in both the c.s.f. and the serum as compared to the control group. The significant increase of lysine, tyrosine, tryptophane, methionine, glycine, and glutamine in the serum is 2 to 3 times greater in uremia than in healthy adults; however the elevation of total free α-amino-nitrogen is only about 50% of the normal. In the c.s.f. of uremic patients the significant increase of glutamine, tyrosine, tryptophane, phenylalanine, glycine, and α-alanine is greater than that of the total free α-amino-nitrogen.

In the 24-hour samples of the control group, the average daily urinary output was 876 ± 244 ml, and contained 110 ± 24 mg of free α-amino-nitrogen (Table III). In renal insufficiency without uremia 1022 ± 371 ml urine and 151 ± 29 free α-amino-nitrogen were daily excreted, but in uremic patients only 439 ± 182 ml urine and 44 ± 23 mg free α-amino-nitrogen. In comparison with the control group, the differences for the free α-amino-nitrogen and most of the free amino acids are statistically significant.

There was a linear correlation between the increase of free amino acids in the serum and c.s.f. of patients with renal insufficiency. This correlation suggests, but does not prove, that the increase of free amino acids in the c.s.f. is secondary to that of the serum.

Discussion. In the course of renal insufficiency the urinary excretion is at first normal and later augmented. Therefore the amino-aciduria is generally increased. Even in this compensated state of renal insufficiency, the free α-amino-nitrogen in serum is 4.3 mg % (normal 4.1 mg %), and in c.s.f. 1.27 mg % (normal 0.76 mg %).

In uremic patients, however, urinary excre-

TABLE III. Free Amino Acids in the Urine of Renal Insufficiency Without and With Uremia. (Mean values in mg in 24 hr urinary excretion.)

	A Normal adults (50)*		B Renal insufficiency without uremia (25)		Statistical significance, B compared with A	C Renal insufficiency with uremia (51)		Statistical significance, C compared with B
	Mean	S.D.	Mean	S.D.	P	Mean	S.D.	P
Aspartic acid	11.6	3.9	16.2	4.0	<.0005	5.5	3.4	<.0025
Glutamic acid	23.3	7.9	24.9	8.2	>.20	9.6	6.7	<.025
Lysine	46.9	13.8	100.0	50.4	<.0005	24.5	14.9	<.025
Arginine	14.6	3.8	20.6	7.9	<.0005	6.2	3.7	<.01
Histidine	71.1	22.5	76.7	21.6	>.15	30.0	19.5	<.0125
Tyrosine	13.6	2.9	21.1	8.1	<.0005	7.0	5.5	<.025
Tryptophane	13.8	3.6	24.6	10.9	<.0005	10.1	6.2	<.05
Phenylalanine	10.7	3.2	15.8	7.5	<.0005	6.6	4.9	>.05
Proline	0	—	0	—	—	0	—	—
Cystine	49.1	12.6	68.3	33.3	<.0005	19.6	14.9	<.05
Methionine	20.5	7.4	26.3	9.6	<.005	8.0	6.7	<.025
Taurine	64.0	26.5	75.9	33.0	>.05	22.3	13.6	<.025
Leucine	12.3	2.9	20.4	8.7	<.0005	10.0	8.9	>.10
Isoleucine	6.9	2.0	11.7	4.4	<.0005	5.8	5.6	>.10
Valine	22.6	6.6	22.9	10.6	>.495	10.1	7.2	>.05
Glycine	207.1	49.3	197.1	56.0	>.100	37.7	24.4	<.0005
α-Alanine	46.2	12.9	62.0	19.5	<.0005	22.1	13.0	<.01
Serine	24.9	7.5	28.1	8.8	>.05	10.3	6.4	<.01
Threonine	15.8	4.6	25.0	8.8	<.0005	8.8	5.3	<.0125
Glutamine	48.3	13.4	58.3	21.2	<.01	21.6	13.7	<.025
α-Amino-N	110.0	23.8	151.0	28.7	<.0005	44.1	23.1	<.0005
24 hr urinary excretion	876	244	1022	371	<.025	439	182	<.025

* No. of patients.

tion is decreased to 439 ml per day, containing 44 mg free α-amino-nitrogen (normal 110 mg). In the serum of uremic patients 6.5 mg % and in c.s.f. 2.23 mg % free α-amino-nitrogen were contained. This means an increase of 50% free amino acids in the serum, but of 300% in the c.s.f. of uremic patients. Glycine, glutamine, and aromatic amino acids are augmented especially. As shown in other investigations(11), metabolites of aromatic amino acids such as indican, indol, scatol, and free phenols including phenol, p-cresol, p-hydroxyphenyllactic acid, and p-hydroxyphenylacetic acid are augmented in the serum and c.s.f. of patients with uremia. Their increase is 2-4 times greater than the increase of aromatic amino acids. The most sensitive indicator for a beginning renal insufficiency is the ratio between the indican content of the serum and that of the 24-hour urine sample(5). Therefore a diminished oxidative deamination of free amino acids is suggested in renal insufficiency. Distinct relationships are observed between the degree of renal insufficiency and the alterations in protein metabolism. In progressive chronic glomerulonephritis, there is a decrease of urinary excretion with a diminished excretion of free α-amino-nitrogen but an increase of free α-amino-nitrogen in the serum, and above all, in the c.s.f. With an improvement of the renal insufficiency, these alterations can be normalized. The early detection of these disturbances is therefore important for the prognosis of renal disease.

Two causes of the increase of free amino acids in c.s.f. of uremic patients are possible: 1. The increased free amino acids in the c.s.f. may be breakdown products from toxic cerebral metabolism. 2. The entry of free amino acids into the c.s.f. may be secondary to an abnormally decreased blood-c.s.f. barrier and/or to increased serum levels of free amino acids in uremia.

In this connection it should be emphasized that in the c.s.f. cell content, protein, NPN, urea, creatinine, potassium, sodium, chloride, and calcium are generally a fraction of the corresponding values in the serum. Glutamic

acid and glutamine are known as products of cerebral metabolism(10), but it can be suggested that in the case of uremia the greater part of free amino acids comes from the blood, because there is a parallel increase in the blood *and* c.s.f. Positive confirmation of this hypothesis may be obtained by injection of labelled amino acids in experimental uremia and detection of their increase in the c.s.f.

Summary. Free α-amino-nitrogen and free amino acids have been measured in the serum, urine, and cerebrospinal fluid (c.s.f.) of 51 patients with severe uremia and 20 patients with renal disease without uremia. The results have been compared with those obtained from a study of 50 patients without renal disease. Severe uremia was found to be associated with a highly significant increase in the levels of almost all amino acids in the cerebrospinal fluid. In particular aromatic amino acids and glutamine are elevated. This increase probably reflects increased blood levels of free amino acids and an increase in the permeability of the blood-brain barrier in uremia.

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Behavior on Transfer of Serum Stimulated Bone Marrow Colonies.* (32563)

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Sera from mice with spontaneous or viral induced leukemia stimulate certain mouse bone marrow cells to proliferate and form cell colonies in semi-solid agar cultures(1,2). Most normal mouse sera exhibit weak or no colony stimulating activity. Developing colonies are initially granulocytic but after 6 days become composed almost entirely of mononuclear cells(3). The present studies have examined the proliferative capacity of developing bone marrow colonies after transfer to new agar plates and the dependence of the colony cells on active serum for continued multiplication.

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Materials and methods. Mice. Three-month-old male DBA/1 mice were donors for bone marrow cells. *Sera.* Sera were obtained from Swiss ICR/Ha mice aged 5-8 months with advanced lymphoid leukemia, induced by neonatal infection with a leukemia-inducing virus whose isolation and properties have been described elsewhere(4,5). Normal Swiss sera were from mice of the same age. AKR sera were from 6-8-months-old mice with lymphoid leukemia. Sera from BALB/c mice with lymphoid leukemia induced by the Moloney leukemia virus were supplied by Dr. J. Moloney, National Cancer Institute, Bethesda. Normal human sera were obtained from the Institute blood bank and sera from patients of similar ages with mononucleosis were supplied by Dr. Britta Wahren, Karolinska Institutet, Stockholm.

Bone marrow culture technique. The technique for induction of bone marrow colony