processes of the early chick embryo to the pathogenic effects of the virus(9).

It is noteworthy that the virus strains which have been shown in this laboratory to produce anomalous development of early embryos following injection through the vitelline membrane were also capable of producing a lethal infection in older (9-10 day) embryos when introduced extra-embryonically. Newcastle disease virus also shares this property(5,6). On the other hand, distemper virus, which did not regularly produce death in older embryos, has not been shown to produce teratogenic effects in early embryos, under the experimental conditions so far employed. The results observed with distemper are quite similar to the data reported for mumps virus(2). The latter agent, although undergoing multiplication in the allantoic sac, did not kill 8-day-old embryos, nor did it prove teratogenic in 48-hour embryos, even though these younger embryos succumbed to the infection within 5 days. It is possible, however, that teratogenesis might also result from infection with these viruses following sufficient modification of such factors as the time selected for inoculation, or the volume and concentration of the inoculum.

Summary. Herpes simplex, vaccinia and influenza-A (NWS) viruses produced teratogenic and lethal effects in the early chick embryo. The primary teratogenic effects of all 3 viruses were micrencephaly and axial flexion, but minor characteristic differences could be detected in embryos infected with each of these agents. Distemper virus failed to produce any gross embryologic changes, although its injection led to the development of ulcerative lesions on the C-A membrane and death of the embryo.

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Free and Apparent Amino Acids in Deproteinized Plasma of Normal and Uremic (Nephrectomized) Dogs.* (22581)

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It would be of physiological and clinical importance to determine whether the disturbances of urea and protein metabolism, known to occur in uremia, are accompanied by abnormal levels of plasma amino acids. Although total amino nitrogen is not elevated consistently in the blood of uremic patients (1,2), it appears possible that individual amino acids may vary markedly. Levenson *et al.*(3) observed that plasma amino nitrogen was near normal in patients with severe battle wounds and renal failure although plasma urea concentrations were as much as 30 times normal. All plasma amino acids were not depressed or elevated at any time but the concentration of some individual

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amino acids rose during the first week. Schmidt *et al.*(4) reported that the concentration of arginine, phenylalanine and tyrosine in uremic plasma was more than double that in normal plasma although considered to be within normal limits. According to Stein and Moore(5), the concentration of glycine was elevated in cystinuria and of phenylalanine in phenylpyruvic oligophrenia although the levels of free amino acids in the plasma of patients suffering from a number of diseases were within the normal range.

Deficiency of even one "essential" amino acid in the blood of uremic individuals could lead to decreased protein synthesis and explain certain clinical features of the uremic syndrome (*e.g.*, poor wound healing, diminished antibody formation and decreased hemoglobin synthesis). On the other hand, excess of an amino acid might contribute to the toxicity of uremia. Plasma from normal dogs and from dogs rendered uremic by nephrectomy was employed in the studies reported here in order to minimize the variability characteristic of human uremic patients.

Choice of analytical method was an important consideration in the present study where the primary objective was to characterize uremic plasma in terms of its normal and any abnormal metabolites with particular emphasis upon free and combined amino acids. Although Redfield and Barron(6) have shown that amino acids may be determined satisfactorily in hydrolysates of a purified protein (bovine serum albumin) by paper chromatography this method, according to Evered(7), is subject to large errors with biological fluids. Stein and Moore(5) have reported that nearly all of the 28 ninhydrin-positive substances in filtrates from normal human plasma were determined by column chromatography using a sulfonated polystyrene resin (Dowex 50-X4) with a calculated yield (4.02 mg %) of α-amino nitrogen in close agreement with the value (4.1 mg %) which Hamilton and Van Slyke(8) found by direct ninhydrin-CO_2 analysis. That the concordance of these overall results may not reflect a similar degree of accuracy for individual amino acids is indicated by the $\pm$ 10% error in recovery of added amino acids and by the possibility that bound amino acids may have been partially hydrolyzed by ion-exchange catalysis(9,10). From their data obtained on acid hydrolysates of normal protein-free plasma Stein and Moore concluded that although major quantities of conjugated amino acids do not appear in plasma, "The possibility still remains that certain forms of conjugated amino acids of unknown nature might exist in plasma and be removed during the deproteinization procedure, either by being bound to the protein-picric acid precipitate or to the Dowex 2 column." Microbiological assay, rather than column chromatography, was selected for the authors' use because of its potentialities for the determination not only of free amino acids but also of conjugated amino acids and possibly other types of substances stimulatory or inhibitory to the growth of the assay organisms.

Experimental. Healthy mongrel dogs, fasted overnight, were exsanguinated from the carotid artery under sodium pentobarbital (25 mg/kg) anaesthesia. The blood was received in siliconed vessels each containing 20 mg of heparin per liter of blood. As soon as possible after exsanguination the heparinized blood was centrifuged, the plasma was pipetted and the residual red cell mass was discarded. The plasma was stored at —20°C, a temperature at which amino acids in plasma are stable(11). For preparation of uremic plasma healthy mongrel dogs were anaesthetized as described and bilateral nephrectomy was performed through a midline abdominal incision in one stage. The animals were returned to their cages and offered 20 ml per kilo of 15% glucose in water daily but no medication, or other fluid or food. Those animals which survived a minimum postoperative period of 72 hours and exhibited signs of uremic toxicity (*e.g.*, twitching vomiting, convulsions) were anaesthetized with the minimum dose of pentobarbital, exsanguinated and the blood processed as described. Amino acids were determined only in those plasma specimens with blood urea nitrogen of at least 200 mg %.

Deproteinization was effected by adding per ml of plasma 1.5 ml of approximately 4%

TABLE I. Concentration of Apparent Amino Acids in Deproteinized Plasma of Normal Dogs.*

Amino acid	Tungstic acid filtrate		Trichloroacetic acid filtrate
	Hier(17), Hier & Bergeim(11,18)	Authors§	Authors
Arginine	33†(23–48)‡	4.1 (1.5–9.9)	19 (16–22)
Aspartic acid		5.1 (1.8–9.2)	4.4
Glutamic "	<6	27 (3.0–65)	12
Glycine		14 (8.4–19)	25
Histidine	12 (9.3–16)	11 (7.2–15)	11
Isoleucine	13 (8.7–20)	14 (11–17)	15
Leucine	21 (14–31)	23 (16–30)	26
Lysine	24 (15–32)	17 (3.6–38)	40 (26–54)
Methionine	12 (8.0–16)	5.3 (1.9–13)	9.4 (7.1–12)
Phenylalanine	12 (7.0–18)	12 (9.9–14)	13
Proline		14 (10–19)	16
Serine		8.8 (2.8–14)	28
Threonine	26 (15–35)	29 (22–37)	31
Tryptophan	10 (7.5–13)		
Tyrosine	11 (4.5–20)		
Valine	22 (15–30)	25 (16–39)	32 (24–39)

* Expressed in μg/ml of plasma. † Avg value. ‡ Range of values. § Samples ranged from 2 pooled or 1 pooled plus 3 individual, to 3 pooled plus 5 individual plasma samples from 13 normal dogs.

sodium tungstate in 0.35 N H_2SO_4 or 1.0 ml of 10% trichloroacetic acid solution. Each mixture was allowed to stand for 30 minutes with occasional shaking, the suspension was filtered on a Buchner funnel, the residue was washed twice with a minimum volume of distilled water and the washings were added to the filtrate. The combined washings and filtrate was extracted with ether to remove excess trichloroacetic acid(12).

Some of the solutions were assayed for amino acids without further treatment. Others were evaporated to dryness under reduced pressure to residual material which was taken up in warm distilled water and, after filtration, was diluted with distilled water to a volume estimated to contain amino acids in a concentration most suitable for the microbiological assays. The assay procedures have been described(13,14).

Results. A factor of critical importance to the present study concerns the capacity or incapacity of deproteinizing agents to precipitate or adsorb proteins, peptides and amino acids from plasma. Hamilton and Van Slyke (8) found that picric and tungstic acids—but not zinc, cadmium or ferric hydroxides—yielded filtrates without loss of amino acids while Christensen and Lynch(15) reported that tungstic acid, picric acid and trichloroacetic acid gave filtrates containing increasing amounts of conjugated amino acids in the order named. On the basis of the latter results Henderson *et al.*(16) adopted tungstic acid as the deproteinizing agent most suitable for the determination of amino acids in plasma by microbiological assay. Stein and Moore (5) concluded that it was more satisfactory to remove proteins with picric acid than by equilibrium dialysis or ultrafiltration.

In the authors' experiments it was found (Tables I and II) that the values for apparent free amino acids were higher for glutamic acid, lower for arginine, glycine, lysine, and serine and about the same for the other amino acids in plasma filtrates deproteinized with tungstic acid compared to trichloroacetic acid. These differences cannot be explained at present.

The concentration of apparent free histidine, isoleucine, leucine, phenylalanine, threonine and valine (Table I) found in tungstic acid filtrates of normal dog plasma agrees (average difference about 8%) with that obtained by Hier(17) and Hier and Bergeim (11,18) under comparable conditions. That these values may represent approximately the concentration of the free amino acids is indicated by the low (average, 3.2%; range, 1.6-7.5%) average mean deviations from the

TABLE II. Concentration of Apparent Amino Acids in Deproteinized Plasma of Uremic (Nephrectomized) Dogs and Its Relationship to That Found for Normal Dogs.

Amino acid	Conc. in plasma*		Ratio of conc. (uremic/normal)†	
	Tungstic acid‡	Trichloro-acetic acid‡	Tungstic acid	Trichloro-acetic acid
Arginine	8.8§(5.7–15)‖	38	2.5§(1.2–3.6)‖ (.16–3.0)¶	2.0 (1.7–2.4) (1.0–2.0)
Aspartic acid	7.8(3.3–19)	7.0	2.7 (1.0–4.5) (1.3–3.0)	1.6 (2.0)
Glutamic "	16 (6.7–47)		.35(.34–.36) (2.8–3.4)	
Glycine	31 (16–50)	40	2.9 (2.2–3.8) (.36–1.3)	1.6 (.40)
Histidine	16 (15–18)	12	1.0 (1.0)	1.1 (.40)
Isoleucine	17 (16–18)	15	1.1 (.65)	1.0 (.55)
Leucine	31 (26–36)	28	1.0 (.60)	1.1 (.50)
Lysine	13 (5.0–28)	16	.99(.71–1.4) (.26–3.0)	.62 (.55)
Methionine	4.4(1.9–12)	5.7	.63(.45–.78) (.24–2.1)	.81 (1.0)
Phenylalanine	16 (14–18)	14	1.2 (.52)	1.0 (.50)
Proline	14 (11–17)	16	1.0 (.89–1.1) (.88–1.3)	1.0 (1.0)
Serine	10 (6.0–18)	43	1.1 (.91–1.3) (.65–.87)	1.5 (.74)
Threonine	11 (9.7–13)	7.9	.22 (.55)	.26 (.58, 1.9)**
Valine	26 (21–32)	19	.94 (.52)	.79 (1.0)

* Expressed in μg/ml of plasma.
† Only at approximately equal concentrations of plasma.
‡ Deproteinizing agent.
§ Avg value. Samples ranged from 2 pooled or 1 pooled plus 5 individual, to 3 pooled plus 6 individual plasma samples from 14 nephrectomized dogs.
‖ Range of values.
¶ Range of plasma concentrations over which comparison was made.
** Relative concentration of normal and uremic plasma samples, respectively. No data at equal concentrations available.

means observed at the different levels of samples. Similarly, the comparable average mean deviations for proline (not determined by Hier or Hier and Bergeim) and glutamic acid were 2.0 and 3.8%, respectively. The relatively low value (<6 γ per ml) for glutamic acid obtained by Hier and Bergeim(18) appears to be explained by conversion of glutamine to microbiologically inactive pyrrolidone carboxylic acid during autoclaving of the plasma filtrate(19). The authors' relatively high value (average, 27 γ per ml, range, 3.0-65 γ per ml) may be due to partial hydrolysis of glutamine (to glutamic acid) during evaporation to dryness of the acidic tungstic acid filtrate prior to autoclaving in the assay. According to Archibald(20) deproteinized plasma from fasting dogs contains 70-130 γ per ml of glutamine. Similarly, the predominance of asparagine rather than aspartic acid in plasma(5) probably accounts for the inability of Hier and Bergeim to find detectable amounts of aspartic acid while the present authors found appreciable quantities (average, 5.1 γ per ml, range, 1.8-9.2 γ per ml).

The authors believe that low mean deviations from the mean of assay values at different levels of test sample signify that significant quantities of microbiologically active compounds other than the test substance were

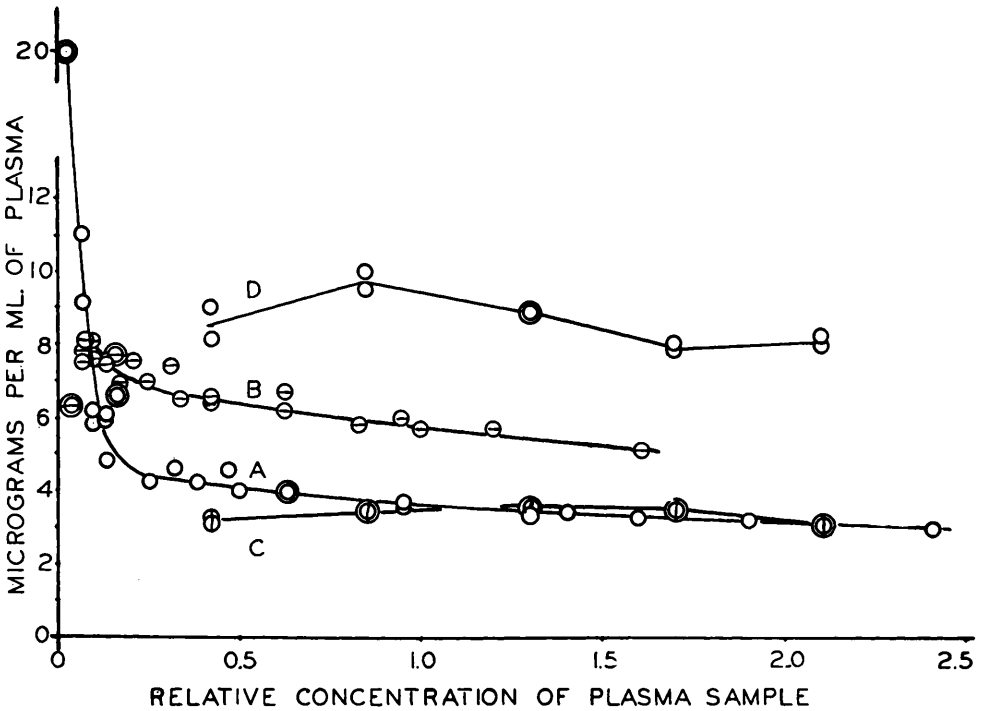

FIG. 1. Concentration of apparent arginine in deproteinized plasma of normal and uremic (nephrectomized) dogs. A—Normal plasma, unhydrolyzed; pooled sample from 3 dogs. B—Uremic plasma, unhydrolyzed; sample from 1 dog. C—Normal plasma, partially hydrolyzed; pooled sample from 3 dogs. D—Uremic plasma, partially hydrolyzed; pooled sample from 5 dogs.

not present. This criterion is believed to be valid, however, only for assays in which the plasma filtrate is assayed over at least 5-fold concentration of which the highest is equal to or greater than that of the original plasma. The discordant values (relatively high average mean deviations from the means) found at the different levels of samples for the other amino acids resulted, presumably, because of the presence in deproteinized plasma of conjugated amino acids and possibly other types of substances stimulatory or inhibitory to the growth of the assay organisms. The markedly different values found for apparent free arginine (Fig. 1) at varying concentrations of plasma sample provide a striking illustration of the point that assay data of this character have only relative significance and that only at the same concentrations of plasma sample.

The ratios (Table II) of the concentrations of apparent amino acids in the plasma filtrates from uremic (nephrectomized) compared to normal dogs were calculated from values obtained *only* at equal concentrations of normal and uremic plasmas. It is of interest, however, that the ratios (Tables I and II) calculated for all of the plasma samples are of the same order of magnitude. It is evident that the uremic to normal plasma ratios are significantly greater than 1 for arginine, aspartic acid and glycine and are significantly less than 1 for glutamic acid and threonine. Although the ratios for glutamic acid and aspartic acid may be fortuitous for reasons mentioned earlier in this paper, those for the other amino acids listed are believed to be valid. Supporting this view is the observation(21) that the activity of hippuric acid towards *L. mesenteroides* P-60 is not markedly different than that of glycine and the present authors' (unpublished) finding that these results cannot be attributed to creatine, creatinine or urea metabolites known to be present in relatively high concentration in uremic blood. Whether or not these different ratios of amino acids plus amino acid-like components are

characteristic of normal compared to uremic human plasma filtrates is now under investigation in the authors' laboratory. Whether or not such altered concentrations of amino acids and any other compounds in plasma filtrates which affect growth stimuli of microorganisms bear any relation to the physiological or clinical aspects of uremia remains to be determined.

The increased concentration of plasma arginine in uremic dogs could be explained by the accumulation of urea with concomitant slowing of the formation of urea and ornithine from arginine. The expectation that there would be an accompanying low concentration of ornithine in plasma is now being investigated. Although there is no obvious explanation for the accumulation of apparent glycine in uremic plasma it is known that excessive glycine produces toxic effects. The low concentration of threonine and, possibly, methionine in uremic dog plasma may be a factor in decreasing protein synthesis.

Summary. 1. Microbiological assay procedures were found to be applicable to the determination of free amino acids in deproteinized plasma but only in the absence of interfering substances. The apparent (relative) values obtained in the presence of interfering substances were shown to be meaningful but only when determined over a wide range of concentration of plasma filtrates. 2. It was concluded that the concentration of some free amino acids (histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine and valine) was not significantly different but that of some apparent amino acids was higher (arginine and glycine) and others (methionine and threonine) lower in the deproteinized plasma of uremic (nephrectomized) compared to normal dogs.

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