tion of water or electrolytes or both appears to increase the incidence and severity of renal lesions associated with jaundice produced by occlusion of the bile duct in rats. The renal lesions under these conditions may vary greatly in extent and severity but usually do not appear sufficient to produce either significant or fatal renal insufficiency. They usually consist of vacuolation of the tubules, especially proximal convoluted tubules, with a few casts and dilated tubules. Necrosis of tubules occasionally occurs. The renal lesions in this study infrequently closely resembled those of hemoglobinuric nephrosis. The casts, however, appeared to be mostly bile-stained casts. Rarely the lesion was extensive enough to cause severe renal damage.

The renal lesions seen in bile nephrosis then may be due in part to or may be superimposed on those due to severe electrolyte and water depletion or other causes of renal tubular degeneration.

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## Concentrations of Nineteen Amino Acids in Plasma and Urine of Fasting Normal Males.* (19757)

HAROLD A. HARPER,† MAXINE E. HUTCHIN, AND JOE R. KIMMEL.
*From the Metabolic Research Facility, U. S. Naval Hospital, Oakland, Calif.*

The data on the concentrations of individual amino acids in the plasma and urine of normal humans are somewhat limited. Furthermore, the findings which are available are scattered, and, until recently, have been confined to reports concerned with one or two of those amino acids which could be determined chemically. Specific quantitative chemical methods have been applied only to the determination of glutamine(1-3), glutamic acid (4,5), glycine(6), alanine(7), arginine(8), and tyrosine(9), and normal levels of these substances have been reported for human plasma and urine. However, since the introduction of microbiological and chromatographic technics, it has become possible to assay biological fluids for almost all of the amino acids of physiological importance. Therefore, it is now feasible to study variations in plasma and urine concentrations of at least 19 individual amino acids both in health and in disease. Some studies of this nature have been reported. Ackermann *et al.* (10,11) have studied plasma levels of 9 amino acids in human subjects of various age groups. Kirsner *et al.*(12,13) and Steele *et al.*(14) have applied microbiological methods to studies of the effect of variations in protein intake on levels of amino acids in human plasma and urine. Hier and Bergeim(15) reported plasma levels of 10 amino acids determined microbiologically in normal human beings. Harper *et al.* have reported on the normal levels of

* The opinions expressed in this paper are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

† Professor of Biology, University of San Francisco, Civilian Consultant in Biochemistry, U. S. Naval Hospital, Oakland, Calif.

methionine(16) and glutamine and glutamic acid(17), also determined microbiologically. It is the purpose of this paper to report the simultaneous determination of the plasma and urine levels of 19 amino acids as observed in fasting, healthy, young males.

*Procedure.* The subjects chosen for this study were young males on active duty in the U. S. Navy. They were, for the most part, staff members of the U. S. Naval Hospital, Oakland, Calif., ranging in age from 18-30 years. Food was withheld after the evening meal and at 7:00 a.m. the following day the bladder was emptied. All urine excreted in the 2-hour period between 7:00 and 9:00 a.m., was collected for amino acid assay and a sample of venous blood was then drawn for determination of the plasma amino acid levels following this 12- to 13-hour fast. Heparin was used as anticoagulant.

Diet is known to have a marked influence on the excretion of a number of amino acids (12-14). For this reason the urinary amino acids were determined on specimens voided after a fast of 12-13 hours. It is considered that this technic minimized individual variations attributable to the previous diet.

Microbiological assays for 19 amino acids were carried out on the urine specimens and on the filtrates of plasma deproteinized by treatment with 5% acetic acid at 100°C. Arginine and threonine were determined using the basal medium described by Henderson and Snell(18) with *Streptococcus faecalis* (ATC, 8043) as test organism. In the assay of the other amino acids the medium described by Steele *et al.*(19) was used. One-half milliliter of basal medium was added to each assay tube with supplements to a total volume of 1 ml except in the case of glutamic acid and glutamine, where double these quantities was used. The acid produced after 72 hours incubation was measured by titration with N/100 NaOH, or N/50 NaOH when a total volume of 2 ml was employed (glutamine and glutamic acid). *Leuconostoc mesenteroides* P-60 (ATC, 8042) was the test organism for the assay of aspartic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, tryptophan, and tyrosine; *Lactobacillus buchneri* (ATC, 4005) for cysteine-cystine; *Leuconostoc citrovorum* (ATC, 8081) for alanine; and *Lactobacillus arabinosus* 17-5 (ATC, 8014) for valine, glutamic acid and glutamine. The method of Harper(17) was utilized for the assay of glutamic acid and glutamine; that of Hutchin *et al.*(20) for cysteine-cystine. In the assay for glutamine and for cysteine-cystine the samples for assay and the standard solutions were sterilized by Seitz filtration and added aseptically to the tubes containing the basal medium which had been previously heat-sterilized and cooled.

It is to be noted that the amino acid levels observed represent only free amino acids, since no attempt was made to derive additional amino acids by hydrolytic procedures.

*Results and discussion.* The mean plasma levels of the 19 amino acids studied are recorded in Table I. The number of subjects, the ranges of plasma concentration, and the standard deviations are also shown in this table. For purposes of comparison the results of other observers have been supplied. With respect to most of the amino acids the data from our study agree well with the results of the authors previously cited. The only noteworthy exceptions were in the cases of glycine, histidine, phenylalanine, and tryptophan (mean varied by more than one standard deviation from that of previous authors). When the large number of physiological processes in which glycine is implicated is considered, the observed variability in the plasma levels of this particular amino acid is not surprising.

Marked variations in the amount of each amino acid excreted during the 2-hour test period were noted. However, with respect to the absolute quantities found in the urine, the amino acids could be conveniently divided into 4 groups. Glycine (3-36 mg/hr, mean 11.7 $\pm$ 9.45) and histidine (2.7-23.0 mg/hr, mean 7.75 $\pm$ 1.33) comprised the first group. These 2 were uniformly excreted in the largest amounts. Glutamine (1.6-4.6 mg/hr, mean 2.87 $\pm$ 0.96), and cystine (0.4-4.3 mg/hr, mean 2.30 $\pm$ 1.22) were intermediate. Leucine and isoleucine, valine, aspartic and glutamic acids, and methionine were excreted in small amounts (maximum, less than 1 mg/hr), while the remaining 9 amino acids were found

TABLE I. Plasma Levels of 19 Amino Acids in Fasting Normal Male Subjects.

| Amino acid | No. of subjects | Observed plasma levels (mg/100 ml) | | | Reported plasma levels (mg/100 ml) | | | |
|---|---|---|---|---|---|---|---|---|
| | | Range | Mean | S.D. | Range | Mean | S.D. | Ref. |
| Glutamine | 14 | 4.6 –10.6 | 7.51 | 1.63 | 5 –12 | — | — | 1 |
| | | | | | 5 –12 | — | — | 2 |
| | | | | | 2.7 – 7.8 | — | — | 4 |
| | | | | | — | 8.3 | 1.5 | 5 |
| | | | | | 6.1 –13 | — | — | 17 |
| Alanine | 17 | 2.4 – 7.6 | 3.96 | 1.47 | — | 3.97 | .7 | 7 |
| | | | | | 3.4 – 4.9 | — | — | 14 |
| | | | | | 3.2 – 6.4 | — | — | 21 |
| Lysine | 17 | 2.3 – 5.8 | 3.68 | 1.15 | 1.8 – 4 | 2.85 | .57 | 11 |
| | | | | | 2.1 – 3.1 | — | — | 13 |
| | | | | | 3.7 – 4.2 | — | — | 14 |
| | | | | | — | 2.95 | .42 | 15 |
| Valine | 16 | 2.5 – 4.2 | 3.23 | .53 | — | 2.86 | .42 | 11 |
| | | | | | 2 – 3.4 | — | — | 13 |
| | | | | | 2.8 – 3.8 | — | — | 14 |
| | | | | | — | 2.83 | .34 | 15 |
| Cysteine-cystine | 13 | 1.8 – 5 | 3.02 | 1.16 | — | — | — | — |
| Glycine | 17 | .8 – 5.4 | 2.91 | 1.36 | — | 1.77 | .26 | 6 |
| | | | | | — | 1.26 | .23 | 11 |
| | | | | | .6 – 1.6 | — | — | 14 |
| | | | | | 1.5 – 2 | — | — | 21 |
| Proline | 17 | 1.5 – 5.7 | 2.61 | 1.26 | 2.6 – 3 | — | — | 14 |
| Leucine | 17 | 1 – 5.2 | 2.48 | .90 | — | 1.89 | .29 | 11 |
| | | | | | 2 – 2.8 | — | — | 13 |
| | | | | | 1.7 – 3.3 | — | — | 14 |
| | | | | | — | 1.91 | .34 | 15 |
| Arginine | 16 | 1.2 – 3 | 2.26 | .47 | 2 – 2.4 | — | — | 8 |
| | | | | | 1.5 – 1.9 | — | — | 13 |
| | | | | | 1.6 – 3.6 | — | — | 14 |
| | | | | | — | 2.34 | .62 | 15 |
| Histidine | 17 | 1 – 3.8 | 2.11 | .61 | — | 1.34 | .17 | 11 |
| | | | | | 1.7 – 2.5 | — | — | 13 |
| | | | | | 2.7 – 3.9 | — | — | 14 |
| | | | | | — | 2.02 | .45 | 15 |
| Threonine | 17 | .9 – 3.6 | 2.06 | .69 | — | 1.88 | .42 | 11 |
| | | | | | 1.7 – 2.5 | — | — | 13 |
| | | | | | 2.7 – 3.9 | — | — | 14 |
| | | | | | — | 2.02 | .45 | 15 |
| Isoleucine | 17 | 1.2 – 4.2 | 2 | .79 | — | 1.33 | .19 | 11 |
| | | | | | 1.6 – 2.1 | — | — | 13 |
| | | | | | 1.6 – 2.3 | — | — | 14 |
| | | | | | — | 1.60 | .31 | 15 |
| Phenylalanine | 17 | 1.1 – 4 | 1.99 | .82 | 1.5 – 2.4 | — | — | 14 |
| | | | | | — | 1.38 | .32 | 15 |
| Tryptophan | 17 | .9 – 3 | 1.74 | .51 | .8 – 1.5 | 1.18 | .20 | 11 |
| | | | | | 1 – 2.3 | — | — | 14 |
| | | | | | — | 1.08 | .21 | 15 |
| Serine | 17 | .3 – 2 | 1.39 | .44 | — | — | — | — |
| Tyrosine | 17 | .9 – 2.4 | 1.32 | .37 | 1 – 1.8 | 1.4 | — | 9 |
| | | | | | — | 1.38 | .22 | 11 |
| | | | | | .9 – 1.5 | — | — | 14 |
| | | | | | — | 1.48 | .37 | 15 |
| Glutamic acid | 17 | .0 – 1.3 | .89 | .39 | 1.3 – 5.9 | — | — | 4 |
| | | | | | — | 1.1 | .5 | 5 |
| | | | | | .0 – 1.4 | — | — | 17 |
| Methionine | 18 | .25– 1 | .57 | .27 | .4 – .7 | — | — | 13 |
| | | | | | .8 – 1.1 | — | — | 14 |
| | | | | | .5 – 1.5 | .85 | — | 16 |
| Aspartic acid | 17 | .0 – 1.2 | .33 | .37 | .6 – .9 | — | — | 14 |

in amounts ranging from 1.0-3.4 mg/hr.

The considerable variability in the normal excretion of any one amino acid which we have noted has also been observed by other workers(12-14). It illustrates the difficulties likely to be encountered in interpreting or ascribing significance to amino acid levels in urine.

*Summary.* The plasma and urine levels of 19 free amino acids as determined microbiologically in fasting, healthy, young males are reported.

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## An Anthrone Procedure for Determination of Inulin in Biological Fluids. (19758)

M. KENDALL YOUNG, JR., AND LAWRENCE G. RAISZ. (Introduced by R. J. Williams.)

*From the Surgical Research Unit, Brooke Army Hospital, Ft. Sam Houston, Texas.*

The use of inulin for extracellular space estimation, as well as clearance measurements, has increased the need for a simpler and more accurate method for the determination of this substance in biological fluids. At present, resorcinol(1,2) and diphenylamine(3) are most frequently used for this determination. The former has a greater specificity for fructose, but is somewhat less reproducible. While greater accuracy may be obtained with diphenylamine, this method is more tedious.

Recently, Morris(4) has described the quantitative determination of carbohydrates with Dreywood's anthrone reagent. The present report describes the application of this method to the measurement of inulin in plasma and urine. A modification of Dreywood's anthrone reagent makes possible the measurement of fructose under controlled temperature conditions and gives highly reproducible results. Glucose and other alkali-labile chromogens are destroyed by sodium hydroxide digestion(5) so that inulin can be determined in the presence of high concentrations of these materials. It has been found that commercial inulin contains a considerable amount of alkali-labile carbohydrate material that can be completely removed by further purification (6).

*Methods.* Reagent: To prepare one liter of anthrone reagent, 500 ml of concentrated sulfuric acid are added to 250 ml of water, and the mixture is cooled to room temperature. Four grams of anthrone (Eastman Organic