Standardization with oxygenated blood prior to each period of use eliminates this source of error. In addition the introduction of a more sensitive galvanometer would reduce the portability of the apparatus.

Agreement within 4.8% saturation between oximeter and manometric values can be expected ninety-five percent of the time. However, we have not used the oximeter other than as a scouting device at the time of catheterization checking all results with Van Slyke analyses prior to calculation of flow components.

Summary. (1) A polythene tubing cuvette is described which adapts the Millikan oximeter for measurement of oxygen saturation of drawn samples of whole blood.

(2) Oxygen saturation values agree with Van Slyke manometric values with a standard error of 2.4%.

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Intravenous Amino Acid Tolerance Studies in Humans.* (17372)

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The introduction of specific and sensitive microbiological methods for the assay of amino acids has made possible the further study of many fundamental problems in the metabolism of these nutrients. The increasing clinical use of amino acids in parenteral nutrition, particularly in surgery, further emphasizes the necessity for more detailed studies on the fate of these substances in the human organism. For this purpose the rates of disappearance of intravenously administered amino acids have been investigated. The problem was first studied with *DL*-methionine as a test amino acid.[1-3] In view of the recent work on the importance of the simultaneous presence of all of the essential amino acids if maximal nitrogen retention is to occur,[4] it was felt that further tolerance studies should be conducted with a mixture of the 10 amino acids originally thought to be indispensable to man. The present paper reports the results of such studies on normal human males in intravenous tolerance to 10 amino acids when injected simultaneously. Included also are the data on the urinary excretion of the injected amino acids.

Experimental. The amino acid preparation used was the VUJ-N mixture kindly supplied by Merck & Company. The product contained varying amounts of the 10 indispensable amino acids plus additional glycine. After a 12-hour fast, blood samples were withdrawn for determination of fasting levels of the 10 amino acids. The amino acid mixture was then injected intravenously as rapidly as possible using 50 ml syringes (about 4-5 minutes) at a level of 1 ml per pound body weight. Blood samples were taken at 15, 30, 60, 120 and 180 minutes after the injection, and urine excreted during the 2-hour period immediately prior to the injection as well as during the period when the bloods were taken was also collected for assay. Ten amino acids were then determined by microbiological methods on heat deproteinized plasma from heparinized blood, and on the urines. The basal medium used was either that of Henderson and Snell,[5] or Steele, Sauberlich, Rey-

* Presented before the Society of University Surgeons, Tenth Annual Meeting, San Francisco, March 24, 1949.

1 Harper, H. A., Kinsell, L. W., and Barton, H. C., *Science*, 1947, **106**, 309.

2 Kinsell, L. W., Harper, H. A., Barton, H. C., Michaels, G. D., and Weiss, H. A., *Science*, 1947, **106**, 589.

3 Kinsell, L. W., Harper, H. A., Barton, H. C., Hutchin, M. E., and Hess, J. R., *J. Clin. Invest.*, 1948, **27**, 677.

4 Geiger, E., *J. Nutr.*, 1948, **34**, 97.

5 Henderson, L. M., and Snell, E. E., *J. Biol. Chem.*, 1948, **172**, 15.

TABLE I.
Plasma and Urine Amino Acid Levels Prior and Subsequent to Injection of 131 ml of a Mixture of 10 Amino Acids (VUJ-N).
Subject, K.H. ♂ 131 Lbs.

Amino acid inj. (mg L isomer)	Leucine 1640	Lysine 1390	Isoleucine 1073	Valine 753	Histidine 524	Methionine 524	Phenylalanine 341	Threonine 245	Arginine 219	Tryptophan 30
				Blood plasma levels (mg per 100 ml)						
Time (min.)										
0	1.6	3.2	0.8	3.7	2.1	0.2	1.3	1.6	1.3	1.3
15	6.2	7.8	4.5	10.4	3.2	2.0	1.8	2.4	3.5	1.8
30	4.7	5.8	2.4	6.7	2.9	1.2	1.7	2.2	2.0	1.7
60	2.9	4.2	1.5	4.9	2.4	1.0	1.6	1.7	1.2	1.4
120	2.2	3.4	0.9	3.8	2.0	0.4	1.4	1.4	1.2	1.2
180	2.1	3.0	0.9	3.7	1.8	0.3	1.3	1.2	1.2	0.9
				Urine levels (μg per hr)						
2 hr prior to inj.	78	200	24	104	4000	24	64	320	440	310
3 hr post inj.	363	2406	91	182	13600	182	647	1816	1020	363
Diff.	285	2206	67	78	9600	158	583	1496	580	53
% of inj. dose excr. in 3 hr	0.05	0.48	0.02	0.03	5.50	0.09	0.51	1.83	0.79	0.53

nolds, and Baumann.[6] *Leuconostoc mesenteroides*, P-60 was employed as the assay organism for all amino acids except threonine and arginine for which *Streptococcus fecalis* was preferred.

Results and discussion. In Table I there are listed the absolute amino acid levels (*L*-isomers, mg per 100 ml plasma) which were observed before and at various intervals after the amino acid mixture was infused in a normal male subject chosen as typical of 5 experiments. This table also lists the quantities of the 10 amino acids (μg per hour) excreted during the 2-hour period preceding the infusion as well as for the 3-hour period during which blood samples were taken. From this data the percentage of the injected dose which was excreted during the period of observation was calculated.

The amino acid pattern infused is also compared (Fig. 1) to that obtaining in the plasma of 2 subjects at various intervals subsequent to the injection. For this purpose each amino acid is first corrected for its fasting level and all concentrations are then expressed as a percentage of the total amino nitrogen measured in the form of the 10 amino acids. In order to compare the excreted with the infused pattern the individual contribution (% amino nitrogen) of each amino acid to the total 3-hour amino nitrogen excretion of the 10 amino acids is also shown in Fig. 1.

It will be noted that while the amino acid levels at 15 minutes after the injection are more or less elevated in proportion to the amount injected, subsequently the levels fall rapidly, in some instances decreasing to substantially lower than the fasting levels. It is possible that this latter effect is referable to a synthetic process which requires some of the original amino acid supply of the body. The comparison of the pattern of 10 amino acids in the plasma at various intervals after the injection with that originally infused reveals some differences between the two subjects in the rate of disappearance of individual acids. In Subject K.H., the preponderance of leucine, valine, and methionine is noteworthy.

[6] Steele, B. F., Sauberlich, H. E., Reynolds, M. S., and Baumann, C. A., *J. Biol. Chem.*, 1949, **177**, 533.

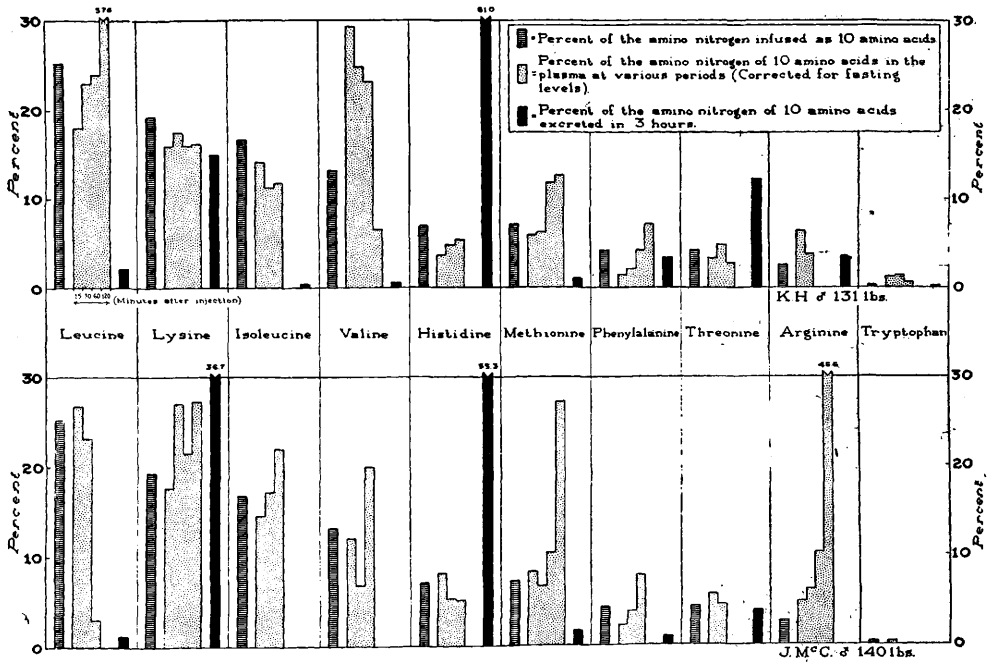

FIG. 1.

Comparison of amino acid pattern infused with that of the plasma at intervals subsequent to injection. The individual contribution of each amino acid to the total 3-hour amino nitrogen excretion of 10 amino acids is also shown. All concentrations are expressed as a percentage of the total amino nitrogen measured in the form of 10 amino acids.

In Subject J. McC., lysine, methionine, and arginine are responsible for the excess over the infusion mixture, in the later periods. Only the persistence of methionine is common to both subjects. The other acids retain a close resemblance to the infusion mixture during most of the period of observation. The effect of a change in the concentration of any one amino acid on the uptake of the others would be of interest. This experiment is planned as an extension of the basic tolerance studies.

The pattern of amino acids excreted bears no relationship to that infused. Very little quantitative data on the renal threshold for amino acid excretion in humans is available. One fact that is well established, however, is that the reabsorptive capacity of the kidney for the natural *L* isomers, in contrast to the *D* isomers, is relatively high. At the infusion levels used in these experiments, histidine, lysine and threonine accounted for the majority of the urinary amino nitrogen measured. One could therefore disregard the losses by the renal route of the other amino acids.

Silber and his collaborators[7] have infused into dogs an amino acid mixture similar to that used in our experiments and studied the pattern of the essential amino acids excreted in the urine. They also point out that the urine pattern did not resemble the pattern infused nor was it greatly altered by protein depletion. However, in a subsequent paper, Silber[8] by infusing 3 different amino acid mixtures in dogs was able to show a relationship between intake and excretion and suggested that this approach might be useful in designing amino acid mixtures for specific types of patients. From our experiments it would seem that because of the high renal threshold for most amino acids, the urine fails adequately to reflect either blood levels or extent of uptake by the tissues. In liver disease we have repeatedly observed plasma levels of *L*-methionine, for example, which far

[7] Silber, R. H., Seeler, A. O., and Howe, E. E., *J. Biol. Chem.*, 1946, **164**, 639.

[8] Silber, R. H., Howe, E. E., and Porter, C. C., *Trans. N.Y. Acad. Sci.*, 1948, Ser. II, **10**, 277.

exceeded normal without any resulting marked increase in excretion. In the present experiments, the rate of disappearance of leucine from the blood is much greater than that of histidine but these differences are not reflected in the urine. It is therefore felt that studies of amino acid changes in the blood plasma may be more valid for the purpose of an investigation of utilization of various mixtures.

A comparison of tolerance to the same quantities of amino acids when infused at a constant rate over a one-hour period is now in progress. The results will be reported in a later communication.

Summary. A mixture of the 10 indispensable amino acids has been injected intravenously in normal human male subjects and the plasma levels of these 10 acids as well as the urinary excretion, prior and at various intervals subsequent to the injection, were determined microbiologically. The pattern of amino acids excreted is shown to bear no relationship to that infused.

The work here reported has been carried out with the active cooperation of Drs. H. J. McCorkle, of the University of California Medical School, and H. L. Silvani, Frank Choy, and their associates at The Veterans' Hospital, Fort Miley.

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Intravenous Administration of Massive Dosages of Estrogen to the Human Subject; Blood Levels Attained. (17373)

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The employment of estrogens in the management of cases of cancer of the breast[1] and prostate[2] necessitates our further knowledge of the biological effects of the estrogens when administered in varying dosage and by various routes.

Few data are available concerning the effects of intravenous administration of estrogen in the human subject. Loeser[3] injected as much as 10 mg estrone, 25 mg estradiol, or 150 mg stilbestrol intravenously in propylene glycol and observed an elevation of intrauterine temperature presumably attributable to increased uterine blood-flow. He noted no toxic side reactions and indicated that the medication was well tolerated. Abarbanel[4] reported the administration of small doses of estrone sulfate for the control of uterine bleeding.

We wish to report on the clinical tolerance of intravenous infusions of massive dosages of an aqueous solution of conjugated natural estrogens.* We have also determined by bioassay the blood levels attained immediately following infusion and at fixed intervals after treatment.

Materials and methods. Thirteen female and 9 male subjects have been studied. Their age, race, and clinical diagnosis are indicated in Table I. All patients were at bed rest when treated. Intravenous infusions in the dose and volume indicated were carried out in the customary manner employing the antecubital vein. A buffered aqueous solution of conjugated estrogens in the form of a concentrate from pregnant mare's urine was mixed with normal saline so that a final concentration of from 0.5 to 2 mg equivalents of estrone sulfate per cc was obtained for injection. The rate of flow was so adjusted that the total dose was administered in from 35 to 60 minutes. Each patient's temperature, pulse,

1 Nathanson, I. T., *Surg. Clin. N. A.*, 1947, **27**, 1144.

2 Huggins, C., Stevens, R. E., and Hodges, C. V., *Arch. Surg.*, 1941, **43**, 209.

3 Loeser, A. S., *J. Obst. and Gynec. Brit. Emp.*, 1948, **55**, 17.

4 Abarbanel, A. R., paper No. 71; read by title; Assn. for Study of Internal Secretions, June, 1949.

* The preparation used was "Premarin" (injectable) kindly supplied by the Ayerst, McKenna & Harrison Ltd., through the courtesy of Drs. G. H. C. McKeown and E. C. Reifenstein, Jr.