TABLE II.
Relation of Protein and Methionine Levels to the Rate of Gain in Chicks.

Protein in diet, %	Methionine added to diet, %	Total methionine in diet, %	Daily rate of gain		
			S.C. White Leghorn, %	New Hampshire, %	New Hampshire-Barred Rock cross, %
20	0	.30	6.3*	7.1*	7.2
20	.10	.40	6.7	7.6*	7.7
20	.20	.50	7.0*	8.0*	7.9
20	.30	.60	7.0	7.9*	7.9
30	0	.45	6.2*	8.2*	—
30	.10	.55	—	8.4*	—
30	.20	.65	6.7	8.5*	8.2
30	.30	.75	7.2*	8.6*	8.5
30	.40	.85	7.2	8.6*	8.4

* Average growth rate of duplicate pens.

These results are analogous to those with sesame meal and lysine.[2]

The results discussed above seem sufficient to justify an extension of the concept previously stated by the writer,[1] that the proportions of indispensable amino acids to each other remain relatively the same for maximal efficiency of utilization under varying conditions of level and source of the amino acids. This concept now includes levels of protein intake considerably above what is usually considered the normal requirement for maximal rate of gain.

Since maximal protein efficiency, as indicated by growth rate, is closely related to proportions of amino acids in the diet, even at super-normal protein levels, it would appear, as previously suggested,[1] that "the proportions of amino acids reaching the synthetic regions in the animal are determined largely by the proportions in the diet." The balance of indispensable amino acids in the nitrogenous increments reaching these cells is more important than the gross protein intake.

Amino acid imbalance in chick diets is not fully correctable by feeding more of the same imbalanced protein. This fact has a bearing on chick assay diets for "animal protein factor," in which approximately 70% soybean meal is used. Even with best qualities of soybean meals, these diets are methionine deficient, and when a material is added which contains both "animal protein factor" and an effective quantity of methionine, as in the case of fish meals, the growth response observed is a resultant of a dual supplementation.

Summary. The methionine requirement of the chick for maximal rate of gain on a 30% protein diet is approximately 0.75% of the diet. This value is in direct proportion of the protein levels to the established methionine requirement of 0.50% in a 20% protein diet.

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Rapid Measurement of the Oxygen Saturation of Whole Blood Samples with the Millikan Oximeter.* (17371)

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During cardiac catheterization it is frequently desirable to obtain a rapid estimation of blood oxygen saturation in order to orient abnormal locations of the catheter and to determine the uniformity of multiple samples. With this purpose in mind, Groom, Wood, Burchell and Parker[1] have developed a whole blood cuvette oximeter which requires a spec-

cial photocell unit. We have adapted the principles outlined by these workers to the use of the compensated circuit Millikan oximeter. The earpiece of this apparatus is clamped over a plastic tube cuvette. Standardization of the red and infra-red-red bias circuits is obtained by galvanometer settings with the neutral filter and with the cuvette filled with oxygen saturated blood respectively.

Apparatus: To utilize the photosensitive area of the Millikan earpiece to the maximum, a length of translucent polythene plastic tubing is looped to pass twice through the earpiece perpendicular to the long axis of the photocell limb. The tubing is compressed slightly by thin bakelite plates. Each plate contains an ovoid window in which a microscope cover-glass has been cemented recessed flush with the inside surface of the plate. The diameter of the window perpendicular to the axis of the tubing is determined by the lateral edges of the lumens of the two segments of the polythene loop lying in close proximity. Such dimensions shield light which would pass laterally to the fluid columns. The ends of the ovoid window are arcs of a circle equal in diameter to the photocell port in the Millikan earpiece. The loop of tubing is compressed to produce a light path distance 1.2 mm through the lumen by a set screw with lock nut at each corner of the bakelite plates. The ovoid window is placed asymmetrically in the plates so that the earpiece is centered over the window when its upright is against one edge of the cuvette plates and the photocell limb is against two of the set screws. This insures a relatively constant location of the earpiece prior to standardization. The plastic tubing is 4.3 mm in external diameter with walls 0.5 mm thick. The tubing, obtained from an ordinary disposable intravenous infusion set (Baxter), may vary slightly in diameter and translucency without affecting the accuracy of the apparatus. When this tubing is compressed to internal diameters

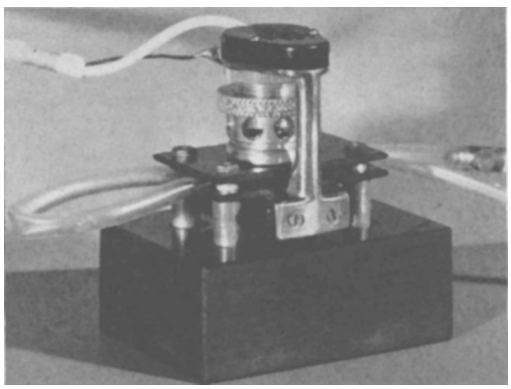

FIG. 1A.
Assembled Cuvette-Earpiece unit. The setscrews controlling cuvette thickness fit into the bakelite base and are not seen.

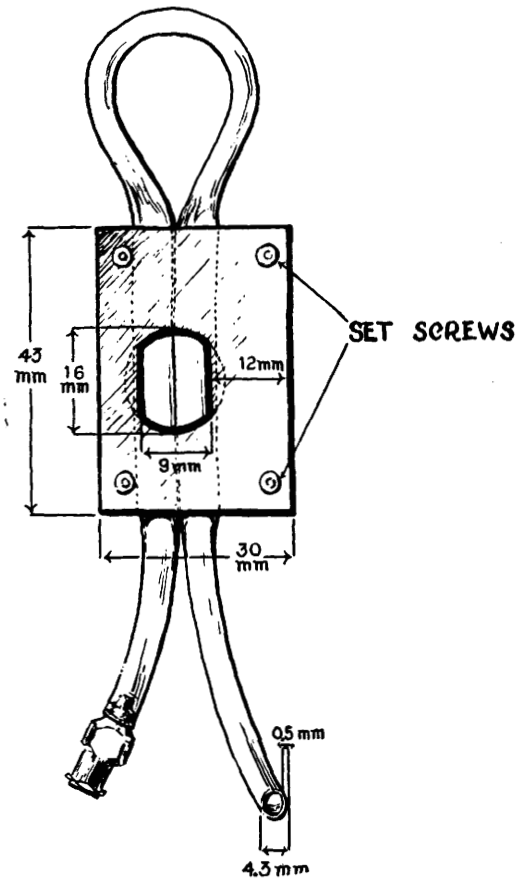

FIG. 1B.
Detail of cuvette light port.

* Aided by grants from the American Heart Association and the Oregon Heart Association. The oximeter was made available by a generous gift from Mr. Henry Chaney to the Doernbecher Memorial Hospital for Children.

1 Groom, D., Wood, E. H., Burchell, H. B., and Parker, R. L., *Proc. Mayo Clinic Staff Meetings*, 1948, **23**, 601.

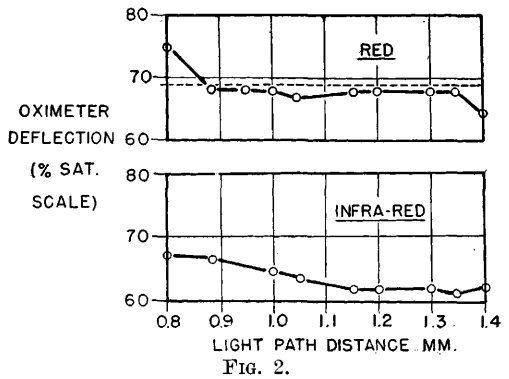

FIG. 2.

Light transmission properties with varying light path distance. At each light path distance the red infra-red bias circuit was standardized with fully saturated blood in the cuvette. Aliquots of a nitrogen desaturated sample of the same blood then produced galvanometer deflection. The lower portion of the diagram shows the galvanometer deflection produced by activation of the infra-red photocell alone. The upper portion of the diagram (RED) shows galvanometer deflection produced by the bias circuit measuring oxygen saturation. The dotted line indicates oxygen saturation of the nitrogenated blood sample as measured by Van Slyke manometer. Between light path distances of 0.88 mm and 1.35 mm a constant half scale galvanometer deflection is produced with desaturated blood having an oxygen capacity of 23.1 volumes %.

shown in Fig. 2, an area of absorbing solution on the order of 120 sq mm is exposed to light. One end of the tubing is fitted with the hub of an 18 gauge hypodermic needle, which has been drilled to larger bore. The set-screws of the cuvette fit into a heavy bakelite base.

A commercial oximeter serves as the recording device and as power unit for the earpiece lamp.† The earpiece utilizes a light filter in the red range and infra-red range rather than the red and green light range of the original Millikan earpiece. The bias circuit of this apparatus results in direct recording of oxygen saturation; such a circuit is described in Millikan's original communication[2] as a single scale oximeter with automatic adjustment for ear thickness. Currents are recorded with a mirror galvanometer (Rubicon 3415). The scale, 10 cm in length, is calibrated directly in percent oxygen saturation from 50% to 100% in 5% scale divisions.

Procedure. A warm-up period of 5 to 10 minutes is required for the earpiece circuits. A standard galvanometer deflection of the infrared cell circuit ("green" circuit) alone is set using variable resistance R_1 with a standard filter in the light path of the earpiece (Coleman A Filter). The earpiece is clamped firmly in place over the cuvette. A blood sample is drawn by catheter from the patient and saturated with tank oxygen at room temperature. With this saturated blood in the cuvette, the red-infra-red bias circuit is standardized at 100% saturation with variable resistance R_2. The oximeter remains stable over a period of hours, but bias circuit standardization may be repeated at intervals. Once standardization is completed, optical arrangements should not be altered by change of position of setscrews, earpiece screw or earpiece. The oximeter will faithfully reproduce saturation values over a wide range of thickness of the whole blood column (Fig. 2) but, following any change of light path distance, new standardization of the bias circuit with fully oxygenated blood is required. A portion of the unknown blood sample, collected in an oiled heparin syringe, is saved in a fluoride tube for Van Slyke manometric analysis. The last 2 ml of blood contained in the syringe are passed into the cuvette. This quantity of blood is sufficient to fill the cuvette allowing 0.5 ml to flush the lighted segments of plastic tubing. Both infra-red and bias circuit galvanometer readings are made. The infra-red deflection records changes in total hemoglobin concentration. The bias circuit reading indicates the oxygen saturation. Constancy of the infra-red galvanometer reading for successive blood samples indicates absence of air or oil bubbles in the light path and constancy of hemoglobin concentration. The entire length of the plastic tubing can be inspected for trapped air or oil at any time during the procedure. Usual room lighting produces no significant error in saturation determinations. Between readings the cuvette is left filled with blood being flushed with isotonic sodium chloride solution and air just prior to introduction of the next sample. This maneuver

† Coleman Anoxia Photometer Model 17A, Coleman Instrument Company, Maywood, Ill.

[2] Millikan, G. A., *Rev. Sci. Inst.*, 1942, **13**, 434.

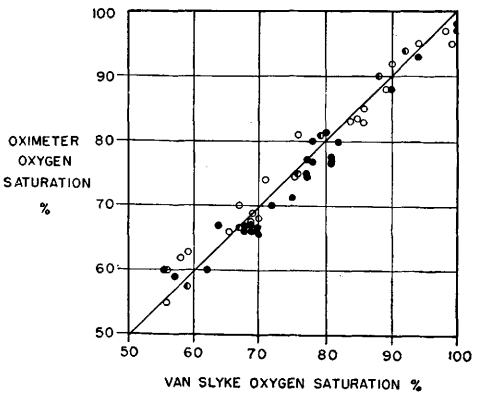

FIG. 3.

Comparison of oxygen saturation determinations on 54 blood samples, as determined by the oximeter and by the Van Slyke manometric method. Black points represent polythemic blood samples having an oxygen capacity from 29.1 to 33.2 volumes %. Half-black circles represent anemic blood samples having an oxygen capacity of 9.0 volumes %. Circles represent blood samples of intermediate hemoglobin concentration (14.0 volumes % to 23.0 volumes % oxygen capacity).

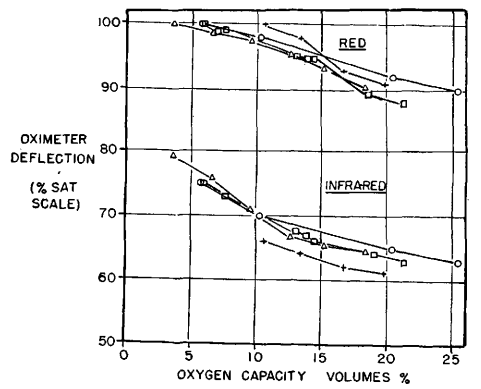

FIG. 4.

Variations of apparent oxygen saturation produced by change of hemoglobin concentration after standardization of the oximeter circuits. Fully saturated blood was diluted serially with plasma. The most dilute blood sample was used to set the bias circuit (RED) at 100% saturation, and, without change of galvanometer settings, the fully saturated samples of higher oxygen capacity measured. Although the true oxygen saturation of all samples was 100%, there was a fall in oxygen saturation as measured by the oximeter with more concentrated blood specimens, indicating incomplete compensation for changes of hemoglobin concentration.

minimizes exhaustion of the barrier layer photocells.

Results. Comparison of blood oxygen saturation determinations by the oximeter and by the manometric method of Van Slyke and Neill[3] is shown in Fig. 3. The standard error between observations by the two methods on 54 blood samples was 2.4% oxygen saturation. Determinations were made using human blood samples taken at the time of right heart catheterization and samples of dog blood desaturated by tonometric equilibration with nitrogen and air. The oxygen capacity of these samples varied from 9.0 volumes % to 33.2 volumes % without affecting accuracy significantly. The effect of wide variations of hemoglobin concentration following standardization is shown in Fig. 4. Blood from stock dogs was serially diluted with dog plasma and equilibrated with air at room temperature. The most dilute blood-plasma mixture was used to set the bias circuit at 100% saturation. The apparent oxygen saturation reading and infrared galvanometer reading were then recorded for the more concentrated fully saturated blood-plasma mixtures. The observations during four such experiments are plotted against oxygen capacity in Fig. 4. A change of oxygen capacity on the order of 10 to 20 volumes % is required to produce a change of 10% in the observed oxygen saturation. Thus although the bias circuit does not completely compensate for wide fluctuations in hemoglobin concentration, any slight variation of hemoglobin concentration due to fluid infusion and serial sampling during catheterization should not affect saturation readings appreciably.

If it can be shown that the light transmission of the empty cuvette remains constant, modification of this recording circuit would permit measurement of the entire length of galvanometer deflection on introduction of blood into the cuvette and consequently would allow direct reading of oxygen saturation without the step of standardization with oxygen-saturated blood. However, the assumption of constancy of light transmission is not warranted since after several hours' use there is gradual fogging of the plastic tube exposed to the 6 v earpiece lamp. After several hours' use, the plastic tubing is replaced.

[3] Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.

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.