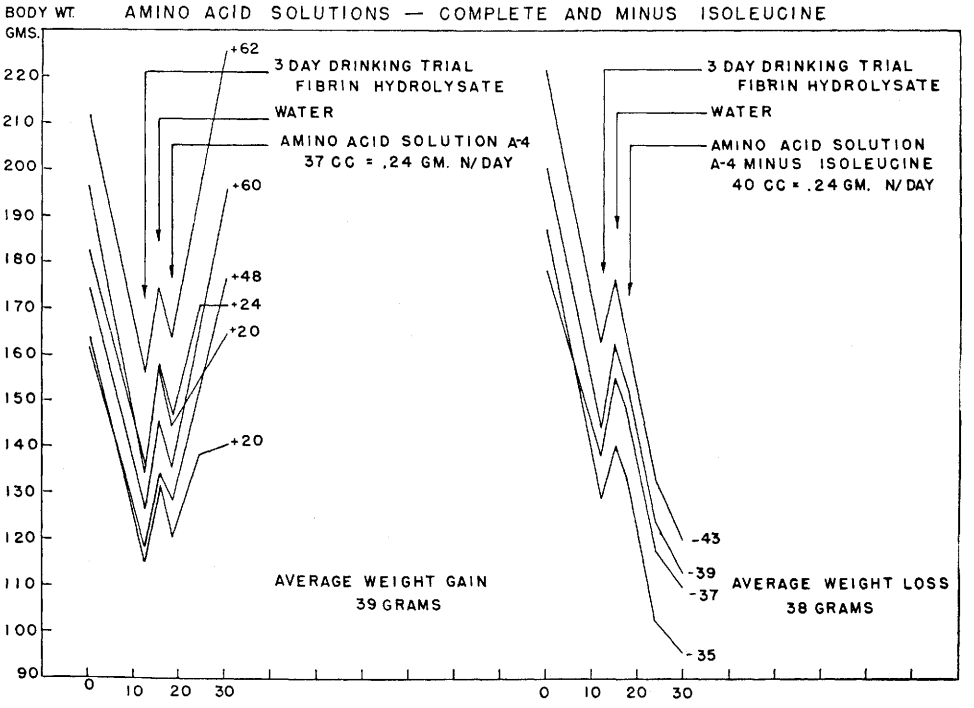

FIG. 2.

Weight changes of individual rats during 12-day depletion, 3-day orientation to liquid feeding, 3-day redepletion and final assay periods. Assay of a 5% amino acid solution patterned after the amino acid composition of casein (corrected for insolubility of tyrosine, cystine, and glutamic acid) versus the similar mixture minus isoleucine.

young adult protein-depleted rats to 5% protein hydrolysates fed as the sole source of amino acid nitrogen provides a basis for rapid assay. The response to liquid hydrolysate feeding approached that of dried hydrolysate or whole protein added to the diet. Pure amino acid solutions of good nutritive composition are readily accepted and support good weight recovery. The effect of the *D*-amino acids in such mixtures requires clarification.

Acknowledgement is made to Eleanor Willerton for microbiological amino acid assays and to E. O. Krueger for the chemical assays. We wish also to thank Dr. Paul R. Cannon for his helpful counsel in these studies.

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Enzyme Studies on Human Blood. II. The Substrate in the Fibrinogen-Thrombin Reaction.*

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Divergent views exist in respect to the influence of fibrinogen concentration on the fibrinogen-thrombin reaction. An optimal range of substrate concentration for the velocity of the fibrinogen-thrombin reaction has been reported.[1,2,3,4] In this communica-

* Fibrinogen fractions were prepared from dried normal human plasma which had been processed from blood obtained from volunteer donors enrolled by the American Red Cross.

tion, the results of 51 consecutive experiments are presented, confirming the existence of an optimum concentration for the substrate. The relationship between this optimum concentration and the nature of the fibrinogen preparation has been investigated.

Methods and Material. Fibrinogen preparations (Fraction I) were obtained from dried human plasma by the low temperature-ethanol procedure, Method 6, of the Department of Physical Chemistry, Harvard Medical School.[5] The precipitate was formed in a low temperature bath at −2.5 to −3.0°C and separated in a refrigerated centrifuge (International PR 1). Subfractions of Fraction I were also prepared by low salt, low temperature procedures. All protein precipitates were lyophilized at an initial temperature of −70°C, and at less than 0.1 mm pressure. The dry powder was stored in a vacuum desiccator. One or 2% solutions of the fibrinogen fractions in a buffer were prepared on the days of the experiment.

The thrombin employed, prior to its expiration date, was Lederle's "Hemostatic Globulin."[6] The activity of this product was quite constant, day to day and vial to vial, as determined by experiments with a standard fibrinogen preparation. For the measurement of clotting times a 1:10 dilution of the material with the appropriate buffer was prepared. This solution retained its potency at room temperature for at least 6 hours. For the estimation of thrombin-clottable protein, a 1:60 dilution of dialyzed thrombin was prepared.

All reagents were dissolved or diluted in either the Michaelis veronal buffer as modified by Owren,[3] pH 7.3, Γ/2 (ionic strength) 0.154, or a citrate-phosphate buffer, pH 7.2, Γ/2 0.129. The latter consists of 250 cc 1/15 M Sorensen buffer, pH 7.1, and 75 cc 0.1 M sodium citrate in one liter solution. The pH was determined with a glass electrode electrometer at 25°C.

The fibrinogen was estimated by two methods. In the thrombin technic, 1 cc of approximately 1% protein solution was added to 7 cc of buffer and 2 cc of 1:60 dialyzed thrombin and allowed to stand for 3 hours; in the heat technic, an approximately 0.1% protein solution was heated at 54-56°C in a water bath for 5 minutes. After centrifugation the macro Folin-Ciocalteu[7] tyrosine equivalents were determined, of the supernatant fluid, the untreated protein solution, and the thrombin reagent. As an additional correction factor, acid soluble "tyrosine" values were obtained on various fibrinogen preparations by the micro-technic. This colorimetric method as employed in this laboratory has an approximate average sensitivity of 0.003 mg tyrosine per 1% T for the macro-technic, and 0.001 mg per 1% T for the micro-technic on the Coleman Junior Spectrophotometer.

The clotting times were measured as follows: The substrate (0.8 cc) was pipetted into a test tube (*i.d.*, 1 cm) and equilibrated in a 37.5°C water bath. The stop watch, with a hidden face, was started at the moment 0.2 cc of the thrombin was blown in. In this study, the criteria for the end-point were: the characteristic gel formation, or the appearance of clumped fibrin granules or threads. The latter appeared in the test tube simultaneously with complete disappearance of any opacity in the solution. The latter criterion was necessary when experimenting with dilute fibrinogen solutions.

Results. The data from two experiments on a fibrinogen fraction (Run 251) are presented in Fig. 1. They are typical of the results obtained in each of the 51 experiments on 34 preparations. The concentration-clotting time curve is U-shaped and the clotting times are

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higher for a given fibrinogen preparation in the citrate-phosphate buffer than in the Michaelis veronal buffer. In Table I the results from all experiments are summarized and grouped according to the range of fibrinogen purity and the buffer used.

Discussion. Our experiments confirm the findings of Wöhlisch *et al.*[1,2] and others[3,4] that there is an optimum substrate concentration for the thrombin-fibrinogen reaction velocity. In any comparison of the range of the optimum concentration reported here and by Ferry and Morrison,[4] with those of other investigators, it must be recognized that fibrinogen fractions, prepared by the low temperature-ethanol procedure, are not "pure" as shown by quantitative data. Ferry and Morrison[4] found the minimum concentration to

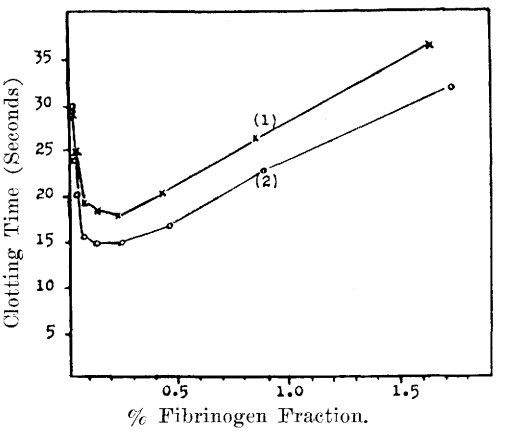

FIG. 1.

Relation of fibrinogen fraction concentration to thrombin clotting time: fibrinogen in fraction, 78.2% (thrombin technic) and 77.2% (heat technic). (1) Citrate-phosphate buffer, Γ/2, 0.129; pH, 7.2. (2) Michaelis veronal buffer, Γ/2, 0.154; pH, 7.3.

be approximately 0.1-0.2% in an experiment with a fibrinogen fraction of 74% purity. Other investigators tested either plasma or fibrinogen prepared by the classical ammonium sulfate principle and of a purity sometimes not given. Thus, Wöhlisch[1,2] demonstrated an optimum fibrinogen concentration, first at 0.2-0.4% and later at 0.05%. Within the limits of our experimental conditions, the over-all optimum range of the substrate was approximately between 0.1-0.3%, calculated as fibrinogen.

TABLE I.

Thrombin Clotting Time of Fibrinogen Solutions at Various Concentrations: A Summary of 51 Experiments.

Fibrinogen purity %	No. of Prep.	No. of Exp.	Buffer	Clotting time* in seconds ± S.D. % fibrinogen fraction in substrate 1.0	0.5	0.25	0.125	0.062	0.031	0.016
60-69	12	18	M.V.	17.2 ± 2.1	13.9 ± 1.4	13.0 ± 1.0	13.6 ± 0.2	16.5 ± 1.0	24.3 ± 2.8	46.4 ± 8.3
		2	C.P.	27.7 ± 0.9	19.5 ± 2.9	17.4 ± 0.8	17.7 ± 0.2	19.2 ± 0.4	23.4 ± 0.8	31.5 ± 2.1
70-79	19	19	M.V.	20.6 ± 3.4	15.4 ± 2.1	13.4 ± 1.2	13.5 ± 0.9	15.4 ± 1.6	19.9 ± 3.0	31.3 ± 8.4
		1	C.P.	35.0	25.0	20.4	18.6	19.2	22.0	30.8
80-84	3	3	M.V.	23.6 ± 3.4	16.3 ± 1.5	14.6 ± 0.3	14.2 ± 1.7	15.3 ± 2.3	21.1 ± 3.1	38.7 ± 9.0
		8	C.P.	26.5 ± 2.3	20.1 ± 1.1	17.7 ± 0.6	17.5 ± 0.7	18.4 ± 1.1	22.1 ± 1.7	31.0 ± 3.1

* 0.2 cc thrombin added to 0.8 cc of substrate, 37.5°C.
M.V., Michaelis Veronal, pH 7.3, Γ/2 0.154.
C.P., Citrate-Phosphate, pH 7.2, Γ/2 0.129.

In general, the level of the clotting times at concentrations of fibrinogen fraction over 0.25% becomes elevated as the purity of the preparation increases. This elevation in the reaction time cannot be accounted for by the absolute increase in the concentration of fibrinogen. A possible explanation is that further purification of Fraction I resulted in preparations containing relatively more anticoagulant and/or less pro-coagulant factors. The minor components of Fraction I are alpha-, beta-, gamma-globulins and albumin.[5] An antihemophilic "globulin"[8] and an active proteolytic enzyme[9] have also been reported in this fraction. Thus far only the antihemophilic globulin has been reported as having a direct influence on the coagulation. The influence of the other minor components of Fraction I upon the clotting time is being studied in our laboratory.

Summary. Fifty-one experiments on 34 fibrinogen fractions prepared by the low temperature-ethanol principle confirm the reports of those investigators who observed an optimum concentration of substrate for the thrombin-fibrinogen reaction. The results also demonstrate that the level and range of this optimum concentration are determined by the nature of the fibrinogen preparation.

The valuable technical assistance of Mary Beth Everhart is gratefully acknowledged.

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Nutrition of the Mouse. VII. Lactation Performance of Four Strains Maintained on Stock Rations.*

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Highly purified diets adequate to support growth of rats and mice are in common use today. The adequacy of such rations for reproduction and lactation (especially under conditions reducing the opportunity for coprophagy) is still open to some doubt.[1-6] The completeness of a diet for reproduction and lactation can be judged by several criteria, among which the weaning weights of the young and the weight changes of the female during the lactation period have been frequently employed. Too often, however, purified diets have been compared, not with excellent, but with mediocre stock rations. In order to indicate the standards which a synthetic diet must meet, we wish to report our observations with mice of 4 highly inbred strains maintained in our breeding colony on various stock diets.

Methods. The procedure for maintaining the breeding colony has been described previously (Fenton and Cowgill).[6] A commercial ra-

* Supported by grants from the Anna Fuller Fund, and the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.

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