Retitration of 123 local strains of *S. hemolyticus* originally isolated from superficial infections of human beings has raised the percentage of recognizable fibrinolytic strains to 35%. About one-eighth of all apparently non-fibrinolytic strains of *S. hemolyticus* from infections in man, therefore, have a demonstrable fibrinolytic potential, recognizable by the 20-fold enzyme-concentration technic.

By the same enzyme-concentration technique the percentage of strains of *S. hemolyticus* from infections of lower animals positively lytic for human fibrin has been raised from 7% to 22%. None of the 40 local strains of *S. viridans,* however, has shown a demonstrable anti-human fibrinolytic capacity.

7718 C

A Modified Method for the Estimation of Tryptophane.*

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This paper deals with a modification of May and Rose's[1] method for the determination of tryptophane. In their method the protein is dissolved in HCl which contains Ehrlich's reagent, incubated at 35° for 24 hours, and then allowed to stand for an additional 40 hours at room temperature. The blue color which is thus produced is matched against a similar color obtained by treating casein in an identical manner.

In this procedure such factors as the particular preparation of casein used for the standard and the room temperature may be uncontrolled variables. May and Rose assumed the tryptophane content of casein to be 1.5%. It is based on the data of Hopkins and Cole.[2] Other values have been reported.[3] The uncertainty as to the tryptophane content of casein makes this protein of questionable value as a standard.

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[1] May, C. E., and Rose, E. R., *J. Biol. Chem.*, 1922, **54**, 213.

[2] Hopkins, F. G., and Cole, S. W., *J. Physiol.*, 1902, **27**, 418.

[3] Herzfeld, E., *Biochem. Z.*, 1913, **56**, 258, 0.51%; Thomas,P., *Ann. Inst. Past.*, 1920, **34**, 701, 1.7-1.8%; Fürth, O., and Nobel, E., *Biochem. Z.*, 1920, **109**, 103, 2.02%; Folin, O., and Ciocalteau, V., *J. Biol. Chem.*, 1927, **73**, 627, 1.4%; Holm, G. E., and Greenbank, G. R., *J. Am. Chem. Soc.*, 1923, **45**, 1788, 2.24%.

In the present work the attempt was made to use tryptophane for the production of the standard color. It was found that the development of the color does not proceed at the same rate when casein and tryptophane are treated with the reagent. Tryptophane reacts more slowly. The color given by tryptophane is not identical with that obtained when casein is used. At 45° both casein and tryptophane react more rapidly than at either 37° or 30°; however, at 45° both substances show only 60-80% of the maximum color intensity of that which was obtained at either of the 2 lower temperatures. Apparently considerable destruction of tryptophane takes place at 45°. At 30° the color is fairly stable and continues so for a period of 48 hours after reaching the maximum intensity. The present data are not entirely in accord with those reported by Holm and Greenbank.[4] Using the color of the copper ammonium complex as a standard, they found that the same maximum color intensity was obtained irrespective of whether tryptophane was treated with Ehrlich's reagent at 25° or 45°. The maximum color intensity in their experiments appeared in 8 days at 45°, in 12 days at 37°, and in 20 days at 25°. In the present experiments the maximum color was obtained in about 80 hours at 37°, and in 120 hours at 30°.

Herzfeld[5] has shown that the color of the copper ammonium complex salt is similar to that obtained when tryptophane is treated with the Ehrlich reagent. An objection to the use of the former color standard are the fumes of ammonium chloride which are formed when the protein or tryptophane which is treated with the hydrochloric acid-containing reagent is brought near the ammonia-containing copper reagent. In the present experiments it was found that the color produced by reduced phosphomolybdate which is made use of in the determination of phosphorus was quite similar to the color of the condensation product formed when tryptophane is treated with the Ehrlich reagent. For the estimation of tryptophane the blue color given by a standard phosphate solution prepared essentially according to Lohmann and Jendrassik's[6] modification of Fiske and Subbarow's method was adopted as a color standard. The preparation of the phosphate standard was carried out as follows:

1. Molybdate solution. 0.2% solution of ammonium molybdate in 5 N sulfuric acid.

2. Eikonogen solution. 0.5 gm. of Eikonogen (aminonaphthol

[4] Holm, G. E., and Greenbank, G. R., *J. Am. Chem. Soc.*, 1923, **45**, 1788.

[5] Herzfeld, E., *Biochem. Z.*, 1913, **56**, 258.

[6] Lohmann, K., and Jendrassik, L., *Biochem. Z.*, 1926, **178**, 419.

sulfonic acid) is dissolved in a mixture of 195 cc. of 15% $NaHSO_3$ solution and 5 cc. of 20% Na_2SO_3 solution.

3. Standard phosphate solution. 0.4394 gm. of KH_2PO_4 is dissolved in a liter of water, *i. e.*, 1 cc. is equivalent to 0.1 mg. P.

The standard color is prepared by pipetting 10 cc. of the standard phosphate solution, 5 cc. of the ammonium molybdate solution, and 1 cc. of the Eikonogen solution into a 25 cc. volumetric flask, the mixture is warmed in a water bath at 37° for 5 minutes, then cooled to room temperature with tap water, and brought to volume by the addition of distilled water. This solution is diluted 10 times and used as the color standard. It can be accurately reproduced and does not change appreciably over a 24-hour period. The color of the phosphate mixture is just equivalent to the color of a mixture which contained 0.860 mg. of tryptophane dissolved in 10 cc. of distilled water to which was added 2 cc. of 0.2% NaOH solution, 90 cc. of 19% HCl, and 1 cc. of Ehrlich's reagent, and incubated at 30° for 5-6 days, *i. e.*, the period which gave the maximum color intensity. The Ehrlich reagent used was a 5% solution of p-dimethylaminobenzaldehyde in 10% H_2SO_4. In estimating the tryptophane content of casein, about 50 mg. of the protein were brought into solution by warming at about 50° with 2 cc. of 0.2% NaOH.[7] After cooling, 100 cc. of 19% HCl and 1 cc. of the reagent were added and the solution was incubated at 30° until it gave the maximum color intensity.

The tryptophane content of Merck's Hammarsten casein was

TABLE I.
Course of Development of the Tryptophane Color in Various Kinds of Proteins when the Inorganic Color Standard Was Set at 20 on the Colorimeter.

Protein	Days of Incubation 3	4	5	6	8	10	12	Tryptophane content %	Folin and Ciocalteaus Values
Cascin	29.7	23.8	21.6	21.4	21.5	21.6	25.0	1.7	1.4
Edestin	28.6	25.6	23.2	23.4	22.2	22.4	23.9	1.5	1.5
Egg Albumin	31.8	28.4	23.7	23.3	21.8	21.0	20.9	1.3	1.3
Protein of									
Soy Bean*	54.6	36.6	31.5	32.7	30.7	30.4	33.6	1.3	
Silk-worm Pupa*	56.8	33.1	26.8	25.6	23.6	22.1	22.6	1.5	
Muscle Protein of									
Bonito	63.8	46.4	38.8	37.4	33.7	31.0	30.0	1.2	
Whale*	54.2	38.4	33.3	33.5	31.3	28.9	38.1	1.2	
Sardine*	62.8	41.0	34.5	33.8	31.1	29.7	29.4	1.4	
Chicken	50.6	44.6	37.2	35.4	33.3	31.1	30.6	1.1	

*The preparation of these mixed proteins is described in *J. Biochem.* (Japan), 1934, **19**, 345.

[7] Matsuyama, Y., and Mori, T., *Nippon Kagaku Kaishi*, 1923, **44**, 377.

found to be 1.7%. This is somewhat higher than the figure reported by Folin and Ciocalteau.[8] Of the other proteins given in Table I, the percentage of tryptophane in edestin and egg albumin agrees with the values obtained by the latter workers. The data given in Table I show that such mixed proteins as those of muscle should be incubated for a longer period than the more simple proteins in order to obtain the maximum color intensity. This is probably due to the difficulty of effecting complete solution of the protein material which contains muscle fibre. In this connection Thomas[9] has reported that the tryptophane value of casein rose from 1.3 to 1.6% when the Herzfeld[5] method was used, and from 0.6 to 1.78% when the Fasal[10] method was employed. It is obvious, in order to obtain accurate results, that complete solution of the protein in question is a necessary prerequisite. In the case of finely ground proteins which are difficultly soluble, about 8 days of incubation are necessary in order to reach the maximum color intensity. It is advisable that daily comparisons of the sample solution be made after the sixth day in order to ascertain the point of maximum color intensity.

7719 C

Experimental Hypersensitivity to Undenatured H. Pertussis Protein.*

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It has been observed that hypersensitivity to a solution of undenatured *H. pertussis* protein[1] may occur during whooping cough.[2, 3] It was therefore considered of interest to determine whether anaphylaxis could be provoked with this agent in guinea pigs. Sensitization of these animals was attempted by injection of living *H. pertussis,* undenatured *H. pertussis* protein, and sera of animals immunized against *H. pertussis.*

[8] Folin, O., and Ciocalteau, V., *J. Biol. Chem.*, 1927, **73**, 627.

[9] Thomas, P., *Ann. Inst. Past.*, 1920, **34**, 701.

[10] Fasal, H., *Biochem. Z.*, 1912, **44**, 392.

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† National Research Council Fellow in Medicine.

[1] Kreuger, A. P., Nichols, V. C., and Frawley, J. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1097.

[2] Stallings, M., and Nichols, V. C., *Am. J. Dis. Child.*, in press.

[3] Frawley, J. M., Stallings, M., and Nichols, V. C., *J. Pediatrics*, 1934, **4**, 179.