the highest ever obtained with bovine material.

1. Seegers, Walter H., *J. Biol. Chem.*, 1940, v136, 103.

2. Seegers, Walter H., Loomis, Eugene C., and Vandenbelt, J. M., *Arch. Biochem.*, 1945, v6, 85.

3. Ware, Arnold G., and Seegers, Walter H., *J. Biol. Chem.*, 1948, v174, 565.

4. Seegers, Walter H. and Smith, H. P., *J. Biol. Chem.*, 1941, v140, 677.

5. Warner, E. D., DeGowin, Elmer L., and Seegers, Walter H., PROC. SOC. EXP. BIOL. AND MED., 1940, v43, 251.

6. Ware, Arnold G., Guest, M. Mason, and Seegers, Walter H., *Am. J. Physiol.*, 1947, v150, 58.

7. Fahey, John L., Ware, Arnold G., and Seegers, Walter H., *Am. J. Physiol.*, 1948, v154, 122.

Received November 26, 1951. P.S.E.B.M., 1952, v79.

Stability of Purified Hyaluronidase. (19290)

MONROE E. FREEMAN AND MARION E. WEBSTER.

From the Department of Biochemistry, Army Medical Service Graduate School, Washington, D.C.

Highly purified bovine testicular hyaluronidase(1,2) has been found to be quite unstable compared to crude fractions containing less than 1000 turbidity reducing units per mg of nitrogen. McCullagh *et al.*(3) reported inactivation of dried enzyme and aqueous solutions at higher storage temperatures and some stabilization by gelatin and gelatin polypeptides. In this laboratory, however, hyaluronidase suspensions purified to 1,000,000 TRU per mg were rapidly inactivated above 0°C unless specific conditions were imposed. Assays(4) were difficult to evaluate and serious losses were encountered in dialysis, dilution, and lyophilization.

Ranges of pH, ionic strength, temperature, and enzyme concentration that seem to be critical for stability of the highly purified enzyme have been included in this report.

Methods. Hyaluronidase was prepared from fresh bovine testes(1,2) and assayed by the turbidity reducing method(4). Enzyme units were defined by this assay method and enzyme purity has been expressed as TRU per mg N. Enzyme solutions contained merthiolate, 1 to 10,000, and were kept in 0.022 M phosphate-citrate buffer at pH 5.2 containing 0.28% NaCl, ionic strength, 0.10, unless otherwise noted.

Hydrogen Ion Concentration, Table I, was not a critical factor for stability between pH 4.0 and 9.5. However, under less favorable conditions, optimum stability appeared to be between 5 and 7.

TABLE I. Effect of Hydrogen Ion Concentration on Stability of Buffered Hyaluronidase Solutions. Results in TRU/ml.

	Purified hyaluronidase, 60000 TRU per mg N					
pH	3.5	5.5	7	8.5	8.5	9.5
0 day	800	750	950	800	750	920
7	290	855	1020	1030	880	1000
56	200	840	990	930	860	920

Enzyme sol. contained .1 mg protein per ml (of .022 M phosphate-citrate-NaCl buffer solution at −2°C).

Total Protein Concentration, Table II, was found to be the most critical factor, with rapid deterioration apparent at concentrations below 1 mg of total protein per ml. At the same total protein concentrations, purified enzyme was inactivated more rapidly than crude. The high dilutions necessary for assay (4), to about 3 TRU per ml, involved serious errors unless dilutions were made with 0.1% bovine serum albumin solution.

Temperature. Above 0°C the rate of inactivation of hyaluronidase, Table III, was increased with the noteworthy exception that purified enzymes were not more labile at higher temperature if total protein concentrations were high enough.

Buffer Salts and Ionic Strength. This factor played a significant role in the stability of hyaluronidase in aqueous solutions, Table III, and in ethanol suspensions at −2°C, Table IV. Phosphate-citrate buffers were most satisfactory in the presence of .9% NaCl.

TABLE II. Effect of Total Protein Concentration on Stability of Hyaluronidase. Results in TRU/ml.

Protein conc., mg/ml	Crude hyaluronidase, 800 TRU/mg N		Hyaluronidase, 3500 TRU/mg N			Purified hyaluronidase, 48000 TRU/mg N		
	.1	.01	.1	.01	.001	.1	.01	.001
0 day	9.3	1.06	50.2	5.5	.60	750	54.5	6
3	11.4	1.02	50.7	5.7	.30	800	50	1
7	11.3	1.02	50	4.7	.28	810	40.5	.5
56	10.2	.93	35	2.4	.0	643	29	.0

(Stock solutions of the enzyme fractions diluted with .022 M phosphate-citrate-NaCl, ionic strength—.10 at pH 5.2).

TABLE III. Effect of Temperature on Stability of Hyaluronidase Solutions. Results in TRU/ml.

Temp.	.01 mg protein/ml, buffered*				.1 mg total protein/ml, unbuffered			
	Crude enzyme, 800 TRU/mg N		Purified enzyme, 49000 TRU/mg N		Crude enzyme, 800 TRU/mg N		Purified enzyme, 55000 TRU/mg N	
	20	38	20	38	20	38	20	38
0 day	1.24	1.16	46.5	45.2	13.5	12.9	855	855
1	1.17	.89	53.5	52.8	12.4	10.7	800	250
3	1.14	.68	45.2	41	6.2	.0	423	94
28	.64	.0	23.4	.0	.0	.0	200	0

* In .022 M phosphate-citrate-NaCl, pH 5.2, ionic strength .10.

TABLE IV. Effect of Buffer Salts on Stability of Ethanolic Solutions of Purified Hyaluronidase. Results in TRU/ml.

Conc.	Phosphate citrate NaCl, .7 M	NaCl, .15 M	Phosphate citrate, .022 M	HOH	NaAc, .1 M	Borate, .1 M	Phosphate, .1 M
0 day	310	330	270	270	270	290	320
3	340	220	200	250	140	160	140

Purified enzyme, 60000 TRU per ml, suspended in buffer sol., .1 mg protein per ml, at pH 5.2, except borate buffer, pH 7. Solutions cooled to −2°C. Cold ethanol added to .3 mole fraction. (Enzyme conc. at 0 time was 280 to 310 TRU per ml).

TABLE V. Effect of Ethanol on Buffered Solutions of Purified Hyaluronidase at 20°C. Results in TRU/ml.

Conc. ethanol	.1 mole fraction	.15 mole fraction	.2 mole fraction
0 hr	900	760	400
2	800	690	100
24	900	210	10
72	870	155	0

.10 mg protein/ml (of phosphate-citrate-NaCl buffer, ionic strength .10, and pH 5.2).

Purified enzymes could not be dialyzed against distilled water at 0°C without losses as high as 50%; but the same enzymes were satisfactorily dialyzed against 0.9% NaCl, shell frozen, lyophilized, and stored at room temperature for two years without significant loss.

Ethanol concentration. Concentrations up to 0.3 mole fraction, commonly used for purification, rapidly inactivated the enzyme at 20°C, Table V; but caused only minor losses at −2°C, Table IV.

Agitation. Shaking, suction filtration with excessive foaming, and other manipulations that favor a large free surface area usually resulted in significant losses. Five ml of enzyme, 800 TRU per mg N and 0.1 mg protein per ml, shaken for 15 minutes at room temperature by machine were completely inactivated in air as well as in nitrogen. Added albumin, 2 mg per ml, or high concentration of crude enzyme, 12 mg per ml, served to reduce these losses to much smaller value.

Summary. Critical conditions, including pH, total protein concentration, temperature, buffer salts, and ethanol concentrations (for puri-

fication), have been indicated for the stability of purified hyaluronidase.

1. Freeman, M. E., Anderson, P., Oberg, M., and Dorfman, A., *J. Biol. Chem.*, 1949, v180, 655.

2. Freeman, M. E., Anderson, P., Webster, M., and Dorfman, A., *J. Biol. Chem.*, 1950, v186, 201.

3. McCullagh, D. Roy, Cassidy, J. W., Valentine, F., and Tolksdorf, S., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 295.

4. Dorfman, A., and Ott, M. L., *J. Biol. Chem.*, 1948, v172, 367.

Received November 1, 1951. P.S.E.B.M., 1952, v79.

Diurnal Variations in Acuity of Sense of Taste for Sodium Chloride.*(19291)

DONA L. IRVIN AND FRANZ R. GOETZL.

From the Institute of Medical Research, Permanente Foundation Hospital, Oakland, Calif.

In normal individuals, diurnal variations exist in acuity of olfaction(1,2) and in acuity of the sense of taste for sucrose(3). The pattern of these variations is remarkably uniform and intimately connected with food intake. Freely selected meals are preceded by a period of increasing and followed by one of decreasing acuity of the senses mentioned. When a meal has been omitted a decrease in acuity cannot be demonstrated. The precibal increase in acuity of olfaction and precibal increase in acuity of the sense of taste for sucrose may represent measurable accompaniments of sensations interpreted as desire for food and the postcibal decrease in acuity of olfaction and postcibal decrease in acuity of the sense of taste for sucrose may represent measurable accompaniments of sensations interpreted as satiety afforded by food. The demonstration of the existence of diurnal variations in acuity of the sense of taste for sucrose made it appear desirable to determine whether or not like variations exist with respect to other taste qualities. The present experiments were undertaken with the purpose of determining whether or not such variations exist with respect to the sense of taste for sodium chloride.

Method and procedure. Gustatory thresholds for sodium chloride were determined by placing a measured volume (0.5 cc) of sodium chloride solution on the tip of the subject's extended tongue. The solutions used had been made up with tap water and contained sodium chloride in concentrations ranging from 0.05% to 0.55% in steps of 0.05%. The lowest concentration of sodium chloride which just sufficed to permit instantaneous recognition of saltiness was interpreted as the measure of threshold for this taste quality for the subject at the time. At each determination the threshold value accepted for the subject was the lowest concentration of sodium chloride which produced the sensation of saltiness 3 times in succession. Before and between successive trials the subjects were requested to rinse their mouths with tap water. In this manner gustatory thresholds for sodium chloride were determined at 10:00 and 11:30 in the morning and at 1:30, 3:00 and 4:30 in the afternoon. Subjects exhibiting coating of the tongue or taking medicine for any reason were excluded temporarily from the experiment. During tests the subjects were kept uninformed as to the concentration of the sodium chloride solutions used. There were 8 individuals, 7 females and 1 male, who served as subjects. They were in apparently good health and ranged in age from 23 to 45 years. They held clerical positions in this institution and worked daily from 9:00 in the morning until 5:00 in the evening. As a rule they had breakfast and dinner at home at customary hours, but ate lunch in the hospital cafeteria which offered a variety of dishes so as to permit reasonably free selection of food. Lunch was served between 12 and 1:00 in the afternoon. On

* This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and assigned No. 361. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.