CRYSTALLINE SALMON PEPSIN*

A GLOBULIN type protein possessing high proteolytic activity has been isolated in crystalline form as colorless needles from the stomach mucosa of the Pacific Coast king salmon (*Oncorhynchus tschawytscha*). The specific proteolytic activity, as measured by the Hb method,[1] is comparable with that of crystalline mammalian pepsin. The activity decreases with lowering temperature, the decrease obeying the Arrhenius equation between 37° and 0° C., which more than covers the temperature range of the salmon's natural environment. The optimum pH for the digestion of Hb is near pH 2.2, which corresponds to that of Northrop's crystalline pepsin.[2] As determined by the minimum solubility of the amorphous form, the isoelectric point lies close to pH 1.9.

Identical potency and crystalline form are obtained either by direct acid extraction or by alkaline extraction of the zymogen and subsequent conversion to pepsin by acid. The latter method has been found to give a much greater yield and is now being used as follows: The ground mucosa is mixed with three times its weight of water and brought to a pH of 8.0–8.2 with $NaHCO_3$ for extraction. This mixture is strained through cloth and filtered; the extract is then acidified with 2N H_2SO_4 to a pH 1.8–1.9 and the solution filtered with the aid of Hyflow Super Cell to remove denatured proteins. Solid $(NH_4)_2SO_4$ is added to 0.6 saturation to precipitate the enzyme, which is filtered off. The precipitate is dissolved in M/10 acetate pH 4.2 and the solution clarified by filtration. Precipitation with $(NH_4)_2SO_4$ at pH 1.8–1.9 is repeated twice more. The final precipitate is dissolved in a minimum volume of M/10 acetate pH 5.0, centrifuged quickly, and allowed to stand at room temperature. Crystals begin to form in a few hours, the process being facilitated by seeding and stirring.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

DETERMINATION OF THE VELOCITY OF SOUND IN A GAS; APPLICATION TO ANALYSIS OF MIXTURES OF HELIUM, OXYGEN AND NITROGEN

SINCE the weight of helium is approximately one seventh of that of nitrogen, variations in the volumetric percentages of these two gases in a mixture affect the density, hence are reflected in changes in the velocity of sound in the mixture. The amount of oxygen present in a mixture of gases can be determined by any standard method. Determination of the velocity of sound in a mixture of helium, oxygen and nitrogen, together with an analysis for oxygen, makes it possible to determine the volumetric percentages of all three gases in the mixture.

An apparatus for determining the velocity of sound in a gas has been constructed which is particularly suited for mixtures of helium, nitrogen and oxygen (Fig. 1). A glass tube 5 feet long, 1 inch in diameter, flared at the top to a diameter of 2 inches, is placed in the vertical position. The upper end is covered with tightly stretched rubber dam. Near the upper end are side tubes for inflow and outflow of gas. The lower end is sealed. Near the lower end is a side tube for inflow and outflow of water, to enable one to alter the length of the column of gas in the tube. A small jar for saturating the incoming gas with water vapor is placed in the side tube through which the gas enters. A rubber bag, provided with a rubber escape valve, is attached to the side tube for outflow of gas. Thorough flushing out of the apparatus is essential. If a sample

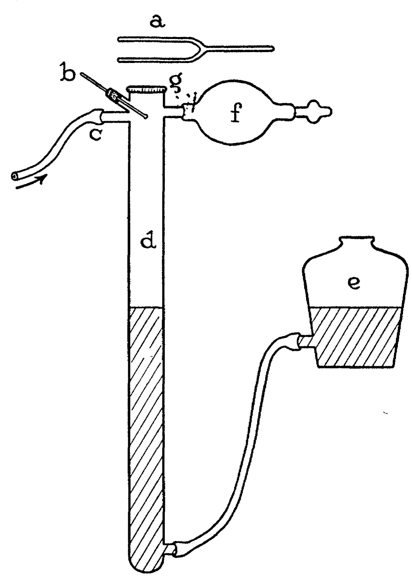

FIG. 1. Diagram of apparatus used in determining the velocity of sound in a gas. *a*. Tuning fork; *b*, thermometer, which can be placed either as indicated or adjacent to apparatus; *c*, side tube for inflow of gas which has been saturated with water vapor by passing it over water; *d*, glass tube. A slight increase in resonance is obtained if the upper end of this tube is flared to a diameter of approximately 2 inches; *e*, water reservoir, connected by rubber tubing to side tube at lower end of apparatus; *f*, rubber bag, provided with rubber escape valve; *g*, rubber diaphragm over upper end of glass tube.

* Contribution No. 89 from the Oceanographic Laboratories.

[1] M. L. Anson, *Jour. Gen. Physiol.*, 22: 79, 1938.

[2] R. M. Herriot and J. H. Northrop, *Jour. Gen. Physiol.*, 18: 35, 1934.

of limited quantity is to be analyzed, the apparatus can be clamped shut at the proper moment. A tuning fork whose frequency of vibration per second is 512 is placed over the upper end of the tube. By striking the tuning fork and raising and lowering the column of water, points of resonance (accentuation of the hum of the tuning fork) are found. The distance between two adjacent points of resonance is half of one wave-length. This distance times two, multiplied by 512 (the frequency of vibration of the tuning fork) equals the velocity of sound per second. A small correction, which is rarely significant, is made in the velocity for variation in laboratory temperature (Table 1);

TABLE 1

Correction of Velocity of Sound for Variations of Temperature from 24° C

Velocity of sound, meters per second	to 650	750	850	900 and over
1° C. equals (meters per second)	± 0.5	±0.14	± 0.3	special corrections (see below)
Add or subtract with rise in temperature	subtract	subtract	add	add

Special corrections for velocities of 900 meters per second and over:

18° C.	19	20	21	22	23	24	25	26	27	28	29	30
−4.1	−3.6	−3.0	−2.4	−1.7	−0.9	0.0	+1.1	+2.2	+3.5	+5.0	+6.7	+8.5

"−" indicates substraction for correction to 24° C.; "+" indicates addition.

changes in the temperature affect the velocity by changing the density of the gases and by changing the amount of water vapor in the mixture.

The formula for the velocity of sound in a gas is

$$V = \sqrt{\frac{\gamma P}{d}},$$

where

V = velocity of sound in centimeters per second;
γ = Cp/Cv = ratio of specific heat at constant pressure to that at constant volume (γ for oxygen is 1.401; for nitrogen it is 1.404; for helium it is 1.66);
P = pressure in dynes per square centimeter; and
d = weight of gas in grams per cubic centimeter.

A chart (Fig. 2) has been constructed on the basis of computation of the theoretical velocity of sound for many different mixtures of helium, oxygen and nitrogen. In using the chart one can read horizontally from the experimentally determined velocity of sound, corrected for temperature, to the determined percentage of oxygen, which may have to be interpolated, thence vertically to the percentages of helium and nitrogen in that portion of the gas remaining after deducting the oxygen. In practice, a large-sized replica of Fig. 2 on graph paper is used.

No attempt has been made to control all the sources of error, most of which are small and may offset one another. It is possible to determine the velocity of sound with our apparatus with an accuracy of approximately ± 0.5 per cent. (range of variability) and to determine the percentages of helium and nitrogen in the whole mixture with an accuracy of approximately ± 1.0 per cent. (range of variability). The apparatus and method are intended for practical use in medicine or industry where this degree of accuracy is satisfactory. The apparatus is inexpensive, mobile, simple and easy to use.

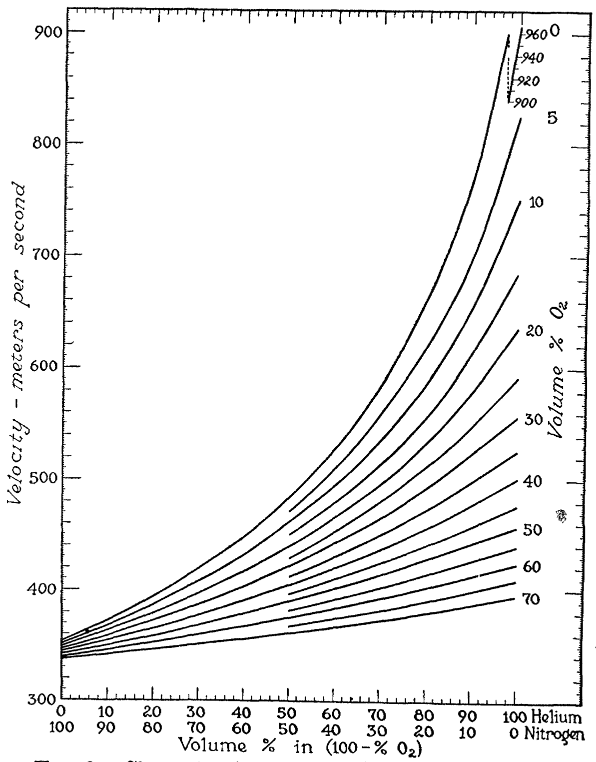

Fig. 2. Chart showing the velocity of sound at 24° C. in mixtures of helium, oxygen and nitrogen, saturated with water vapor.

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