

## 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 tshawytscha*). The specific proteolytic activity, as measured by the Hb method,<sup>1</sup> 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.<sup>2</sup> 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<sub>3</sub> for extraction. This mixture is strained through cloth and filtered; the extract is then acidified with 2N H<sub>2</sub>SO<sub>4</sub> to a pH 1.8–1.9 and the solution filtered with the aid of Hyflow Super Cell to remove denatured proteins. Solid (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 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<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 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

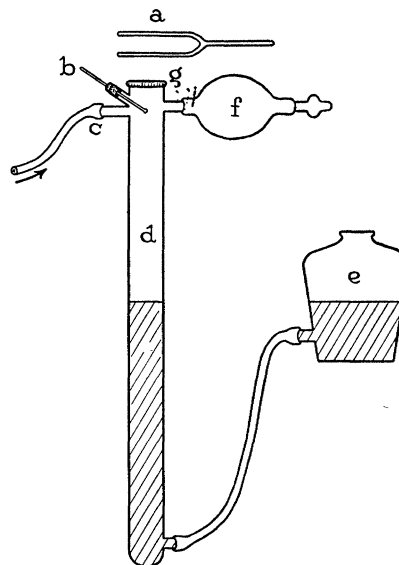


FIG. 1. Diagram of apparatus used in determining the velocity of sound in a gas. *a*, Tuning fork; *b*, thermometer, which can be placed either as indicated or adjacent to apparatus; *c*, side tube for inflow of gas which has been saturated with water vapor by passing it over water; *d*, glass tube. 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.

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<sup>1</sup> M. L. Anson, *Jour. Gen. Physiol.*, 22: 79, 1938.

<sup>2</sup> R. M. Herriot and J. H. Northrop, *Jour. Gen. Physiol.*, 18: 35, 1934.