

ponent with those of the virus complex^{4,6} reveals pronounced differences. Further, in the degradation of the virus complex by any means yet studied, no material with $S_{20}^0 = \text{ca } 70 \times 10^{-13} \text{ cm sec}^{-1} \text{ dynes}^{-1}$ has been seen. It appears reasonable to conclude that the lighter component in diseased embryo tissue is identical with that of normal chick embryo tissue and as such is not specifically concerned with the disease process associated with the virus.

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CRYSTALLINE CATALASE FROM BEEF ERYTHROCYTES

As early as 1911 Wolff and de Stoecklin¹ obtained rather highly purified catalase solutions from erythrocytes (species not stated). In 1923 Tsuchihashi² described a method for the purification of the horse erythrocyte catalase.

In our laboratory Tria³ made considerable progress in purifying the catalase of beef blood, and recently A. L. Dounce⁴ obtained this catalase in crystalline condition. But Dounce's method is time-consuming and the yield is very small.

We have prepared crystalline catalase from washed and laked cow erythrocytes by the following steps:

1. Adsorption on aluminum hydroxide⁵ at pH 5.7, repeated washing of the adsorption complex with dilute acetate buffer pH 5.5, and elution with M/10 phosphate buffer at pH 8.0.

2. Precipitation at pH 5.7 with 30 gm ammonium sulfate for every 100 cc of enzyme solution. This

precipitation was then repeated, using one tenth of the initial volume.

3. Dialysis, followed by precipitation at pH 5.5 by enough alcohol to make 40 per cent. concentration.

4. Solution in 1.25 per cent. NaCl and adsorption at pH 5.5 on $\text{Al}/\text{OH}/_3$ followed by elution with phosphate buffer pH 8.0 (no preliminary washing).

5. Precipitation of the eluted catalase with 30 gm of solid ammonium sulfate per 100 cc, at pH 5.7. The precipitate is mixed with a minute amount of water, centrifuged and allowed to stand in the ice-chest. Crystals form. The yield is increased by adding saturated ammonium sulfate slowly.

The catalase obtained by this method had a "Kat.f" of 43,000 and the crystals had the shape of small needles. After a second recrystallization from ammonium sulfate solution the crystals were mostly plates, while the "Kat.f." was 48,000. The iron content was 0.12 per cent. The visible absorption spectrum is identical with that for liver catalase.

Unlike crystalline beef liver catalase, erythrocyte catalase gives no blue color upon treatment with acetone and hydrochloric acid, but a reddish-brown color. The reddish-brown material has been identified as hemin.

These preliminary findings indicate that beef erythrocyte catalase differs from beef liver catalase in possessing a greater activity and in lacking prosthetic groups which furnish the "blue substance" or biliverdin.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A GRINDER FOR HOMOGENIZING BACTERIAL CLUMPS OR INFECTED TISSUES

THE problem of preparing homogeneous suspensions of acid-fast bacteria, infected tissues or small amounts of various substances in the laboratory has long since resulted in the production of various grinders to facilitate the process. Recently, the need arose for a considerable supply of sterile grinding devices for homogenization of leprosy nodules to recover the bacilli. It was found that remarkably com-

plete homogenization and suspension of subcutaneous nodules, after they were cut down to small pieces by means of scissors, could be brought about in Pyrex grinders of the type illustrated in Fig. 1.

The important features in the construction of such grinders are: (a) selection of pairs of tubes so that one fits closely within the other, (b) careful boring of the holes in the rubber stoppers which join the inner grinding tube to the glass-shaft, and (c) grinding of the paired tubes with emery powder in water to produce roughened surfaces which rotate against each other without letting the small bits of tissue lodge where they may escape grinding. Due to the type of joint used, the inner grinding tube never rotates perfectly on its axis, but has a wobble which causes it to scour the walls of the outer tube. The rotating shaft

¹ J. Wolff and E. de Stoecklin, *Compt. Rend. Acad. Sci.*, 152: 729, 1911.

² M. Tsuchihashi, *Biochem. Zeitschr.*, 140: 63, 1923.

³ E. Tria, unpublished.

⁴ A. L. Dounce, unpublished.

⁵ G. Tracey and W. H. Welker, *Jour. Biol. Chem.*, 22: 55, 1915.

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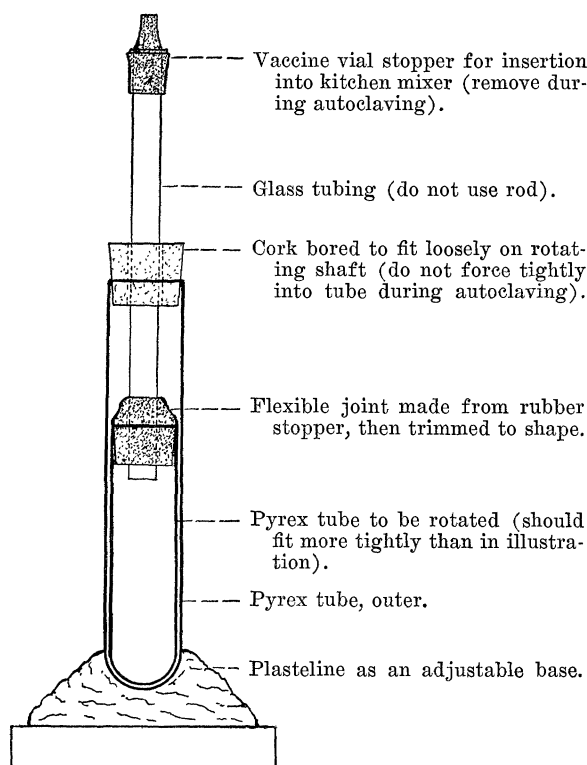


FIG. 1. Grinding Device ($\frac{1}{2}$ natural size). The outer tube is plugged with cotton and sterilized separately to facilitate loading with cultures or tissues. The inner tube assembly is sterilized in a full-length test tube and transferred into the outer tube aseptically when the materials are ready for grinding. Since each pair of inner and outer tubes is ground together, the tubes are given the same number to prevent accidental interchanges.

is hollow to permit expansion of gases during autoclaving, which should be done with the small rubber cap removed. A standard kitchen mixer is used to rotate the grinding tube, but the terminal fittings for this tube can readily be adapted to any source of power. The size of the tubes and the relative length of the inner tube can be varied to suit almost any amount of material.

Due to the large grinding surfaces, the diluting fluids can be added rapidly by simply sliding the sterile cork up the shaft, rotating at low speed while fluid is added, and then giving a final brief spin while the inner tube is raised and lowered in the outer one a few times. Removal of the inner tube and substitution of sterile stoppers into the flamed mouth of the outer tube permits ready access to the suspensions.

The amount of silica liberated through the abrasive action of the two grinding tubes varies with the conditions of operation. If the two tubes are separated by water only, they produce suspensions of silica which are fine enough to remain in virtually complete

suspension for considerable periods of time. When cultures of acid-fast bacteria are being ground to a smooth paste, the tubes are lubricated by the bacteria and do not liberate perceptible amounts of silica. The amount that is liberated in the grinding of tissues is influenced by the amount of tissue (more tissue, less silica) and by the period of grinding. Best results are obtained with just enough fluid or material to fill the space between the tubes. For some purposes silica suspensions may be desired and for others they must be guarded against.

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DENATURATION OF EGG ALBUMIN BY PRESSURE

BRIDGMAN'S¹ observation that the white of egg becomes coagulated by high hydrostatic pressure suggested that egg albumin is denatured by pressure. Late in 1939 and early in 1940 the writers made a preliminary study of the subject of the exposure of -SH linkages by high pressures to verify the effects of denaturation by pressure.

Twenty grams of Merck's impalpable powdered egg albumin were dissolved in 500 milliliters of water and the mixture dialyzed under toluene, filtered four times through a filter cell, twice through filter paper and stored finally under toluene. Ten cc samples were subjected to pressures ranging from 1,000 to 7,500 kg/cm², the samples being submerged under sterile mineral oil in the pressure chamber to separate from the pressure transmitting liquid. In every case the solutions were coagulated by the pressure treatment, the amount of coagulation becoming more copious the higher the pressure.

The exposure of -SH linkages was verified in two ways after the pressure treatment. Using the 2-6 dichlorophenolindophenol dye in the manner of Todrick and Walker² it was observed that the bleaching effect increased with the magnitude of the pressure used for the treatments. Some of the tests were made using the Kassel and Brand³ modification of Folin and Marenzi's⁴ technique. These also showed exposure of -SH groups. Circumstances did not permit further quantitative study of these interesting preliminary observations.

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¹ P. W. Bridgman, *Jour. Biol. Chem.*, 19: 511, 1914.

² Todrick and Walker, *Biochem. Jour.*, 31: 292, 1937.

³ Kassel and Brand, *Jour. Biol. Chem.*, 125: 115, 1938.

⁴ Folin and Marenzi, *Jour. Biol. Chem.*, 83: 103, 1929.