

parasitization, soon led to the development of more profound intravascular pathology, namely, the formation of sticky masses or clumps of red cells. These clumps or agglutinations were seen first only in the venous stream, were small (three to four red cells) and possessed but little intrinsic cohesiveness. As these clumps flowed into larger veins the shearing forces to which they were exposed broke them up. During the early stages of clump formation transient thromboses occurred and the flow rate was markedly retarded to sluggish in many areas.

In the subsequent 24 to 48 hours the usual picture was that of progressive intravascular clumping with the formation of larger agglutinated masses, more permanent thromboses and increasing tissue damage. The clumps or agglutinations now could withstand intravascular stresses as they circulate throughout the organism, appearing occasionally in arterioles. Streamlining was completely disrupted even in the large veins draining the area under observation. The viscosity of the plasma increased. This was demonstrated by the appearance of resistance met by a free red cell as it turned over in its path down stream and entered successively larger currents. These intravascular changes together with the intermingling of broken red cell clumps, free red and white cells and a rare white cell clump reminded one investigator of "sludge." This term vividly pictures the very marked intravascular

pathology little evidence of which would be found with routine autopsy methods.

The circulatory damage resulting from these intravascular pathological changes together with the increasing parasitization precipitated a stage of generalized clumping which represented a status of irreversible pathology progressive to eventual circulatory failure. The clumps or agglutinated red cell masses, which were initially formed by parasitized red cells only and later by both infected and normal red cells, were less fragile than previously and showed marked intrinsic cohesiveness. The clumps stuck to one another and would, therefore, stick to the phagocytes of spleen, liver and bone marrow. However, their great size presented a mechanical difficulty that the phagocytes could not overcome (this fact has been demonstrated by M. H. Knisely in unpublished data). White blood cells stuck to the endothelium in layers, plasma leaking and skimming were marked, the paste-like blood flowed very sluggishly and thromboses became numerous. There was a further increase in the viscosity of the plasma subsequent to the marked plasma leaking. These circulatory changes were regularly followed by the death of the bird within a few hours.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE PREPARATION OF A SUCRASE-FREE TAKA-MALTASE

TAKA-DIASTASE is, as is known, a mixture of different enzymes including maltose and sucrose-splitting factors.

Leibowitz¹ advanced the theory of a specific glucumaltase in taka-diastring distinct from taka-sucrase.² This view was opposed by Weidenhagen,³ who postulated the existence of only one maltase identical with glucumaltase. Leibowitz and Hestrin^{4,5} found a way to differentiate between the two disaccharide-hydrolyzing capacities of the taka-diastring owing to the greater thermostability and acid stability of the maltose-splitting factor.

The present paper describes a simple method for preparing maltase free from sucrase from the commercial taka-diastring (Parke, Davis and Company). The lability of taka-sucrase to reducing agents $N_2S_2O_4$, reported in a previous communication,⁵ is exploited for this purpose.

¹ J. Leibowitz, *Zs. physiol. chem.*, 149: 184, 1925; J. Leibowitz and P. Mechlinsky, *ibid.*, 154: 64, 1926.

² Cf. Sh. Hestrin, *Enzymologia*, 6: 193, 1940.

³ R. Weidenhagen, *Ergeb. Enzymforsch.*, 1: 169, 1932; *Zs. physiol. chem.*, 216: 255, 1933.

2 gr of the commercial sample of taka-diastring were dissolved in 15 ml distilled water in a Cellophane bag and the solution under toluene dialyzed at room temperature for one day against running tap water and one day against distilled water, which was changed several times, until the solution is free of reducing substances.

The dialyzed solution (about 30 ml) was filtered into a glass-stoppered flask and mixed with 0.3 gr of sodium hydrosulfite. After 24 hours at room temperature, the $Na_2S_2O_4$ is removed by dialyzing the solution in a Cellophane bag for one day against running tap water and one day against distilled water, which is changed several times.

The dialyzed solution, showing only maltase activity, was highly diluted. To concentrate, dialysis against a 45 per cent. dextrine solution according to the method of Guy E. Youngburg⁶ was attempted, but this method proved generally impracticable because of the reducing substances diffused in the concentrated solution from the dextrine. We succeeded

⁴ J. Leibowitz and Sh. Hestrin, *Nature*, 141: 552, 1938; 143: 339, 1939.

⁵ J. Feigenbaum, *Biochem. Jour.* (1942) in press.

⁶ Guy E. Youngburg, *SCIENCE*, 94: 498, 1941.

in finding a simple method of concentration, using 96 per cent. ethyl alcohol as the dialyzing liquid or "outside solution."

According to this method, 40 ml of the taka-diastase solution free from $\text{Na}_2\text{S}_2\text{O}_4$ is concentrated by dialyzing through a Cellophane bag for about six hours against 96 per cent. alcohol as "outside solution," the alcohol being changed two or three times.

During the concentration, part of the enzyme was precipitated and deposited on the Cellophane. After concentration the small residual solution (about 3 to 5 ml) was precipitated by the same volume of absolute alcohol, centrifugated and, together with the Cellophane bag (containing very active substance) dried in the desiccator over H_2SO_4 . The yield was about 50 mg, *i.e.*, 2.5 per cent. of the original substance.

Using the same method, we concentrated solutions of the commercial taka-diastase without any treatment by reductants (which required only half the time of the concentration of the taka treated by $\text{Na}_2\text{S}_2\text{O}_4$). The yield was about 3 per cent. of the commercial product. Owing to the small quantity of the taka-diastase it sometimes happened that all the substance was precipitated and deposited on the Cellophane. In this case, the substance after drying was either carefully separated from the Cellophane, or pieces of the Cellophane containing the enzyme were placed in water and filtered off after the substance had dissolved. The substance precipitated on the Cellophane was even more active than the substance precipitated from the concentrated solution by absolute alcohol.

The dried product was tested for activity on maltose and sucrose. It was found that the product retained the full maltose activity splitting power of the original preparation but was practically inactive on sucrose.

This confirms the theory that taka-maltase and taka-sucrase are two distinct enzymes.

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A PLATINUM SCOOP FOR TRANSFERRING STERILE POWDERS

THE transfer of small quantities of sterile powder or chemicals to another container or a medium usually is accomplished with a loop or with a pipette having a wide bore. By such a procedure some powder usually is spilled or scattered on the table, which is obviously undesirable. To overcome this inconvenience, the writer has devised a platinum scoop (shovel) which will accomplish conveniently the transfer of powder from a container or test-tube to another container or a culture medium.

The scoop is made by folding a piece of platinum

sheet into a U-shaped shovel which is attached with a platinum wire, a copper wire or lead glass to an inoculating needle holder. Fig. 1 illustrates three sizes: (a) $24 \times 5 \times 2$ mm; (b) $25 \times 5 \times 3$ mm; (c) $20 \times 10 \times 3$ mm. Scoop (a) will hold about 0.17 grams of starch powder; (b), 0.23 grams; and (c), 0.45 grams.

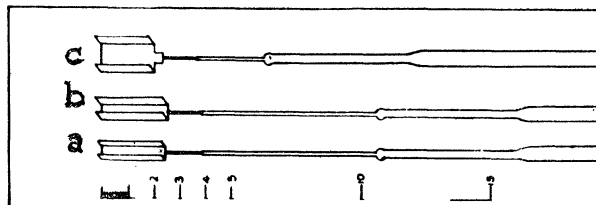


FIG. 1. Platinum scoops (a) $24 \times 5 \times 2$ mm; (b) $25 \times 5 \times 3$ mm; (c) $20 \times 10 \times 3$ mm.

This scoop has been successfully used by the writer in adding sterile rice starch and starch and charcoal to various media for culturing *Endamoeba histolytica* and *Trichomonas vaginalis*. These scoops can be used also in analytic chemical weighings, and have several advantages over glass or metal spatulas.

This simple apparatus has the advantages of being readily sterilized by flame and requiring only a few seconds for cooling. Because of this, the transfer of sterile powder and chemicals is conveniently and neatly accomplished for a large number of culture tubes or containers in a short period of time.

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