A PURIFIED ENZYME PREPARATION OBTAINED FROM SOVIET PANCREATIN AND ITS USE FOR TISSUE "TRYPSINIZATION"

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The preparation trypsin-Difco [4, 5], which is not highly purified trypsin, but a mixture of the latter, chymotrypsin and certain other pancreatic enzymes, is usually used in the preparation of cell suspensions for tissue cultures to be used for tissue "trypsinization". Therefore, the terms "trypsin" and "trypsinization" are in this case conditional. Recently, an extract of nonpurified pancreatin [1], the dosage of which was not determined according to its activity, has been used for tissue "trypsinization", but, as our works have demonstrated, the activity and the protein nitrogen content of pancreatin extracts vary considerably from series to series. For example, we found that the activity of 10% aqueous extracts of pancreatin, determined according to the Chow-Peticolas method [3], ranged from 2,000 to 15,000 Chow/ml (Chow units per 1 ml).

We took on the task of developing a simple, quick and practicable method of obtaining purified preparations containing trypsin and other pancreatic enzymes, the activity of which would only vary slightly.

We proposed a scheme for obtaining a purified enzyme preparation by processing the pancreatin extract twice with activated charcoal (brand A, All-Union State Standard 4453-48).

We used the Chow-Peticolas method [3], which is based on turbidimetric determination of the degree of casein proteolysis, to demonstrate proteinase activity. As the Chow unit, we took the amount of the enzyme which could in 15 minutes (at 37-38°) half of the equal volume of a 0.25% casein solution into a state of unprecipitability by 5% trichloracetic acid.

The casein concentration was determined according to the absorption of light by its suspensions. The protein nitrogen was determined by Kjeldahl's method.

The original pancreatin preparation (series No. 35) was obtained from A. I. Mikoyan's Myasokombinat [Meat combine, literally].

Method of Preparing Preparation. One hundred and twenty-five g dry pancreatin was suspended in 1 liter distilled water; the suspension was shaken thoroughly for an hour, and the sediment was separated by centrifugation. The supernatant liquor containing 100-150 mg % protein nitrogen and 2000-2500 Chow/ml.

We added 30 g activated charcoal (first washed with distilled water) to 1 liter of the supernatant liquor, shook the mixture for 10 minutes and separated the sediment by centrifugation. The operation with the charcoal was then repeated. After the second separation of the charcoal, the solution was clarified by filtering through a 2-3 cm layer of paper pulp on a Buchner's funnel. The clarified solution contained 30-50 mg% protein nitrogen and 750-1800 Chow/ml; the pH of the solution was 5.0-6.5.

The solution was subjected to sterilizing filtration through F-5 candles, after which it was poured into ampules and stored at +4°. Neither the sterilizing process nor 6-month storage caused any significant decrease in the proteinase activity.

The activity of this enzyme preparation is essentially the same as that of crystalline trypsin and chymotrypsin. Each purification cycle requires only one workind day and a few readily available reagents. The preparation can be stored for 6 months (observation period). Before use, the enzyme preparation is poured out of its ampoule and diluted with a sterile Henke's saline solution (pH = 7.2-7.6) to the necessary activity (50-100 Chow/ml). This working solution will remain suitable for use for 2-3 weeks at +4°.

For tissue "trypsinization", small pieces of organs taken from animals under sterile conditions are rinsed free of blood in a Henke's saline solution to which 50-100 units of penicillin per 1 ml of fluid has been added, cut into pieces 1 mm^3 in size with a razor and then again rinsed in the saline solution. The pieces are then transferred to a flask; the working solution of "trypsin" is poured over them in a ratio of 5:1, and the combination is shaken up in a shuttle-apparatus for 15 minutes at 36-37°. After the pieces have settled to the bottom of the flask, the liquid portion containing blood and scraps of tissue is removed and replaced with the same amount of fresh "trypsin" solution; the flask is then shaken again for 30-60 minutes. The length of the "trypsinization" process depends on the age and type of the animals, as well as on the structure of the organ. One and a half to two hours are required to "trypsinize" the tissues of adult animals (the kidneys of an adult rabbit, for example).

Human embryonal tissue breaks down into cells in 30-60 minutes. After the "trypsinization", the contents of the flask are centrifuged for 5 minutes (1000 rpm) at room temperature. The supernatant liquor is discarded, and the sediment is resuspended in a fluid nutrient mixture containing, besides Henke's saline solution (50%), human or horse serum (25%) and rabbit extract of the spleen and bone marrow in a dilution of 1:50 (25%). The nutrient mixture, in which cells are suspended individually and in complexes of 10-20, is poured in amounts of 1.5 ml into test tubes. Test tubes of neutral glass are preferable for this purpose. Mica flakes, 5 x 1.5 cm in size, are bent into a U-shape so that they conform almost exactly to the test tube walls and inserted into the test tubes before the sterilization of the latter to aid in the ensuing detailed morphological study of the "trypsinized" tissue cultures.

During the first 24 hours, cultivation at 36-37° is carried out with the test tubes in a semi-horizontal position. During this period, the cells settle on and adhere to the test tube walls. On the second day of cultivation, the test tubes are put into a drum [see 2] rotating at a rate of 16 rev/hour. When prolonged cultivation of the tissues is required, the "trypsin" solution is also used to produce transplants. For this purpose, the solution is heated to 36°, poured into the test tubes with the cells, from which the nutrient mixture has been first removed, and left for 5 minutes at 36° (can also be left at room temperature if the period of incubation with the "trypsin" solution is lengthened correspondingly). The test tubes are shaken up so that the cells are dislodged from the test tube walls, in sheets or cell complexes in the case of epithelial tissue growth, and usually in single cells when there is fibroblastic growth. The suspension is poured off into centrifuge test tubes and centrifuged under the conditions described above. The supernatant liquor is discarded, and the sediment is transferred to the nutrient mixture, the material of each test tube being divided into 2 or 3 parts depending on the previous growth of the cells.

The use of an enzyme preparation, the dosage of which can be exactly determined according to its proteolytic activity, makes it possible to obtain reproducible results.

Our "trypsin" enzyme preparation is as strong as trypsin-Difco in ability to "disperse" cells and can be effectively used in the practice of tissue cultivation.

SUMMARY

The authors obtained an enzyme preparation - a mixture of tryspin, chymotrypspin and other pancreatic enzymes from the extract of Soviet pancreatin by subjecting it to double purification through activated charcoal. The activity of the resulting preparation approaches that of the crystalline tryspin and chymotryspin (750-1800 Chow Units/m and 30-50 mg% protein nitrogen). The enzyme preparation may be stored in solution in ampules for a period of 6 months at a temperature of +4°C without any noticeable loss of its effectiveness. The preparation successfully replaces trypsin-Difco in tissue cultivation. Its dosage was determined not by the weight of the dry pancreatin, but by the activity expressed by the proteolytic "Chow" Units, which enabled the authors to obtain reproducible results.

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