the medium was buffered with phosphate, phosphite and Tris. Vogt, Dulbecco, and Wenner(11) found that avirulent strains of poliovirus produced a smaller number of plaques than did virulent strains when plated under an agar overlay with reduced amounts of bicarbonate which they presumed was due to the lower pH. Hsiung and Melnick(12) also found this to be so but indicated that low bicarbonate concentration was the responsible factor. Both groups of workers (11,12) could not find any difference in the growth of the various strains of poliovirus in liquid medium with different bicarbonate concentrations. On the other hand, Sabin(13) and Lwoff(14) have obtained data in liquid medium which was comparable with that of Gifford *et al*(10). Mosley and Enders(15) have obtained evidence that low bicarbonate concentrations rather than low pH was responsible for inhibition of some poliovirus strains. In the present study the amount of bicarbonate added to the medium was rather high and the pH was adjusted by varying the proportion of Na_2HPO_4 and NaH_2PO_4. Similar effect on the plating efficiency of vaccinia virus was also obtained by altering the concentration of bicarbonate to change the pH. It is possible that the effects reported here, and related to pH, are due to alterations in bicarbonate concentration but more critical studies would have to be made to determine the relative importance of the two factors.

Summary. Changes in temperature, oxygen tension and pH were shown to have an effect upon the plating efficiency of vaccinia virus on chick cells. These changes had no significant effect on interferon action when the percentage inhibition of plaque formation was used as the parameter of interferon action.

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Dissociation and Cultivation of Chick Embryo Cells with an Actinomycete Protease. (28758)

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During studies on the growth and metabolism of chick embryonic pectoral muscle it was found that crystalline trypsin and ethylenediaminetetraacetic acid (EDTA) did not successfully separate the cells of this tissue. Consequently, a method was sought to dissociate rapidly chick embryonic muscle with physiological salt solutions of known chemical composition. Mintz(2) reported that 1 g/100 ml solutions of a crystalline enzyme obtained

from the actinomycete *Streptomyces griseus*, marketed under the name of Pronase, quickly freed mouse ova from their follicle cells in salt solutions containing monovalent cations. This note reports some experiments conducted to determine whether this enzyme would be useful for dissociating embryonic muscle.

Materials and methods. Crystalline Pronase, research grade, was obtained from the California Corp. for Biochemical Research. Medium 199 was purchased from Microbiological Associates; chicken serum was obtained from Colorado Serum Co. Fertile eggs were obtained from commercial and New Hampshire chicken lines and incubated under standard conditions. Growth media and Hanks'(1) physiological salt buffer were prepared with triply distilled water and sterilized by filtration through a 0.25 μ membrane filter (Millipore Co.). The cells were axenically cultured in a medium similar to that used by Resseguie(4); it contained 50 parts 199; 40 parts Hanks' salt solution and 10 parts serum. Antibiotics were not added. The cells were grown in 0.5 ounce pharmaceutical flasks and Rose chambers at pH values of 7.0 and 7.2 and a temperature of 38°C. Solutions were refrigerated and discarded after one week. The increase in cell number was measured by dispersing the cells from replicate flasks with 10 mg/100 ml Pronase in Hanks' solution, fixing with Bouins fixative, and counting them with a Segwick-Rafter counting chamber and a Whipple ocular micrometer.

Results. Crystalline Pronase solutions successfully dissociated cells from chick embryo pectoral and leg muscles. For example, good results were obtained by treating minced pectoral muscles from 3 or 4 14-day old chick embryos with 30 ml of 50 mg/100 ml Pronase in Hanks' solution. The minced muscles were washed in Hanks' solution and incubated with Pronase for 30 minutes at 38°C with stirring. The suspensions were then passed through a 120 mesh stainless steel screen set into a Swinny hypodermic adapter. Over 90% of the cells in these suspensions were separated from each other by this treatment. There was little debris. Cell counts taken 12 and 24 hours after such cells has been inoculated into growth medium indicated that up to 50% of the cells attached to the glass of the growth flasks. After a few days, fibroblast type cells predominated in the cultures. Cells from these primary cultures could be subcultured after dissociating the monolayers with 10 mg/100 ml Pronase solutions. Experiments to determine how long these cells could be subcultured have not yet been performed. The cells increased over 10 times in number, attaining populations as high as 800,000 cells/ml. The generation times of logarithmically growing cultures ranged from 12 to 60 hours.

Discussion. The criteria applied to judge tissue dissociation techniques are simple ones, since the methods are less developed than, for example, those used to fractionate subcellular constituents. Perhaps the best test of a technique is whether it yields a large number of viable single cells from a given tissue. Using this criterion it is apparent that the enzyme Pronase should be added to the list of cell separating techniques, since chick embryonic muscle tissue may be dissociated by treatment with balanced salt solutions of the crystalline enzyme, and the cells are viable enough to be cultured by standard procedures.

In addition, the use of crystalline enzymes such as Pronase is a step towards defining further the conditions of tissue dissociation. For example, it is known that impure mixtures possessing tryptic activity are more suitable for dispersing cells than crystalline trypsin, and that such solutions are most active when dissolved in salt solutions lacking divalent cations(5,6). Crystalline preparations of Pronase readily dissociated embryonic pectoral tissue in balanced salt solutions. This may have been due to the enzyme's lack of specificity. Nomoto *et al*(3) found that the properties of Pronase differed from those of all other proteases. They stated that it "possesses the lowest specificity on record."

In brief, the activity of crystalline Pronase in balanced salt solutions, and the low specificity of its proteolytic action suggest that this enzyme may be useful for dispersing tissues. Its action deserves further study.

Summary. Cells are liberated rapidly from minced pectoral muscles of 14-day chick embryos by treatment with a crystalline protease from *S. griseus* marketed under the name of Pronase. The treatment is carried out in a physiological salt solution. The dissociated cells grow in a standard tissue culture medium and fibroblast type cells predominate in the cultures.

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The Proteins of Eel Serum: Starch Gel Electrophoresis and Immunological Studies. (28759)

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Comparative studies on animal proteins show a relationship between the number of protein components in the plasma and the level of organization and evolution in the species investigated(19). The recent development of new biochemical technics, such as bidimensional starch gel electrophoresis(1) and immuno-electrophoresis(2,3), has permitted a new approach to the knowledge of plasma proteins. These different technics give information concerning electrophoretic behaviour, molecular size and immunological structure of proteins.

Material and technics. The eel (*Anguilla anguilla*), *Teleostei apod euryalin,* has long been studied in our laboratory(4,5,6,7). Numerous specimens can be obtained in European rivers, hence it has been possible to study individuals of exactly the same weight, the same gonado-somatic index, and the same state of development. Studies of the osmoregulation, ionic equilibrium and endocrine functions of this fish led us to study the nature of the serum proteins. Our work was done on fresh water eels weighing 650 to 800 g and presenting a gonado-somatic index between 1 to 1.5.

Starch gel electrophoresis(8,9). The Smithies technic is used with minor modifications(10,11). The gel is obtained with hydrolyzed Connaught starch and is poured into plexiglass trays (internal dimensions: 234 × 80 × 6 mm). The discontinuous system of buffers (Tris borate buffer) described by Poulik(12) is preferred to the borate buffer because it gives good resolution of the fast globulin components. The samples are placed into slots of 10 × 1 mm as follows: A 30% solution of soluble starch in Tris buffer is heated to boiling, and the transparent fluid obtained is cooled to 40°C, mixed in equal parts with serum sample, and applied in slots. The electrical connection with the end of the gels is obtained by means of thick filter paper (Cofram MAB C^3). A potential of 7 volts/cm is applied for 5 hours. After cutting and staining with amido black, it is possible to transform the gel into plastified and transparent film(13). The gel is placed for one hour in an aqueous solution containing 5% acetic acid and 5% glycerol. Then the gel is heated using an infra-red lamp for 2 to 3 hours on a glass plate. Direct photometry can be done with an extinction photometer (Zeiss, for example).

Lipids are detected by staining with Sudan Black.

Double starch gel electrophoresis. A variant of technic of Poulik and Smithies(1) has been used in our experiments. Eel serum is concentrated 2-fold by dialyzing against Sephadex G50. One ml of concentrated eel