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

serum is submitted to paper electrophoresis on large strips, in barbital buffer pH 8.6, using Whatman No. 3 paper previously subdivided in zones of 5 mm, perpendicular to the migration direction. Protein from these zones is eluted and analyzed by starch gel electrophoresis and immuno-electrophoresis in agar.

Immuno-electrophoresis in agar. Scheidegger's technic is employed(14), using 1.5% agar in veronal calcium lactate buffer(15).

Antisera. Rabbit antisera against eel serum proteins were produced by subcutaneous injection of 1 ml twice a week for 6 weeks. The antigen used was a mixture of equal parts of a pool of 20 eel sera and 25% polyvinyl pyrrolidone.

Results. Proteins and lipoproteins of the eel sera. The amount of total serum protein is lower than in human sera, varying from 62 to 70 g per liter.

In boundary electrophoresis, at pH 8.6 in barbital buffer, 4 fractions are observed with mobilities similar to the Albumin, α, β, and γ globulins of human sera. In paper electrophoresis, 5 zones are visualized in the normal eel serum. In comparison to the mobility of the human serum components, these zones are designated as Albumin, α_1, α_2, β, and γ_1 zones. Most globulin protein is found in the α and β zones. The distribution of lipoproteins is different from that found in human serum. The main component of human lipoproteins is the β lipoprotein; in the eel, the main fraction is an α lipoprotein. Electrophoresis in agar in veronal buffer pH 8.4 yields the same distribution of protein as was obtained in paper electrophoresis. A better resolution was observed in the zone of β globulins and prealbumins. In agar, the β globulins show variation from one animal to the other. Starch gel electrophoresis gives a higher resolution than paper and agar-gel electrophoresis. In starch gel, migration is not only a function of the electric charges of the protein molecules, but also of their molecular size and tends to decrease with increasing molecular size. A mixture of globulins having the same electrophoretic mobility in conventional electrophoresis shows a pattern of

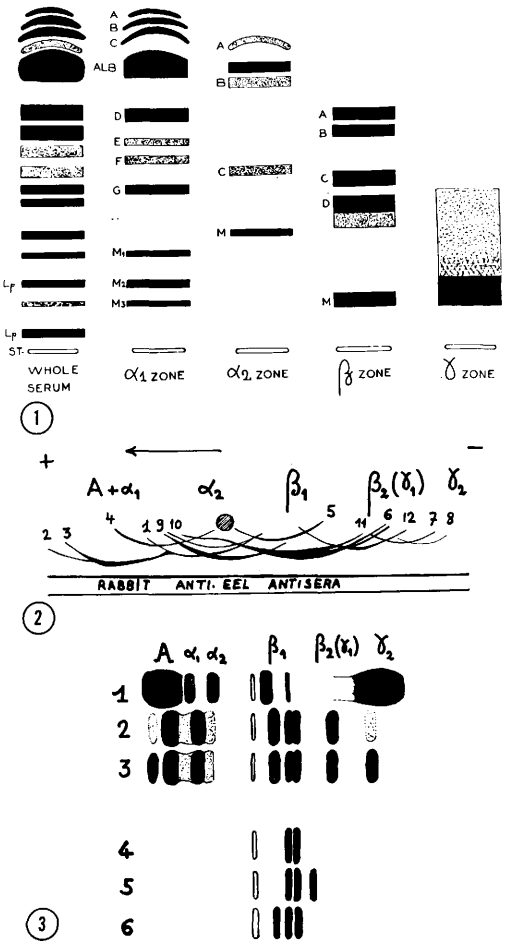

FIG. 1. Double starch gel electrophoresis. Columns show components recovered from whole serum, and the α_1, α_2, β and γ zones of paper strip separations. Components of equivalent mobility are arranged in rows.

FIG. 2. Immuno-electrophoresis of eel serum revealed by a rabbit anti-eel serum.

FIG. 3. Agar-gel electrophoresis (protein staining) showing the increase in the γ_2 globulins in the eel immunized against human proteins (No. 1, 2 and 3). No. 1 = normal human serum; No. 2 = normal eel serum; No. 3 = eel serum after immunization. No. 4, 5 and 6 = agar-gel electrophoresis showing the individual variations in β globulin zone.

different mobilities in starch gel; the slower components are the macromolecules. Whole eel serum has a minimum of 15 components. Fig. 1 gives evidence of 21 components: Albumin, 10 α_1, 4 α_2, 5 β_1 and 1 diffuse γ_1 zones.

A comparison was made of the zones observed in concentrated eel serum using the

paper strip method and using the starch gel method. Segments of paper strips corresponding to the locations of the a_1, a_2 β and γ zones were eluted and run in starch gel. Fig. 1 is a diagrammatic representation of the results.

The double electrophoresis technic gives interesting information on the great heterogeneity of the a_1 zone. The molecular sizes of a_1 constituents are very different. Three a_1 components (A, B, C) have a faster mobility than the Albumin, corresponding to small molecules, but other a_1 components (M1, M2, M3) are macroglobulin (slow mobility); one of these (M2) takes the lipid stain. Similar observations have recently been observed in the rat serum(16,17) and the nature of macroglobulin of the slow a_1 zone has been established in ultracentrifuge(18) (S20 = 18). The β globulins, which represent one of the main fractions in paper and agar electrophoresis, are heterogeneous and one of these components is stained by Sudan Black (the slower β_1).

Gamma$_1$ globulins appear as a diffuse zone corresponding to the position of the γ_1 globulins in man.

Gamma$_2$ globulins of cathodic mobility are absent in this animal.

Ultracentrifugal analysis. Molecular heterogeneity as shown by starch gel electrophoresis has since been confirmed by analysis in the Spinco ultracentrifuge.* Three dilutions have been used:

($\Delta n = 1.14$ $\Delta n = 3.10$ $\Delta n = 5.04$ Phosphate buffer pH 7.3). Table I shows the results at $\Delta n = 3.10$.

Five components of different molecular weight are obtained from eel serum, whereas normal human serum gives only 3 components.

Immunological structure of eel serum proteins. Specificity of the eel proteins. Rabbit anti-eel serum gives no cross reactions with many sera of other species of fish by the Ouchterlony diffusion technic. Sera of the cyclostome *Petromyzon marinus* (lamprey), the chondrichthid *Scyllorhinus caniculus* (dogfish), the teleosts *Conger vulgaris* (conger eel), *Esox lucius* (northern pike), *Labrus bergylta* (ballan wrasse), *Salmo irideus* (salmon trout) *Lota lota* (Turbot) and the ganoid *Acipencer sturio* (sturgeon) were all negative.

TABLE I

	%		%
S_1 = 3.07	(69)	S_4 = 8.20	(2.50)
S_2 = 4.07	(12.35)	S_5 = 11.40	(4.65)
S_3 = 6.11	(11.50)		

Immuno-electrophoresis. A rabbit anti-eel serum revealed 12 precipitin lines (Fig. 2). In previous experiments, we found only 8 components. The β_1 zone precipitin lines are predominant, and the γ zone showed 2 precipitin lines. The slight diffusion of the antigens corresponding to lines 4 and 5 suggests a macromolecular structure of these components. One component (line 8), not demonstrated in the normal sera, appears in the sera of eel immunized against human plasma proteins. This line has a mobility of γ_2 globulin and corresponds to a component of eel sera existing in very low quantities in normal animals. The increase of these γ_2 molecules has been visualized in the serum of immunized eels by agar-gel (Fig. 3).

Immuno-electrophoresis performed upon the eluates from paper electrophoresis strips allows analysis and identification of each line in the zones of various mobilities.

Individual variations in the eel sera. The electrophoretic patterns of eel sera show individual variations which are easily visualized in agar-gel. The variations are located primarily in the β_1 zone (Fig. 3). One individual may have one, 2 or 3 components of differing mobilities. These results are obtained by experiments on 20 various eel examined on repeated occasions and in the same run.

Summary. Eel serum *(Anguilla vulgaris)* has been examined using several electrophoretic technics such as paper, agar gel, starch gel, and double electrophoresis, and using immunological studies such as immunoelectrophoresis and the Ouchterlony diffusion test. Twenty-one components were observed. No γ_2 globulin was seen in the normal sera but it appears after immunization of eel

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against human proteins. The serum of eels contains at least 12 immunologically different antigens to rabbits. We observed most individual electrophoretic differences in the β region of the protein pattern.

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Relationship Between Maximal Secretory Output and Weight of the Pancreas in the Dog.* (28760)

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The maximal rate of secretion of an exocrine gland, by definition, is a function of the number of its secretory units and their rate of activity. In the dog and human stomach, a very close correlation has been established between maximal rate of acid secretion in response to histamine and number of parietal cells, the parietal cell mass(1,2).

We have recently described the maximal bicarbonate and enzyme (amylase) output of the dog pancreas in response to secretin and pancreozymin(3,4). Since the cell of origin of water and bicarbonate of pancreatic juice has not been established, in contrast to the acinar cell of origin for secreted enzymes, we attempt in this report to establish a relation between maximal bicarbonate and amylase output and weight of the pancreas in dogs.

Material and methods. Fifteen female mongrel dogs were prepared with chronic duodenal fistulae, and the maximal 15 minute volume, bicarbonate and amylase output in response to secretin and pancreozymin determined as previously described(3,4). The maximal 15 minute output was obtained with doses of secretin of 4 to 8 units/kg as single intravenous injections, or 4 to 12 units per minute as continuous infusions. The dose of pancreozymin required to produce the maximal output was 5 to 15 units/kg as a single injection or 0.5 unit/kg/minute as continuous infusion. Three of the animals then had a partial pancreatectomy with removal of from 17 to 22 g of pancreas. Following this, these dogs were again stimulated with the same maximal dose of secretin and pancreozymin, and the fluid, bicarbonate and amylase outputs determined.

All the animals were subsequently sacrificed by an overdose of Nembutal, and the pancreas carefully dissected free of surrounding tissues and weighed.

Results. The results are presented in

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