the growing list of mammalian cells which survive "freezing" in presence of glycerol. However, "freezing" may only mean survival in presence of extracellular ice without intracellular ice formation. Evidence for egg survival after intracellular ice formation would have to be based on observation of ice formation under the microscope, followed by transplantation of the same individual intact eggs and their normal reproductive performance.

Summary. This investigation has established the possibility of survival of unfertilized mouse eggs with extracellular ice formation in glycerolated modified Locke's solution at –10°C for periods up to 3½ hours. Survival of eggs so treated was evaluated by progeny development after transplantation into foster mothers.

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Plasma Cells and Serum Proteins in Marine Fish.* (24225)

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There is evidence that in human beings and other mammals plasma cells may participate in the production of gamma globulins and antibodies(1,2). Studies on plasma cells and gamma globulins in lower forms were initiated(3-5) to determine whether or not they are associated. This report describes observations on occurrence of plasma cells and on electrophoretic properties of blood serum proteins in some elasmobranchs and teleosts.

Materials and methods. From one to 10 members of each species were examined shortly after capture. The fish were trapped in the region of Woods Hole, Mass., the majority from Buzzard's Bay. Blood was obtained from living animals by cardiac puncture with silicone-treated equipment. Collection of blood was greatly facilitated by anesthetizing the fish in a tank of sea water containing 1 part per thousand of M.S. 222§ before obtaining the sample. The blood was transferred to plastic tiubes, was allowed to clot, was centrifuged, and the serum withdrawn. It was either analyzed on the day of withdrawal or stored at –10°C for a few days. One specimen, serum of the lemon shark, *Negaprion brevirostris,* was obtained from the Serological Museum, Rutgers University.‖ This sample had been sterilized by filtration and stored at 4°C for several months. The serum proteins were analyzed by zone electrophoresis in starch gel. Soluble starch was produced by acetone-HCl treatment of po-

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‡ Serological Museum, Rutgers Univ., New Brunswick, N. J. Aided by grant from National Science Fn.

§ Meta-aminobenzoic acid-ethylester methane sulfonate obtained from Sandoz Pharmaceutical Co., Hanover, N. J.

‖ We are indebted to Dr. Alan Boyden, for making this specimen available.

TABLE I. Cell Size Summary. Size of cells as measured on smears and touch preparations of blood and spleen.

	Red blood cells	Lymphocytes	Eosinophils	Neutrophils	Plasma cells
Human	8.3 μ	9.9 μ		13.0 μ	14.6 μ
Smooth dogfish	12.5 × 18.2	10.9	15.6 μ		14.6
Dusky shark	12.0 × 17.7	11.5	12.5		14.6
Sting ray	12.5 × 16.2	9.4	12.0		14.1
Goosefish	9.4 × 12.5	6.3		9.9	6.3
Bonita	8.3	7.3	11.5		9.4
Flounder	7.3	6.8		9.9 (?)	9.0
Tautog	7.3	6.3		10.4	8.3

tato starch¶ at 36.1°C for 70 minutes. Gels were prepared from this soluble starch in 0.030 M sodium borate buffer of pH 9.05, and electrophoresis was carried out at 10°C for 16 hours with a potential of 4.5 volts/cm(6.7). Blood smears were obtained, and touch preparations of the fresh spleens were made by lightly touching a newly cut surface to glass slides. The touch preparations and blood smears were dried, treated with Wright-Giemsa stain, and studied at a magnification of 1000x. Occasionally touch preparations of the liver were also studied. Criteria for identification of plasma cells were the same as for their recognition in mammalian lymph nodes, bone marrows, and spleens. They were identified by the occurrence of: (a) an eccentric nucleus showing varying degrees of clumping of the chromatin; (b) abundant basophilic cytoplasm, having a somewhat granulated appearance without the presence of true granules; and (c) usually a paranuclear clear zone. The frequency of occurrence of plasma cells was estimated, using a scale of 0 to 4 plus. Among the species sampled, *Mustelus canis* demonstrated the greatest frequency of plasma cells in the spleen and thus was assigned the 4 plus designation. Relative sizes of white blood cells in smears and imprints of some specimens were determined using a micrometer.

Results. All of the white blood cells in the spleens of the elasmobranchs were about the same size as their counterparts in human tissues (Table I). Structures having the characteristic appearances of plasma cells were readily identified (Fig. 1-C.D.E).

Among the teleosts, however, the white blood cells of the spleens were considerably smaller than those of human beings (Table I), and identification of plasma cells was less certain. Nevertheless, a few small cells from spleens of each of the teleosts appeared to possess some of the morphologic features of plasma cells (Fig. 1-F,G,H,I,J): the nuclei were eccentric; the cytoplasms were basophilic and usually not granulated in appearance; and the paranuclear clear zones were less prominent than in typical mammalian plasma cells. It was possible to identify plasma cells in the spleen of *Sarda sarda* (bonita) with greater certainty than in the other species of teleosts studied (Fig. 1-J).

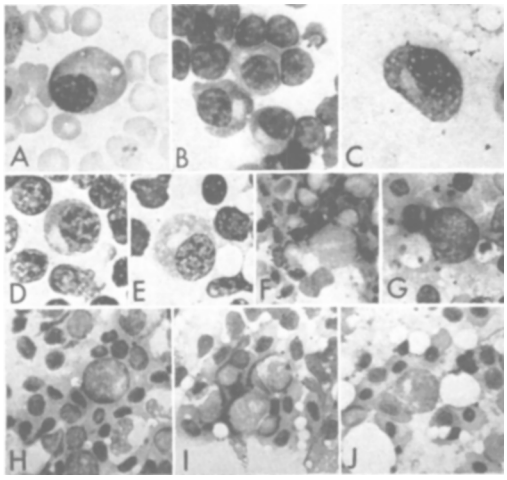

FIG. 1. Plasma cells in smears and touch preparations stained with Wright-Geimsa, 500 ×.
A. Bone marrow, human. B. Lymph node, rabbit. C. Liver, smooth dogfish. D. Spleen, smooth dogfish. E. Spleen, dusky shark. F. Spleen, ramoura. G. Spleen, goosefish. H. Spleen, tautog. I. Spleen, flounder. J. Spleen, bonita.

The serum from each species of elasmobranch and teleost had its own characteristic zone electrophoretic pattern, but within a

¶ Lot No. 584 potato starch obtained from Morningstar-Nicol.

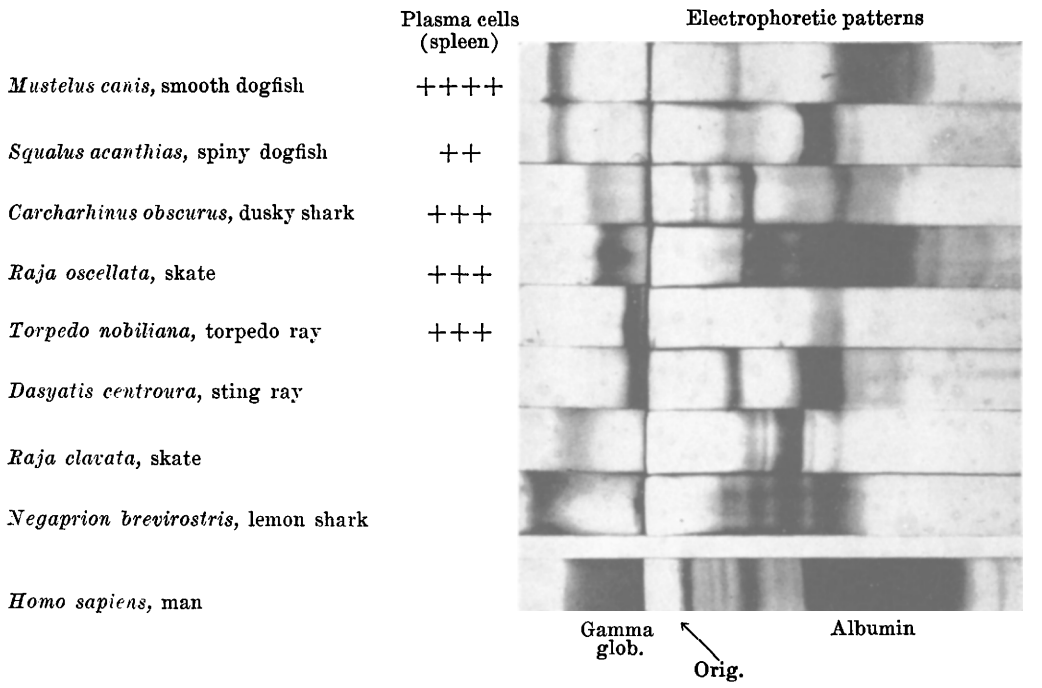

FIG. 2. Starch gel electrophoretic patterns of sera from some elasmobranchs. The position at which samples were applied is indicated by arrow below figure. Cathodic area is left of the origin and anodic area is toward the right.

species there was only slight variation. Zone electrophoresis of serum from each of the elasmobranchs resulted in migration of proteins into the gamma globulin region (cathodic region) of the starch gel. These proteins, migrating toward the cathode, became isolated into well-defined, narrow zones (Fig. 2) in contrast to the diffuse, continuous zone characteristic of human gamma globulin.

Among the teleosts, proteins corresponding electrophoretically to human gamma globulins were either greatly reduced or absent (Fig. 3) Only in the case of *Sarda sarda* (bonita) were there detectable bands in the gamma globulin area, and these were very faint. The occurrence of a protein with a mobility corresponding approximately to that of human serum albumin, and of another which was slightly slower than human B globulin, was consistently observed in every species of bony fish studied.

Figs. 2 and 3 summarize the results of our observations on the occurrence of plasma cells in the spleens and the presence of gamma globulins in the samples of serum from each species. Among the elasmobranchs and teleosts, whenever the frequency of occurrence of plasma cells in the spleens was 3 plus or 4 plus proteins corresponding to mammalian gamma globulin were found in the serum. Whenever the frequency of plasma cells in the spleens was 0 to 2 plus, such proteins could not be detected in the serum.

Discussion. Utilizing boundary electrophoretic studies Deutsch and McShan(8) noted the absence of gamma globulin in the sera of fish. They identified up to 12 components in the electrophoretic patterns of a single species. Variation from one individual to another within the same species was slight; however, variation from one species to another was extreme and easily recognized. They did not study any elasmobranchs. Becker *et al.*,(9) using paper electrophoretic methods, and Drilhon(10) found that gamma globulins were diminished or absent in the sera of teleosts.

Jakowska(11) found a few doubtful plasma cells in teleosts. No observations were found in the literature on the occurrence of plasma

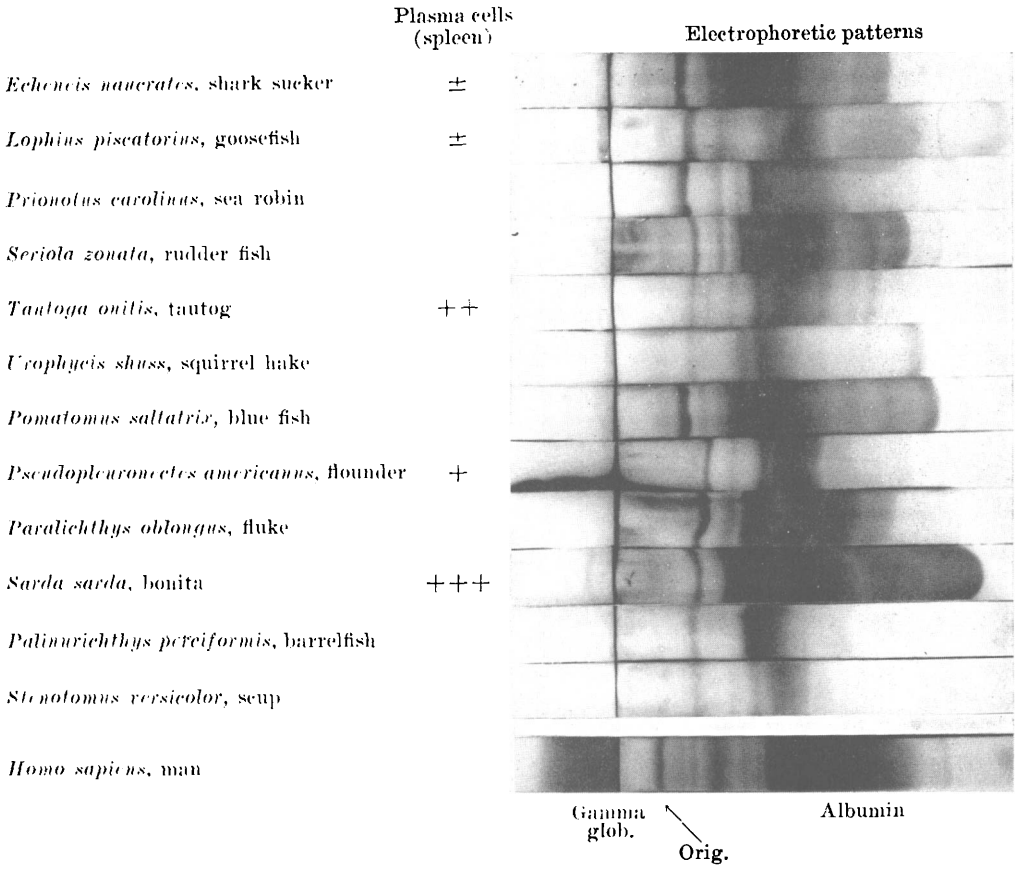

FIG. 3. Starch gel electrophoretic patterns of sera from some marine teleosts. Position at which samples were applied is indicated by arrow below figure. Cathodic area is left of the origin and anodic area is toward the right.

cells in elasmobranchs. Plasma cells have been found in the lymphoid tissues of the ganoid fish, *Polyodon spathula*(12) and in the spleen of the lung fish, *Protopterus ethiopicus*(13).

The presence of plasma cells and gamma globulins in the elasmobranchs and the paucity of plasma cells and absence of gamma globulins in the teleosts as found in the studies reported here suggests that plasma cells may be related to gamma globulins.

It is possible that in teleosts proteins serving the functions of antibodies do not migrate electrophoretically like mammalian gamma globulins. The electrophoretic pattern of each species was complex and some of the bands in the anodic region of the gel might function as antibodies. It is true that in human beings proteins, with the immunologic properties of antibodies, migrate electrophoretically as beta or gamma globulins(14). Attempts should be made to hyperimmunize fish and to follow the response of the serum proteins and plasma cells.

Summary. 1) Serum proteins of 18 species of fish have been analyzed by means of starch gel zone electrophoresis. In 10 of these species, observations were also made on occurrence of plasma cells in spleens. 2) Elasmobranchs had typical plasma cells in their spleens and proteins in their serum migrating like gamma globulins upon electrophoresis. Teleosts, however, generally had very few plasma cells in their spleens and no demonstrable gamma globulin in the serum. These observations suggest that among marine fish, there may be an association between plasma cells and gamma globulins.

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Origin and Fate of Cells in the Medulla of Rat Thymus.* (24226)

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Recently, counts were made of the resting and dividing nuclei of the 4 main cell types present in the cortex of rat thymus (reticular cells, large, medium, and small lymphocytes) and a scheme based on these counts was proposed to explain the production of lymphocytes in this tissue (Fig. 1). Briefly, mitoses of reticular cells would yield an equal number of large lymphocytes and of new reticular cells. The large lymphocytes would then begin a series of successive divisions, so that a total of 8 generations of smaller and smaller lymphocytes would be produced and thus, each initial large lymphocyte would yield 128 "mature" small lymphocytes(1). The present work consists of counting cells and mitoses in the medulla of thymic lobules, using procedures devised to study the cortex(1). The immediate aim was to find out whether or not the pattern of lymphocyte formation observed in the cortex also applied to the medulla. Furthermore, since the investigation of the cortex had given no clues as to how lymphocytes leave the thymus, it was hoped that examination of the medulla would clarify this problem.

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Methods. The same thymus sections as were used in investigating the cortex of young adult rats (Bouin-Hollande fixation, Dominici staining) were utilized in the present study. Resting and dividing cells were counted in 625 μ^2-fields of medulla. It had been previously found that the cortex could be divided into 2 layers: peripheral cortex making up 9%, and deep cortex making up 66% of the parenchymal thymus(1). The medulla accounts for the remaining 25%.

Results. Cytological observations. The 4 cell types seen in the cortex (Fig. 2) were also present in the medulla, but with a few minor differences in their appearance. The cytoplasm of *reticular cells* stained more intensely and was more distinct in medulla (Fig. 7) than in cortex. The nuclei of some cells contained granules of chromatin-like material often present within vacuoles (Fig. 7, lower center)—a picture interpreted as degeneration(2). Moreover, other stages of degeneration up to complete dissolution of nuclear material were seen, particularly in some reticular cells associated with, or making up Hassall's corpuscles (Fig. 7, upper left).

A few of the *large* and *medium* lymphocytes in medulla had irregularly shaped nuclei. Such irregularities were most pronounced in