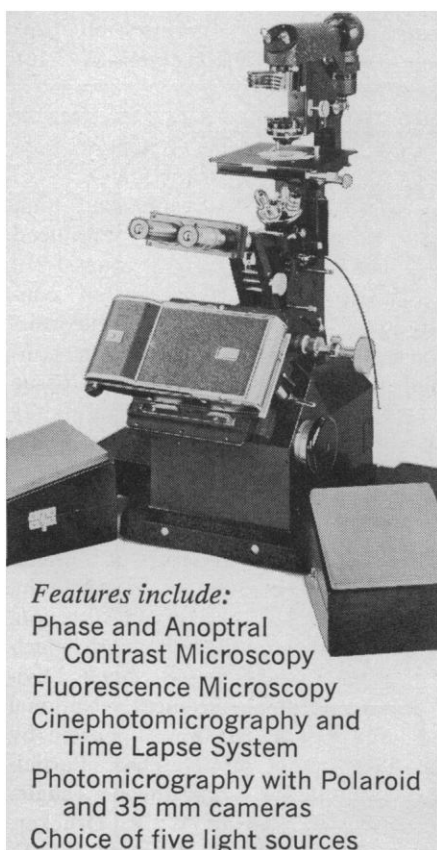


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Immunologic Phenomena: Cold-Blooded Vertebrates

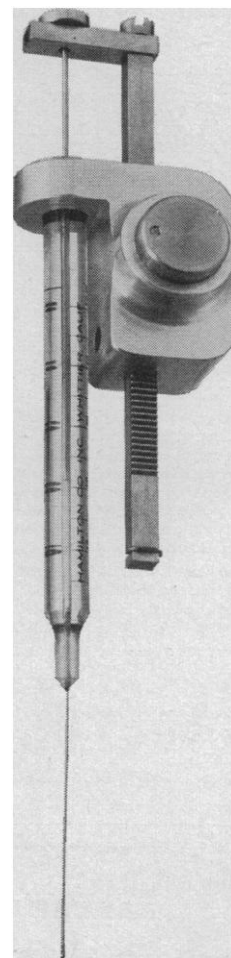
The symposium on immunologic phenomena in cold-blooded vertebrates, held at the recent meeting of the Federation of the American Society for Experimental Microbiology, April 1963, included both reviews and new information on the behavior of fishes, amphibians, and reptiles. The major emphasis was centered on immunological comparisons and regulatory or reaction-controlling factors with references to phylogenetic development.

E. Edward Evans (University of Alabama Medical Center) described his long-range study on the antibody synthesis in reptiles and amphibians. Since the lower vertebrates are poikilothermic, the immune response is greatly influenced by ambient temperature. The reptiles *Sauromalus obesus* and *Dipsosaurus dorsalis* produced a good antibody response to the antigen *Salmonella typhosa* H at 35°C, but at 40°C titers were somewhat lower and not all animals survived. In groups maintained at 25°C, serum titers were very low or not detectable. The marine toad (*Bufo marinus*) responded well at 25° or 35°C, but not at 15°C.

Although the animals immunized at sub-optimal temperatures produced little or no detectable antibody, they acquired the potential for antibody synthesis and when warmed to an optimal temperature they produced antibodies without further immunization. Both synthesis and release of antibodies were inhibited at the sub-optimal temperatures used.

Antibody formation in response to injections of soluble proteins, such as bovine serum albumin and rabbit gamma globulin, was demonstrated by precipitation tests in fluid media or by immunodiffusion in agar. Through the use of fluorescein-labeled anti-bovine serum albumin, antibody-forming cells were shown within the spleen, liver, and kidney of *B. marinus* and the spleen and liver of *D. dorsalis*. These cells resembled plasmablasts. Parallel sections stained with methyl green-pyronin confirmed the presence of plasma cells and their increase in number during immunization.

Studies of antisera from *B. marinus*, *D. dorsalis*, and *S. obesus* by paper electrophoresis revealed that antibodies were located in the slowest migrating component at pH 8.6. Although electrophoretic patterns of these species may be quite different from those of



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mammals, the fraction containing antibody seems to be analogous to γ -globulin of higher forms with respect to electrophoretic mobility. Immuno-electrophoretic patterns of antisera from reptiles and amphibians contain multiple lines resembling those seen with mammalian sera. Antibody activity appears to be associated with lines comparable to the 7S and 19S γ -globulins of man.

The critical role of temperature was also one of the major issues discussed by W. H. Hildemann (UCLA) in his paper entitled "Immunogenesis of homograft reactions in fishes and amphibians," co-authored by E. L. Cooper. The profound temperature effect on the rejection rates of skin homografts in goldfish was clearly illustrated in experiments at temperatures of 10°C and 25°C. At the lower temperature the median survival times for the first and second sets were 40.5 and 19.5 days, respectively, while at the higher temperatures the corresponding values were only 7.2 and 4.7 days. The authors studied the kinetics of this reaction as a function of temperature. They calculated the Q_{10} quotients over several temperature ranges and noted that the Q_{10} values decreased markedly with the increase in temperatures, that is, 20°C:10°C to 32°C:22°C. Furthermore, activation energies obtained from the Arrhenius equation were found to be 11 kcal/mole for 32°C:22°C and 23 kcal/mole for 20°C:10°C. The conclusion was that the Q_{10} and activation energy values decreased with increase in temperature for both 1st and 2nd set homografts. At lower temperatures the rejection process was reduced. The anamnestic responses to the second set grafts were less affected by temperature. Although homograft survival was greatly prolonged at 10°C, immunization occurred within 7 days. Transfer of the homografted hosts to a temperature of 25°C brought about acceleration in the completion of the rejection phenomenon. In the interpretation of these observations Hildemann stressed the need for consideration of factors other than the immune reaction which contributed to the rejection process. These factors include inflammation and wound healing, both of which are influenced by temperature.

X-irradiation prolonged survival of homografts in a teleost fish (*Fundulus heteroclitus*). At 28°C this fish would reject homografts after 3 to 4 days. Radiation at 500 roentgens was without significant effect, but at 1000 to 3000 r transplant survival was ex-

tended. Homografts were also enhanced in the fish by the injection of Cycloheximide (Acti-dione) and Stylomycin (Puromycin). While several nucleic acid analogues and steroids prolonged the survival time of the grafts, they also produced toxic effects. Methyl bis-(β chloroethyl)-amine (Mustargen) and triethylene melamine were also effective, but the highest effectiveness in suppressing the rejection mechanism was obtained with A-methopterin (Methotrexate) and aminopterin.

In studies of the development and maturation of the lymphomyeloid systems, bull frogs served as the most useful animal because they have a prolonged period of larval development associated with slow acquisition of immunologic competence. All types of definitive leukocytes other than small lymphocytes could be demonstrated during the period when larvae could still be made completely tolerant to homograft. Small lymphocytes increased about tenfold and mature eosinophils three to fourfold during the critical period of 40 to 50 days of age. It is at this time of development that the transition from homograft tolerance to the immune type of response occurs at 25°C. Among the most recent findings were some observations relevant to the role of the thymus of bullfrogs. The results indicated that thymus was not crucial to the immunologic competence of larvae during most of the period preceding the adult stage. However, the growth rate in thymectomized larvae decreased regularly, thus suggesting that the vertebrate thymus may have at least two distinct developmental roles; one may promote growth and one may govern lymphopoiesis.

L. W. Clem and M. Michael Sigel of the University of Miami School of Medicine and the Variety Children's Research Foundation reported on comparative immunochemical and immunological reactions in marine fishes with soluble, viral, and bacterial antigens. In order to obtain basic knowledge about the immune mechanism and immunological responsiveness of marine vertebrates, the authors used the lemon shark (*Negaparon brevirostris*) and the margate (*Haemulon album*) to represent the elasmobranchs and teleosts, respectively. The subcutaneous inoculation of PR8 influenza virus into sharks caused a significant production of hemagglutination-inhibition antibodies, the levels of which, at times, exceeded those found in land animals. When tested against a variety of other



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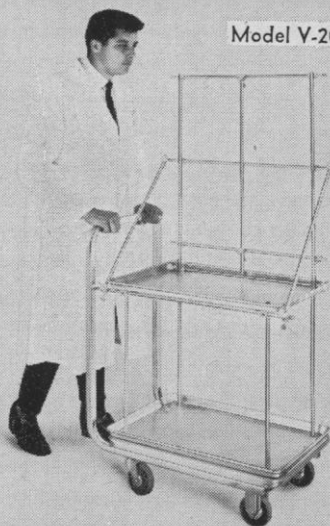
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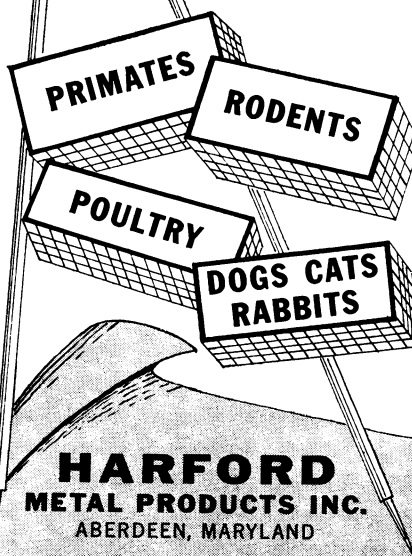
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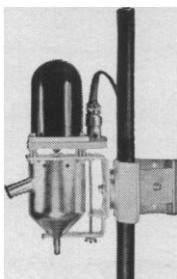
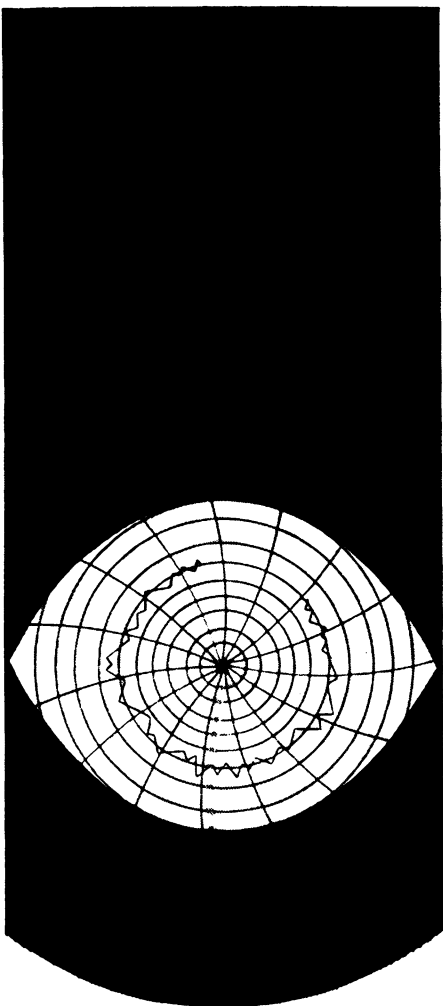


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myxoviruses, the shark anti-PR8 sera displayed a high degree of specificity. The sharks responded poorly to poliovirus; only one of three animals produced an adequate amount of neutralizing antibodies and in this instance the shark had been given combined injections of poliovirus and influenza virus. After the injection of bovine serum albumin there was a relatively small induction of antibodies as shown by the agglutination of red blood cells treated with tannic acid and coated with bovine serum albumin; such antibodies were not detectable in the agar diffusion precipitation test. The immunization with polio and the bovine serum albumin antigen were carried out by the intraperitoneal route which was shown subsequently to be considerably less effective than the subcutaneous route for immunization with influenza virus. Therefore, it is possible that the lack of response was due to the suboptimal route of immunization.

The margates produced antibody to influenza, *Salmonella*, and bovine serum albumin antigens; the latter was detectable by the Ouchterlony technique. The response to influenza antigen was of lower magnitude than observed in the sharks and the degree of specificity was below that of the shark. While no complement-fixing activity could be detected in the sera of either group of animals, significant neutralizing activities were found. The antibody of fish was found to possess several physicochemical properties similar to those described for mammals. Immunochemical investigations of the serum protein showed, however, certain pertinent differences. The shark serum contained a component resembling the gamma 2 globulin of mammals. Such a component could not be demonstrated in the sera of margates; these sera contained proteins which behaved in a manner similar to the fast-moving gamma or slow-moving beta globulin.

Research by a group of investigators (B. W. Papermaster, R. A. Good, R. M. Condie, J. K. Finstad, and A. E. Gabrielsen of the University of Minnesota and Stanford) on the immunologic responsiveness and immunomorphologic characteristics of two cyclostomes, the hagfish and the lamprey, has illustrated the phylogenetic development of adaptive immunity. (These animals represent two of the lowest surviving vertebrate forms.) In the hagfish no antibodies were produced even when adjuvant was added to certain antigens; some of the antigens were found to



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circulate in these animals for as long as 30 days. There was also some indication that the hagfish lacked homograft immunity. Adult lampreys were somewhat more capable of making antibody as judged by a limited primary response. The tests of the secondary response could only be carried out in a few animals because the spawning lamprey does not survive long enough to permit extended observation. The investigators also studied the response of the holostean fish, *Amia*, and the guitarfish, a primitive elasmobranch. Variable degrees of competence exist in these fishes and the secondary response was usually more vigorous than the primary. The hagfish appears to lack lymphocytopoietic tissue. Hagfish serum has no globulin of gamma mobility, but the lamprey serum contains a small amount of component comparable to mammalian gamma globulin. The thymus appears to be totally lacking in the hagfish and only an epithelial thymus is present in the larval lamprey. In sharp contrast, elasmobranchs and teleosts possess lymphopoietic tissue, circulating lymphocytes, gamma globulin, and a thymus. These fishes are also immunologically competent with respect to antibody production and homograft rejection. On the basis of these findings, it was concluded that adaptive immunity developed in parallel with phylogenetic development of the thymus and lymphoid system.

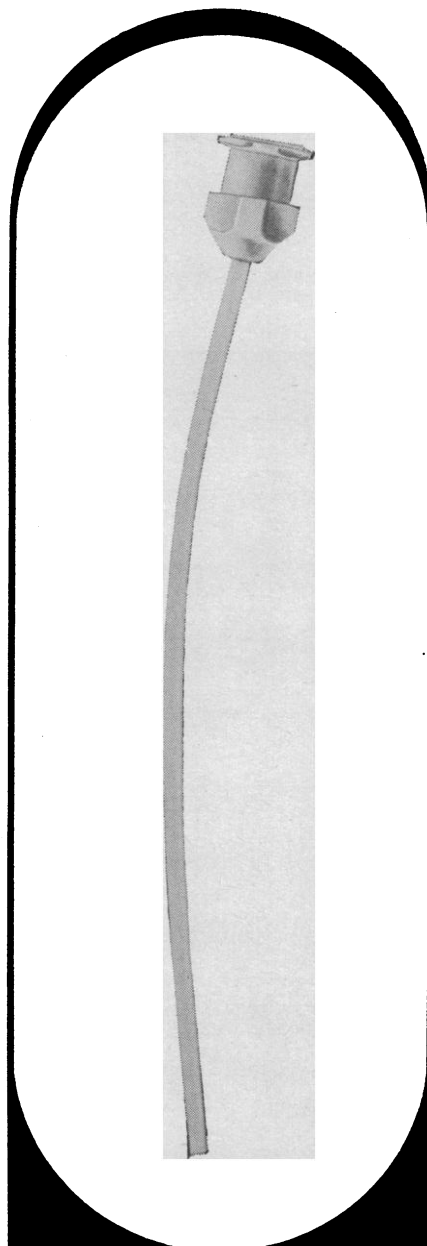
M. MICHAEL SIGEL

*University of Miami School of
Medicine, Coral Gables 34, Florida*

Cell Life Cycle: Macromolecular Aspects

A symposium on the macromolecular aspects of the cell life cycle was the subject of the annual symposium sponsored by the Biology Division of the Oak Ridge National Laboratory and the Division of Biology and Medicine of the Atomic Energy Commission at Gatlinburg, Tennessee (8-11 April).

The major emphasis throughout the meeting was placed on mechanisms controlling the initiation and maintenance of DNA replication and the regulation of DNA function in relation to other events of the cell life cycle. The discussion of both DNA polymerase and the conversion of non-primer DNA to the primer state, with respect to the initiation of DNA synthesis, pointed up the current gap in information about



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