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STUDIES IN DEVELOPMENTAL IMMUNOGENETICS
Annual Progress Report

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ABSTRACT

This contract provides the sole research support, external to the Institution, for a research group concerned with a relatively large range of problems. The integrating thread that runs through it is that of an interest in development, mainly in complex organisms and with an emphasis on the immune system as a model for developmental analysis and a tool for following the development of other systems. It includes studies of biochemical genetics, primarily from a developmental viewpoint, and of immunologic interactions between generations with particular regard to brain development, the flow of information through the nervous system, and cellular aspects of the development of the immunologic system.

PROGRESS REPORTSTUDIES ON THE BURSA OF FABRICIUS IN PIGEONS
AND ITS CONTRIBUTIONS TO THE IMMUNE RESPONSE

In chickens, development of complete immunological competence requires that both the thymus and the Bursa of Fabricius be present in late embryonic and early neonatal life. The thymus-dependent immune functions include delayed hypersensitivity and graft rejection; the bursal functions include reactions mediated by humoral antibody. By performing bursectomies in ova, one can further dissect the antibody response with respect to classes of immunoglobulin molecules (7S IgG and 19S IgM) elaborated.

Reconstitution studies of X-irradiated mice similarly indicate 2 cell lines involved in the immune response. Moreover, they indicate that at least 2 cell-types interact in the sequence of reactions leading to antibody elaboration. Current theories designate the thymic-dependent cell as the antigen reactive cell (ARC), important in the recognition of antigen and the induction of the antibody response. The actual antibody-forming precursor cell (AFPC) is of bone marrow origin in mice and presumably of bursa-dependent origin in chickens.

There exists no precise mammalian equivalent to the bursa and for this reason birds offer opportunities for experimental work so far impossible in mammals. Experiments are in progress to study the development of the bursa, bursal activation in vitro and the possible interaction between bursal and thymic cells.

A STUDY OF THE EFFECT OF IRRADIATION ON THE PIGEON KARYOTYPE

Pigeons are remarkably tolerant to X-irradiation. In this laboratory pigeons have survived 1750 r whole body irradiation, a dose nearly 3 times that which is fatal to man. The irradiated birds are somewhat runted, have unusual feather structure and show abnormal serum gamma-globulin patterns on electrophoresis. However, one preliminary experiment has shown no significant effect on the rate of primary feather growth in 6 pigeons which received from 1250 to 1750 r whole body X-ray.

It is of interest to see whether effects of high doses of radiation on the pigeon chromosome complement can be detected. The chromosome squash technique was modified so that squashes could be made from pulp in the quills of regenerating primary feathers. Karyotypes of normal, unirradiated pigeons were made, concentrating mainly on the first 10 pairs of chromosomes, the macrochromosomes. Others of the 80-chromosome complement are impossible to karyotype accurately. So far one irradiated bird, which received 1750 r, has been compared with the normal, and no chromosomal abnormalities have yet been observed.

MULTIPLE EFFECTS OF ANTI-LYMPHOCYTIC SERUM

Based on demonstrations that anti-lymphocytic serum (ALS) could substantially delay graft rejection, ALS has been used in human transplants and in animal studies of immune rejection. Besides prolonging survival

of transplanted skin and organs, ALS treatment has weak suppressive effects on humoral antibody responses and improves growth of transplanted tumors in hamsters. Human transplant patients treated with ALS show an increased incidence of spontaneous tumors over non-ALS-treated patients. Different preparations of ALS differ in these respects and, to date, there are no in vitro tests which correlate closely with the in vivo effectiveness of ALS.

In this investigation, mice that had received graft-prolonging ALS treatment were studied for their antibody responses to serum antigens and attempts were made to solubilize lymphocyte antigens for in vitro assays of ALS preparations. ALS-treated mice responded to serum antigens in much the same way as control mice although differences in the timing of the response may be revealed by further studies. ALS yielded a precipitin band against thymic extracts in immunoelectrophoresis. The antigen appears to be a serum contaminant of the thymocyte preparations. At 6 months after receiving their last serum injections, some ALS-treated mice show spontaneous tumors. The control mice are tumor-free. Further work on the antigenic properties of these tumors may elucidate the mechanism of spontaneous tumor induction.

AN ATTEMPT TO DEVELOP A TECHNIQUE FOR THE IDENTIFICATION OF INDIVIDUAL CELLS PRODUCING ANTI-BACTERIOPHAGE ANTIBODY

The Jerne plaque technique demonstrates antibody production by individual cells in the center of a focus of hemolyzed red blood cells. Drawbacks of this technique are that erythrocytes have many antigenic sites;

they cross-react with bacterial antigens; there exist natural antibodies to red blood cells; and the direct technique reveals IgM, but not other classes of antibodies.

An assay utilizing phage would minimize these problems. We have attempted to develop such an assay by plating immune cells with bacteria and enough bacteriophage to clear the plate. A cell producing anti-phage antibody should protect the bacteria surrounding it from infection and subsequent lysis by the phage.

Early experiments showed a clear difference between cell suspensions from immunized and from unimmunized mice. Up to 50% of the cells from immunized mice were surrounded by stained, roughly circular areas, 2 or 3 cell diameters across, interpreted to be micro-colonies of *E. coli*.

Later attempts to duplicate the above results were not successful. No difference was found between cells from an immunized and an unimmunized mouse. The question of the success of the technique remains in doubt.

Further study of this problem may involve: 1) finding more compatible agars, such that cell growth is favorable while growth of bacteria at the interface is enhanced; and 2) increasing the sensitivity of the assay by using minimal amounts of phage and bacteria to carpet the plate.

MAINTENANCE OF POLYMORPHISM FOR TRANSFERRINS IN THE PIGEON

Transferrin is a non-heme, iron-binding protein. In pigeons, it is found in serum, egg white, egg yolk, pigeon milk and probably semen. Only 2 alleles are known at this locus. In our pigeon colony and in the feral

population sampled, the 2 alleles occur in approximately equal frequency. The distribution of the genotypes in the population fits the Hardy-Weinberg equilibrium, with no excess of heterozygotes.

The polymorphism has been investigated developmentally by using matings which produce only heterozygotes. We found that the young squabs, for several days after hatching, have only the maternal type of transferrins in their serum, even though the squabs are fed crop milk from both parents and the milk contains both transferrin types. This indicates that transferrin is transferred via the egg, not via the milk.

The fecundity of the female pigeons in the colony was examined. Overall hatchability of the eggs was 37%. Tabulated according to maternal transferrin genotype, the hatchabilities were: Tr^a/Tr^a , 29.2%; Tr^B/Tr^B , 31.4%; Tr^A/Tr^B , 46.0%. These differences are significant.

Adult mortality in the flock shows no association with transferrin type. If selection maintains the polymorphism consistently observed at this locus, it appears to act by favoring heterozygosity for the maternal contribution to the egg, embryo, and young squab.

Several microorganisms can infect adult pigeons asymptotically, but cause death of large numbers of embryos. We are investigating a possible differential inhibition of microbial growth by egg whites of the different transferrin types.

BEHAVIORAL BIOLOGY OF ROTIFERS

Rotifers offer some special advantages for biological studies, based on their rapidity of reproduction, the limited and fixed number of nuclei involved in the mature micro-metazoan, the ease of developing asexual clones in which particular genotypes can be multiplied without going through the gene-reshuffling processes of meiosis and fertilization, and the recent development of a method for inducing sexual reproduction at will in *Asplanchna brightwelli*, which should greatly facilitate genetic work.

In spite of past frustrations, we are continuing work with rotifers. Our emphasis is now on studying their relatively simple nervous system and behavior. This work is just getting started; our plan is to alter the nervous system (by genetic changes or microbeam irradiation) and study the effects on behavior.

THE USE OF ANTIBODIES DIRECTED AGAINST CELL SURFACES TO
INTERFERE WITH NORMAL DEVELOPMENT OF THE PIGEON BRAIN

An unsolved and challenging question is: how do the developing neurons of higher animals reach their proper destinations and establish correct connections? One way of attacking this question is by interfering with the normal development of the nervous system. This might be done by the treatment of developing embryos with antibodies directed against brain cell surface antigens.

Cell membranes from cerebellar tissue taken from 14-day pigeon embryos were injected with Freund's complete adjuvant into New Zealand White rabbits. The rabbits received a booster injection of antigen after 1 month, and were bled 2 weeks later. The resulting antiserum is to be injected into normal embryos which will later be examined for behavioral and physiological abnormalities.

A study of the blood-brain barrier of newly-hatched squabs revealed that for the dye trypan blue this barrier is completely established by the first day after hatching. None of the injected dye penetrated into the brain beyond the choroid plexus. Intravenous administration of antiserum into squabs does not, therefore, appear to be a promising procedure in the above context.