

(such as norepinephrine release from the chromaffin tissue which is characteristic in this species). There is a suggestive relationship that warrants further study of changes in plasma osmolality as a candidate for the role of the unknown stimulus.

This research was supported by a Grant-in-Aid from the Monmouth County Affiliate of the New Jersey Heart Association.

1972 #36

#### THE MORPHOLOGY OF THE BLOOD CELLS OF THE ELASMOBRANCH *Squalus acanthias*

D.J. Prieur, G.N. Trosclair, R.L. Reagan, and A.M. Guarino, Laboratory of Toxicology, National Cancer Institute, Bethesda, Maryland

The microenvironment of the bone marrow appears to play an essential role in hematopoiesis in higher vertebrates. That the study of the production and release of blood cells in an elasmobranch with neither bones nor bone marrow would aid in the more exact delineation of the role of the marrow microenvironment is obvious. The effects upon the hematopoietic system of elasmobranchs of several antineoplastic agents that in mammals are selectively toxic to hematopoietic tissue are of special interest to us. The spiny dogfish *Squalus acanthias* should be an ideal species for this type of study. It is readily available and blood can be collected easily from it. A perusal of the literature however reveals a paucity of information on the blood of this species. This is a preliminary report of a study designed to identify and describe the blood cells of the spiny dogfish prior to extending the studies of Rall, Ratner, and Roomet (Bull. MDIBL 9: 51-52, 1969) on the toxicity of antineoplastic agents upon the hematopoietic system of this species.

Blood was collected from the caudal vein of dogfish and mixed with heparin. Smears were made and stained with Wright's stain. The remaining blood was centrifuged to concentrate the leukocytes, and the pellets were fixed in glutaraldehyde, processed, and examined in an electron microscope.

Examination of the Wright-stained smears revealed oval-shaped erythrocytes 13 to 17  $\mu$  in width and 23 to 27  $\mu$  long. These cells averaged approximately 15 by 25  $\mu$ . A centrally positioned elongated nucleus with dense granular chromatin, averaging 6 by 10  $\mu$  was present in each red blood cell. Many immature erythrocytes are present in the blood. They are recognized by nuclei that are larger and rounder and by cytoplasm that is more bluish but less abundant than mature erythrocytes. Upon ultrastructural examination, the erythrocytes, both mature and immature, reveal mitochondria in the cytoplasm. Thrombocytes in the peripheral blood of spiny dogfish are nucleated and fairly numerous. They are oval-shaped and approximately 8 by 14  $\mu$  in the blood smears. The nuclei are large and elongated, approximately 6 by 13  $\mu$ , and comprise the major portion of each cell. Some of the nuclei contain a cleft along one side near the center. A scant amount of very faint cytoplasm is present around the nuclei. An occasional thrombocyte contained a single red granule in its cytoplasm. Only one type of granulated leukocyte is found. Based on morphology and staining characteristics these polymorphonuclear cells should probably be called heterophils. They are round in the smears and varied in diameter from 16 to 21  $\mu$ . Most of the nuclei are bilobed, some oval, and

some have three to five lobes. In none of the cells are the lobes sharply segmented as in mammalian granulocytes. The cytoplasm of the heterophils is filled with approximately 125 red, rod-shaped granules about  $0.5 \mu$  in diameter and  $1.0$  to  $1.5 \mu$  in length. Electron microscopy revealed mitochondria and a single type of osmiophilic elongated granule in the cytoplasm. A moderate number of cells approximately  $14 \mu$  in diameter are present in the smears. A scant rim of dark blue cytoplasm arranged in a polar fashion on opposite sides of the nuclei is present in these cells. Although they resemble morphologically the lymphocytes of higher vertebrates, the dense chromatin pattern and the consistent polarity suggest to us the probability that these cells are derived from the erythrocytic or thrombocytic lines.

The presence of apparently only one type of granulocyte with only one type of granule is quite interesting from a phylogenetic standpoint. Higher vertebrates generally have three types of granulocytes and the predominate type of cell usually has at least two types of granules.

1972 #37

#### DDE-INDUCED EGGSHELL THINNING IN WHITE PEKIN DUCKS *Anas platyrhynchos*: STRUCTURAL, PHYSIOLOGICAL, AND BIOCHEMICAL STUDIES

John B. Pritchard, David B. Peakall, Robert W. Risebrough and William B. Kinter, Medical University of South Carolina, Charleston, South Carolina, Cornell University, Ithaca, New York, Canadian Wildlife Service, Ottawa, Canada and Mount Desert Island Biological Laboratory

While the occurrence of eggshell thinning due to DDT and its metabolites has been widely noted in many species of birds, the physiological consequences of this thinning have received little attention. Egg breakage as a result of thinning has been noted but no systematic studies of the relationship of shell thickness to breaking strength has been made for eggs from birds exposed to DDT and its metabolites. Changes in shell permeability during eggshell thinning have not been studied. Finally studies on the biochemical basis for DDT-induced thinning have indicated that direct effects on shell gland enzymes seem to be involved (Risebrough, et al., *The Biological Impact of Pesticides in the Environment*, J.W. Gilbert, editor, Oregon State Univ., Corvallis, 1970, pp 40-53).

White Pekin layers weighing 7-9 lb (C. & R. Duck Farm, Westhampton, Long Island) were kept in two flocks of about 10 birds each and fed *ad lib* duck breeder mash (kindly donated by Agway, Inc.). The experimental flock had 40 ppm p,p -DDE (Aldrich Chemical Co.) added to the mash before pelletization. Eggs were collected daily and washed briefly in warm water to remove surface dirt. Length and breadth were then measured and shell thickness was later measured at the waist of the egg (average of 10 readings) using an Ames Model 25E thickness gauge. Permeability to water vapor was measured by maintaining unfertilized eggs in a constant temperature desiccator containing anhydrous  $\text{CaSO}_4$ . Addition of an open dish of  $\text{P}_2\text{O}_5$  did not increase the rate of water loss. The eggs were weighed daily for four consecutive days. Breaking strength of eggs was determined using an Instron Universal Testing machine and scanning electron micrographs of the shell surface were prepared with a Cambridge Stereoscan Model 2A microscope. Biochemical studies were carried out on freshly scraped mucosa from functioning shell glands (contained an egg undergoing