Cancer Research, Columbia University, New York, were examined in many series of cultures made in chicken plasma and combinations of chicken and rat plasma. The comparison of the living malignant cells with one another and with normal cells revealed visible differences between them. Although the sarcoma cells from the five tumors differ from one another they have the following charactertistics in common which distinguish them from normal ones, (1) their cytoplasm is less transparent and more granular, (2) the fat globules are more refractive, (3) the mitochondria are smaller, (4) fewer neutral red stainable granules accumulate, (5) the nucleus seems to be larger in proportion to the size of the cell, (6) the nuclear membrane is thicker, (7) the nucleolar material is increased, and (8) the nucleus appears to be more granular. Slight variations of these characters together with the size and form of the cells enables one to distinguish the cells of different tumors from one another.

Malignant cells migrate more readily in the cultures than do normal connective tissue cells in cultures of adult tissues. The general characteristics of their outgrowths differ from one another and from those of normal cells. Although the shapes of cells undergo many changes as they move about in the cultures, those from each tumor have certain features in common which distinguish them from one another and from normal ones.

These cytological characteritstics of malignant cells are probably associated in some way with the peculiar functional characteristics which distinguish them from normal ones as they grow in the body, such as uncontrolled growth, transplantability from animal to animal, disorderly growth, lack of useful function, injurious effects on normal tissues, rapid cell death as well as multiplication and acid metabolism.

SOME STUDIES ON THE SELACHIAN BLOOD IN VITRO

CHUN CHANG,* *National Medical College of Shanghai, China*

The blood of the skate (*Raia erinacea*) and of the dogfish (*Acanthias vulgaris*) was taken from the heart, and hanging drop cultures of whole blood and buffy coat were made. Vital stains with neutral red and brilliant cresyl blue were employed to bring out the detailed structure of the blood cells. Wright and Giemsa stains were used in dry spreads made at intervals during the period of cultivation. Chemicals that lower surface tension such as chloroform (three times diluted saturated aqueous solution) or calcium chloride (0.24 gm. to 100 c.c. of sea water) were added to the blood (equal parts) to activate the pseudopod formation and increase the viscosity of the cytoplasm of the leucocytes in the hope that phagocytosis might be produced in the leucocytes other than the monocytic macrophages. Calcium free artificial sea water was also added to some cultures and fresh plasma or old plasma left with blood cells for several days was added to older cultures.

The blood of both skate and dogfish contains erythrocytes, lymphocytes, monocytes, thrombocytes and granulocytes, and in addition in

* Fellow of Rockefeller Foundation.

the dogfish, cells of the size and shape of the granulocytes but with few or sometimes no granules are found. The granulocytes of both the skate and the dogfish are of two types; those that contain bright granules and those with pale granules both of which are eosinophilic in stained preparation. The bright granuled type of both fishes appear alike in living cells but differ when vitally stained with brilliant cresyl blue which stains the bright granuled cells of dogfish bright blue, but that of skate light purple, or not at all. The pale granuled cells are the same in both fishes and even when stained. The granules of the bright granuled cells are spherical or oval in shape, closely packed together and exhibit little movement whereas those of the pale granuled ones are of nearly equal size but of various shapes ranging from round, oval, triangular to spindle, loosely arranged and move around in the cytoplasm by the cytoplasmic current, individually or en masse. They usually move in rows away from or towards the clearer centrosphere which is situated close to the nucleus, more or less near the center of the cell. When the granules approach the area of a pseudopod which is usually free from them but with some very fine dancing particles, some of them may enter it and travel freely as wandering granules, but soon fall back to one of the rows. In dead or resting cells the granules arranged themselves in a definite parallel linear pattern, the rows crossing each other in an oblique direction due to their occupying a minimum space when the cell contracts and lines which are simply an optical effect seem to join the adjacent granules into rows. The granules of both bright and pale types are arranged in one layer on the surface of the cell except the region of the nucleus. The pseudopod is formed by the flowing out of the cytoplasm of the more central area which pushes aside the granules of that part and is usually devoid of them. When the pseudopod is withdrawn the granules cover that part again.

Many immature erythrocytes of different stages ranging from small spindle shaped thrombocytes to that of large oval basophilic erythrocytes are found in the blood. Mitotic figures of immature erythrocytes are frequently seen. Haemoglobin is first laid down around the nucleus and from there extends towards the periphery, the two ends of the oval erythrocytes being the last regions to be endowed with haemoglobin.

Chloroform and calcium chloride cause the pale granuled cell of both fishes to spread out and glide on the coverslip with membranous pseudopods resembling the epithelioid cell. Their cytoplasm becomes apparently more adhesive. When two cells move together they are liable to be stuck to each other and pull out to a long strand of cytoplasm when they try to move apart. A part of cytoplasm may be thus cut off from the main cell body without any noticeable injury. Carmine particles were observed first to stick to the surface of the cell and then taken up into it. No engulfment has been observed. This stickiness is reversible by changing the medium.

Fresh plasma has similar but very slight action on the older cultures causing the leucocytes which are already stuck to the coverslip to spread out and move around. Old plasma which was left with the

blood cells has a stronger effect showing a slight pull of the cytoplasm of the joined cells moving apart. This difference may be caused by the additional calcium salts freed from the decomposed cells in the old plasma. The bright granuled cells are little affected by these chemicals, being only slightly activated by showing pseudopods. Calcium free sea water inhibits the growth of the cells from the explant. The presence or absence of urea which is contained, in large quantity, in the selachian blood has no effect on this phenomenon. As a like effect of chloroform and calcium did not occur in the blood of other animals, including teleost (sculpin), amphibia (toad, frog), reptile (snake), bird (chick), and mammal (mouse) it seems to indicate a special property in these granulocytes of the selachian blood. The pale granulocytes of both fishes gradually lose their granules and gain vacuoles and fat globules, coming to resemble macrophages but the bright granulocytes remain unchanged for a long period, then break down as the culture degenerates.

The selachian blood thus contains erythrocytes, thrombocytes, granular and non-granular leucocytes. The lymphocytes and monocytes belong to the non-granular type, while the granular type consists of pale granuled and bright granuled cells and in addition in the dogfish another variety with few or no granules.

GLOMERULAR FUNCTION IN THE SCULPIN

ROBERT W. CLARKE, *New York University*

The rate of glomerular filtration in the longhorn sculpin, *M. octodecimspinosus*, has been studied by means of xylose as used in the dog (4), in the dogfish (2) and in man (1). Grafflin (3) has presented data on four sculpins which indicate that its use is not invalid in this species as well.

A method was developed by which the necessary blood sample could be drawn at the end of the urine collection period, and the bleeding could therefore have no effect on the kidney function. A 50 per cent solution of xylose was injected into 22 fish, using a 24 gauge needle two inches long, inserted just under the skin for its full length to minimize leakage. The dose was approximately 2 grams of the sugar for each kilogram of fish. At the end of 24 hours about 0.5 cc of blood was drawn from the tail vessels. A second sample was taken at the end of 48 hours, and the xylose concentration was determined in each. There was considerable variation among the fish in the concentration of xylose in the plasma at 48 hours (85 to 183 mg per cent). When the rate of fall of plasma xylose concentration in the interval from 24 to 48 hours after injection is related to the absolute concentration at the 48th hour there is good agreement among the data. This is shown, when the data are plotted, by the general parallelism of the individual slope lines, but with increasing steepness at the higher absolute xylose levels. These data were smoothed graphically, and served as a means of estimating the average plasma xylose concentration during a short urine collection period from an analysis of a blood sample taken at its termination.