

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

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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.

For the measurement of glomerular filtration xylose was injected as described above. At about the 40th hour the bladder was emptied and the urinary papilla was tied. As near to the 48th hour as possible the fish was killed, bled, the abdomen opened and the urine drawn into a narrow graduated tube, in the end of which a fine hypodermic needle was cemented. The plasma was analyzed for xylose, and the urine, after appropriate dilution, for xylose and chloride.

Since it is known (3) that sculpins when under experimental conditions show a marked diuresis and a progressive increase in the concentration of urinary chloride, certain precautions were taken to obtain fish free as far as possible from these effects. The fish were caught on hand lines but rejected if there was any injury from the hook. They were never removed from water, and xylose was injected at the time of catching to reduce the number of handlings. When emptied and tied up they were handled under water with bare hands, and in the meantime were kept in all-glass aquaria. According to Grafflin, the urine of freshly caught and presumably normal sculpins is free of chloride or contains only traces, and such fish, if handled with extreme care, may be expected to have urine flows of not more than 4 cc per kilo per day. My group contains 8 fish having no urinary chloride or only a trace (max. 29, av. 10 mM per liter), with an average urine flow of 3.4 and glomerular clearances from 9 to 24 cc per kilo per day (av. 14.6). Among the other fish there is a general rise of glomerular filtration as higher urine flows appear. Fifteen fish with flows between 4 and 6 cc have an average glomerular filtration of 20.8 cc. The injection of xylose and its presence in the blood (to 0.2%), and in the urine (to 1%), does not necessarily prevent a sculpin from satisfying the present criteria for normality.

In order to determine the nature of the most extreme diuresis, a number of the sculpins were repeatedly handled by taking short successive urine collection periods. When the rate of flow had apparently reached a maximum the fish were bled and the rates of glomerular filtration computed. In this group there were 13 fish with flows from 37 to 101 (av. 50) cc per kilo per day and corresponding glomerular clearances of 35 to 131 (av. 66) cc per kilo per day. All of these fish had urinary chloride concentrations (169 to 285, av. 236 mM per liter) in excess of the normal chloride concentration of the plasma, about 160 mM per liter. These experiments show that an increase in glomerular filtration and a diminution in the relative tubular reabsorption of water are both responsible for the high diuresis found in handled and confined fish.

#### REFERENCES

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