ON THE HÆMOGLOBINOMETRY AND HÆMACYTOMETRY OF THE BLOOD OF THE SKATE.

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THE observations that follow were preliminary to a study of the effects of splenectomy in fish—a research on which I engaged in the light of Dr Noël Paton's interesting work on splenectomy in mammals[1]. It was obviously essential to establish at the outset the percentage of hæmoglobin, and the number of corpuscles in unit volume of the blood of normal skates. As the fish possesses so little blood in relation to its body-weight, the method of obtaining blood by puncture of the skin at the time of splenectomy is not feasible, nor is the drawing off of blood from the splenic artery or vein attended with any greater success, as there is barely enough blood in either vessel in a small or medium-sized fish, to fill the pipette of an Oliver hæmoglobinometer. Furthermore, the method of puncturing the skin is rendered very uncertain by blocking of the pipette by the cutaneous mucus. To establish the richness of pigment and of cells in the normal blood, I therefore decided to use heart-blood, and to sacrifice a fish each time that estimations were made. There is probably only about 15—20 c.c. of blood in the body of a large skate, *e.g.* of one weighing from 600 to 900 grammes. The blood-films were fixed by heat (Thermostat at 50°C. for 24 hrs).

The fish used were: Raia batis (the grey skate, or "Dinney"), and Raia clavata (the Thorny-back).

Skates do not feed at all heartily in captivity, and do not display much activity in the tanks. The fish were killed by an overdose of ether stirred up in sea-water in a tub to which they had been transferred. The pericardial cavity was rapidly opened, and from the incised

[1] This *Journal*, XXIX. p. 411, 1903 and XXVIII. p. 83, 1902.

heart enough blood for all the above processes was obtained, but only by using the utmost expedition; speed is necessary, seeing that skate-blood coagulates with considerable rapidity (5′—6′).

The percentage of hæmoglobin. Oliver's tintometer[1] was used. In this instrument, blood, having 15·5 % of HbO_2 will, when diluted to 1 in 100, match the disc marked 100: (the pipette holds 5 cub. mm., and to fill the mixing cell, 495 c.mm. of distilled water are added). The lowest reading recorded was 20 % of this scale, which is $\frac{20}{100} \times 15{\cdot}5 = 3{\cdot}1$ % HbO_2; and the highest was 40 % = 6·2 HbO_2; giving a mean of $\frac{9{\cdot}3}{2} = 4{\cdot}65$ % HbO_2.

By far the greatest number of readings ranged from 20 to 30 % of Oliver's scale, *i.e.* from 3·1 % to 4·65 %; and as the result of estimations extending over two years and made at all the four seasons, I am able to say that the average percentage of HbO_2 is between 3·5 % and 3·8 % of the blood of a normal skate. Estimations were in nearly all cases also made by v. Fleischl's hæmometer; the agreement between this instrument and Oliver's was very close. In about half the number of estimations, I also checked the results by the spectrocolorimetric method of Rollet[2]. Quite different in principle, its readings were in agreement with those of the two tintometers, thus:

	Hæmoglobin %		
By Oliver's hæmoglobinometer	3·10	3·1	2·80
By the spectrocolorimetric method	3·08	3·4	2·13

Seeing, however, that much more blood is required for the spectrocolorimetric method, and that the two tintometers agreed more closely with each other than it did with either, I gave up this method, which had been a useful corroboration in the earlier estimations.

The specific gravity of the blood was taken on several occasions by the diluted glycerine method: it ranged between 1038 and 1035.

The number of erythrocytes per cubic mm. was determined by the aid of a Thoma-Leitz hæmacytometer: in this instrument 16 squares form a field, and 16 squares were always counted (the factor of multiplication is 400,000 when the dilution with Hayem's fluid is 1 in 100). The average number of erythrocytes per 16 squares is 14, or just less than 1 per square: $(\frac{14}{16} \times 400{,}000) = 350{,}000$ number per cub. mm. I believe there

[1] Oliver. *Lancet*, June 20, 1896.

[2] Rollet. "Physiologie des Blutes." *Hermann's Handbuch der Physiologie*, Bd. IV.

are seasonal variations, but I have not sufficiently numerous observations throughout all the seasons to establish certainties. It seems that the minimum is reached about February (275,000), while in June and in October the figures have reached 400,000. The HbO_2-burden per corpuscle is thus $\frac{3{\cdot}5}{350{,}000}$ or $\frac{1}{100{,}000} = {\cdot}00001$. The similar ratio in man is $\frac{15}{5{,}000{,}000} = {\cdot}000{,}003$, these are as $3\frac{1}{3} : 1$, or the burden of hæmoglobin per erythrocyte is, in the skate, $3\frac{1}{3}$ times as great as in man.

The number of leucocytes per cub. mm. varies from a minimum of 12,500 to a maximum of 40,000 (readings at all seasons and in all conditions of nutrition of the fish). The instrument used was a Thoma-Leitz hæmacytometer (dilution 1 in 10).

The fact that skates will not feed freely in captivity on their food of "small green cod" and molluscs, no doubt influences the number of leucocytes. I frequently counted 40,000 or 16 per 16 squares (with a dilution of 1 in 10) when the fish were newly brought in from the sea, and had presumably been recently feeding, as their stomachs contained semi-digested material. After some days' captivity, such fields as 9, 10, and 12 per 16 squares are common, *i.e.* 22,500, 25,000, 30,000 respectively, and of these the last two are by far the most common. I believe the leucocytes per cub. mm. in an average skate (500—600 grms wt.) are between 25,000 and 30,000.

Thus the red to white ratio is 350,000 : 300; or 12 : 1 (about); or 10 : 1.

Amongst the leucocytes to be seen in perfectly fresh blood and in fish which have been in captivity for not more than an hour or two, are a few large very coarsely granular cells. They vary between ·012 and ·015 in diameter: their granules which are strongly eosinophil are large and refractive: the cells in films have a tendency to burst. The isolated nucleus and the deep red granules around it are very distinct. These large cells, which appear extremely dark or opaque in the field of the hæmacytometer, are present on an average to the extent of 4 per 16 squares, *i.e.* $(\frac{4}{16} \times 40{,}000) = 10{,}000$ per cub. mm.

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