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Effect of Low Pressure on the Blood Picture of Necturus Maculosus.

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This problem was undertaken for three reasons. (1) There are no well controlled experiments, so far as I know, in which an aquatic form has been subjected to a constant low pressure and examined for changes in its blood picture. Necturus was chosen as the experimental animal because of the readily available source of blood in the afferent branchial arteries of the external gills and because the normal blood picture has been thoroughly worked out by Dawson.[1] (2) Experiments such as those described below supply a method for determining the nature of the red cell ancestors in Necturus by causing them to leave the erythrocytopoietic loci and enter the circulating blood. (3) It is interesting to compare the intensity and duration of the stimulus required to send out erythroid cells into the blood stream of an aquatic form with the intensity and duration necessary to evoke an analogous response in mammals (*i. e.*, increase the reticulocyte and total red cell count).

Groups of Necturi were placed in jars of water and subjected to a constant pressure of 330 mm. Hg. in a specially devised low pressure chamber for varying periods up to 9 weeks of continuous exposure. (For the construction of the apparatus, see Dubin.[2]) The animals were removed once a day for about an hour, and the water in which they were kept was replaced by fresh water. All animals, including the controls, were fed live earthworms twice weekly. In order to minimize the effects of hemorrhage, the small quantity of blood necessary for total counts and 2 smears was drawn on 2 occasions only, once, before the animals were placed in the tank, and later, when they were examined to determine the effects of the reduced pressure. Because of the relatively small red cell count in Necturus (about 42,000 per mm^3. as determined in 28 normal animals), total red cell counts were made by drawing blood from the afferent branchial artery up to the 0.1 mark in a white cell pipette, diluting with Hayem's solution or 0.65% saline containing methylene blue, and counting the cells on 800 squares of a Levy-Hausser counting chamber. By this method the maximum error incurred in counting

* C represents a complete, P a preliminary manuscript.

1 Dawson, Alden B., *J. Morph.*, 1933, **55**, 349.

2 Dubin, Max, *Quart. J. Exp. Physiol.*, 1934, **24**, 31.

red cells in consecutive samples drawn from trial animals was ±6%. White cell counts were made on the same preparation (since they are easily distinguishable from the reds) except that the cells on an area equivalent to 4,000 squares were counted. In making the white cell counts by this method, it is difficult to distinguish between haemocytoblasts and true leucocytic elements, and so both were included in the total count. The average in 16 animals was found to be 440 per mm^3. with a maximum error of ±20%. These counts must be made immediately after the extraction of the blood, for the cells sediment rapidly in the pipette and then tend to disintegrate.

No significant changes in the circulating blood picture were noted until about the 4th or 5th week, when the total red cell count began to show a significant increase. By the end of the 6th week the presence of considerable numbers of red cell progenitors (*i. e.*, haemocytoblasts, proerythroblasts, and erythroblasts) were observed in dry smears stained with Wright's or Pappenheim's stain. The 4 animals examined at the end of the 7th week of exposure showed a 25 to 35% increase in red cell count (average before exposure, 44,000 per mm^3.; average after exposure, 58,000 per mm^3.). Autopsies performed at this time showed that the spleens were no longer dark red (as in the controls), but a pale pink. The white cell counts of these animals at this period also showed a significant increase (average before exposure, 350 per mm^3.; average after exposure, 1520 per mm^3.) This increase was due almost entirely to the increased number of haemocytoblasts observable in the stained dry smears. The thrombocytes also increased in number. The neutrophile, eosinophile, and basophile numbers, however, did not alter significantly.

The red cell counts of the animals examined after 9 weeks of exposure did not show as great percentage increases as did those of the 7-week animals. Neither were the differentiating elements, the haemocytoblasts, proerythroblasts, and erythroblasts as numerous. In fact, it appears that the maximum reaction was reached at the end of the 7th week of exposure and then tended to diminish in intensity with continued exposure. Unpublished data show that rabbits react in the same way to low pressures except that the time taken to produce a response is considerably shorter. Thus, in 3 rabbits exposed to a pressure of 350 mm. Hg. the red cell count reached a maximum in from 7 to 10 days, and then began to fall despite the continued application of the stimulus.

Control animals examined at frequent intervals during the entire

course of the experiments showed none of the changes described above.

The definitive lymphocytes which Jordan[3] has described as occurring in the circulating blood of *Proteus anguineus* (in a prolonged state of inanition) and which Dawson[1] has noted in the blood of *Necturus maculosus* beginning with about the 12th day of immersion in water containing lead were not observed in Necturus even after the 9th week of exposure to the low pressure. The absence of these cells is not necessarily opposed to Dawson's claim that they act as erythroid progenitors when "the demand for new cells is excessive and prolonged". The increased demands in these experiments (due most likely to the decreased oxygen tension at the low pressure employed) are apparently adequately met by an outpouring from the spleen, first of immature red cells which divide by mitosis in the blood stream, and then of haemocytoblasts which proliferate and differentiate intravascularly. The demands do not become great enough to result in a release of lymphocytes from the haemocytopoietic loci.

It is interesting to note that Charipper[4] has shown that the quantity of thyroid extract necessary to stimulate the granulocytopoietic centers is much greater in Necturus than in mammals, and that the maximum point of the response (*i. e.*, the greatest deflection of the polynuclear count to the left) is reached considerably later. The above results demonstrate, in still another way, the sluggishness of the response of Necturus when compared with the response in mammals. It is only after Necturus has been kept for about 5 weeks at the pressure of 330 mm. Hg. that a response is evoked in the form of an outpouring of erythrogenic elements; Dubin,[2] on the other hand, has observed that rabbits need only to be subjected to a pressure of 411 mm. Hg. for 5 days to show a 7 to 10% increase in reticulocytes and a 30 to 40% increase in total red cell count.

[3] Jordan, H. E., *Am. J. Anat.*, 1932, **51**, 215.

[4] Charipper, H. A., *Quart. J. Exp. Physiol.*, 1928, **19**, 109.