ERYTHROCYTE DEGENERATION IN THE ATLANTIC HERRING, *CLUPEA HARENGUS HARENGUS* L.

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ABSTRACT

Cytoplasmic inclusions, associated with erythrocytic degeneration, were found in the circulating blood of herring from Boothbay Harbor, Maine, and from Passamaquoddy Bay at Deer Island, N.B., Canada, in 1969. Except in one instance, when inclusions occurred in herring from water of 2°C, all herring from Boothbay Harbor having inclusions were taken from seawater temperatures of 13.8°C or above. A relationship appears to exist between inclusions in herring erythrocytes and stress factors, especially temperature extremes. At a temperature of 16°C, 96% of a sample of herring were affected with inclusions. Herring sampled at the highest temperature (16°C) were markedly different from all other samples in their blood morphology and had the highest incidence of inclusions. Inclusions were found in the Passamaquoddy Bay area in 2 of the 50 herring sampled from a seawater temperature of 9.8°C, the highest temperature sampled in that area.

Inclusions rarely occurred more than one to a red cell and varied in size from 1.3 to 3.9 μ. In herring containing a high incidence of inclusions, the larger inclusions were usually in the youngest red cells. Cells containing inclusions generally appeared rounded and swollen. Either an abnormally high percentage of up to 90% immature red cells or a low of 1 to 5% immature red cells generally characterized herring containing inclusions.

The blood of herring has been studied at the National Marine Fisheries Service Laboratory at Boothbay Harbor to find physiological indicators of environmental stress that may help us to determine causes of fluctuations in success of year classes. During this investigation I observed inclusion bodies in the cytoplasm of the red cells in many of the herring. In this report I describe these inclusion bodies, their incidence, and the abnormal blood cell morphology associated with these bodies.

Nonspecific cytoplasmic inclusions have been reported in *Fundulus* sp. (Gardner and Yevich, 1969) occurring in wet smears in May and July prior to, and at the beginning of the new breeding season, but not evident in fixed smears. The cytoplasm of erythrocytes from chinook salmon, *Oncorhynchus tshawytscha,* sockeye salmon, *Oncorhynchus nerka,* and adult rainbow trout, *Salmo gairdneri,* contained granular material following fixation procedures (Ridgway, 1956) that the author thought were of mitochondrial origin.

Laird and Bullock (1969) reported finding a distinctive inclusion body formed in the cytoplasm of infected cells associated with piscine erythrocytic necrosis which is responsible for massive red blood cell destruction in *Gadus morhua* from Passamaquoddy Bay. *Liparis atlanticus* from Kent Island, N.B., Canada, and *Myoxocephalus octodecemspinosus* from Portsmouth Harbor, N.H. were lightly infected.

MATERIALS AND METHODS

The 355 herring examined in this study from February through October 1969 consisted of 201 wild herring and 154 captive herring in 12 samples. The herring ranged in length from 12.5 to 30.4 cm and in weight from 10.6 to 214.5 g. The wild herring were taken from four fishermen's catches between central Maine and Canada. Three categories of herring are considered

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in this report: 1) long-term captive herring held 6 months before sampling began in February and terminated in June when the supply of test fish was exhausted, 2) short-term captives which consisted of herring held 2 weeks before being bled, and 3) wild herring that were taken when available. The captive herring were held in seawater which was pumped from the ocean through the tanks and which approximated the temperature of natural seawater. The water temperature was recorded at the site of capture in each instance.

A blood sample was taken from the heart of each herring and preserved in a modified Alsever's solution for serological studies; a microhematocrit was determined and a morphology slide made for each herring. The herring were measured for total length, weighed, sexed, marked, and frozen for reference. All herring were examined for gross parasitism.

The hematocrits and morphology slides were made of blood taken by direct heart puncture with a heparinized 75 mm × 1.3-1.5 mm outside diameter capillary tube. A small drop of blood from the tube was placed on a microscope slide, the tube sealed with plastic clay, and the smear made. The tubes were centrifuged in a microhematocrit centrifuge for 3½ min at 11,000 rpm and read in a microcapillary reader. Slides were air-dried and stained by either the Wright's or Wright-Giemsa staining method. Distilled water was used as a diluent for the Wright's and Giemsa stains. Cells were examined under oil immersion and photographed at 800 and 1250 powers. Hematocrits were measured as the volume percent of packed red cells to the total blood column. (The term "hematocrit" is used in this paper, although Widmark (1970) has suggested the term be replaced with "packed cell volume").

I classify herring erythrocytes according to the stage of development in the peripheral blood as erythroblasts, early polychromatics, middle polychromatics, late polychromatics or mature cells, depending upon their size and the amount of polychromasia present. These stages are described in Table 1. Reticulocytes cannot be identified readily without vital staining so are not included in Table 1. There are variations in individual herring in the size and shape between and within cell stages and the amount of polychromasia present is the best indicator as to the series to which the cell belongs.

RESULTS

The sample source, date of sampling, incidence of inclusion bodies, mean length, standard deviation and range in lengths, mean weight,

TABLE 1.—The developmental stages and the average size of erythrocytes in the peripheral blood of wild herring.

Stage	Description	Cell measurements[1] (microns)	
		Cytosome	Nucleus
Erythroblast	Round, slightly larger cell than the early polychromatic. Has a dark blue staining cytoplasm with lightly stained spaces. *The round purple-red staining nucleus* takes up most of the cell. Erythroblasts are scarce in normal samples.	7.8 × 7.3	5.9 × 6.2
Early polychromatic	The smallest immature red cell that is normally seen in any quantity. Has a light blue to gray staining cytoplasm and appears round. The nucleus takes up most of the cell.	7.8 × 7.1	4.6 × 3.3
Middle polychromatic	Round to slightly oval cell with a gray to light gray-orange staining cytoplasm. Cell is larger than the early polychromatic.	9.5 × 7.0	4.8 × 3.0
Late polychromatic	Slightly oval, has a larger cytoplasm and a *smaller nucleus* than the middle polychromatic. The cytoplasm appears light orange-yellow.	10.0 × 7.7	4.6 × 2.9
Mature erythrocyte	Oval, has a *slightly larger cytoplasm and a slightly smaller* nucleus than the late polychromatic. The cytoplasm appears orange-yellow to reddish. Late polychromatic and mature cells have essentially the same appearance with Wright's stain.	10.3 × 7.7	4.2 × 2.8

[1] Measurements based on 25 cells in each stage from a normal wild herring in March.

TABLE 2.—The occurrence of inclusion bodies in the cytoplasm of herring erythrocytes, 25 February-30 October 1969.

Sample source and category[1]	Date	Incidence in sample	Percent incidence	Water temp. (°C)	Mean length, SD, and range of sample (cm)	Mean weight, SD and range of sample (g)
Long-term captives	25 Feb.	0/25	0.0	1.3	15.3 ± 0.79(14.0-17.2)	20.2 ± 4.3(14.1- 29.0)
Wild, Sheepscot River, Boothbay Harbor	13 Mar.	1/35	2.9	2.0	16.4 ± 2.0 (13.5-19.0)	26.2 ± 9.7(14.0- 42.0)
Long-term captives	24 Mar.	0/25	0.0	3.3	16.0 ± 0.99(13.2-17.5)	20.7 ± 4.5(10.6- 29.4)
Long-term captives	21 Apr.	0/20	0.0	4.9	16.3 ± 0.99(14.3-17.7)	23.0 ± 5.0(14.9- 33.1)
Wild, Eastport, Maine	10 June	0/40	0.0	7.7	22.2 ± 3.0 (14.5-30.4)	80.3 ± 41.3(18.0-214.5)
Long-term captives	23 June	2/12	16.7	15.2	16.2 ± 1.4 (13.1-18.0)	22.8 ± 5.8(14.1- 34.7)
Wild, Spruce Point, Boothbay Harbor	8 July	5/76	6.6	13.8	15.5 ± 1.4 (12.5-18.5)	23.7 ± 6.6(12.8- 43.4)
Wild, Deer Island, N.B., Canada	16 July	2/50	4.0	9.8	21.0 ± 1.9 (13.9-25.2)	81.0 ± 21.1(13.6-133.0)
Short-term captives	22 July	24/25	96.0	16.0	16.0 ± 1.2 (14.4-18.0)	25.3 ± 5.7(17.6- 36.4)
Short-term captives	21 Aug.	3/25	12.0	14.0	16.1 ± 1.3 (13.2-18.6)	22.1 ± 6.2(11.7- 40.9)
Short-term captives	25 Aug.	0/10	0.0	15.5	17.7 ± 1.2 (15.3-20.0)	29.9 ± 7.4(18.5- 48.1)
Short term captives	30 Oct.	0/12	0.0	9.2	16.2 ± 1.1 (15.1-19.0)	20.6 ± 4.2(16.3- 27.6)

[1] Long-term captives—Boothbay Harbor herring held 6 months before being bled.
Short-term captives—herring from wild 8 July sample held 2 weeks before being bled.

and standard deviation and range in weights of all herring included in this study are given in Table 2.

DESCRIPTION OF INCLUSION BODIES

The inclusions are round, granular, intracytoplasmic and appear acidophilic with Wright's stain. The inclusions generally occur singly in the affected cells and vary in size with the largest inclusions usually in the youngest cells. A few red cells contained two inclusions. The bodies characteristically range in size from 2.3 to 3.3 μ in early polychromatics, 1.7 to 1.9 μ in middle polychromatics, and 1.3 to 1.6 μ in late polychromatics and mature erythrocytes. The inclusions vary from bright red to reddish-purple in contrast with the blue-gray cytoplasm of the young cells and the dull orange-yellow cytoplasm of the mature cells. Many inclusions have a dark-purple periphery with a light central zone; other inclusions are the same color throughout. Some of the larger inclusions appear to have at least four small, dense-staining particles within or along the periphery of the inclusion.

Inclusions were not found outside the red cells, nor were inclusions observed in any white cells of the 355 herring examined in this study.

MORPHOLOGY

Wild herring that did not contain inclusions ranged from 3 to 35% with an average of 20% immature erythrocytes, while captive herring without inclusions ranged from 2 to 25% with an average of 14% immature erythrocytes in their peripheral blood.

Two types of morphology usually characterized the blood of herring that contained inclusions: either upward to 90% immature red cells or a low of 1 to 5% immature red cells. The single herring with inclusions in March had the highest percentage of immature erythrocytes I had found in wild herring to that date. Eighty percent of the red cells were immature, with 12% of the immature and 90% of the mature cells affected with inclusions. Erythroblasts, rare in a normal blood sample, were abundant on this slide. The inclusions occurred singly in the cytoplasm and varied in size; the largest were in the youngest cells. The bodies ranged in size from 2.3 to 3.1 μ in early polychromatics, 1.7 to 1.9 μ in middle polychromatics, and 1.3 to 1.6 μ in late polychromatics and mature erythrocytes. The nucleus of the affected cells exhibited vacuolization and pyknosis. Abnormally large immature red cells (macrocytes) were evident with atypical cells present in all developmental stages (Figure 1). The remaining 34 herring in the sample had normal red cell morphology (Figure 2).

Inclusions first appeared in long-term captive herring in June in 2 out of 12 specimens. These two herring had the lowest hematocrits of the sample. The blood morphology of the two affected herring differed. One herring had 60%

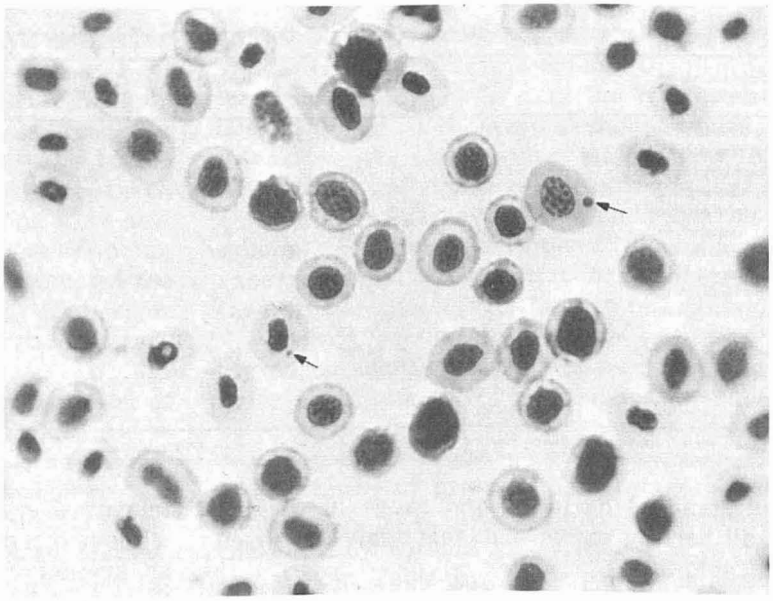

FIGURE 1.—13 March 1969. Photomicrograph of wild herring blood showing macrocytosis of the young cells. Early polychromatics are prevalent. Arrows point to an inclusion in a middle polychromatic and in a mature red cell.

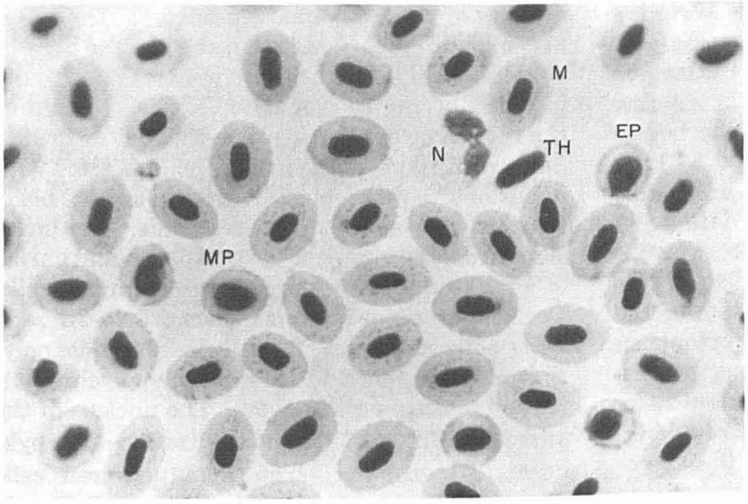

FIGURE 2.—13 March 1969. Photomicrograph of normal wild herring blood showing the absence of inclusions.

EP — early polychromatic erythrocyte
MP — middle polychromatic erythrocyte
M — mature erythrocyte
N — neutrophil
Th — thrombocyte

immature red cells with inclusions found in only 6% of the mature red cells; the other affected herring had 12% immature red cells with inclusions in 50% of the immature and 20% of the mature cells.

Nearly 7% (5/76) of the wild herring sampled on 8 July from Boothbay Harbor contained inclusions, and a few cells in several herring contained two inclusions. Four of the five affected herring contained over 70% immature red cells, the other 15%. Both abnormally large and small erythrocytes and many disintegrated cells were present. Anisopoikilocytosis (abnormal cell sizes and shapes) of all red cell developmental stages was evident. The nuclei of many affected erythrocytes contained two or

three large vacuoles. The affected mature cells were rounded instead of the usual oval (Figure 3); a typical rounded mature cell measured 10.1 × 9.4 μ for the cytosome, 3.7 × 3.4 μ for the nucleus, and 1.2 × 1.5 μ for the inclusion body. Vacuolization of the cytoplasm was evident in many red cells. Inclusions were present in some microcytic mature erythrocytes as small as 4 × 4 μ for the cytosome (less than one-half normal size). Inclusions in a few early polychromatics were larger than usual. One of the largest inclusions in a young cell was nearly as large as the cell nucleus—the cytosome measured 9.2 × 8.0 μ, the nucleus 4.7 × 3.7 μ, and the inclusion 3.9 × 3.6 μ (Figure 4). Otherwise inclusions in the wild herring of March and July were of the same size.

A relationship appears to exist in the occurrence of inclusions and abnormal red cell morphology with temperature extremes. The short-term captive herring sampled on 22 July at 16°C, the highest temperature at which samples were taken, were markedly different from all other samples in their morphology and incidence of inclusions. Ninety-six percent (24/25) of the herring had inclusions, and of those over half had inclusions in at least 90% of their red cells. A majority of the smears in this sample showed 5% or less intact immature red cells. Anucleated "balloon" cells were evident in all smears in this sample, some smears had up to 50% of these cells (Figure 5). The balloon cells appear pale red with Wright's stain, are similar in size, and range from 9.4 × 9.4 μ to 10.9 × 10.9 μ. Some of the cells appear to show diffusion of nuclear material into the cytoplasm. The smears with the greatest incidence of inclusions generally had the most balloon cells. The most heavily affected herring from the 8 July sample also showed these cells. In the smear free of inclusions a few balloon cells were seen, the intact cells appeared normal and 10% immature red cells were present (Figure 6). Such balloon cells are seen in apparently normal blood samples only occasionally and in very low frequency.

The short-term captive herring sampled on 21 August at 14°C showed a substantial decrease in inclusions with 12% of the sample affected, but many nonaffected fish had abnormal cells (Figure 7). Higher than normal seawater temperatures of up to 20.5°C (68.9°F) during August may account for the abnormal cells in herring without inclusions.

Inclusions were found in 2 of the 50 herring

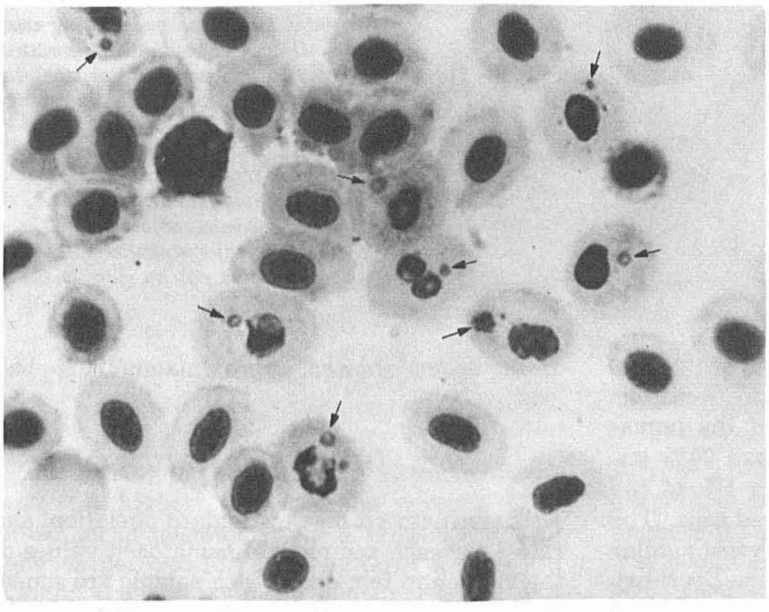

FIGURE 3.—8 July 1969. Photomicrograph of wild herring blood showing intracytoplasmic inclusions associated with nuclear degeneration and a ballooning of the red cells.

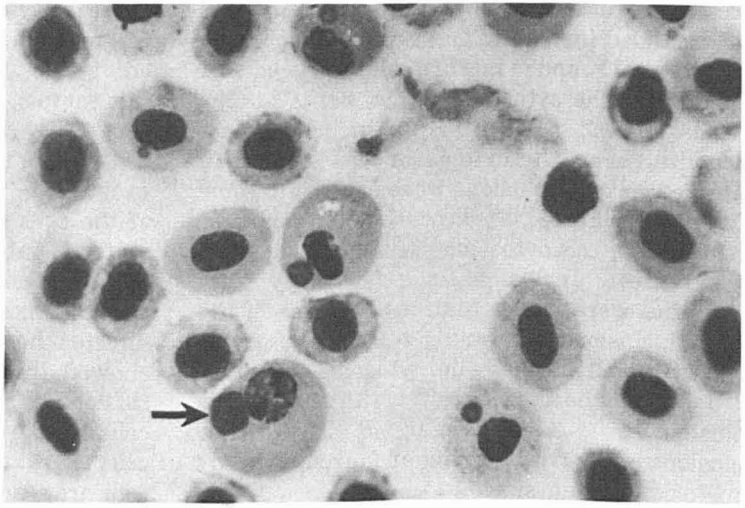

FIGURE 4.—8 July 1969. Photomicrograph of wild herring blood showing one of the largest inclusions seen in this study. The inclusion measures 3.9 × 3.6 μ, the cell nucleus 4.7 × 3.7 μ, and the cytosome 9.2 × 8.0 μ.

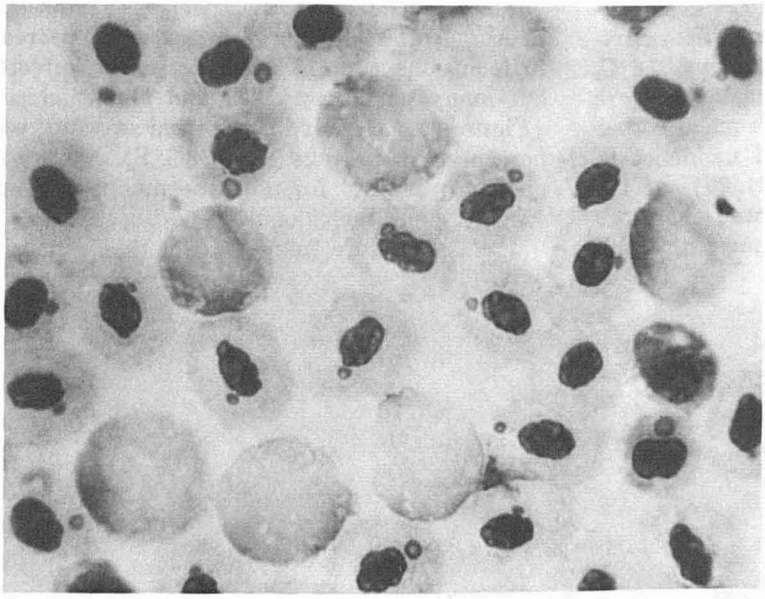

FIGURE 5.—22 July 1969. Photomicrograph of herring blood from a short-term captive, 2 weeks after placing wild fish from the 8 July sample in the tanks, showing nearly all of the red cells affected with inclusions, abnormal nuclei, and anucleated "balloon" cells.

sampled on 16 July from Deer Island, N.B., Canada. One herring had 25% immature red cells with inclusions in less than 1% of the immatures; the other affected herring had 90% immature red cells with inclusions in 1% of the immature and 90% of the mature red cells. The morphology and size of inclusions were similar to that of the 8 July samples from Boothbay Harbor. The smear with the greatest incidence of inclusions showed approximately 20% balloon cells.

HEMATOCRITS

The hematocrit mean, standard deviation, and range for each sample and hematocrit values of the males and females in each sample are shown in Table 3. The lowest hematocrit for an indi-

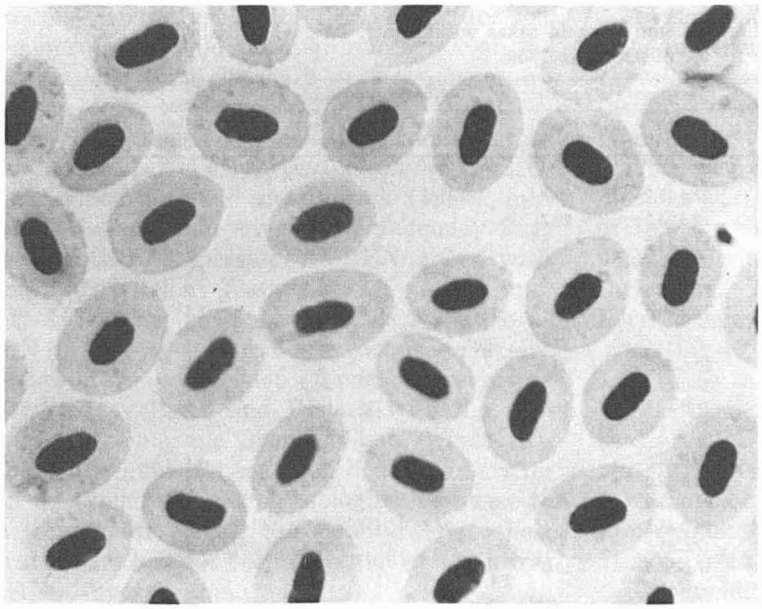

FIGURE 6.—22 July 1969. Photomicrograph of normal red cells from the only herring not affected with inclusions from a sample of 25 short-term captives.

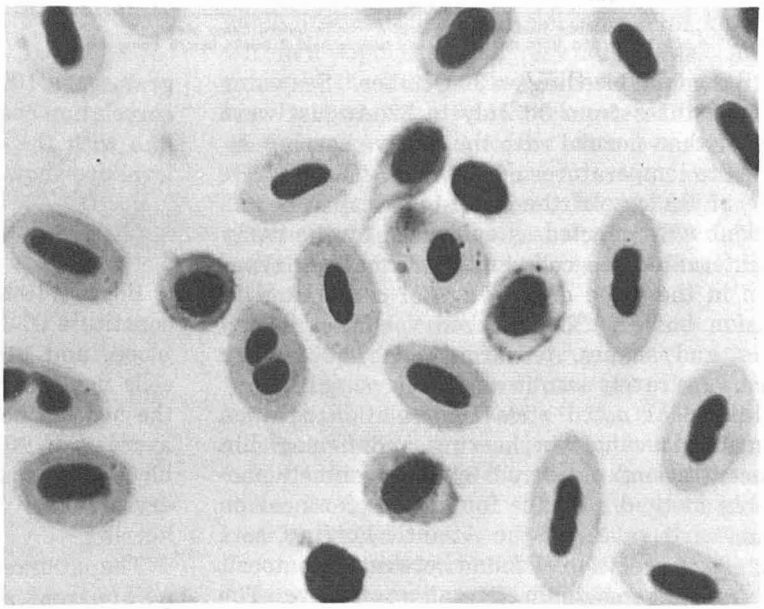

FIGURE 7.—21 August 1969. Photomicrograph of abnormal cells in short-term captive herring. Higher than normal natural seawater temperatures of up to 20.5°C (68.9°F) during August may account for the abnormal cells in herring not affected with inclusions. This herring had one of the lowest hematocrits of the sample (21 volumes percent); the scarcity of cells on the slide reflects this finding.

vidual herring in this study was 17 volumes percent; the highest, 54.5 volumes percent. The lowest mean hematocrit for a sample was 28.7 volumes percent for the long-term captives in March; the highest mean hematocrit was 41.4 volumes percent for a sample of wild herring in July. The *t*-test analysis revealed no significant differences in hematocrit values between sexes in these immature herring.

A consistent decrease is evident in the mean hematocrit values of the wild herring from the time they were placed in captivity on 8 July

TABLE 3.—Hematocrits of herring samples and sexes within each sample, 25 February-30 October 1969.

Date	Water temp. (°C)	Herring sampled (Number)	Hematocrits of samples		Mean hematocrits of:		Standard deviation
			Range (vol %)	Mean (vol %)	Males (vol %)	Females (vol %)	
Long-term captives[1]:							
25 Feb.	1.3	23	22.5-38.0	29.7			4.1
24 Mar.	3.3	25	22.5-36.0	28.7			3.6
21 Apr.	4.9	20	23.0-42.5	31.2			4.3
23 June	15.2	5	22.0-42.0		34.9		7.8
		7	22.5-43.5			36.3	7.9
		12	22.0-43.5	35.7			7.5
Wild, Spruce Point, Boothbay Harbor:							
8 July	13.8	27	27.0-54.5		42.2		6.0
		49	31.0-49.5			40.9	4.5
		76	27.0-54.5	41.4			5.0
Short-term captives[2]:							
22 July	16.0	13	25.0-47.0		40.5		6.1
		12	34.0-53.0			39.6	5.4
		25	25.0-53.0	40.1			5.7
21 Aug.	14.0	12	21.0-46.5		36.5		6.9
		13	17.0-52.5			35.4	8.9
		25	17.0-52.5	36.0			7.9
25 Aug.	15.5	4	23.0-31.5		28.6		3.8
		6	24.0-39.0			33.5	6.2
		10	23.0-39.0	31.6			5.7
30 Oct.	9.2	12	23.0-39.0	30.3			5.2

[1] Boothbay Harbor herring held 6 months before being bled.
[2] Herring from the wild 8 July sample held 2 weeks before being bled.

until the final bleeding on 30 October. Seawater temperatures from 30 July to 22 August were higher than normal with the captive herring exposed to temperatures of up to 20.5°C (68.9°F). The physiology of the short-term captives was undoubtedly affected as evidenced by the many disintegrated red cells and abnormal cell types seen in the blood of herring not containing inclusion bodies. The marked variation in cell sizes and shapes, teardrop cells and bizarre forms are rarely seen in normal herring blood.

In 1965 I noted a close correlation between hematocrit values in herring and hemoglobin concentrations measured by the cyanmethemoglobin method. I have found no references on hematocrit values of the Atlantic herring, so I include the relations I found between hematocrit values and hemoglobin concentrations here. The herring sampled in 1965 were long-term captive herring 12.7-25.4 cm in length. Hematocrits were taken as described in the present study. Blood for hemoglobin measurements was obtained from the heart and placed in a small test tube to which a drop of liquid heparin had been added. Hemoglobins were measured as grams per 100 ml. Regression analysis gave a correlation coefficient of 0.9333. The regression line with the confidence limits of Y at the 0.05 level are shown in Figure 8.

DISCUSSION

Boyar (1962) reported that mature red cells constitute 97-100% of all blood cells in herring blood, and the immature red cells plus white cells made up less than 3% of the total cells in the herring he examined. However, I found an average of 20% immature erythrocytes in the blood of normal wild herring and 14% immature erythrocytes in the blood of normal captive herring.

The occurrence of cytoplasmic inclusions had no apparent relationship to sex, length, weight, or hematocrits, nor did herring with inclusions show, on cursory examination, more than the usual parasites observed in samples without inclusions. The occurrence of inclusions is associated with other hematological abnormalities in the peripheral blood including upward to 90% immature red cells or a low of 1 to 5% immature

red cells in contrast to the 20% immature red cells normal for wild herring; microcytic erythrocytes less than one-half normal size; and varying degrees of anisocytosis and poikilocytosis. The affected red cells have some characteristics of piscine erythrocytic necrosis (PEN) as described by Laird and Bullock (1969), in a cod, *Gadus morhua*, from Passamaquoddy Bay. These authors associated the PEN in cod with viruslike particles. Walker (1971; pers. comm., July 1972) has confirmed the viral nature of PEN in cod by electron microscopy. He also confirmed the correlation of nuclear lesions as described by Laird and Bullock with the presence of cytoplasmic viroplasm and virions. Although I believe the inclusion bodies in herring can be explained as a physiological response to environmental stress, the possibility of their viral nature has not been ruled out and requires further investigation.

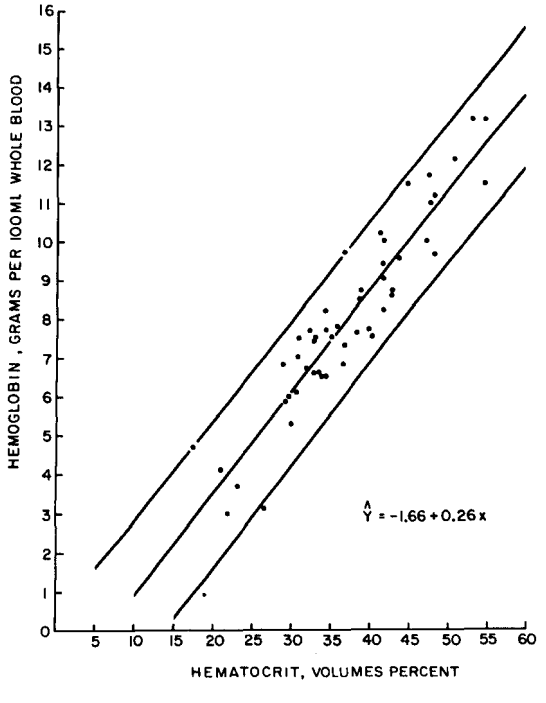

FIGURE 8.—Relation of hematocrit values to hemoglobin concentrations in captive herring during late winter, 1965.

A relationship appears to exist between inclusions in herring erythrocytes and stress factors, especially temperature extremes. Except in one instance when inclusions occurred in herring from water of 2°C, all herring from Boothbay Harbor (lat 43°50'N, long 69°40'W) having inclusions were taken from seawater temperatures of 13.8°C or above. At a temperature of 16°C, 96% of a sample of herring were affected with inclusions. Inclusions were found in 2 of 90 herring sampled from the Passamaquoddy Bay area (lat 45°00'N, long 67°00'W). These herring were taken from a seawater temperature of 9.8°C, the highest temperature sampled in that area. During the months of June and July water temperatures in the Passamaquoddy Bay area have, over a number of years, averaged approximately 4°C lower than in the Boothbay Harbor area (Colton and Stoddard, 1972).

The incidence of inclusions within a population can change rapidly, apparently with changing environmental conditions, and they are capable of affecting a high percentage of herring within a population in a very short time. As an example, the wild herring on 8 July from Boothbay Harbor had a 6.6% incidence of inclusions (5/76); however, 2 weeks after herring from this population were placed in the laboratory tanks, 96% of the herring sampled (24/25) were affected with inclusions, and over 90% of the red cells in individual herring contained these bodies.

These bodies, associated with erythrocytic degeneration characterized by necrotic nuclei, a ballooning degeneration of the red cells and the appearance of unusual cells in the blood, may be indicative of stress situations for immature herring in the wild. If the stress factors causing these inclusion bodies affect enough herring, they could conceivably have an adverse affect on the population structure endemic to certain areas. The erythrocytic degeneration found in herring may be due to a viral infection as described in other fishes by Laird and Bullock (1969) and confirmed by Walker (1971). The occurrence of such a viral infection in epidemic frequency would certainly be no less important to our understanding of fluctuations in abundance of herring populations.

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