



Erythroplastids of Duck Blood Produced by Cytokinesis, Lysis, and Amitosis

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ABSTRACT

The aim is to describe anuclear erythrocytes (erythroplastids), pyrenocytes (small nucleated daughter erythrocytes), and amitosis (division without chromosomes or a spindle apparatus) of the commercial duck. Wright-Giemsa-stained blood samples came from ducks between 2 and 22 weeks of age. The erythroplastids and pyrenocytes were produced by fully hemoglobinized (normochromic) erythrocytes, and their earlier developmental stages (polychromatic erythrocytes). The cytokinesis results indicated a process beginning with constriction of the cell membrane, and continuing with constriction of the nucleus; followed by its polar displacement and expulsion. Instances of intermediate stages in which both the erythroplastid and the pyrenocyte remained attached by a thin cytoplasmic isthmus were also found. Erythroplastids may be produced by a second mechanism where the RBC nucleus lyses rather than being expelled. Furthermore, there were examples of erythroplastids produced during amitosis, occurring in mature erythrocytes, and at earlier (polychromatic) stages. The causes of erythroplastid formation and amitosis remain obscure, and it is possible that they result from distinct stimuli. As Goncalves et al. (2020) reported, recently erythroplastids were used to measure the effects of air pollution in passerine birds. However, as is the case for other atypical erythrocytes they could be the consequence of toxins, DNA damage, vitamin deficiencies, or immune dysfunction. Erythroplastids and amitotic cells were present along with evidence of fungal infection in some ducks and in others deliberately exposed to aflatoxin B₁ supporting a case for toxicity. Accordingly, these atypical cells may serve as sensitive cytological indicators and bio-markers useful in the study of diseases or toxin exposure.

Keywords: Amitosis, Bio-marker, Erythroplastid, Mycotoxin, Pyrenocyte

INTRODUCTION

Unlike mammals, the majority of avian erythrocytes retain a nucleus during their lifespan. However, anuclear forms, “erythroplastids” can also be found in the circulation (Emmel, 1924). Clark and Raidal (2014) identified erythroplastids in 30 of 70 birds representing 15 orders and 24 species. According to their estimate, based on 2 observations, they occur in Anseriformes and Galliformes between 0 and $\sim 5.3 \times 10^9/L$. In the author’s experience, erythroplastids occur in the presence of atypical leukocytes as described in Cotter (2015a, b, c).

Formation of erythroplastids by fully hemoglobinized cells (normochromic erythrocytes) is portrayed by several illustrations appearing in Lucas and Jamroz (1961). The process begins with cell membrane constriction followed by condensation of the nucleus and

its displacement toward a pole. Expulsion (abscission) at the narrow end of the elliptical erythrocyte results in two products. One, a larger anuclear daughter is the “erythroplastid”. The other nucleated fragment surrounded by a thin cytoplasmic rim, is the “pyrenocyte”. Collectively these stages illustrate the “cytokinesis” process. However, erythroplastids could result from mechanisms other than cytokinesis.

Erythroplastids are rare relative to nucleated normochromic cells, typically comprising < 0.1% of erythrocytes, however, other atypical red blood cells (RBCs) are more common (Cotter, unpublished observations). Lucas and Jamroz (p. 210, 1961) indicated they were numerous in the blood of Mallards, but this was not found by the present author (Cotter, unpublished observations). In an extreme case, the blood of a captive cockatoo contained 47% erythroplastids (Clark et al.,

2013). Moreover, plastid forms (anuclear cells) are not restricted to cells of the erythrocyte lineage (Cotter and Bakst, 2017). Studies reviewed by Lucas and Jamroz (1961) suggest erythroplastids do not form by amitosis, cell division without chromosome condensation, or spindle apparatus formation. He hypothesized that a firm establishment of amitotic nuclear division might require *in vitro* study. He provides a rare example of embryonic amitosis but no post-hatch examples are given. On the other hand, Macklin (1916) demonstrated amitotic nuclear division of “normal” cultured chick heart cells. He differentiated this process from nuclear fragmentation considered as pathologic. Bloom et al. (1970) summarized early amitosis literature and described the role of the turkey (*bn*) gene in the formation of binuclear erythrocytes and “other abnormal erythrocytes”. He emphasized the importance of such investigations in learning about the control of cell division.

With this in mind, the current study aimed to provide evidence for erythroplastid production by lysis of the nucleus in addition to cytokinesis, and also by amitosis. This occurred in both young (polychromatic) and mature (normochromic) erythrocytes.

MATERIAL AND METHODS

Ducks

White Pekin ducks of Maple Leaf Farms (commercial strains) between 2 and 22 weeks of age were the sources. Duck welfare is monitored under the Maple Leaf Farms Trident Stewardship Program for Duck Well Being and procedures were reviewed by a PAACO certified auditor and licensed Veterinarian. Table 1 provides information about the ages, gender, treatments, and microbiology of the investigated ducks.

Table 1. Age, treatment, and microbiology of Maple Leaf Farms ducks providing figures

| Figure | Age (wk) | Gender | Treatment | Microbiology |
|----------|----------|--------|--------------------------|----------------------|
| 1 | 2 | M | .02 ppm AFB ₁ | - |
| 2 | 20 | M | Restricted feed | - |
| 3A, C, D | 2 | M | .02 ppm AFB ₁ | - |
| 3B | 20 | M | Restricted feed | Fungemia |
| 4A | 16 | F | Restricted feed | Fungemia |
| 4B | 2 | M | .02 ppm AFB ₁ | Low grade bacteremia |
| 5A, B | 20 | M | Restricted feed | - |
| 5C, D | 2 | M | .02 ppm AFB ₁ | - |
| 6A, B | 20 | M | Restricted feed | Fungemia |
| 6C, D | 5 | M | Restricted feed | - |

M: Male, F: Female, AFB₁: Aflatoxin B₁

Blood and stain procedures

Blood sample (~ 1 mL) from the hock joint vein by needle prick was drawn into tubes containing EDTA anticoagulant; ~ 3 µL was spread directly onto alcohol-cleaned microscope slides. After drying in a warm air stream and post-fixing in EtOH, slides were stained using an in-house version of Wright’s method followed by brief secondary exposure to Giemsa (Hewitt, 1942; Smith, 1947). Erythroplastids and amitotic cells were located by microscopy at 40x magnification.

Microscopy and photomicrographs

Olympus CX-41 light microscope (Olympus America, Center Valley, PA) equipped with Plan N 40x,

0.65 numerical aperture (high dry) and Plan N, 1.25 numerical aperture 100x (oil) objectives. Images were photographed at either 40x or 100x (oil) with an Infinity-2 1.4-megapixel CCD USB 2.0 camera, and captured with infinity analyze software, Release 5.0.2 (14); Lumenera, Inc. Ottawa, Ontario, CA.

Statistics

Means were separated by a two-tailed t-test with significance level of $p < 0.05$ using Minitab Statistical Software (Release 17 for Windows, Minitab Inc., State College, PA).

RESULTS

Erythroplastid variation

As erythroplastids occur in the context of both normochromic (mature, nRBC) and polychromatic RBC (immature, pRBC) examples are presented using a “canvas approach” in which an atypical cell is featured among a group of neighbors. Examples of erythroplastids as seen at 40x magnification among nucleated neighbors are given in Figures 1 (pRBC) and 2 (nRBC). Both types of erythroplastids are products of cytokinesis. The 40x canvas of Figure 1 shows a field with a mixed age RBC population. Mature nRBC (N) whose average length is 12.2 (+/- 1.0 μm) are distributed among the younger grayish pRBC differentiated from fully hemoglobinized cells by their graded amounts of basophilic cytoplasm. Lengths of pRBC are only slightly less (12.0, +/- 0.6 μm , $t = 0.48$, NS) than nRBC. The tapered end of the gray polychromatic erythroplastid (E) marks the place of nuclear exit (abscission); the complementary daughter (pyrenocyte) is absent from the field. Several nuclear remnants devoid of cytoplasm are possibly from disintegrated thrombocytes (Th).

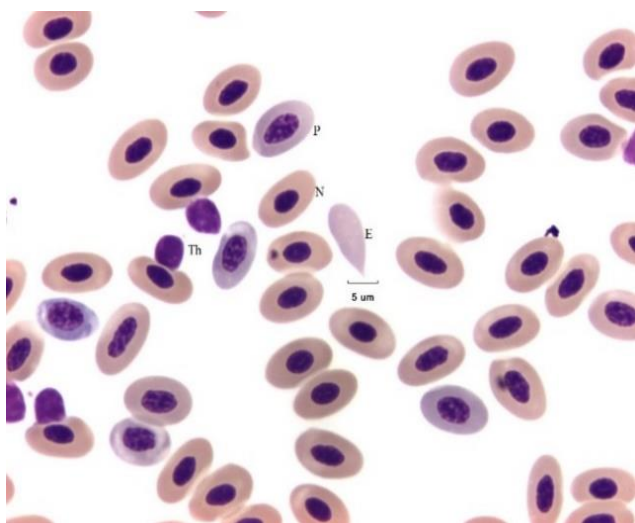


Figure 1. 40x field with a mixed age RBC population containing an erythroplastid product of a pRBC (P). N: erythroplastid from a nRBC; P: polychromatic RBC, Th: thrombocyte nuclei. Detailed descriptions are in the text.

The 40x canvas in Figure 2 shows a polychromatic RBC at an intermediate stage of amitosis (A). Its daughter cells will be asymmetric; one will have a micronucleus (bottom) the other will be a microcyte (top). An erythroplastid (E) that arose by cytokinesis of a mature RBC is located to the left side of A. The remainder of the

field is populated by early and late (P) RBC and nRBC (N).

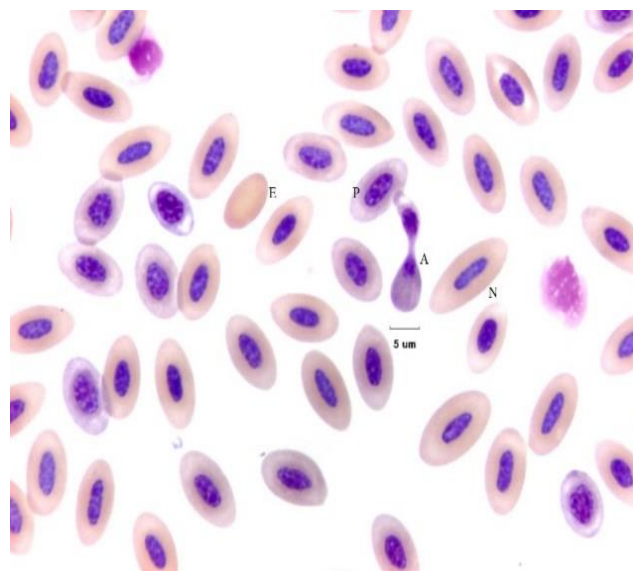


Figure 2. A 40x field with a mixed age RBC population containing an amitotic pRBC (A) and an erythroplastid (E) product of a nRBC, N, normochromic RBC. Detailed descriptions are in the text.

Amitosis

Lucas (1961) stated that to establish amitosis “...find a series of stages in which the nucleus was first involved and divided into halves and each half moved to opposite poles when the cytoplasm divided.” Examples of these stages are presented in Figures 3 and 4.

The stages of amitosis as seen at 100x are shown in Figure 3. Panel A shows early constriction of the nucleus (arrow) without cytoplasm constriction (nRBC). The cell of panel B shows constriction of the both nucleus and cytoplasm (arrow, pRBC). Panel C shows the separation of parent cell nuclei into nascent daughters. The nuclear membrane remains intact but has thinned. Panel D shows extreme thinning of the isthmus prior to separation. The daughter cells will be of unequal size.

Although the cell at an advanced stage of amitosis in Figure 4A (100x) appears to be a late pRBC, one of its daughters contain a large central vacuole reminiscent of atypical late erythroblast types long ago described by Murray (Figure 13, p. 520, 1932). Panel B shows a late-stage amitotic giant pRBC (100x). Its length at 25.3 μm is twice the length of nearby standard size nRBC, ~ 12 μm suggesting polyploidy. The daughter cells that remain attached by a thin isthmus containing chromatin will likely be pseudodiploids.

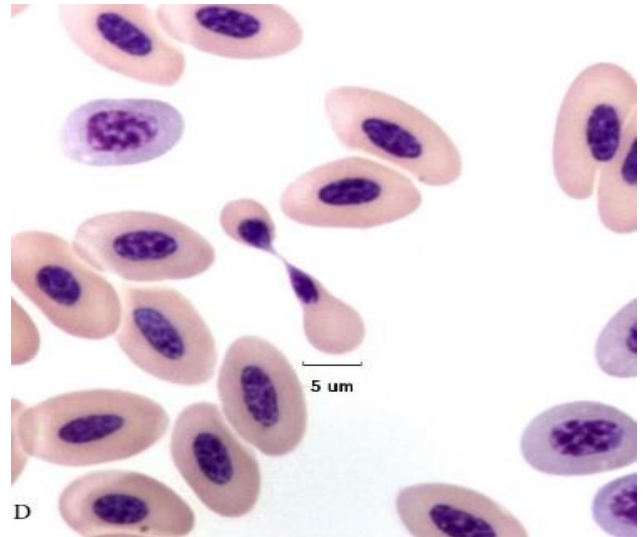


Figure 3. The early (panels A, B) and later stages (panels C, D) of amitosis as seen at 100x. Detailed descriptions are in the text.

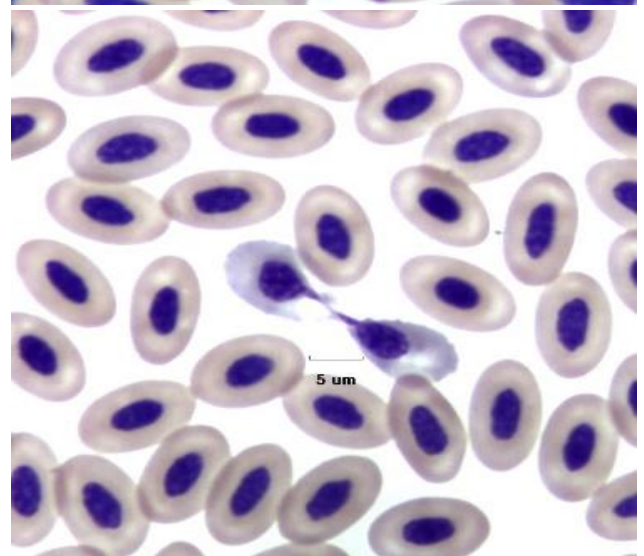
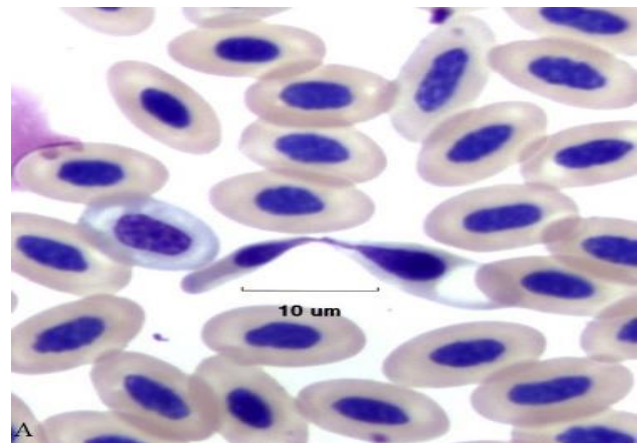
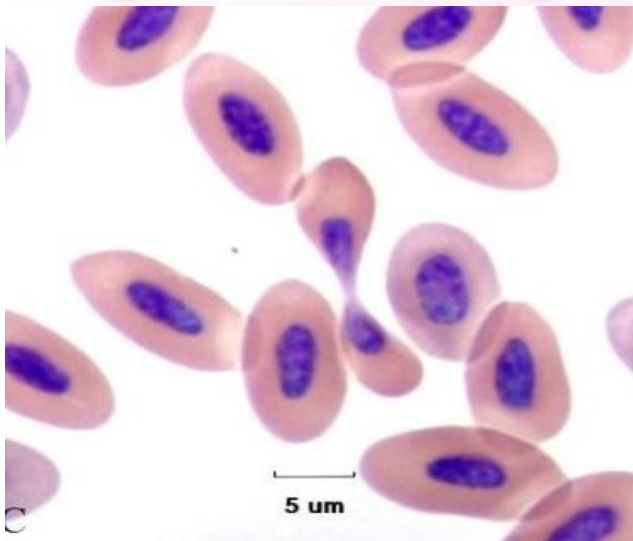
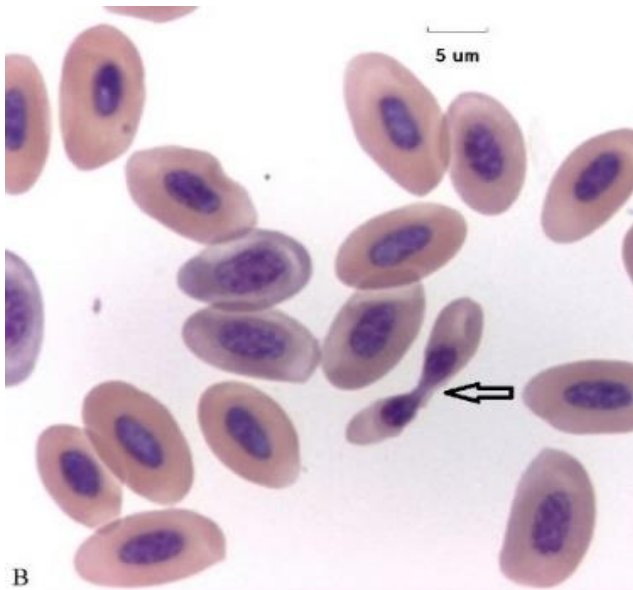


Figure 4. Examples of an early stage (panel A) and a later stage (panel B) of amitosis by pRBC as seen at 100x. Detailed descriptions are in the text.

Complex amitosis

Complex amitosis is herein defined as amitotic cell division accompanied by erythroplastid production. Examples are shown in Figure 5 (100x). The fully hemoglobinized RBC of Figure 5, panel A is already at a late stage of amitosis. It is also producing an erythroplastid by cytokinesis seen near the center of the microscopic field. The nascent erythroplastid remains attached to its parent cell by an isthmus not containing chromatin (top) while the isthmus of the nascent pyrenocyte contains chromatin (left bottom). When separation has been completed, the erythroplastid will have an umbilicus, a small cytoplasmic protuberance. After amitosis is completed the left-hand daughter cell will resemble a microcyte, and the central daughter will be a pyrenocyte. The slightly basophilic cytoplasm of the amitotic RBC of Figure 5, panel B indicates they represent late polychromatic stages. The amitotic cell will produce 3 polychromatic daughters comparable to the products of the cell of panel A.

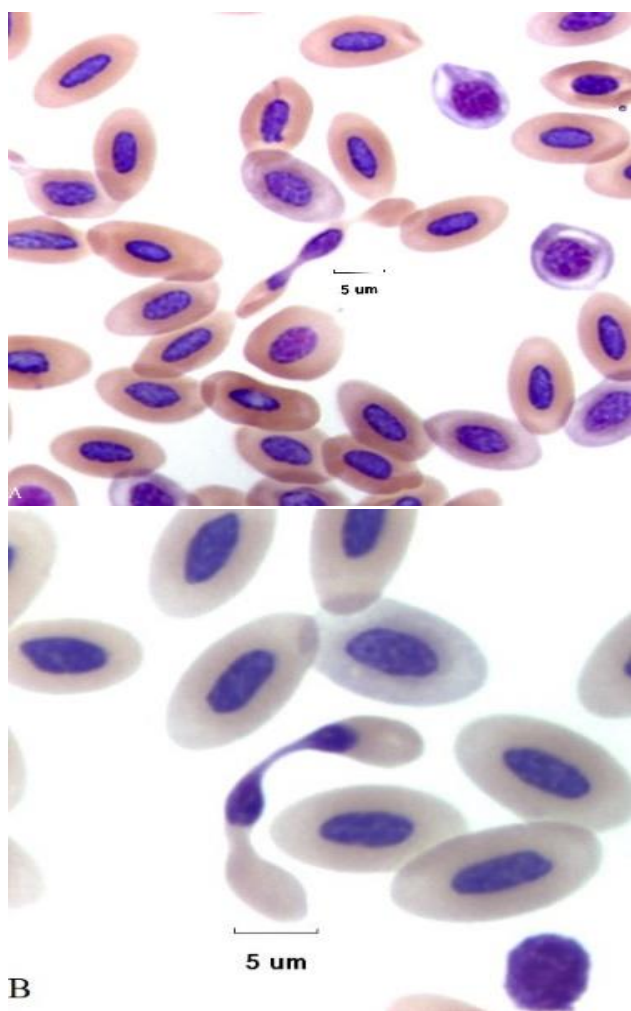
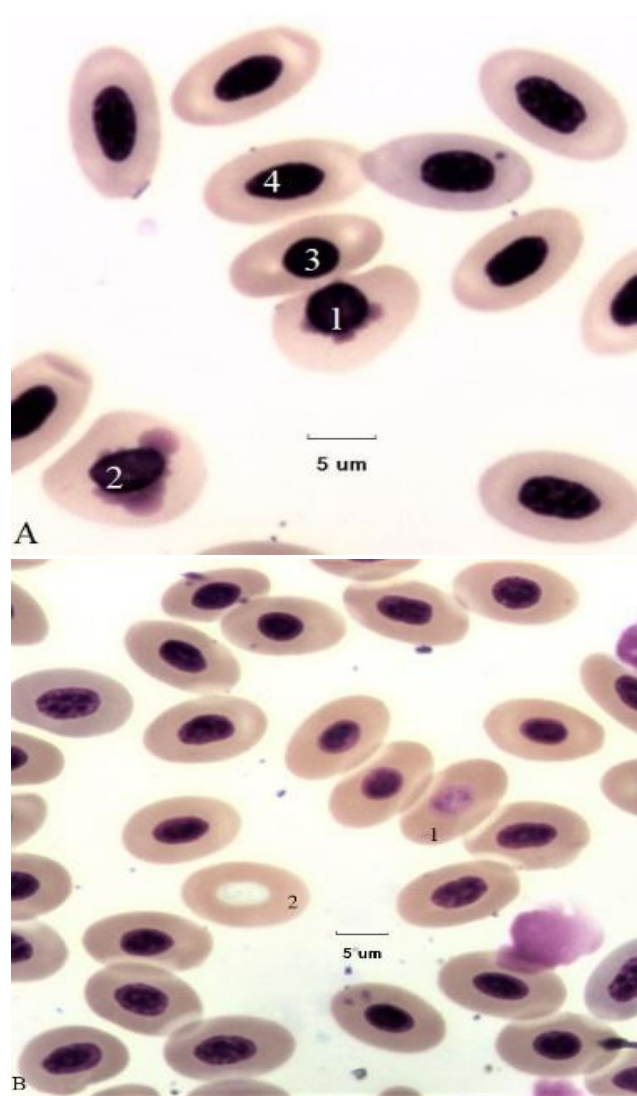


Figure 5. Complex amitosis (division also with erythroplastid production) of nRBC (panel A) and pRBC (panel B) at 100x. Detailed descriptions are in the text.

Erythroplastid production by karyolysis

Karyolysis will be defined as dissolution (lysis) of the nucleus *in situ*. Examples are shown in Figure 6. (100x). Early karyolysis means the integrity of the nuclear membrane has been compromised and the nucleoplasm is beginning to leak into the cytoplasmic space (Figure 6, cell 1). Leakage is further evident in cell 2. Cell 3 has a condensed nucleus seen sometimes at an early stage of the cytokinesis process. The intact nucleus of cell 4 can be used for comparison with nuclei showing leakage. In Figure 6, panel B the nucleus of cell 1 is at an early stage of karyolysis *in situ* without apparent leakage. The chromatophobic nucleus of cell 2 is nearly fully dissolved. Completion of karyolysis by either leakage or dissolution *in situ* will produce an erythroplastid. The erythroplastids of Figure 6, panels C and D retain nuclear residua (arrows) similar to Howell-Jolly bodies (nuclear chromatin remnants) sometimes seen in atypical mammalian erythrocytes. To enhance visibility panels C and D were photographed without the use of an LBD-IF (blue) condenser filter.



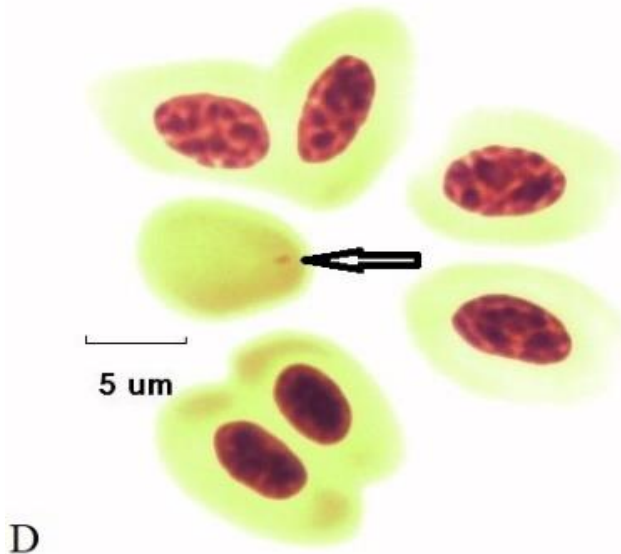
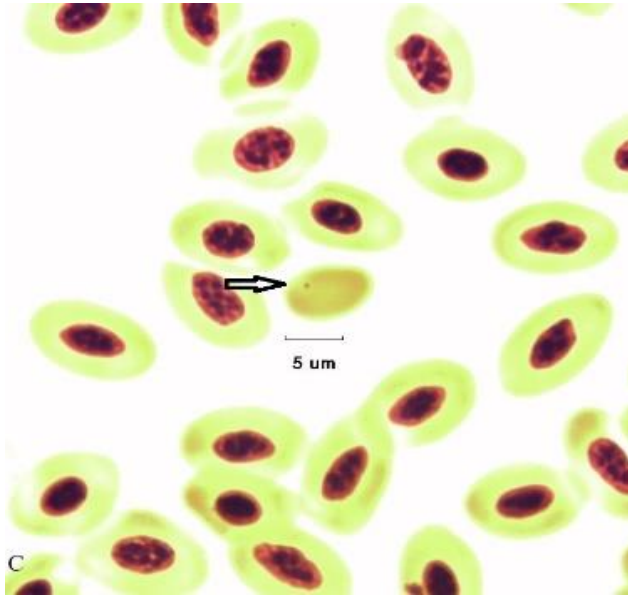


Figure 6. Examples of erythroplastids produced by progressive karyolysis; cells 1, 2, 3; cell 4 has a condensed nucleus. Panel B. Further karyolysis; cells 1 and 2. Panels C and D. Howell-Jolley like bodies in erythroplastids as seen at 100x (arrows). Detailed descriptions are in the text.

DISCUSSION

The aim of the research was to show evidence for erythroplastid production by cells other than fully hemoglobinized (mature) RBC. A second objective was to show that they may arise by processes other than cytokinesis (Lucas and Jamroz, 1961). The gray erythroplastid of Figure 1 indicates that it originated by cytokinesis from an early polychromatic nucleated, pRBC. This cell contrasts with the fully hemoglobinized

erythroplastid of Figure 2 that arose by enucleation of a nRBC.

The cells of Figure 5 indicate erythroplastids may rarely be produced by lysis of nuclei (karyolysis), and this may occur in pRBC as well as nRBC. Occasionally a remnant resembling a Howell-Jolly body (nuclear chromatin remnants) may be found in such cells (Figure 6, panels C and D). Moreover, erythroplastids may be a result of complex amitosis occurring in either nRBC or pRBC. The products are microcytes or pyrenocytes, as is seen in Figure 4, and erythroplastids.

The present observations establish the occurrence of amitosis in mature erythrocytes (nRBC, Figure 3) and at earlier stages (pRBC, Figure 4) an indication that amitosis is not restricted to cells at an early developmental stage (Lucas and Jamroz, 1961). Giant cells (polyploid) are also capable of amitosis (Figure 4B). It is highly unlikely that amitosis is solely a consequence of senescence. If that were the case, it might be expected to occur at higher frequencies, and be more regularly observed in blood samples. Whether amitosis and erythroplastid production occur in the spleen or bone marrow is the subject of further investigation.

The cells described here were located during standard differential counts (400 leukocytes per standard differential count, SDC) performed at 40x magnification. Although atypical erythrocytes are not usually included in an SDC finding erythroplastids was taken as an indication of a remarkable hemogram. In every instance, these atypical RBC were found in the presence of atypical leukocytes. Medium-sized and large reactive lymphocytes, including plasma cells, and atypical heterophils were often found during the SDC (Cotter, personal observation). A description of atypical heterophils of ducks occurring along with bacteremia and appears in previous study of Cotter (2021).

CONCLUSION

In conclusion, the present observations lengthen the list of atypical erythrocytes, expand the mechanisms of erythroplastid production, and demonstrate amitosis occurring in post-embryonic erythrocytes. Collectively, these observations add to the basic knowledge of erythrocyte biology.

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Competing interests

None declared.

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, double publication and/or submission, and redundancy) have been checked by the sole author.

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