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Histochemical observations on chicken blood and bone marrow cells

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INTRODUCTION

In the cells of mammalian blood and bone marrow the distribution of lipid, glycogen and various enzymes has been studied fairly extensively (McManus, 1946; Sheehan & Storey, 1947; Hayhoe, 1953; Schmalzl & Brannsteiner, 1971). However, very little information is available about the histochemical properties of avian blood and bone marrow cells (Merkal & Mora, 1962). Weiss & Fawcett (1953) reported some histochemical studies of chicken monocytes, macrophages and giant cells in tissue culture, stating that lipid, glycogen and acid phosphatase are not demonstrable in developing monocytes but are demonstrable when monocytes transform into macrophages. Against this background, the authors decided to examine cell populations in smear preparations of peripheral blood and bone marrow from young chickens for lipid, glycogen, peroxidase, alkaline and acid phosphatase.

MATERIALS AND METHODS

Thirty five young white Leghorn chickens, 10–30 days old and weighing 50–250 g were used.

Preparation of bone marrow and blood smears

Chickens were killed by exsanguination. Blood was collected into centrifuge tubes which were spun at 3000 r.p.m. for 10 minutes. A standard volume of the serum was pipetted into a small tube. A standard segment of femoral bone marrow was placed in the serum and the tube was shaken thoroughly. The resulting suspension was used in the preparation of the bone marrow smears. Smears of peripheral blood were obtained from the dripping blood before it clotted.

Incidence of damaged cells in marrow smears

The incidence of damaged cells was high in the initial stages of this study. However, damage was reduced considerably when marrow and serum were shaken together very gently. Attempts to obtain teased marrow smears were abandoned because of the high incidence of damaged cells.

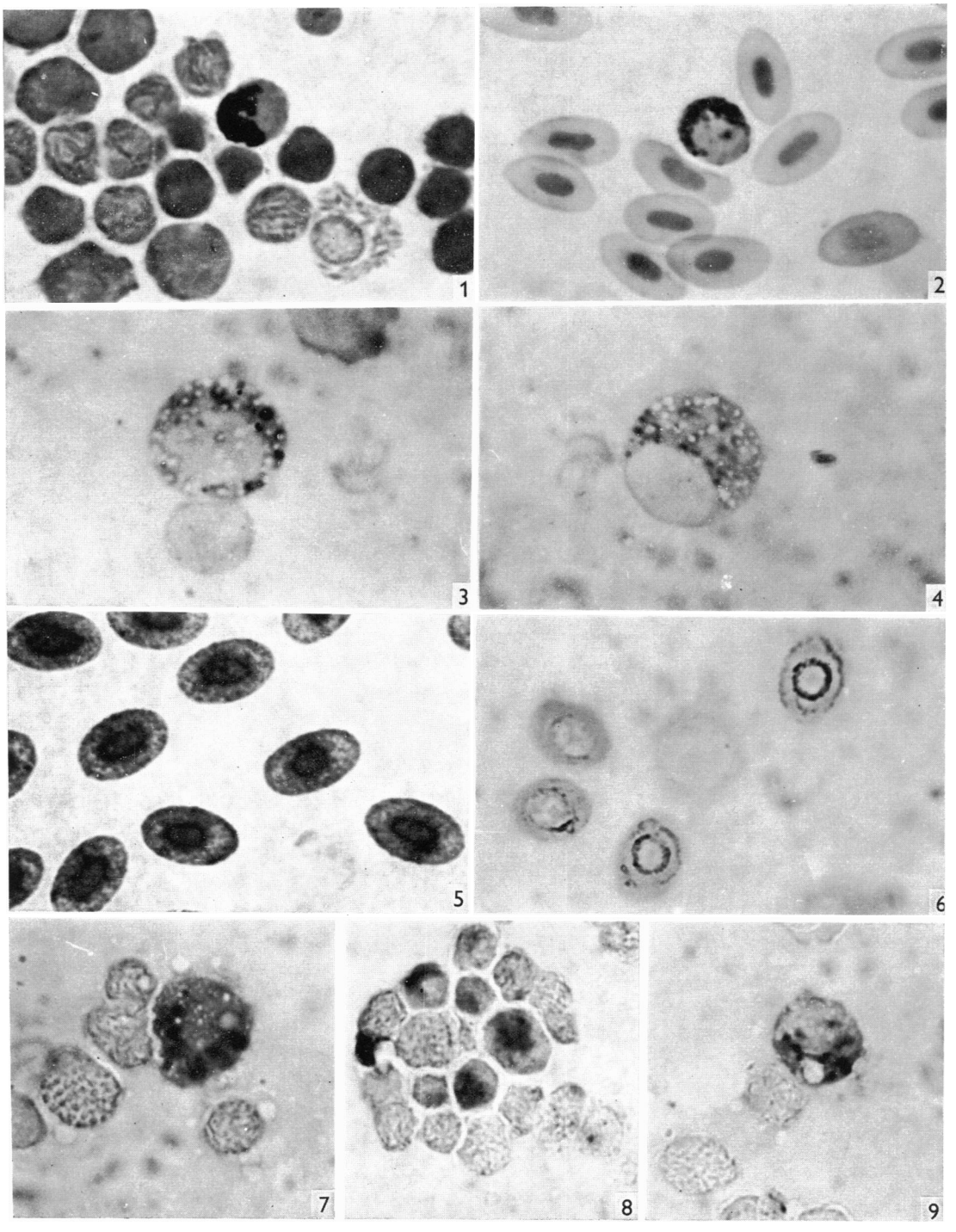
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Table 1. *Summary of histochemical reactions of chicken blood cells*

Cell type	Histochemical techniques				
	For peroxidase	SBB	PAS	For Alk. P.	For Acid P.
Mature erythrocyte	+ → + + +	0	0	0 → +	0
Developing erythrocyte	+ → + +	0	0	+ → + +	0
Mature heterophil	0	0	+ + +	0	0 → ±
Developing heterophil	0	0	+ +	0	0 → +
Lymphocyte	0	0	0	0	0 → +
Monocyte	0 → ±	0 → + + +	0 → + +	0	+ → + +

0, no reaction; ±, faint reaction; +, definite positive reaction; + +, strongly positive reaction; + + +, very strong positive reaction; SBB, Sudan black B; PAS, periodic acid Schiff; Alk. P., alkaline phosphatase; Acid P., Acid phosphatase.

Routine examination of blood and bone marrow smears

Freshly prepared air-dried smears were fixed in absolute methanol (10 minutes), stained in Giemsa solution (10 minutes), washed in tap water (5–10 minutes), and then mounted in neutral medium.

References for the histochemical techniques employed

(i) For lipid: Sudan black B (Sheehan & Storey, 1947).
(ii) For glycogen: PAS (McManus, 1946; Hayhoe, 1953).
(iii) For peroxidase (Washburn, 1928; Robbins, Fahimi & Cotran, 1971).
(iv) For alkaline phosphatase (Kaplow, 1955).
(v) For acid phosphatase (Barka & Anderson, 1962).

RESULTS

Lipid (Sudan black B)

A grey–black precipitate was seen in some monocytes in bone marrow smears (Fig. 1): some of these cells stained intensely. No other cell type stained (Fig. 1). Sudanophilic monocytes were also found in blood smears (Fig. 2).

(All magnifications × 1200.)

Fig. 1. A monocyte containing grey-black precipitate (Sudan black B).

Fig. 2. Many monocytes show grey-black precipitate but erythrocytes are unstained (Sudan black B).

Figs. 3 and 4, show glycogen in monocytes (Fig. 3) and in developing heterophils (Fig. 4) (PAS).

Fig. 5. Erythrocytes are strongly reactive for peroxidase.

Fig. 6. Developing erythrocytes in bone marrow. Many alkaline phosphatase positive granules are seen around the nucleus.

Figs. 7–9. Monocytes (Figs. 7 and 9) show intense, while developing heterophils (Fig. 8) show moderate acid phosphatase activity.

Glycogen (PAS)

Heterophils and monocytes showed bright red granules. Other cell types were negative (Figs. 3, 4).

Peroxidase

In bone marrow smears the erythroid series of cells showed intense peroxidase activity. In peripheral blood smears erythrocytes were strongly reactive (Fig. 5). Monocytes showed faint reactivity.

Alkaline phosphatase

Some mature and developing erythrocytes contained a few positive reactive granules localized around the nucleus (Fig. 6). No other cells reacted.

Acid phosphatase

Enzyme reactivity was visualized as red granules. Monocytes reacted strongly, while some developing and mature heterophils showed moderate or weak reactivity. Some lymphocytes showed a very few (one or two) positive granules (Figs. 7, 8, 9).

These results are summarized in Table 1.

DISCUSSION

Our findings on the distribution of Sudanophilia conflict with those of Merkal & Mora (1962) who observed it in all cell types in the blood and bone marrow smears of the chickens they examined. It may be significant that these authors used the method of Zugibe, Fink & Brown (1959) while we used the method described by Sheehan & Storey (1947) which has reportedly given reliable, consistent and reproducible results (McManus, 1946; Wislocki & Dempsey, 1946; Hayhoe, 1953; Caxton-Martins, 1973).

Weiss & Fawcett (1953) reported Sudanophilia in macrophages in tissue cultures of chicken blood cells. It seems probable that the Sudanophilic monocytes in our own preparations are equivalent to Weiss & Fawcett's macrophages.

Sudanophilia is readily demonstrable in human and other mammalian granulocytes and monocytes, the staining intensity varying with cell maturity (Sheehan & Storey, 1947; Hayhoe, 1953; Caxton-Martins, 1973). It would appear, therefore, either that there are species differences or that the granulocytes and monocytes of young chickens have not attained a degree of functional differentiation comparable with that of mammals.

The presence of glycogen in chicken heterophils was first observed by Weiss & Fawcett (1953). Our findings are in general agreement. However, we did not observe an increase in glycogen content with cell maturity as reported by Hayhoe (1953) for mammalian neutrophils.

The peroxidase of developing blood and bone marrow erythrocytes in the chicken is probably haemoglobin-associated. In mammalian smears, this enzyme, demonstrated by the same technique, is usually restricted to granulocytes and monocytes (Washburn, 1928; Cohn & Hirsch, 1960; Vercauteren, Roela-De Schrijver &

Dellair, 1967; Ackerman, 1968; Bainton & Farquhar, 1968; Jacobs, 1958; Caxton-Martins, Harris & Kugler, 1973).

Alkaline phosphatase activity was confined to some developing and mature erythrocytes. This observation is especially interesting because the enzyme is not demonstrable in mammalian erythrocytes but is confined to mature granulocytes (Kaplow, 1955; Hayhoe, Quaglino & Doll, 1964; Bainton & Farquhar, 1968). This appears to be another species difference.

Weiss & Fawcett (1953) were unable to demonstrate acid phosphatase activity in their preparations. Using the azo-dye method of Barka & Anderson (1962) we observed intense enzyme activity in bone marrow monocytes. Some lymphocytes showed a few positive granules and some heterophils showed a moderate reactivity which decreased with cell maturity. Merkal & Mora (1962) reported acid phosphatase activity in immature erythrocytes in the circulating blood of chickens.

As a result of our observations we would like to suggest that chicken heterophil granules, unlike mammalian neutrophil granules (Cohn & Weiner, 1963; Vercauteren *et al.* 1967; Ackerman, 1968; Bainton & Farquhar, 1968) lack peroxidase and alkaline phosphatase. It is also worth emphasizing that intense peroxidase reactivity is found in erythrocytes in chickens, but in granulocytes and monocytes in mammals. This again underlines species differences with regard to histochemical reactions.

SUMMARY

The distribution of lipid, glycogen, peroxidase, alkaline phosphatase and acid phosphatase has been studied in the cells of blood and bone marrow smears from young chickens. Chicken heterophil granules react differently from those of mammalian neutrophils. A strongly positive peroxidase reaction was given by developing erythrocytes in chickens, unlike mammals. The significance of these species differences is not yet clear.

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REFERENCES

ACKERMAN, G. A. (1968). Ultrastructure and cytochemistry of the developing neutrophil. *Laboratory Investigations* **19**, 290–302.

BAINTON, D. F. & FARQUHAR, M. G. (1968). Differences in enzyme content of azurophil and specific granules of polymorphonuclear leukocytes. I. Histochemical staining of bone marrow smears. *Journal of Cell Biology* **39**, 286–298.

BARKA T. & ANDERSON, P. J. (1962). Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. *Journal of Histochemistry and Cytochemistry* **10**, 741–753.

CAXTON-MARTINS, A. E. (1973). Some histochemical studies on transitional cells in normal and irradiated guinea-pig bone marrow and in the bone marrow of the human foetus. M.Phil. Thesis, University of Nottingham, England.

CAXTON-MARTINS, A. E., HARRIS, P. F. & KUGLER, J. H. (1973). Histochemical studies of 'transitional' cells in bone marrow and blood of human foetus: evidence for differentiation into granulocytes and monocytes. *Journal of Anatomy* **114**, 300.

COHN, Z. A. & HIRSCH, J. G. (1960). The isolation and properties of the specific cytoplasmic granules of rabbit polymorphonuclear leukocytes. *Journal of Experimental Medicine* **112**, 983–1004.

COHN, Z. A. & WEINER, E. (1963). The particulate hydrolases of macrophages. I. Comparative enzymology, isolation and properties. *Journal of Experimental Medicine* **118**, 991–1008.

HAYHOE, F. G. J. (1953). The cytochemical demonstration of lipids in blood and bone marrow cells. *Journal of Pathology and Bacteriology* **65**, 413–421.

HAYHOE, F. G. J., QUAGLINO, D. & DOLL, R. (1964). The cytology and cytochemistry of acute leukaemias. *Medical Research Council, Special Report Series, No. 304.*

JACOBS, A. (1958). Staining for leucocyte peroxidase. *Lancet* 7022, 697.

KAPLOW, L. S. (1955). A histochemical procedure for localizing and evaluating leucocyte alkaline phosphatase activity in smears of blood and marrow. *Blood* **10**, 1023–1029.

MCMANUS, J. F. A. (1946). Oxidation of mucin with periodic acid producing the capacity of stain with Schiff's reagent. *Nature* **158**, 202.

MERKAL, R. S. & MORA, E. C. (1962). Cytochemistry of erythrocytes and leukocytes of the White Leghorn chicken. *Experimental and Molecular Pathology* **1**, 497–508.

ROBBINS, D., FAHIMI, H. D. & COTRAN, R. S. (1971). Fine structural cytochemical localization of peroxidase activity in rat peritoneal cells: Mononuclear cells, eosinophils and mast cells. *Journal of Histochemistry* **19**, 571–575.

SCHMALZL, F. & BRANNSTEINER, H. (1971). The application of cytochemical methods to the study of acute leukemia. *Acta Haematologica* **45**, 209–217.

SHEEHAN, H. L. & STOREY, G. W. (1947). An improved method of staining leucocyte granules with Sudan Black B. *Journal of Pathology and Bacteriology* **59**, 336–337.

VERCAUTEREN, R. E., ROELA-DESCHRIJVER, M. P. & DECLAIR, W. (1967). Further studies on the specific granules of leukocytes. *Enzymologia* **33**, 279–298.

WASHBURN, A. H. (1928). A combined peroxidase and Wright's Stain for routine blood smears. *Journal of Laboratory and Clinical Medicine* **14**, 246–250.

WEISS, L. P. & FAWCETT, D. W. (1953). Cytochemical observations on chicken monocytes, macrophages and giant cells in tissue culture. *Journal of Histochemistry and Cytochemistry* **1**, 47–65.

WISLOCKI, C. B. & DEMPSEY, E. W. (1946). Observations on the chemical cytology of normal blood and haemopoietic tissues. *Anatomical Record* **96**, 249–277.

ZUGIBE, F. T., FINK, M. L. & BROWN, K. D. (1959). Carbowax 400: A new solvent for oil red O and Sudan IV for staining carbowax embedded and frozen sections. *Stain Technology* **34**, 33–37.