PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Emma-4 Analysis of Iron in Cells of the Thymic Cortex of a Weaver-Bird (Quelea quelea)

Marion D. Kendall

Phil. Trans. R. Soc. Lond. B 1975 **273**, 79-82
doi: 10.1098/rstb.1975.0105

Phil. Trans. R. Soc. Lond. B. **273**, 79–82 (1975)
Printed in Great Britain

EMMA-4 ANALYSIS OF IRON IN CELLS OF THE THYMIC CORTEX OF A WEAVER-BIRD (*QUELEA QUELEA*)

By MARION D. KENDALL†
Department of Anatomy, St Thomas's Hospital Medical School, Lambeth Palace Road, London SE1 7EH

(*Communicated by E. C. Amoroso, F.R.S. – Received* 24 *February* 1975)

[Plate 6]

CONTENTS

Combined morphological and analytical studies with the EMMA-4 analytical electron microscope have enabled very early erythroid cells to be identified within the cortex of enlarging thymic lobes of *Quelea quelea*. These early erythroid cells have pale cytoplasm (sometimes with ferritin-like crystals present), slightly pachychromatic nuclei and have fewer cell organelles (mitochondria) than lymphocytes. Counts for iron were approximately 70 % lower than counts from mature erythrocytes found free in the cortex. Iron was also recorded from some epithelial reticular cells and pyknotic nuclei; no iron was recorded from small lymphocytes (thymocytes) in the cortex. The presence of very early erythroid cells is a further indication that erythropoiesis occurs *in situ* in the avian thymus.

Introduction

Erythropoiesis has been demonstrated in the thymic lobes of the red-billed quelea, *Quelea quelea* L. (Kendall & Ward 1974). While invasion of the thymic lobes by immature erythroid cells produced elsewhere in the body cannot be entirely ruled out, the presence of large numbers of very immature cells suggests that the cells were developing *in situ*.

If the erythroid cells were developing within the cortex of the gland very early erythroid cells should be identifiable on electron micrographs. During the examination of electron micrographs (to be reported elsewhere, Kendall & Frazier, in preparation) pale cells with sometimes elongated and slightly pachychromatic nuclei were found close to clearly recognizable immature erythroid cells. While some of these cells could be described as lymphocyte-like

† Previous address: Centre for Overseas Pest Research, College House, Wrights Lane, London W8 5SJ.

(Yoffey & Courtice 1970), the comparative lack of cell organelles and general shape suggested early erythroid cells prior to the deposition of haemoglobin in the cytoplasm.

It was thus decided to analyse these cells and others in the cortex of the thymus of *Quelea quelea* for the presence of iron. This was done with the analytical electron microscope EMMA-4 which allows the simultaneous viewing and analysis of ultra-thin sections. The results are reported here.

Materials and methods

The thymus glands were taken from wild *Quelea quelea* captured in mist nets in Tanzania and Kenya between 1969 and 1972.† The glands were excised and immediately fixed in ice-cold 2.5 % glutaraldehyde in phosphate buffer, pH 7.4, for 2 h or 5 % glutaraldehyde in veronal buffer, pH 7.3, for 4 h. They were washed in the respective buffers with 10 % added sucrose, dehydrated and embedded in Araldite. Silver and gold sections were collected for routine electronmicroscopy, blue and 1 μm thick sections were used for analysis. The sections for analysis were collected onto large mesh carbon-coated grids and stabilized with a thin film of carbon. EMMA-4 was operated at 80 kV for the analysis with a probe current of 0.04 μA. The standard was a stainless steel grid. The counts for iron and total white radiation were made over a 100 s period. To enable the results to be compared statistically 10 cells of each type were analysed from one specimen where the white radiation count was consistently between 8000 and 12500; these counts are expressed with reference to a white radiation value of 10000 and are given ± standard deviations.

Results

The cell types analysed are shown in figure 1, plate 6. Fully mature erythrocytes were characterized by having fully condensed pachychromatic nuclei and electron-dense homogeneous cytoplasm. Less mature erythrocytes showed larger, less condensed nuclei and the cytoplasm was often less electron dense. Both types of mature cell sometimes contained profiles of degenerating cell organelles, and neither of these may have been as mature as cells normally found circulating in the blood. Pale cells had slightly pachychromatic nuclei, fairly homogeneous cytoplasm and sometimes exhibited figures resembling ferritin crystals. Epithelial reticular cells had large nuclei with peripherally dispersed euchromatin, fairly extensive cytoplasm, always contained tonofibrils in the cytoplasm and desmosomes could be seen along the plasma membrane. Pyknotic cells were very variable in morphology and the two shown on figure 1 are typical of some. Other pyknotic nuclei were very rounded with clearly clumped chromatin and scant cytoplasm.

Table 1 gives the results of an analysis of cells from an adult in postnuptial moult, when the thymus was found to be enlarging. Smaller numbers of cells from six other specimens were also examined and in all individuals pale cells gave much lower counts for iron than the mature cells, and iron was recorded from almost all pale cells analysed.

There was no significant difference between the iron counts from fully mature and less mature erythrocytes although the cytoplasm often showed a slight difference in electron density.

† The Tropical Bird Pest Research Project instituted jointly by the East African Community's Tropical Pesticides Research Institute in Arusha, Tanzania and the Centre for Overseas Pest Research in London 1969–71.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

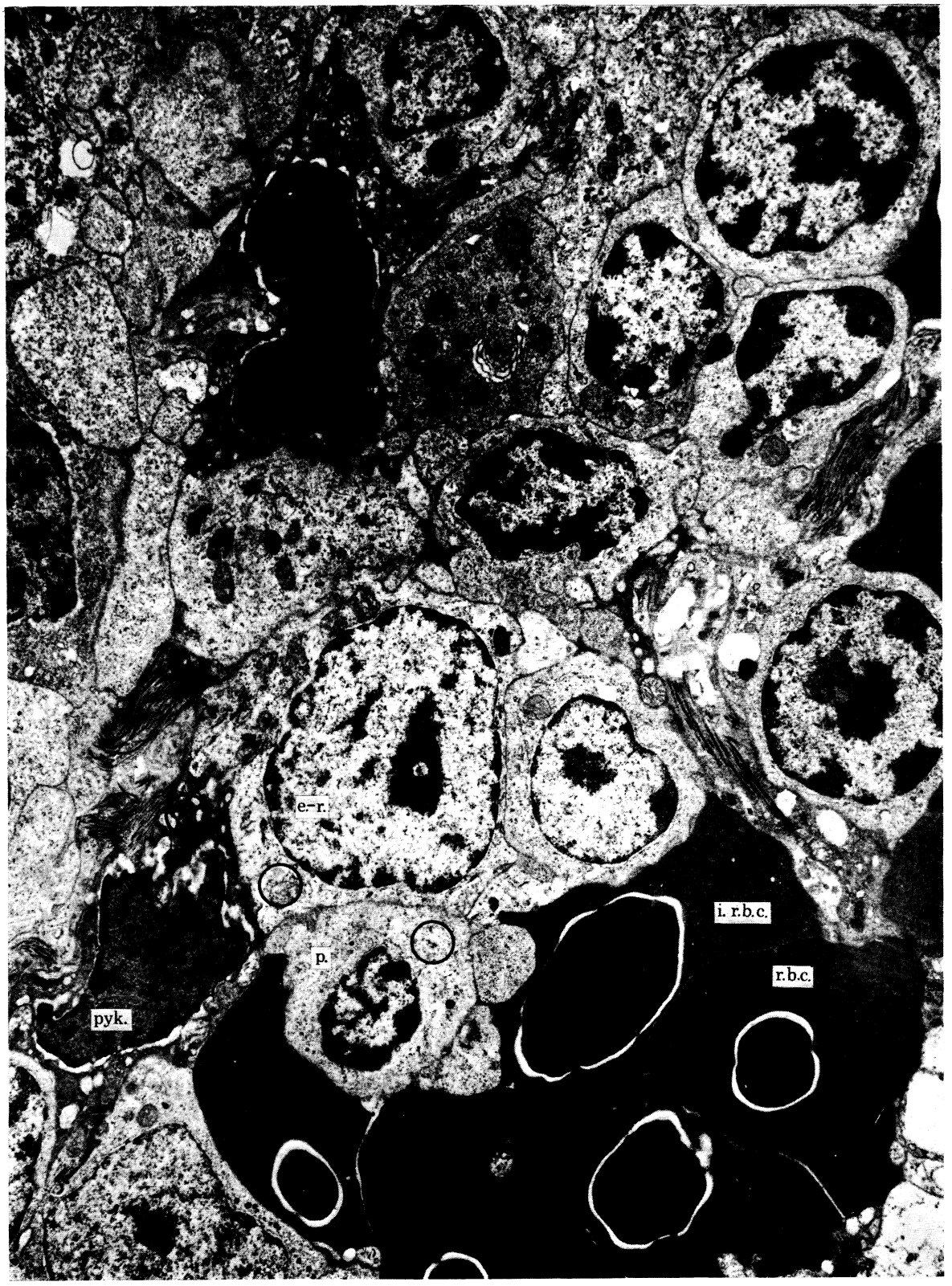

FIGURE 1. The cortex of an enlarging thymus of an adult quelea in postnuptial moult. Mature erythrocytes (r.b.c.), immature erythrocytes (i. r.b.c.), pale cells (p.), pyknotic cells (pyk.), epithelial reticular cells (e.-r.) are shown. Circles indicate the type of region analysed for iron. (Magn. × 9000.)

(*Facing p.* 80)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Epithelial reticular cells were variable and some had no iron and none gave iron counts as high as those from pale cells.

There were few pyknotic cells present in the specimen analysed above and no iron was found in them. Some pyknotic cells from other specimens gave counts for iron but the white radiation counts were of a different order so the counts cannot easily be compared with those given in table 1. (The count for white radiation can be used as a measure of specimen thickness, but the results were so different that another source of variation must be suspected.)

TABLE 1. THE IRON COUNT (CORRECTED FOR BACKGROUND) OBTAINED FROM 1 μm THICK CARBON-COATED SECTIONS OF THE THYMUS OF AN ADULT QUELEA

cell type (10 cells)	cytoplasmic iron	t	P
mature erythrocytes	138.0 ± 15.47		
		6.78	> 0.001
pale cells	95.5 ± 12.39		
		8.83	> 0.001
epithelial reticular cells	25.1 ± 21.95		
thymocytes	0		

DISCUSSION

Most cells will contain a little iron in haem-containing compounds such as the cytochromes, but it is not expected that such small quantities of iron would be easily detectable. The counts for iron in this study are presumed to come from haemoglobin or ferritin (or possibly haemosiderin in some cells). The cytoplasm of the mature cells is electron dense and may be presumed to be haemoglobinized; the highest counts were obtained from these cells.

Quite high counts for iron were also recorded from the pale cells. It may therefore be concluded that these cells were early erythroid cells. Since the cytoplasm was not yet electron dense the iron was presumably not incorporated into haemoglobin and may be present as ferritin or the haem precursors of haemoglobin. It is interesting to note that in some of these cells nuclear condensation had already started.

The presence of iron in epithelial reticular cells is interesting. Are these cells perhaps acting as an iron store for the process of erythropoiesis? The classical picture of epithelial reticular cells acting as nurse cells and supplying iron for haemoglobin formation (Bessis 1961; Bessis & Breton-Gorius 1962) has not yet been seen on electron micrographs of this quelea thymus material. When epithelial reticular cells are present they are only rarely close to groups of developing erythrocytes, and do not form well marked erythropoietic islands.

The presence of iron in some pyknotic cells and the light microscope observations of Bacchus & Kendall (1975) that both erythrocytes and pyknotic nuclei stain with Krag's stain for erythrocytes support the view that pyknotic nuclei are sometimes derived from erythroid cells. Pyknotic cells very closely resembling erythrocytes have been seen within macrophages on electronmicrographs (Kendall & Frazier, in preparation). The pyknotic cells need further analytical and morphological investigation.

In conclusion the presence of pale erythroid cells close to foci of developing erythrocytes is further evidence in support of *in situ* erythropoiesis within the thymus of quelea. Such immature cells are very unlikely to be circulating in the blood and to have invaded the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

tissue. The next step is undoubtedly further refinements of the technique to identify even earlier erythroid cells.

I am greatly indebted to Dr P. Ward for all his help in studying the quelea thymus glands. I also wish to thank members of the staff of both the Centre for Overseas Pest Research and the Anatomy Department of St Thomas's Hospital Medical School for technical help. Preliminary studies on the feasibility of using EMMA-4 for this work were possible due to the courtesy of A.E.I.

References

Bacchus, S. & Kendall, M. D. 1975 Histological changes associated with enlargement and regression of the thymus glands of the red-billed quelea *Quelea quelea* L. (Ploceidae: Weaver-birds). *Phil. Trans. R. Soc. Lond.* B **273**, 65–78.

Bessis, M. 1961 The blood cells and their formation. In *The cell* (eds J. Brachet & A. E. Mirsky). New York: Academic Press.

Bessis, M. & Breton-Gorius, J. 1962 Iron metabolism in the bone marrow as seen by electron microscopy: a critical review. *Blood* **19**, 635–663.

Kendall, M. D. & Frazier, J. A. F. Ultrastructural studies on erythropoiesis in avian thymus glands. (In preparation.)

Kendall, M. D. & Ward, P. 1974 Erythropoiesis in an avian thymus. *Nature, Lond.* **249**, 366–367.

Yoffey, J. M. & Courtice, F. C. 1970 *Lymphatics, lymph and the lymphomyeloid complex*. London and New York: Academic Press.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

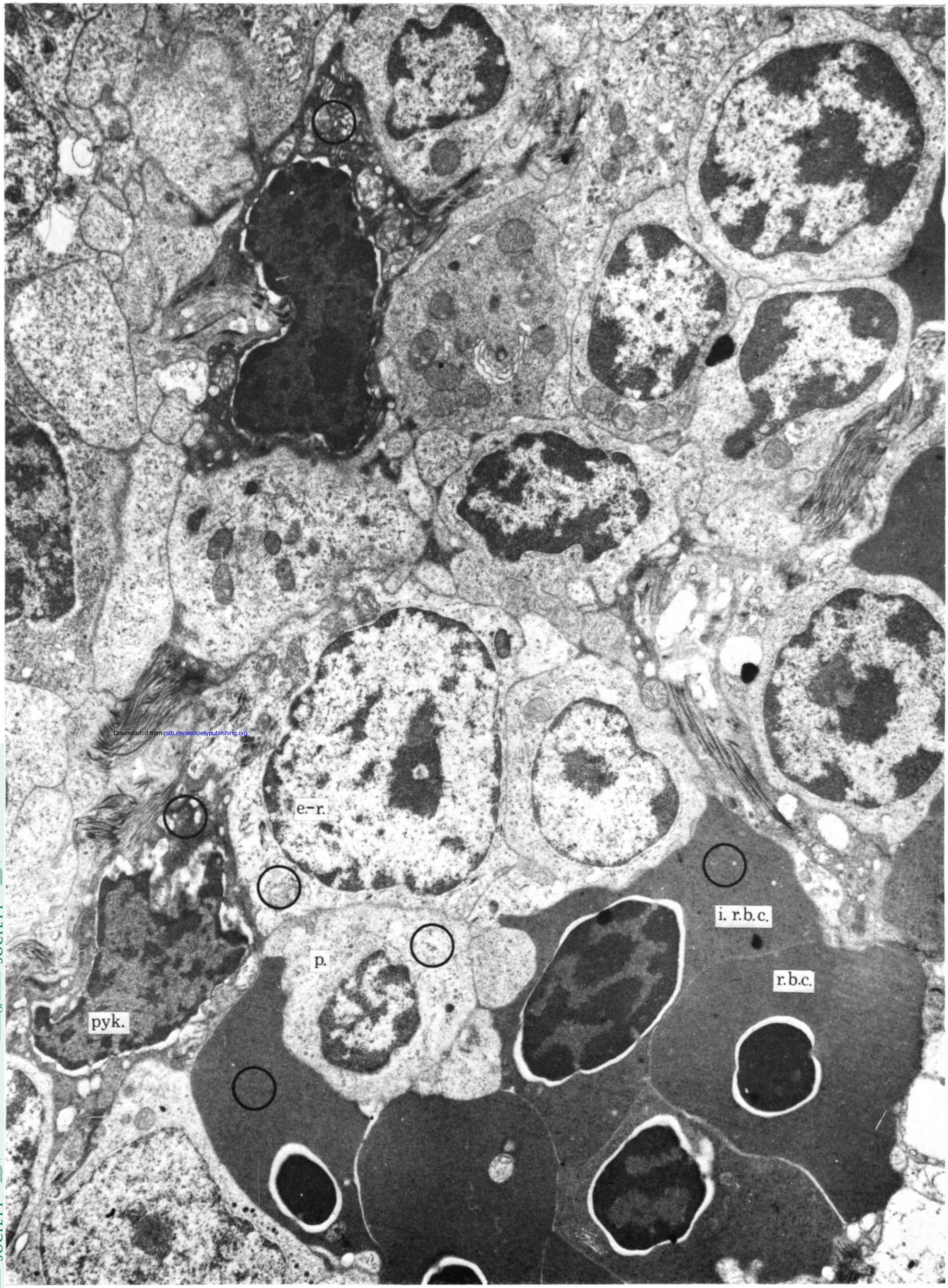

FIGURE 1. The cortex of an enlarging thymus of an adult quelea in postnuptial moult. Mature erythrocytes (r.b.c.), immature erythrocytes (i. r.b.c.), pale cells (p.), pyknotic cells (pyk.), epithelial reticular cells (e.-r.) are shown. Circles indicate the type of region analysed for iron. (Magn. × 9000.)