

## Submammalian Erythropoiesis: Endothelial Cell Transformation by Mammalian Hypophyseal Intermediate Lobe<sup>1</sup>

Bovine hypophyseal intermediate lobe materials induce certain cells of mesodermal origin in homeotherms to proliferate and differentiate along the erythroid cell line<sup>2-5</sup>. It is unusual that factors stimulating erythropoiesis in homeotherms should do so in poikilotherms<sup>6-10</sup>. Nevertheless, intermediate lobe materials caused endothelial cells lining the cavities of the turtle heart and endothelial cells lining coronary vessels to reach erythropoietic maturity.

It is essential that the term, intermediate lobe materials, as used in this study be clearly understood. Although the regulatory mechanisms are not as yet apparent, the bovine hypophyseal intermediate lobe is cyclic in nature (unpublished data) exhibiting 3 phases: first, desquamation and autolysis; second, regeneration and third, resting. In concert with an increase in cellular size and cytoplasmic vacuolization, intermediate lobe cells in the marginal half of the lobe desquamate and break away from the remainder of the lobe, thus converting what was once a potential space between the anterior and intermediate lobe into an intraglandular lumen. Within the lumen, the cells undergo autolysis resulting in the formation of colloid. The intraglandular lumen opens directly into the venous cavernous sinuses by way of well-defined channels passing through the capsule of the gland. This

system provides a means by which the lumen expels its colloid contents into the venous circulation. Direct division of the remaining intermediate lobe cells re-establishes the lobe which now enters a period of rest. The intraglandular lumen returns to its original status, that of a potential space. Thus, intermediate lobe materials in this study refers to intermediate lobe cells and colloid.

The presence of erythropoietin has not been established in poikilotherms, yet erythroid cell maturation is said not to occur unless erythropoietin is present<sup>11</sup>. The results of recent investigations involving intermediate lobe cells suggests that they may be synthesizing ACTH or ACTH-like substances thought to be inherent in intermediate lobe MSH<sup>12-19</sup>. Indeed, ACTH is said to stimulate erythropoiesis in mice<sup>20</sup>, in rats<sup>21, 22</sup> and in rabbits<sup>23</sup> and its presence in intermediate lobe materials may be directly affecting certain cells of mesodermal origin. Of further significance is the suggestion by LEWIS et al.<sup>24</sup> that colloid of intraglandular lumen origin is only active when there is desquamation or autolysis of intermediate lobe cells. Since it is known that factors usually stimulating erythropoiesis have no effect on animals in the classes Pices, Amphibia and Reptilia<sup>6-10</sup>, it seemed worthwhile to test intermediate lobe materials on poikilotherms. The turtle (*Terrapene carolina*) was chosen as the experimental animal to represent the group of poikilotherms.

Fresh bovine pituitary glands and blocks of striated muscle from 2-year-old steers and heifers were secured within 10 min after slaughter and placed immediately in saline. Since the gland is enclosed in a dense fibrous capsule, sterility was maintained. After several washings in sterile saline, the glands were cut in the midsagittal

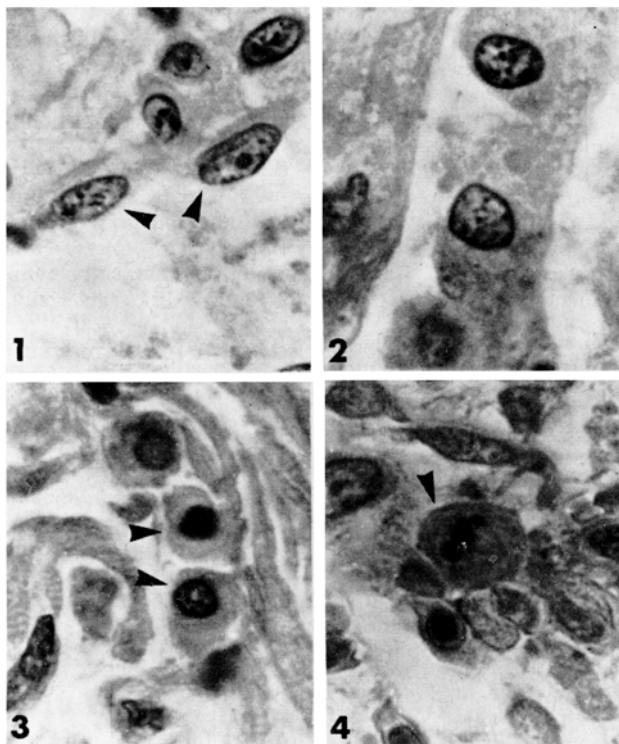


Fig. 1. Tissue from animal killed on day 2 showing progressive nuclear enlargement of endothelial lining cells with a prominent nucleolus. Hematoxylin-eosin-azure II.  $\times 0,900$ .

Fig. 2. Tissue from animal killed on day 5 showing a rounding up of endothelial lining cells. The cytoplasm is basophilic staining Hematoxylin-eosin-azure II.  $\times 0,900$ .

Fig. 3. Endothelial lining cells (day 5) showing prominent nuclear chromatin clumps. Hematoxylin-eosin-azure II.  $\times 0,800$ .

Fig. 4. Mitotic figure of an endothelial cell (day 5) in late metaphase showing granular cytoplasm. Hematoxylin-eosin-azure II.  $\times 0,900$ .

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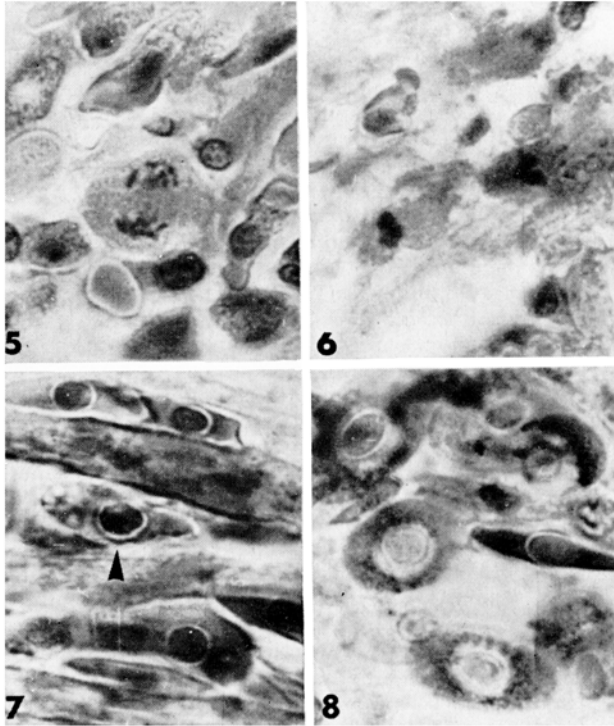


Fig. 5. Mitotic figure of an endothelial cell (day 5) in late anaphase. Hematoxylin-eosin-azure II.  $\times 0,900$ .

Fig. 6. Daughter cells developing into immature erythrocytes. Hematoxylin-eosin-azure II.  $\times 0,900$ .

Fig. 7. Immature erythrocytes showing 'halo' around the nucleus. Benzidine method.  $\times 0,900$ .

Fig. 8. Mature erythrocytes showing 'halo' around the nucleus. Benzidine method.  $\times 0,900$ .

plane exposing the intraglandular lumen and the lobes of the gland. Within 1 h after slaughter, inocula were prepared from muscle tissue, anterior and posterior lobe material, and from intermediate lobe materials, i.e., intermediate lobe cells from glands in which no intraglandular lumen is present (resting phase), and desquamating intermediate lobe cells and intraglandular colloid from glands in which an intraglandular lumen is present (active phase). The slight avascularity of the intermediate lobe differentiates it quite clearly from the posterior lobe to which it is intimately attached. With careful handling, intermediate lobe tissue can be easily dissected away from the posterior lobe.

Inocula were prepared by mincing the hypophyseal materials and muscle tissue, each within its own group, into fine aggregates (0.25 mm or less). The aggregates in each group were divided into compartments of 3 mm<sup>3</sup>. To each compartment was added 2 ml of H-597 balanced medium (Connaught Medical Research Laboratories, Toronto, Canada). 5 separate inocula were prepared. A 21 gauge hypodermic needle was used to inject 0.2 ml of an inoculum into the turtle heart. A rectangular piece of the plastron, directly over the region of the heart was removed for this procedure and then replaced. The turtles were killed on days 2, 5, 7, 9 and 11. Even numbered microscopic sections were stained by the Benzidine method while odd numbered sections were stained with Hematoxylin-eosin-azure II. Turtle hearts injected with desquamating intermediate lobe cells and intraglandular colloid, only, showed changes in the endothelial cells

lining the coronary vessels and heart cavities. No changes were observed in hearts injected with the other inocula.

Endothelial cells lining the cavities of the heart in tissue from animals killed on day 2 show a progressive nuclear enlargement with a large prominent nucleolus (Figure 1). Tissue from animals killed on day 5 show a rounding up of endothelial lining cells (Figures 2 and 3) together with the appearance of basophilic staining cytoplasm. Mitotic figures are frequent (Figures 4–6). By day 7, daughter cells assume a spindle shape (Figure 7) and contain distinct nuclear chromatin clumps. As the cells reach maturity (day 9) they again round-up. In concert with this development, there is an increase in cytoplasmic acidophilia, and the mature erythrocytes contain an abundance of cytoplasmic granules (Figure 8). At this stage, clumps of cells are drawn into the general circulation. Turtles killed after day 9, show no striking changes in the endothelial lining cells. Sections stained by the Benzidine method, show that incorporation of iron into the cell occurs at the time the spindle shape is achieved (Figure 7), and is readily demonstrated in the more mature cells (Figure 8).

It has been reported<sup>8</sup> that the turtle erythrocyte matures very slowly and that increased erythropoiesis in response to bleeding lasts at least 21–50 days. These data rule out internal bleeding as the possible erythropoietic stimulant in the present study for a single inoculation of intermediate lobe materials produce mature erythrocytes from endothelial cells within 2–9 days followed by a cessation of erythropoiesis. This study detects an erythropoietic-like stimulating factor in intermediate lobe materials which has a specific effect on cells of mesodermal origin.

The relationship between red cell production and intermediate lobe activity was thought to be settled since studies by VAN DYKE et al.<sup>25</sup> showed a maintenance of circulating red cell volume in rats to be related entirely to the anterior lobe of the hypophysis. Since recent evidence associates ACTH and ACTH-like substances with intermediate lobe materials, bovine ACTH is now being utilized as a test material in continuing studies. The objective is to determine whether endothelial cells will respond in a manner similar to that observed when intermediate lobe materials are utilized as the test substance.

**Zusammenfassung.** Nachweis, dass Zellen und Kolloid des Hypophysenzwischenlappens des Rindes, in Herzkammern von Schildkröten überführt, deren Endothelzellen zu primitiven Blutzellen und schliesslich zu reifen Erythrozyten transformieren. Es wird angenommen, dass ACTH-ähnliche Substanzen aus dem Hypophysenmittellappen die Erythropoese anzuregen vermögen.

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