maturation, we can conclude that sexual maturation of the Oregon females begins earlier than in males and that Ebony females are late.

Results showed that the prothoracic glands of all stages, sexes, and strains remain unchanged. This conforms to the suggestion[4] that the change in the activity of the prothoracic gland actually only accommodates growth of the larvae.

For the wild type, results conformed to previously reported data[2]. We assume that the less active corpus allatum found consistently in our studies correlates with the decreased juvenile hormone titer. It would appear that with a less active corpus allatum, the ecdyson would be uninhibited and free to carry out the metamorphosis. This suggests that the juvenile hormone suppresses the activity of the prothoracic gland at second instar since the prothoracic gland activity was constant between second and third instars.

With respect to the Ebony strain, the corpus allatum increased in relative protein concentration from the second to the third instars. These results showed the prothoracic gland to be unchanged in protein activity from second to third instars. If the corpus allatum increased in activity and this is related to hormone activity, there would be no molt. The hormone then is not being secreted to the circulatory system. Since there is still a larval molt, we know the corpus allatum is continuing to release juvenile hormone[2]. As long as this juvenile hormone does not exceed the ecdyson level in the circulation, there will be a molt. Since the corpus allatum of the Ebony is so active, we can suggest that the corpus allatum is storing quantities of juvenile hormone or producing it at a greater rate[11].

Résumé. Le contenu protéique des cellules glandulaires circulaires des larves de 2 races de *Drosophila melanogaster* est examiné en détail. Les résultats de cette étude montrent que la production et l'activité hormonale de ces 2 races diffère.

W. H. BECKERT and JUDITH JOHANSEN

Biology Department, St. John's University, Jamaica (New York 11432, USA), 7 July 1969.

[11] This investigation was supported in part by Project No. B-1, St. John's University.

Influence of Hypophyseal Intermediate Lobe Tissue and Colloid on the Reticuloendothelial Cells of the Liver

Although a vast amount of literature has been devoted to the proliferation of reticuloendothelial cells of the liver under the influence of various agents[1–8], only a few doubtful mitotic figures, yet unpublished, have ever been observed in livers known to be synthesizing DNA[9].

The dirth of even questionable mitotic figures in the face of experimental results indicating rapidly proliferating reticuloendothelial cells gives the impression that there may be either a recruitment of cells from other sources or an activation of cells potentially phagocytic.

An invasion of the liver with external macrophage precursors has been reported[10], and furthermore, it is suggested that an increase in the number of actively phagocytic cells might be due to dormant cells rather than to mitosis of existing histiocytes[11].

In a continuing study of the effect of bovine hypophyseal substances on target organs, it was discovered that intermediate lobe tissue undergoing desquamation and autolysis as well as its resultant product, colloid, has a peculiar affinity for certain cells of mesodermal origin. Investigations showed that neither anterior nor posterior lobe tissue produced a similar effect.

In order to demonstrate the influence of intermediate lobe tissue and colloid on mesodermal tissue, reticuloendothelial cells of the liver proved to be a suitable model.

The liver of 8 adult dogs, both male and female were divided equally into 2 groups. 4 others were used as controls. The hypophysis from 2-year-old steers and heifers were collected within 15 min after slaughter and placed in Tyrode's solution for transport to the laboratory. After rinsing in several changes of Tyrode's solution, the glands were cut mid-sagittaly into 2 equal halves, exposing the lobes of the gland and the residual lumen (intraglandular cleft). Only glands with a colloid filled lumen were utilized. The colloid was collected, and the avascular intermediate lobe tissue, readily identifiable, was carefully scraped from the rostral surface of the posterior lobe. Neither intermediate lobe tissue nor colloid was pooled.

An inoculum was prepared by mincing separately intermediate lobe colloid and tissue into fine aggregates ($^1/_4$ mm or less) which were then divided into compartments 3 mm^3. Each compartment contained approximately 29.6 mg/ml of protein. The inoculum from each compartment was fed into the pointed end of an 18-gauge hypodermic needle and the opposing end of the needle was fitted with a metal wire plunger.

The experimental animals were lightly anesthetized with ether and the inoculum was injected directly into the liver of each animal, transabdominally, below the twelfth rib in the right mid-axillary line. 2 male and 2 female animals received colloid, the others received intermediate lobe tissues. One animal from each group was sacrificed on day 3, 5, 7 and 9. By following the needle tract into the

[1] J. H. HELLER, R. M. MEIER, R. ZUCKER and G. W. MAST, Endocrinology *61*, 235 (1957).
[2] D. L. J. BILBEY and T. NICOL, Nature *182*, 674 (1958).
[3] B. BENACERAF, B. N. HALPERN, G. BIOZZI and S. A. BENOS, Br. J. exp. Path. *35*, 97 (1954).
[4] E. R. GABRIELI and H. HOLMGRAN, Acta path. microbiol. scand. *31*, 205 (1952).
[5] J. G. HOWARD and A. C. WARDLAW, Immunology *1*, 338 (1958).
[6] N. R. DI LUZIO, Am. J. Physiol. *181*, 595 (1955).
[7] S. J. RIGGO and N. R. DI LUZIO, Am. J. Physiol. *200*, 297 (1961).
[8] T. NICOL and D. L. J. BILBEY, Nature *182*, 192 (1958).
[9] L. S. KELLY, E. L. DOBSON, C. R. FINNEY and J. D. HIRSCH, Am. J. Physiol. *198*, 1134 (1960).
[10] J. L. BOAK, G. H. CHRISTIE, W. L. FORD and J. G. HOWARD, Proc. R. Soc. B. *169*, 307 (1968).
[11] J. G. HOWARD, J. Path. Bact. *78*, 465 (1959).

liver, the injection site was located. Tissue taken from the site was fixed in 10% formalin, and prepared in the usual manner for microscopic study. The tissue was serially sectioned at 3–5 μ and stained with hematoxylin-eosin.

In the control animals, 2 types of cells were seen in the sinusoids of the liver. The endothelial cells had little structural detail and appeared as thin, compact, elongated cells with a small dark nucleus. The distinctly larger and well defined Kupffer cells had what appeared to be numerous cytoplasmic processes which were intimately connected to the wall of the sinusoids. The Kupffer cells contained a large oval nucleus with a rather small prominent nucleolus.

The microscopic picture of both autolysis colloid and intermediate lobe tissue injected liver was similar. Animals sacrificed on days 3, 5 and 7, showed autolysis of intermediate lobe tissue and colloid in the sinusoids of the liver. Sections from animals sacrificed on day 3, showed an increase in number and size of both the Kupffer and the endothelial cells lining the sinusoids. The cells were equally distributed throughout the perilobular and centrilobular regions of the liver.

Under the conditions of the present experiment, the Kupffer cell showed the most striking changes. The swollen cell almost completely blocked passage through the sinusoids (Figure 1). The cytoplasmic protrusions proved to be more than just stellate processes. Indeed, the processes now appeared as cytoplasmic tubules extending from the surface of the cell to project into the parenchyma of the liver between hepatic cells. Here they diminished in size and branched into many small channels which made direct contact with the hepatic cell membrane. In serial sections, the tubules were found to open directly into the hepatic cells. This arrangement suggests an

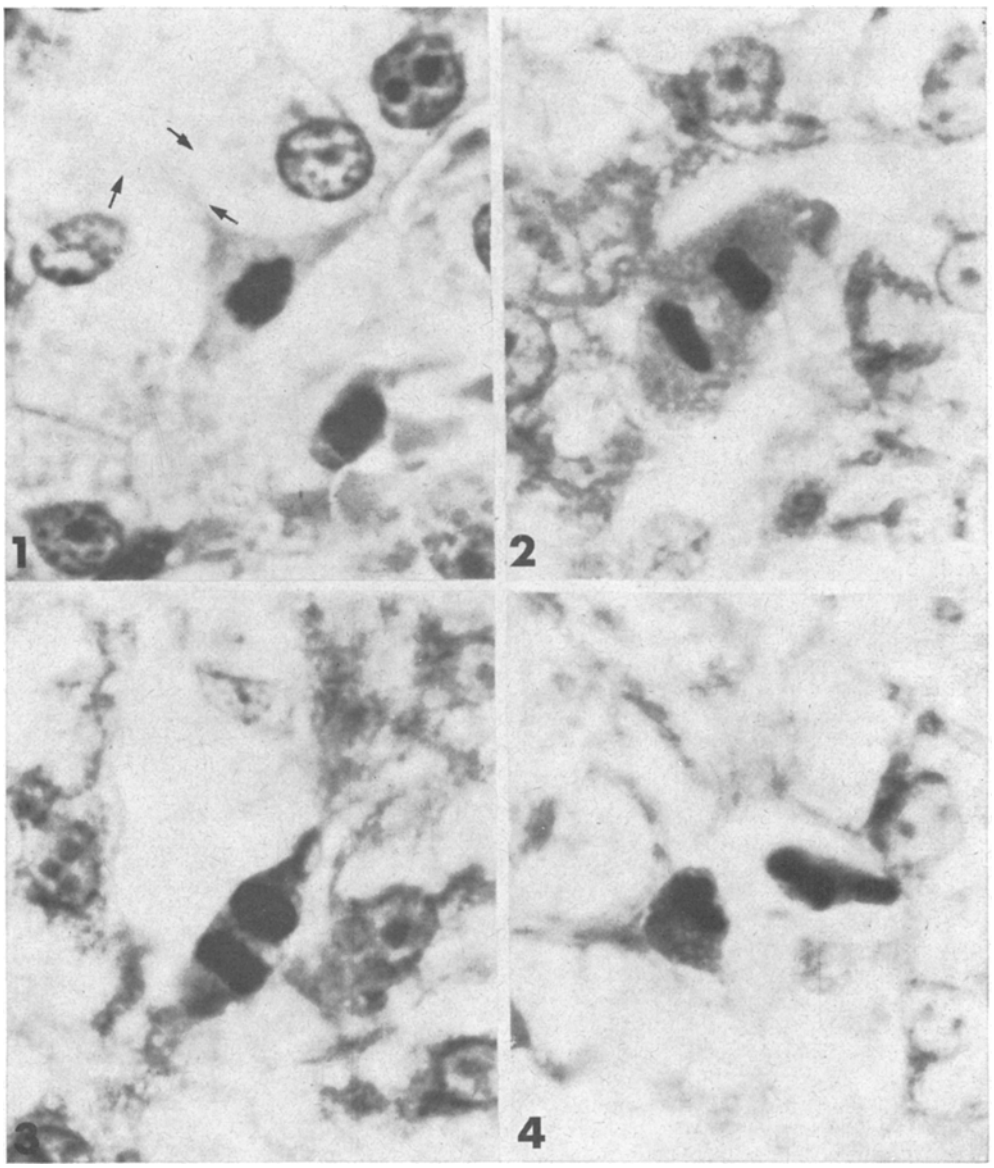

Fig. 1. Kupffer cell from liver injected with intermediate lobe tissue. Note branching cytoplasmic tubules. × 1400.

Fig. 2. Kupffer cell during late anaphase. × 1400.

Fig. 3. Endothelial cell during late anaphase. × 1400.

Fig. 4. Daughter cells taking up new positions on opposite sides of the sinusoid. × 1400.

intimate relationship between the sinusoid, the Kupffer and the hepatic cells. At this time, the Kupffer cell showed signs of preparing for division; the nucleus with its uneven membrane was filled with dark staining chromatin clumps.

Microscopic sections of the liver from animals sacrificed on day 5, showed both dividing Kupffer and endothelial cells. By late anaphase, the Kupffer cell had lost its cytoplasmic tubules. The cell, now considerably swollen with foamy cytoplasm, had elongated, extended from one wall of the sinusoid to the other (Figure 2). It is interesting that during the mitotic phase, the Kupffer cell never lost complete contact with the wall of the sinusoid.

Although there was considerable swelling of the endothelial cell during its mitotic phase, it too never lost contact with the wall of the sinusoid, nor did it ever attain the size of the Kupffer cell. Notwithstanding these facts, during late anaphase, the cell managed to extend itself across the sinusoid from one wall to the other (Figure 3).

After the reticuloendothelial cell had completed its division, the daughter cells took up new positions, one attached to either side of the wall of the sinusoid (Figure 4). The new cells, both Kupffer and endothelial, repeated this process, accounting for the great increase in reticuloendothelial cells observed in the liver on day 7. On day 9, the process had greatly diminished and no dramatic increase in reticuloendothelial cells per se were seen.

Amid the activity of these cells on days 5, 7 and 9, the newly formed Kupffer and endothelial cells were engaged in a most unique process, revealing their differentiation in the direction characterized as cells belonging to the erythroid series.

Under the conditions of the present study, the increase in reticuloendothelial cells of the liver is entirely a local phenomenon exhibited by existing cells, with no contributions from outside sources[10]. These results lend credence to evidence maintaining that mature macrophages can undergo division[12–16], and demonstrates that the responsiveness of reticuloendothelial cells to intermediate lobe materials is an important bearing on the role of endocrines in the regulation of the reticuloendothelial system[17–21].

Although the reticuloendothelial cells of the liver are unstable, are multipotential and behave according to the nature of the stimulus employed, there is no doubt about their mitotic capabilities.

Zusammenfassung. Gewebe des Hypophysenzwischenlappens und das Kolloid wirken auf Endothelzellen ein. Sie vermögen die retikuloendothelialen Zellen der Leber zur Mitose anzuregen. Diese Zellen scheinen eine wichtige Rolle bei der Übermittlung von endokrinen Stoffen und der Regulation des retikuloendothelialen Systems zu spielen.

W. H. BOYD

Department of Anatomy, University of Guelph, Guelph (Ontario, Canada), 8 July 1969.

[12] W. R. WOOLES and N. R. DI LUZIO, J. Reticuloendothelial Soc. *1*, 160 (1964).
[13] J. L. EDWARDS and R. E. KLEIN, Am. J. Path. *38*, 437 (1961).
[14] R. S. SPIERS, Y. WENCH and V. JENSEN, Proc. Soc. exp. Biol. Med. *106*, 251 (1961).
[15] J. I. FORBES and G. B. MACKANESS, Lancet *2*, 1203 (1963).
[16] C. A. MIMS, J. exp. Path. *45*, 37 (1964).
[17] A. S. GORDON and G. F. KATSCH, Ann. N.Y. Acad. Sci. *52*, 1 (1949).
[18] P. S. TIMIRAS, Acta endocr. Buc. *12*, 1 (1953).
[19] W. B. RAWLS, J. GOLDZIEHEV, J. B. TICHER and E. BAKER, J. Lab. clin. Med. *44*, 512 (1954).
[20] S. M. REICHARD, A. EDELMANN and A. S. GORDON, J. Lab. clin. Med. *48*, 431 (1956b).
[21] F. POPHILLAT, Annls. Inst. Pasteur *103*, 43 (1962).

The Relationship Between Cell Division and Cell Specialization in the Mouse Intestinal Epithelium

The steady state concept which characterizes renewing cell populations has been formulated on a statistical basis. Thus, to maintain an equilibrium between cell birth and cell death, one daughter cell of a progenitor (or stem) cell division remains in the proliferative pool, while the other progeny migrates towards the functional compartment and takes on special functions. Therefore, in order to account for this mechanism, several stochastic models depicting the relationship between cell birth and cell specialization have been suggested[1–8]. Until recently, the most widely accepted model has been predicated on an asymmetrical mitosis[1–3,6].

Such a concept, however, does not allow for considerable variation in the life history and function of individual cells within a population. Moreover, recent evidence obtained from a variety of renewing epithelial cell populations, esophagus[5], jejunum[7–9], duodenum[10], has suggested that 3 models may be operating in concert to maintain the steady state, none of which use an asymmetrical division to explain the relationship between division and the subsequent specialization of progeny. Therefore, the present study was undertaken in an attempt to demonstrate the role of individual progenitor cells in the renewal of the duodenal epithelium.

Materials and methods. A total of 46 male Swiss albino mice were utilized. Each animal received a single dorsal s.c. injection of tritium thymidine (hereafter, 3HTdr) labeled at the methyl position, New England Nuclear Corporation, specific activity 6.4 C/m*M*, at a concentration of 0.5 μc/g body weight. The mice were sacrificed by cervical dislocation, 2 per time interval, from $^1/_4$ to 30 h

[1] E. ROLSHOVEN, Verh. anat. Ges., Jena *49*, 189 (1951).
[2] E. E. OSGOOD, J. Natn. Cancer Inst. *18*, 155 (1957).
[3] E. E. OSGOOD, Ann. N.Y. Acad. Sci. *95*, 828 (1961).
[4] M. R. LORAN and T. T. CROCKER, J. Cell Biol. *19*, 285 (1963).
[5] R. C. GREULICH, in *The Epidermis* (Eds. W. MONTAGNA and W. C. LOBITZ JR.; Academic Press, New York 1964), p. 117.
[6] D. W. TALMAGE and H. N. CLAMAN, in *The Thymus in Immunobiology* (Eds. R. A. GOOD and A. E. GABRIELSEN; Hoeber Medical Division, New York 1964), p. 49.
[7] A. B. CAIRNIE, L. F. LAMERTON and G. G. STEEL, Expl. Cell Res. *39*, 539 (1965b).
[8] A. B. CAIRNIE, L. F. LAMERTON and G. G. STEEL, Expl. Cell Res. *39*, 528 (1965a).
[9] A. B. CAIRNIE and R. E. BENTLEY, Expl. Cell Res. *46*, 428 (1967).
[10] J. D. THRASHER, in *Methods in Cell Physiology* (Ed. D. M. PRESCOTT; Academic Press, New York 1966), vol. 2, p. 323.