References and Notes

1. Ecdysterone (crustecdysone, 20-hydroxyecdysone, β-ecdysone) isolated from the seawater crayfish *Jasus lalandei* caused puparium formation in an insect [F. Hampshire and D. H. S. Horn, *Chem. Commun.* **2**, 37, (1966)].
2. L. M. Passano, in *The Physiology of Crustacea*, T. H. Waterman, Ed. (Academic Press, New York, 1960), vol. 1, p. 473.
3. C. Zeleny, *J. Exp. Zool.* **2**, 1 (1905).
4. D. E. Bliss, *Biol. Bull.* **104**, 275 (1953); D. M. Skinner, thesis, Radcliffe College (1958).
5. D. M. Skinner, *Amer. Zool.* **6**, 235 (1966).
6. D. E. Bliss, personal communication.
7. ———, in *Bertil Hanstrom: Zoological Papers in Honour of his Sixty-fifth Birthday, November 20th, 1956*, K. G. Wingstrand, Ed. (Zoological Institute, Lund, Sweden, 1956), p. 56.
8. Autotomy: In certain Crustacea, muscles at the base of an injured limb are contracted, breaking off the limb at a preformed plane (that is, autotomy plane).
9. D. M. Skinner, *Biol. Bull.* **123**, 635 (1962).
10. Ecdysterone was purchased from the Mann Research Laboratories or was the kind gift of Dr. D. H. S. Horn. We thank Dr. E. Mattingly for checking the efficacy of the preparations by injecting 50 μg/g into *Rhyncosciara*; the expected chromosomal puffs appeared.
11. A. Krishnakumaran and H. A. Schneiderman, *Nature* **220**, 601 (1968).
12. C. M. Williams, *Biol. Bull.* **134**, 344 (1968).
13. P. Karlson and D. M. Skinner, *Nature* **185**, 543 (1960).
14. M. E. Lowe, D. H. S. Horn, M. N. Galbraith, *Experientia* **24**, 518 (1968).
15. D. Adelung, *Zool. Anz.* **30**, suppl., 264 (1967); A. C. Warner, J. R. Stevenson, C. Church, *Amer. Zool.* **9**, 1085A (1969).
16. N. Gabrusewycz-Garcia and E. Mattingly, personal communications.
17. R. B. Nickerson, F. J. Ossiander, G. C. Powell, *J. Fish. Res. Board Can.* **23**, 729 (1966).
18. K. Niwa and H. Kurata, *Bull. Hokkaido Reg. Fish. Res. Lab.* **28**, 51 (1964).

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Differentiation of Immature Mucous Cells into Parietal, Argyrophil, and Chief Cells in Stomach Grafts

Abstract. *Microscopic and submicroscopic studies on regenerating gastric mucosa neonatally grafted in the subcutaneous tissue of littermate mice have revealed that immature mucous cells are totipotent; ultimately they transform into mature mucous, parietal, argyrophil, and chief cells in the gastric glands.*

Since Bensley's description of specialized cell types in the gastric mucosal epithelium much uncertainty has existed concerning their origin in the fundic glands. Several investigators have studied their reappearance in gastric mucosa following surgical or thermal injury (*1*). It was stated that the cell types of the stomach are fixed and cells of the foveolar surface do not transform to mucous neck cells, nor do the latter transform into parietal and chief cells (*2*). On the other hand, it has been postulated that such transformations do occur and the mucous neck cells serve largely to replenish short-lived parietal cells (*3*). Much of the difficulty in visualizing these transformations has arisen from the lack of techniques adequate for the demonstration of transitional forms. The regression of specialized cell types in glands bordering a lesion to more nondifferentiated type also makes it extremely difficult to identify newly regenerated glands as opposed to old regressed ones. A technique for the creation of a glandular stomach in the subcutaneous tissue has been established in mice (*4*). By coupling this technique with electron microscopy we have analyzed which cells constitute the precursors for parietal, argyrophil, and chief cells.

The gastric mucosa of newborn mice of the C57BL/6Ms and dd/I strains consisted of surface epithelium, pits, and a few simple tubular glands. It was ascertained in these mice that the surface and pits were covered with mucous cells which contained fully formed secretory granules, and that fundic glands contained numerous parietal cells which had well-developed intracellular canaliculi and a few argyrophil cells. Mice of both strains, less than 24 hours old, were grafted subcutaneously with the glandular segment of the stomach of littermates of the same sex. Two to three days after the operation the grafts formed tumors that were 1 to 2 mm in diameter, which consisted mainly of degenerating epithelial and mesenchymal cells covered with a single layer of regenerating epithelial cells (Fig. 1A). Examination of serial sections from 12 such grafts revealed that all the regenerating epithelial cells were positive for the periodic acid–Schiff reaction and for mucicarmin staining. Electron micrographs taken from four other grafts also demonstrated that only mucous cells survived as epithelial cells (Fig. 1B). The majority of the cells were characteristic in their possession of many intramitochondrial globules between the inner mitochondrial membranes. During this period bundles of fibroblasts of host origin accompanying numerous capillaries infiltrated the central parts of the necrotic masses of the grafts. The bundles became thicker and turned inside out the epithelium covered masses. Thus, the tumors transformed into small branched canals 4 to 6 days after grafting. A few immature parietal cells with undeveloped intracellular canaliculi and mucous granules appeared in the epithelium during this period (Fig. 2A). These cells may secrete hydrochloric acid (*5*), and this might be correlated with transformation of grafts from tumors into canals to isolate the acid from the subcutaneous tissue of the hosts. Between day 6 and day 14 the canals developed

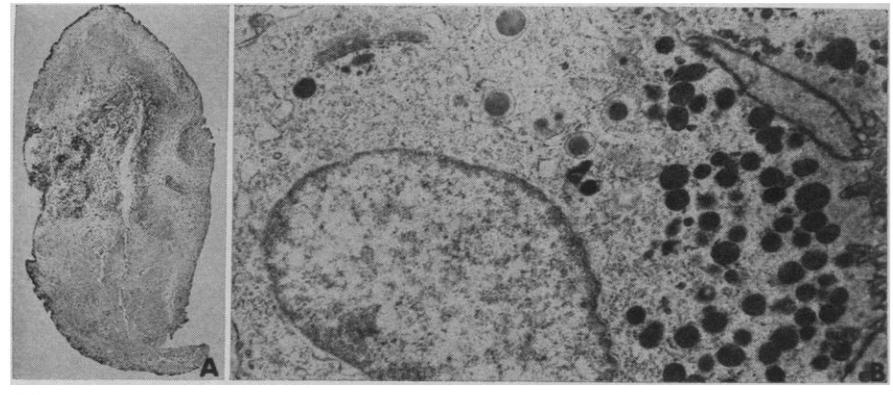

Fig. 1. Stomach graft implanted neonatally, 3 days before, in the subcutaneous tissue of a littermate mouse. (A) Single layer of regenerating epithelial cells covers degenerative epithelial and mesenchymal tissues and central regenerating mesenchymal tissue which contain newly infiltrated vessels from the host (× 29). (B) Immature mucous cells in the regenerating epithelium in a stomach graft as shown in (A). Fully formed mucous granules are present in the apical cytoplasm and mitochondria which contain a few intramitochondrial globules are scattered in the perinuclear area (× 9230).

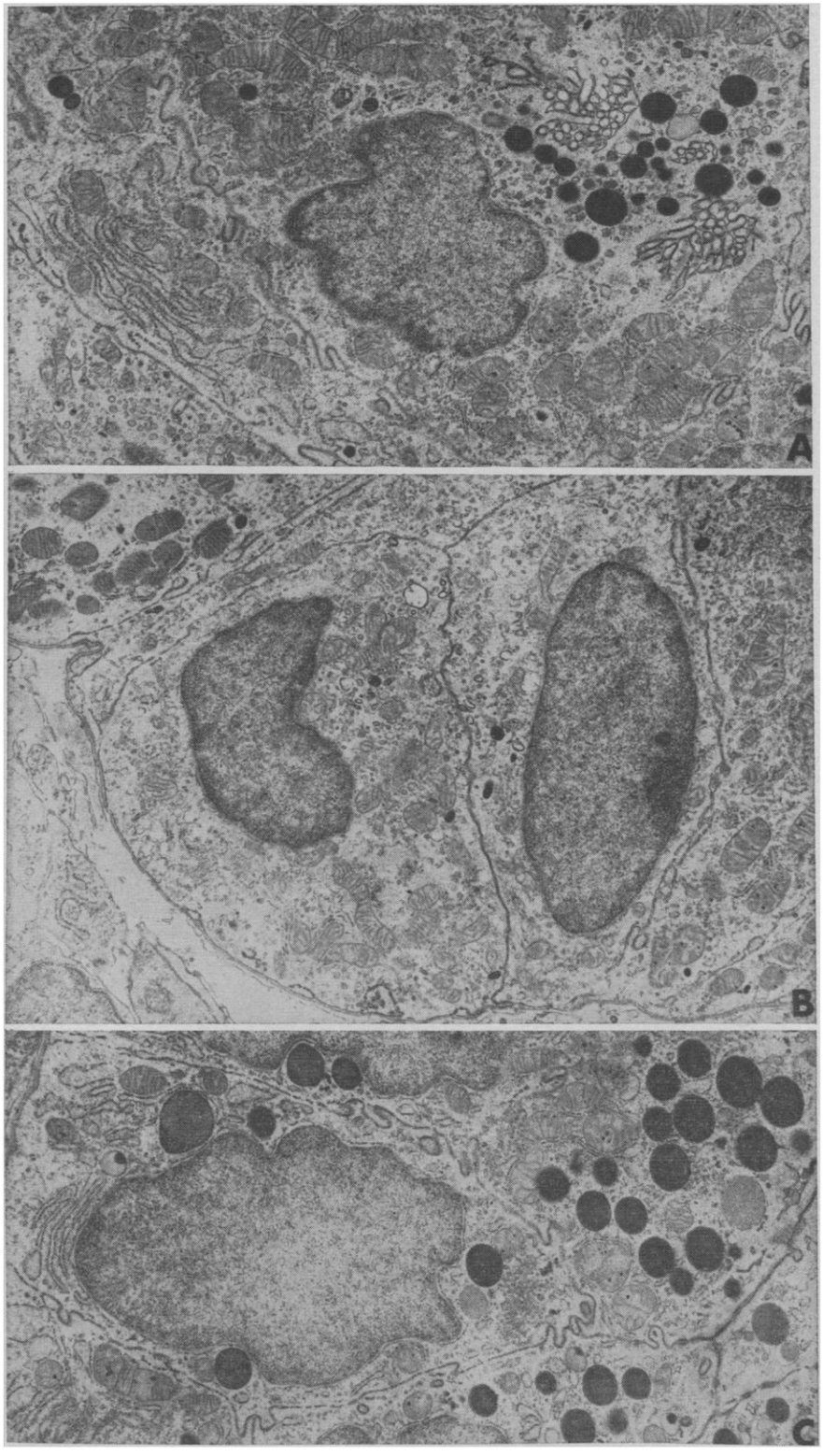

Fig. 2. Ultrastructures of intermediate forms of cells for the specialized cells in the gastric glands, found in grafts implanted 6 to 14 days before. (A) The immature parietal cell bears undeveloped intracellular canaliculi and many mucous granules in the apical area. Large mitochondria and tubulovesicles are characteristic of this type of cell (× 7820). (B) The immature argyrophil cells abut on the basement membrane and have a lighter cytoplasm than those of other types of cells, but darker than those of mature argyrophil cells, in which numerous small vacuoles containing amorphous material are scattered. Oval nuclei are sometimes situated apically and a few very small mitochondria are scattered in the cytoplasm (× 6960). (C) The mucous neck cells have large granules of lesser density than those of surface mucous cells in the apical area and moderately developed rough-surfaced endoplasmic reticulum in the basal area (× 7920).

into cysts, and immature argyrophil cells (Fig. 2B), mucous neck cells (Fig. 2C), and mature parietal cells were seen in the epithelium (*6*). Thereafter these cysts grew in size parallel with the growth of the body. Mature argyrophil cells characterized by membrane-enclosed, secretory granules were scattered in the glands after 3 weeks. One month later the cyst wall consisted of a fully developed, thick mucous membrane that contained glands, submucosal tissues, and muscle layers. Mature parietal and chief cells were a majority in the deeper portion of the glands in this period. The luminal content of the cysts had a *p*H from 2.5 to 2.7 and had high peptic activity (*4*).

It has been believed that parietal and chief cells rarely divide by mitosis. Nevertheless it has been disputed that these differentiated cells are replaced (*1, 2*). However, we have shown that the immature mucous cells can differentiate into the mature mucous, parietal, chief, and also argyrophil cells. The mucous cells which have the intramitochondrial globules might be immature and rapidly proliferative, since we have found mitotic cells containing both mucous granules and the intramitochondrial globules in the stomachs of fetal mice (*7*). In general development of the mucosa of the stomach grafts in the subcutaneous tissue does not differ from that of regeneration of stomach mucosa after surgical or thermal injury. There is a formation of a thin regenerating epithelial layer consisting only of mucous cells after degeneration. Subsequently pits and glands differentiate from this layer. The sequence of cellular regeneration is completed with the appearance of parietal, argyrophil, and chief cells in that order. This sequence of events during development provides that one cell type, the immature mucous cell, changes into others (Fig. 3). Histological and functional studies with a technique, in which exteriorization and subsequent reformation of subcutaneous gastric pouches associated with the vascular pedicles was performed in dogs, also indicated that the specialized cells arose from undifferentiated mucous cells rather than from specialized cells of like kind (*8*).

Information concerning the origin of argyrophil cells has been lacking. It was assumed that only the cells normally found in the connective tissue and in the blood stream were probable sources for argyrophil cells, since mucous cells have never been histo-

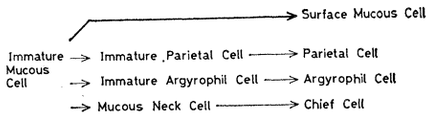

Fig. 3. Developmental relations of the specialized cells in the gastric glands.

logically observed in transition to this cell type (*9*). However, many electron microscopists have classified the cells as epithelial cells, because the cells abut on the basement membrane and are connected with the adjacent cells by the desmosomes (*10*). Our submicroscopic observation of immature argyrophil cells, as transitional cells, in the epithelial wall of the subcutaneous gastric grafts clearly demonstrated the epithelial nature of the cells. This was supported by the findings that rosette, acinar, or tubular structures in the human argentaffin cell tumor were formed by cells with microvilli (*11*) and that the neoplastic process in the stomach of the *Praomys* (*Mastomys*) *natalensis* began as a focal proliferation of argyrophil cells deep in the mucosa and infiltrated into the submucosa (*12*). Reported cases of human intestinal neoplasms with both carcinoid pattern and mucous secretion are also suggestive (*13*).

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References and Notes

1. A. W. Williams, *Brit. J. Surg.* **41**, 319 (1953); E. S. Finckh and G. W. Milton, *J. Pathol. Bacteriol.* **80**, 143 (1960); G. S. Gunter, *Gastroenterology* **15**, 708 (1960).
2. C. E. Stevens and C. P. Leblond, *Anat. Rec.* **115**, 231 (1953); B. Messier and C. P. Leblond, *Amer. J. Anat.* **106**, 247 (1960).
3. T. E. Hunt, *Anat. Rec.* **127**, 539 (1957); ——— and E. A. Hunt, *ibid.*, p. 311; T. E. Hunt, *ibid.* **131**, 193 (1958); H. Ragins, F. Wincze, S. Liu, M. Dittbrenner, *ibid.* **162**, 99 (1968); W. Rubin, L. L. Ross, M. H. Sleisenger, G. H. Jeffries, *Lab. Invest.* **19**, 598 (1968).
4. M. Matsuyama, H. Suzuki, W. Nakamura, *Nagoya Med. J.* **14**, 97 (1968); M. Matsuyama and H. Suzuki, *Gann* **60**, 333 (1969).
5. H. F. Helander, *Gastroenterology* **56**, 35 (1969).
6. Diagnosis of the immature argyrophil cells based mainly on the presence of numerous vacuoles which have an amorphous content of low-electron opacity, since the maturation of beta-cell granules, from an amorphous material to dense granules, in the pancreatic islets of neonatal rabbits was established [S. S. Lazarus, S. H. Shapiro, B. W. Volk, *Lab. Invest.* **16**, 330 (1967)]. The assumption that the mucous neck cells are precursor for the chief cells has been made by several workers [R. E. Corpron, *Amer. J. Anat.* **118**, 53 (1966); H. F. Helander, *Gastroenterology* **56**, 53 (1969)].
7. M. Matsuyama and H. Suzuki, unpublished data.
8. D. M. Coder, R. G. Shorter, D. C. McIlrath, *Surgery* **64**, 769 (1968).
9. S. F. Townsend, *Amer. J. Anat.* **109**, 133 (1961).
10. K. Kurosumi, in *Int. Rev. Cytol.* **11**, 1 (1961); H. F. Helander, *J. Ultrastruct. Res. Suppl.* **4**, 1 (1962); S. Ito, in *Handbook of Physiology*; *Alimentary Canal*, C. F. Code, Ed. (American Physiological Society, Washington, D.C., 1967), vol. 2, p. 705.
11. S. A. Luse and P. E. Lacy, *Cancer* **13**, 334 (1960); W. C. Black and H. E. Haffner, *ibid.* **21**, 1080 (1968).
12. K. C. Snell and H. L. Stewart, *Science* **163**, 470 (1969).
13. F. J. Hernandez and J. D. Reid, *Arch. Pathol.* **88**, 489 (1969).
14. We thank Prof. T. Nagayo and Dr. M. Hoshino for their advice, and the Lady Tata Memorial Trust for a fellowship to M.M.

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Divergent Biological Effects of Adenosine and Dibutyryl Adenosine 3′,5′-Monophosphate on the Isolated Fat Cell

Abstract. *Adenosine 3′,5′-monophosphate stimulated production of carbon dioxide and lipid from glucose, whereas its dibutyryl derivative inhibited this conversion. Addition of the dibutyryl derivative to the isolated fat cell further stimulated lipolysis induced by adrenocorticotropic hormone, whereas addition of adenosine 3′,5′-monophosphate inhibited this lipolysis. Hence, measured by these two parameters, the biologic properties of adenosine 3′,5′-monophosphate and its dibutyryl derivative are distinctly different.*

Adenosine 3′,5′-monophosphate (cyclic AMP) has been indicated as a "second messenger" in multiple biologic systems. In particular, the reaction of multiple hormones has been shown to involve, at least in part, cyclic AMP and adenyl cyclase (*1*). Both cyclic AMP and its dibutyryl derivative (dibutyryl cyclic AMP) have been studied with regard to their ability to promote lipolysis (*2*). Although the biological effects of dibutyryl cyclic AMP were studied in fat (*3*) and muscle (*4*), the comparative effects of dibutyryl cyclic AMP and cyclic AMP have not been previously reported. This report presents new data on the effect of these two nucleotides in the isolated fat cell and compares their lipolytic and antilipolytic properties with those of glucose transport and oxidation. The insulin-like activity of the cyclic compounds was measured by incorporation of glucose, uniformly labeled with ^{14}C, into $^{14}CO_2$ and lipids in the isolated fat cell of the rat by the method of Gliemann (*5*) as modified by Kitabchi (*6*). As an index of lipolysis, the release of glycerol from the isolated fat cell was determined enzymatically by the method of Chernick (*7*).

Table 1. Effects of dibutyryl cyclic AMP and cyclic AMP on lipolysis in the presence and absence of ACTH.

Addition	Concentration per milliliter	Nanomoles of glycerol* released per 10^5 cell/1 hour
Base		7.5 ± 3.2
Dibutyryl cyclic AMP	1.0 μmole	299.1 ± 18.5
Cyclic AMP	2.5 μmole	31.4 ± 9.1
ACTH	0.2 μg	741.6 ± 7.3
ACTH + dibutyryl cyclic AMP	0.2 μg + 1 μmole	916.7 ± 57.3
ACTH + cyclic AMP	0.2 μg + 2.5 μmole	456.3 ± 21.4

* Condition of experiment is the same as in Fig. 1, except that the results are reported as nanomoles of glycerol released per 10^5 cells per 1 hour. The values are an average of three observations, ± standard error of the mean.

Figure 1, A and B, depicts the results of experiments in which the effect of varying concentrations of cyclic AMP and its dibutyryl derivative were tested on glucose oxidation and uptake. The dose-response curve of cyclic AMP on glucose conversion to CO_2 follows a pattern showing marked stimulation of this conversion at low concentrations of cyclic AMP, with more gradual stimulation at higher concentrations. In contrast, dibutyryl cyclic AMP does not act in the same way as cyclic AMP, but instead inhibits the conversion of glucose to CO_2 below the base line in a manner proportional to the concentration of dibutyryl cyclic AMP in the solution. Essentially similar results were obtained when the conversion of glucose carbon into lipids was measured, that is, cyclic AMP stimulated, whereas dibutyryl cyclic AMP inhibited, lipogenesis from glucose.

The lipolytic effects of cyclic and dibutyryl cyclic AMP were measured at both high and low concentrations of nucleotide and compared to high and low concentrations of insulin. The data in Table 1 show that both dibutyryl cyclic and cyclic AMP promote endogenous lipolysis and that dibutyryl cyclic AMP potentiates the lipolytic effect of adrenocorticotropic hormone (ACTH), but cyclic AMP inhibits lipolysis induced by ACTH. The antilipolytic property of cyclic AMP on ACTH-induced lipolysis has not hitherto been reported. To study this mechanism of the two