sol tyrosine hydroxylase activities and melanin biosynthesis. These changes did not occur in control cultures carried on in parallel in the standard medium.

In soft agar, melanotic and amelanotic melanoma cells produce 2 major morphologically distinct types of colonies. The 2 major colony variants were: a) groups of 50–250 dark (melanotic) small (5–20 µm diameter) cells, and b) groups of 5–50 light, large (20–40 µm diameter) cells. Control cultures in standard DMEM maintained their dark small morphology for over 30 subcultures. On the other hand, on the 3rd subculture, each of the experimental cultures, i.e. cultures grown in media supplemented with either M-THI inhibitor or L-DOPA, gave a mixture of the 2 types of colonies, and on the 7th subculture they gave mostly the light, large colonies.

Human melanoma cells contain 2 types of endogeneous tyrosine hydroxylase (TH) inhibitors. By filtration through Amicon membranes, the data in table 2 indicate that the inhibitors differed in molecular size. The M-THI inhibitor from supernatants of the cloned hybrids had the highest molecular weight. All the 3 inhibitors are protein in nature, but only M-THI in presence of the enzyme produced a precipitin band in agar suggesting that it is an antibody to tyrosine hydroxylase.

Whilst the method in somatic hybridization, introduced by Köhler and Milstein[20] has proved entirely successful for the production of antibodies against particulate antigens such as those on the cell surface[21,22] and viruses[23], it has only recently been successfully applied to soluble tumor markers such as CEA[2], hCG[25] and alpha-fetoprotein[26].

Inhibition of tyrosinase activity produces regression of abnormal cell growth in human and mouse melanoma[27–29]. The described experiments showed the binding of estrogen to tyrosine hydroxylase present in cytosols of melanotic melanoma cells, thus confirming reports by other investigators[17,18]. They also demonstrated the production of an enzyme inhibitor by somatic cell hybrids. The role of these inhibitors in the modulation of melanin biosynthesis, differentiation, morphology and oncogenicity of human malignant melanoma are under current studies.

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Close relationship of mitochondria with intercellular junctions in the adrenaline cells of the mouse adrenal gland

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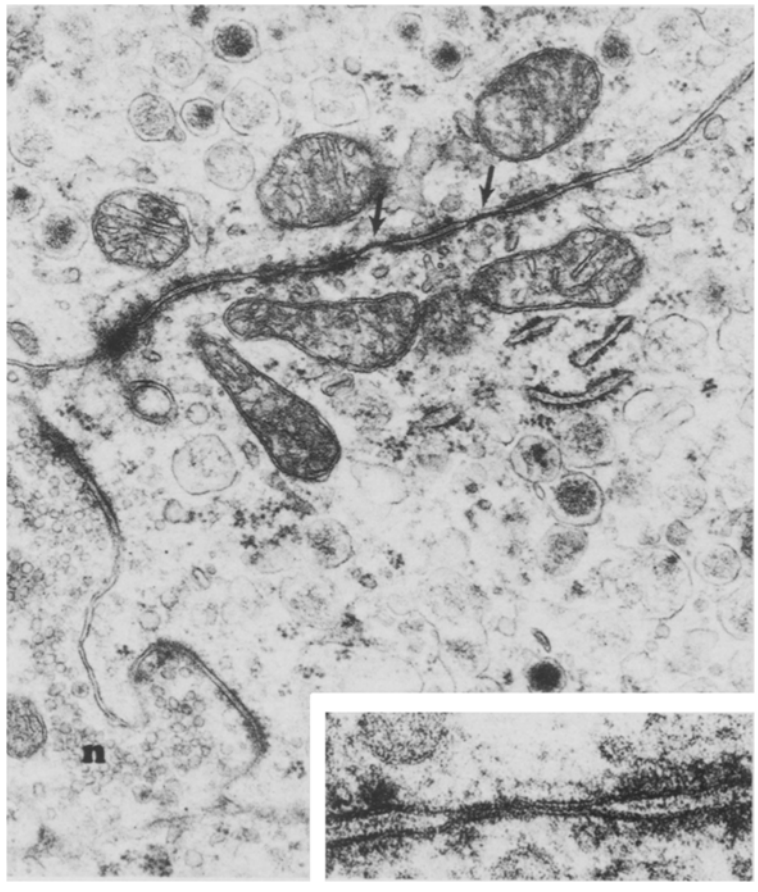
Summary. In adrenaline cells, junctional complexes formed by alternating gap junctions and attachment plaques were identified in close proximity to bilateral clusters of mitochondria. It is suggested that this proximity is related to a role of gap junctions in metabolic coupling.

Gap junctions have been structurally identified between contacting cells of most tissues especially glandular epithelia. Only recently, however, freeze-fracture studies have revealed the presence of gap junctions in the adrenal medulla[1]. Gap junctional particle aggregates were shown to be relatively scarce, with many linear and loop-like configurations. The possibility existed therefore that adrenomedullary cells might possess small and non-macular gap junctions previously missed in studies of thin sections, revealing only attachment plaques. In contrast to freeze-fracture preparations, where cell types could not be identified, adrenaline and noradrenaline cells are easily distinguished in thin sections by the differential electron density of their storage granules after glutaraldehyde fixation[2]. Thus intercellular junctions were carefully reinvestigated in thin sections of mouse adrenal medulla. The mouse adrenal medulla was chosen because its cellular composition is well known[3]. Moreover, freeze-fracture studies showed that no tight junctional fibrils were present in this species. 3 mice were perfused through the heart with buffered 2.5% glutaraldehyde for 10 min. Small pieces of tissue were immersed in the same fixative for 1–3 h, post-fixed in osmium tetroxyde and 'en bloc' treated by uranyl acetate.

Small or linear gap junctions are expected to appear as



Junctional complex between 2 adrenaline cells. 2 gap junctions (arrows) are seen, each one immediately adjacent to attachment plaques. Mitochondria are in close proximity to junctional membranes in both cells. A synaptic junction is seen between the lower cell and a nerve terminal (n). Synaptic junctions are fairly often found near intercellular junctions (× 35,000). Inset. 1 gap junction at a higher magnification to show the septilaminar profile comprised by the 2 apposing plasma membranes (× 142,000).

punctate or short septilaminar profiles consisting of the 2 apposing plasma membranes separated by a space of 2–4 nm. Indeed, septilaminar profiles not exceeding 200 nm in length (fig., inset) were found between adrenaline cells (fig.), noradrenaline cells and cells of both types. The largest and most numerous gap junctions were found between adrenaline cells (fig.) which constitute 70% of the total chromaffin cell population[3]. Profiles of 39 gap junctions between adrenaline cells were collected and are examined in this report.

Gap junctions are closely adjacent to attachment plaques, thus forming together a junctional complex. The junctional complex appears as one or several septilaminar profiles alternating with attachment plaques (fig.). Interestingly, the junctional complexes of several neighbouring cells are found within a short distance of each other, in an area far away from the free surface of the cells. Junctional complexes of paired cells are localized on cell bodies or, fairly often, on small cell processes.

The most striking feature of adrenaline cell junctional complexes is the presence of mitochondria along each side of the junctional membranes. The narrow band of cytoplasm separating junctional membranes and mitochondria is about 120–200 nm wide and contains microtubules and small vesicular and tubular elements; no storage granules are found. Bilateral clusters of mitochondria were found in 32 profiles. In the 7 other cases, where no mitochondria or only unilateral mitochondria were found, the junctional complex seemed to have been sectioned through too small a portion of the complex to be regarded as significant without semi-serial sections. Searching for bilateral clusters of mitochondria along plasma membranes of contacting cells was found to be an efficient way of detecting junctional complexes, otherwise requiring painstaking examination.

It is now generally accepted that attachment plaques play a role in cell-to-cell adhesion. The precise functional significance of gap junctions cannot yet be ascertained. In excitable cells, they are regarded as the low-resistance pathway for electrotonic coupling. In non-excitable cells, gap junctions have been implicated in metabolic coupling (for a review, see Hertzberg et al.[4]). Chromaffin cells are electrically excitable[5,6]. However, there is no clear need for intercellular propagation of excitation[7], since chromaffin cells have individual synaptic junctions (fig.)[8,9]. It is therefore suggested that the close and bilateral proximity of mitochondria to junctional complexes in adrenaline cells is in agreement with a role of gap junctions in metabolic coupling.

Work is in progress to find out whether mitochondria-associated junctional complexes are as numerous in noradrenaline cells as they are in adrenaline cells.

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