

A case of carcinoid tumor of the duodenum: Light and electron microscopic observations

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SUMMARY

A case of carcinoid tumor of the duodenum associated with peptic ulcer was recorded. Histologically, the tumor showed a trabecular pattern in a major portion of the tumor nodule with numerous small glandular structure in the peripheral area. The glandular lumens in question contained eosinophilic substances, which showed a positive staining with PAS and mucicarmine. The outstanding feature was that a large part of these substances were recognized as neurosecretory granules of the carcinoid tumor under electron microscopy. Therefore, these observations suggest that this tumor is not a mucus-secreting carcinoid; such as "composite carcinoid" or "mixed carcinoid", in spite of the fact that the substances in the lumen were stained positive with PAS and mucicarmine. And also, it was suspected that the glandular formation of the tumor revealing the neurosecretion of granules into the lumen was a result of dysdifferentiation of the tumor cells derived from the neural crest cell.

Carcinoid tumors of gastrointestinal tract have been discussed in many reports¹⁾ from histological, ultrastructural biochemical and histogenetical points of view. However, reports on duodenal carcinoid tumors are scarce. The incidence of duodenal carcinoid is very rare. Orloff²⁾ reported that duodenal carcinoid was only seen in 1.3% of the 3,000 gastrointestinal carcinoid cases collected from the literature. Kuiper *et al.*³⁾ recorded that of 72 cases of gastrointestinal carcinoids only 2.8% was duodenal carcinoids. Abe⁴⁾ found 9 cases of duodenal carcinoid out of 107 cases of gastrointestinal carcinoid in Japan up to 1970. Furthermore, electron microscopic examinations of carcinoids of the duodenum are almost not available in the literature, because these tumors were less malignant in most cases and were found only by chance after surgical operations of duodenal ulcer or

in incidental postmortem examinations. In this paper, it will be discussed the nature of the glandular structure or the rosette-like structure and of the neurosecretory granules of the duodenal carcinoid with the aid of electron microscopy, and the possible correlation of the tumor and the peptic ulcer of the duodenum observed in this case.

CLINICAL FINDINGS

The patient was a 28 year-old male complaining of a sudden upper abdominal pain and nausea one morning. There were almost no signs of digestive disorder in his pre-history, although surgical removal of the left renal calculus was undertaken 3 years prior to the present examinations. Clinical data showed that the blood pressure was 140/80 mm. Hg, the pulse was 80/min., and the leucocytes in the peripheral blood were 12,400/cub. mm. He complained again of a sever stomach-ache at 9:00 p.m. on the same day and underwent a surgical removal of the duodenum at midnight with a diagnosis of acute perforative duodenal ulcer. The ulcer was found at a distance of about 1 cm. from the pyloric ring and was covered with white pustule and with the omentum.

There were on signs of carcinoid syndrome before and after the operation of the duodenal ulcer. After histological diagnosis of carcinoid was made, biochemical analyses of blood and urine were done, but no active substances such as serotonin, 5-hydroxytryptophan and hisamine were detected.

HISTOLOGICAL FINDINGS

Ulceration of the duodenum was relatively small and perforative. Neutrophilic leucocytes were abundant and granulation tissue was not well developed in the perforative wound. A carcinoid tumor nodule, about 3 mm. in diameter, was found in the submucosa just beside the perforative wound of the duodenum and was encapsulated with a thin collagenous wall.

Histologically, the tumor generally showed a trabecular pattern in the central area of the nodule, but there were small glandular lumens or rosette-like structures formed by tumor cells in the peripheral area. Some of these contained eosinophilic substances. The tumor cells showed no atypism or pleomorphism, but a limited amount of the cells was seen invading the neighboring duodenal glands (Fig. 1). The substances in the lumen were stained positively with PAS (Fig. 2) and mucicarmine, and were stained blue with alcian blue and Azan stainings (Fig. 3). But no substances were recognized in the cytoplasm of tumor cells with these stainings.

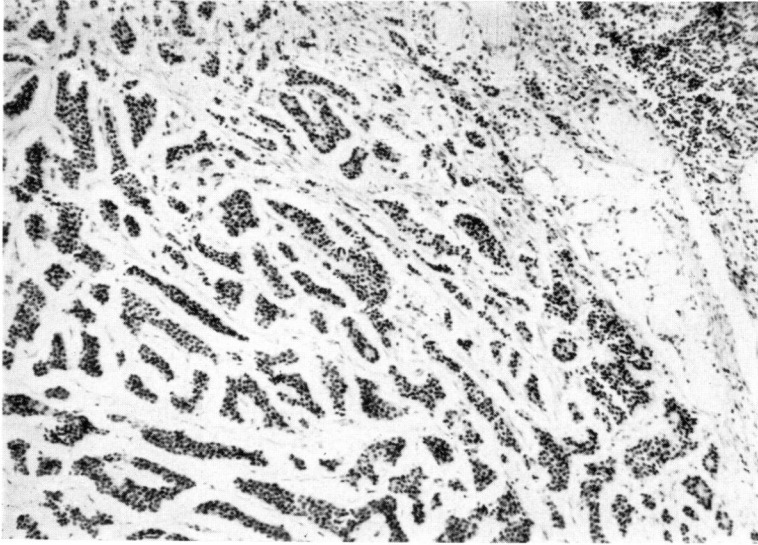


Fig. 1. Carcinoid tumor and duodenal ulcer. The tumor shows a trabecular pattern in the mid zone of tumor nodule and glandular formation in the peripheral zone (H-E, $\times 100$).

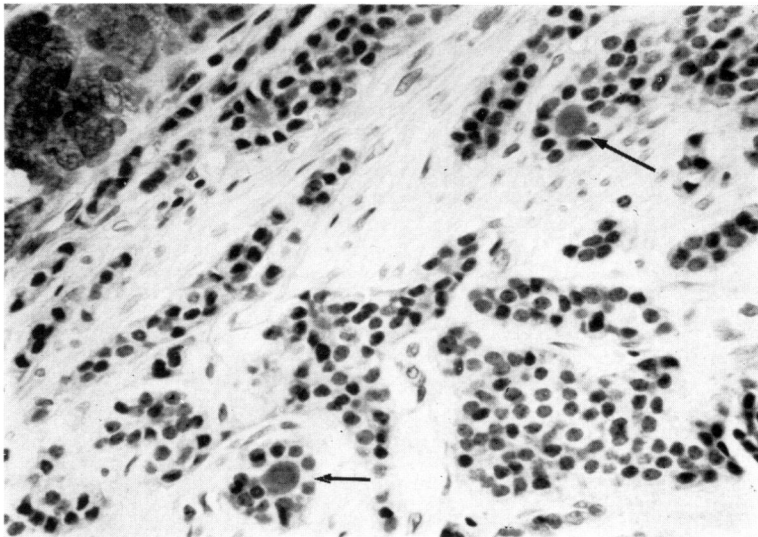


Fig. 2. Two glandular lumens containing dense PAS positive substances are seen in the peripheral area of the tumor nodule as indicated by arrows (PAS, $\times 400$).

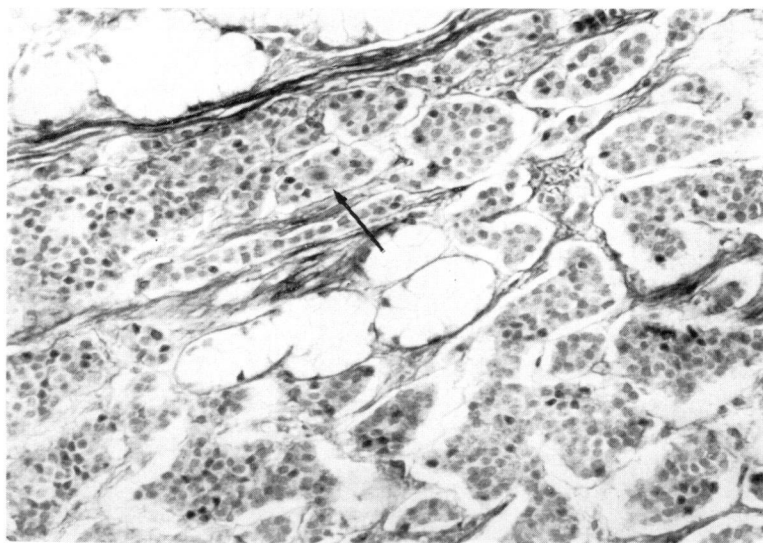


Fig. 3. Similar findings as in Fig. 2. The contents in the lumen are stained blue with Azan stainings (arrow). Tumor cells are seen invading the neighboring duodenal glands (Azan, $\times 200$).

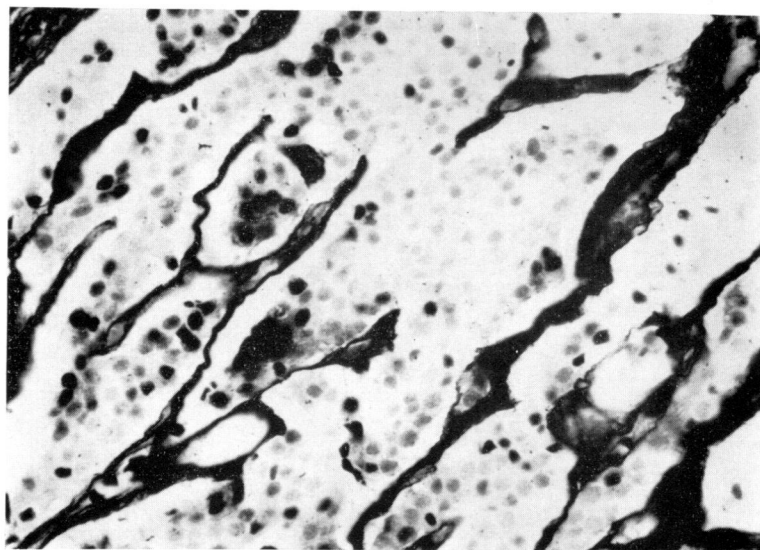


Fig. 4. None of tumor cells are stained positive with silver impregnation (Sevier-Munger, $\times 400$).

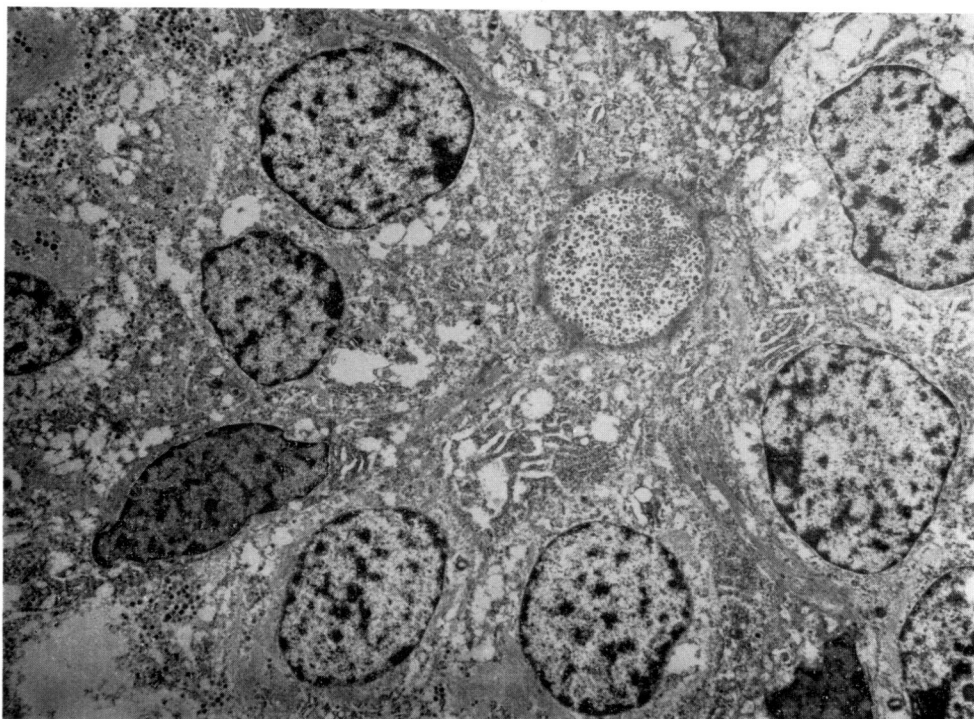


Fig. 5. The lumen is surrounded by several tumor cells with the terminal bars. There are many neurosecretory granules in the lumen and a few granules in the cytoplasm ($\times 4,000$).

Fig. 5~Fig. 8 are electron micrographs obtained from a deparaffinized block; Post-fixed with osmium tetroxide, Stained with uranyl acetate and lead citrate.

Silver impregnations by the method of Sevier-Munger⁵⁾ for argyrophile cells and of Fontana-Masson for argentaffine cells were negative in all of the tumor cells (Fig. 4). Furthermore, reactive proliferation of argyrophile cells associated with duodenal ulcer was not seen in the region of the ulcer.

ELECTRON MICROSCOPICAL OBSERVATIONS

Materials for the electron microscopic examinations were obtained from paraffin embedded blocks and were studied after deparaffinization and post-fixation with osmium tetroxide. Thin sections were stained with uranyl acetate and lead citrate.

The lumen mentioned above was recognized more clearly under an electron microscope than under a light microscope. These lumens were surrounded by several tumor cells and showed distinct terminal bars be-

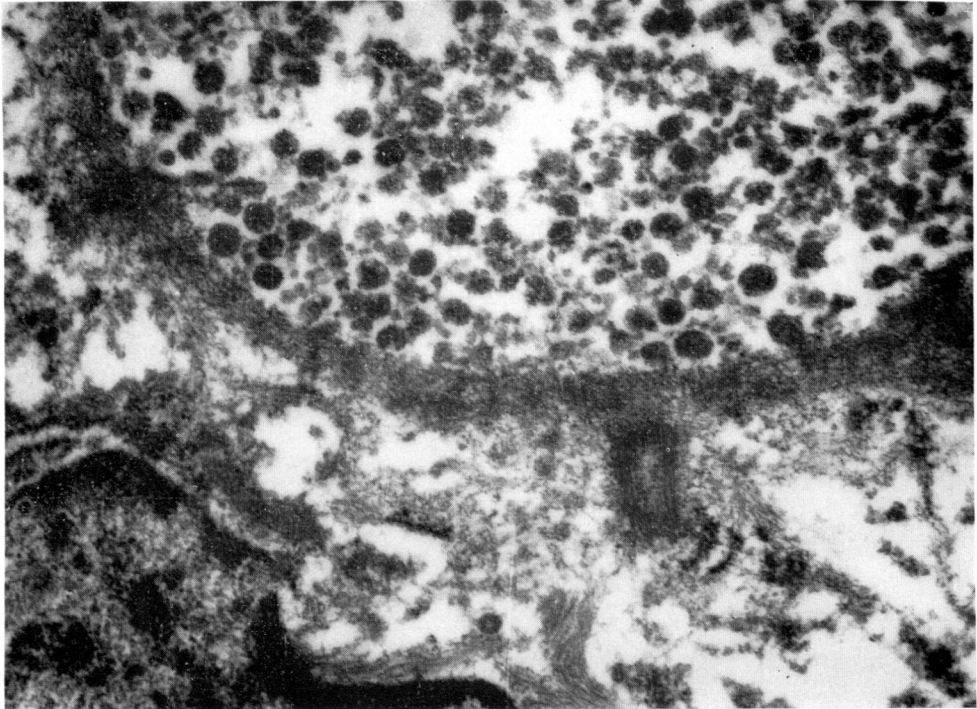


Fig. 6. Similar findings of the lumen as seen in Fig. 5. Neurosecretory granules are clearly observed in the lumen. Terminal bars and tonofibrils are also seen ($\times 30,000$).

tween the cells. It appeared that these cells did not have basement membranes but were surrounded by other tumor cells (Fig. 5). A remarkable finding was that there were a large number of neurosecretory granules in the lumen and a few in the cytoplasm of the inner tumor cells (Fig. 6). On the other hand, when the tumor cells were located in the outer portion of a tumor nest and were associated with the basement membrane, secretory granules were seen in the basal portion of the cells (Fig. 7). Although the limiting membrane of granules disappeared, because of cell damage due to formalin fixation of the specimens, the granules were relatively uniform in size and shape; they were small, round and dense (Fig. 8). The size of the granules was about 100–150 $m\mu$ in diameter. Although cytoplasmic architectures were not clear, but an incomplete microvilli were observed in some cells and fibrillar structures were seen in the cytoplasm. Some of fibrils were identified as tonofibrils. Desmosome-like structures were also seen between the cells. The nuclei were relatively round but some were irregular in shape.

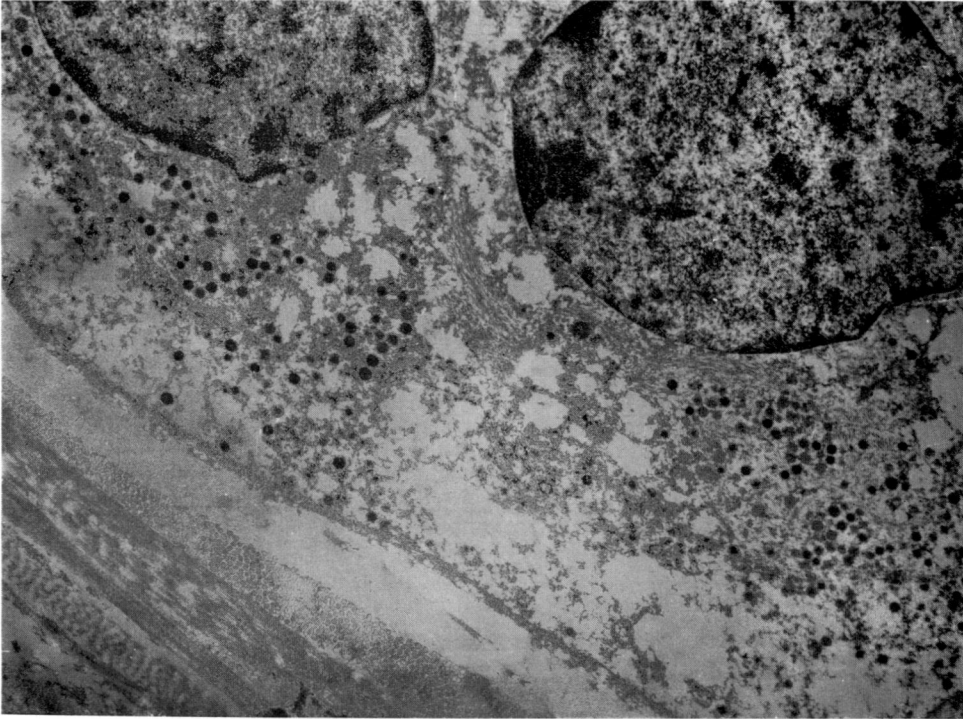


Fig. 7. Many secretory granules in the basal portion of cells are seen. A thin basement membrane is also seen ($\times 10,000$).

DISCUSSION

Histological features of this tumor showed a trabecular pattern without an anastomosing in major portion of the tumor nodule, but some nests showed a solid nodular pattern with narrow fibrocapillary stroma. While in the peripheral region of the tumor nodule, many small glandular lumens were observed in these nests. Although there were minor variations of nests, this tumor seemed to be a classical type of the carcinoid tumor of the duodenum.

Outstanding feature was that the glandular lumens were clearly observed, showing the distinct margin of the tumor cells of inner layer of the nest, and that the eosinophilic substances were observed in the lumen. These substances were stained positively with PAS and mucicarmine stainings and were stained blue with alcian blue and Azan stainings. But in the cytoplasm of the tumor cells the substances were not stained with these stainings. Similar findings of the carcinoid tumor were observed in the "composite carcinoid"^{6,7)} or the "mixed carcinoid"⁸⁾ with respect to

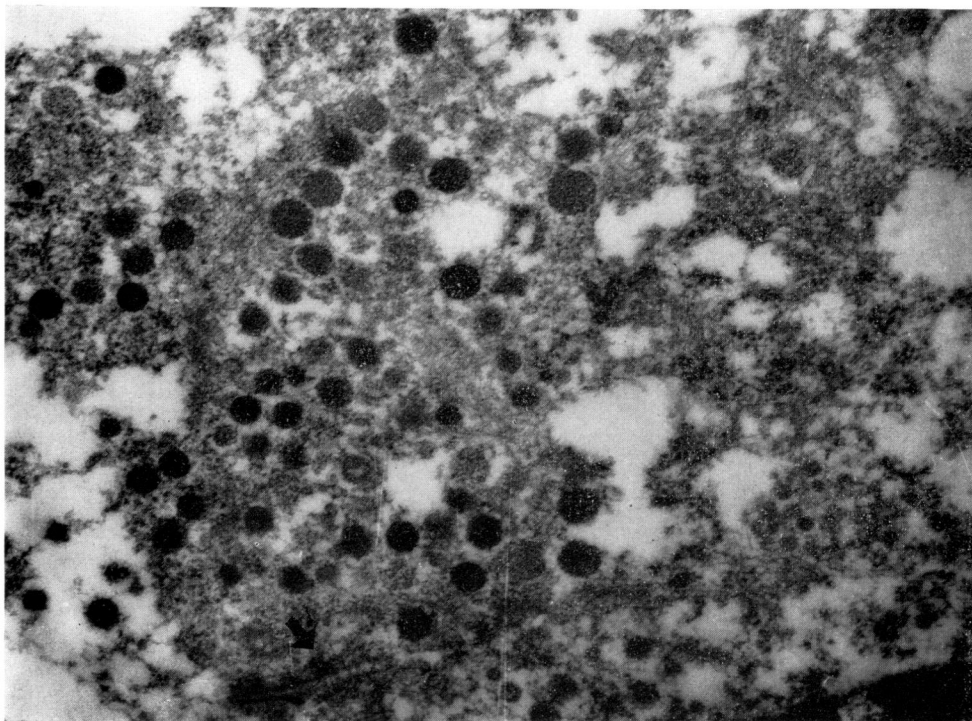


Fig. 8. Neurosecretory granules in the cytoplasm. They are small, round and dense, although the limiting membranes have disappeared. Desmosome-like structure is seen between cells as indicated by arrow ($\times 30,000$).

mucus secretion. But these tumors had not been examined with an electron microscope. In our case, numerous neurosecretory granules were recognized in the glandular lumen by the aid of electron microscopy. Therefore, this does not mean that the tumor with glandular lumen containing PAS and mucicarmine positive substances is always mucus-secreting carcinoid. In such a case, electron microscopic examinations are useful for determination whether the substances are mucus or not, especially when the lumens are so small and silver impregnations are not positive.

It seems that there was a certain disorder of polarization of the tumor cells, which showed two directions of flow of the secretory granules. One direction was that the granules flowed to the apical portion of the cells when the cells formed the lumen (Fig. 5). The second was that the granules reached the basal portion of the cells when the cells were associated with the basement membrane and with fine stroma (Fig. 7). The former may assumably be considered as a reversion of the polarity of the secre-

tion, and the latter seems to be a usual form in these endocrine cells. Concerning the histogenesis of carcinoid tumor, the neural crest cell have been placed as an ancestor of the Kultschitzky cells⁹, although Matsuyama & Suzuki.¹⁰ reported that the immature mucus cell might transfer to the argyrophiles in the gastric glands of mice. In relation to these considerations, we suspect that the glandular formation and the neurosecretion of the cells into the lumen, which were observed in our case, may not be due to the differentiation of the tumor cell arising from the mucus cell but to the dysdifferentiation of the tumor cell derived from the neural crest cell.

Observation of the granules in the cytoplasm of tumor cells have been reported in some papers. Orloff² subdivided the granules and the secretory products of carcinoid tumors into three types¹¹ derived from the foregut, midgut and hindgut. According to his schematic diagram, our observations of granules agreed with those of the foregut type and the granules were not identified as serotonin from their shape. And also the granules corresponded with those of the type 1 cell reported by Black¹² and of the G cell by Solcia *et al.*¹³, or somewhat resembled to the granules of the intestinal D-cell of Fossmann *et al.*¹⁴ Some authors suggested that some of the secretory products from these cells of foregut origin were 5-hydroxytryptophan^{12,15}, histamine^{11,16,17} and gastrin^{13,14,18}. It is well known that the carcinoid tumor and the islet-cell tumor are often associated with peptic ulcers of the stomach or the duodenum. From consideration of the Zollinger-Ellison syndrome, Cavallero *et al.*¹⁸ concluded that the D-cell of the pancreas might produce gastrin. Weichert & Creach.¹⁹ reported 16 cases of duodenal tumors and suggested that the duodenal carcinoid and the islet-cell tumor might have a common cell origin. Therefore, the neurosecretory granules in our case may possibly correspond to gastrin, because the carcinoid tumor and the peptic ulcer seem to exist closely in the duodenum.

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