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Abstract

The activities of five hydrolytic enzymes, alkaline and acid phosphatase, beta-esterase, leucine aminopeptidase and beta-glucuronidase, of human gastric carcinomas from 180 patients were investigated histochemically. Alkaline phosphatase activity was almost negative in the carcinoma but was weakly positive in this tumor at times (about 10 to 20 per cent). Acid phosphatase activity which displayed a slightly increasing tendency of the reaction in poorly differentiated tumor was variegated and mainly from feeble to moderate in activity. Beta-esterase reaction was in varying degrees with each case, but more malignant the carcinomas, the weaker was the activity. Leucine aminopeptidase was positive in about 30 to 60 per cent of the specimens observed but the reaction was founded to be localized often in some areas and generally similar to alkaline phosphatase reaction. The activities of leucine aminopeptidase, alkaline phosphatase and beta-esterase were positive at a higher rate in mucinous carcinomas than in non-mucin producing one. Beta-glucuronidase activity was slight or moderate in general but rather strong in the early stage of carcinomas.

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**HISTOCHEMICAL STUDIES ON ENZYME ACTIVITIES
OF GASTRIC CARCINOMA
I. HYDROLYTIC ENZYMES**

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Enzyme activity of human gastric carcinomas has been studied histochemically by many authors in the past 20 years, and recently some electron microscope observations on some enzyme activities have been made by several investigators. However, there are as yet no report that the enzyme activities of gastric carcinomas were arranged systematically from the view point of statistics which may serve for the ideal classification of carcinomas being directly connected to the biological activities of cancer cells.

In the present paper the histochemical observations on five hydrolytic enzymes, alkaline phosphatase, acid phosphatase, beta-esterase, leucine aminopeptidase and beta-glucuronidase are reported.

MATERIALS AND METHODS

The human gastric carcinomas removed from 180 patients, who were admitted to the Department of Surgery, Okayama University Medical School from 1960 to 1965, were used as materials. Small tissue blocks from the fresh materials were frozen at -20°C immediately after the removal, and serial sections of 20 microns thick were prepared in a cryostat. For the histochemical demonstration of hydrolytic enzymes, the tissue sections were fixed in 10 per cent cold formalin for 10 minutes, rinsed in distilled water, and then they were incubated by the following media. For alkaline phosphatase 10 mg of sodium alpha-naphthyl phosphate were dissolved in 20 ml of CLARK and LUB's buffer at pH 9.2 and 20 mg of fast blue B were added. The sections were incubated at 20°C for 30 minutes and dehydrated and mounted on balsam. For acid phosphatase 10 mg of sodium alpha-naphthyl phosphate were dissolved in 20 ml of acetate buffer at pH 5.8, to which 20 mg of fast blue B were added. The incubation time was one hour at 20°C . Sections were dehydrated and mounted

on balsam. For beta-esterase 10 mg beta-naphthyl acetate were dissolved in 1 ml of acetone, and 20 ml of MICHAELIS buffer at pH 7.2 and 20 mg of fast blue B were added. Incubation was carried out at 20°C for 30 minutes. The slides were mounted on glycerin. For leucine aminopeptidase the method of NACHLAS *et al.* (1957) was used. For beta-glucuronidase the method of SELIGMAN *et al.* (1954) was applied, in which 6-bromo-2-naphthyl-beta-D-glucuronide was used as substrate.

In order to establish the generalized standard of biological activity of cancer cells in connection with the morphologic structure of carcinomatous tissues the authors observed the tumors dividing them according to the histological classification of gastric carcinomas established by the Japanese Pathological Society in 1962 (Table 1). In this classification the carcinomas are divided into five types :

Table 1

	CAT	SAT	INF	Structural pattern	Functional pattern	Stromal quantity
Adenocarcinoma	I, II, III	1, 2, 3	α, β, γ	tubular papillary acinar	muconodular mucocellular	medullar scirrhous
Carcinoma solidum simplex (simple carcinoma)	I, II, III	1, 2, 3	α, β, γ	macro- meso- micro- alveolar	muconodular mucocellular	medullar scirrhous
Epidermoid carcinoma	I, II, III	1, 2, 3	α, β, γ	macro- meso- micro- alveolar	keratoid	medullar scirrhous
Adenoacanthoma	I, II, III	1, 2, 3	α, β, γ	Mucosal carcinoma, early Ulcer carcinoma, early Polyp carcinoma, early Double carcinoma		
Miscellaneous carcinoma	I, II, III	1, 2, 3	α, β, γ			

CAT: Cellular atypism

I, 1 and α : slight grade

SAT: Structural atypism

II, 2 and β : moderate grade

INF: Carcinoma cell infiltration

III, 3 and γ : heavy grade

namely, adenocarcinoma, simple carcinoma, epidermoid carcinoma, adenoacanthoma and miscellaneous carcinoma. Each of these five types of the carcinomas is further sorted into cellular atypism (CAT), structural atypism (SAT) and the mode of infiltration (INF) as shown in Table 1.

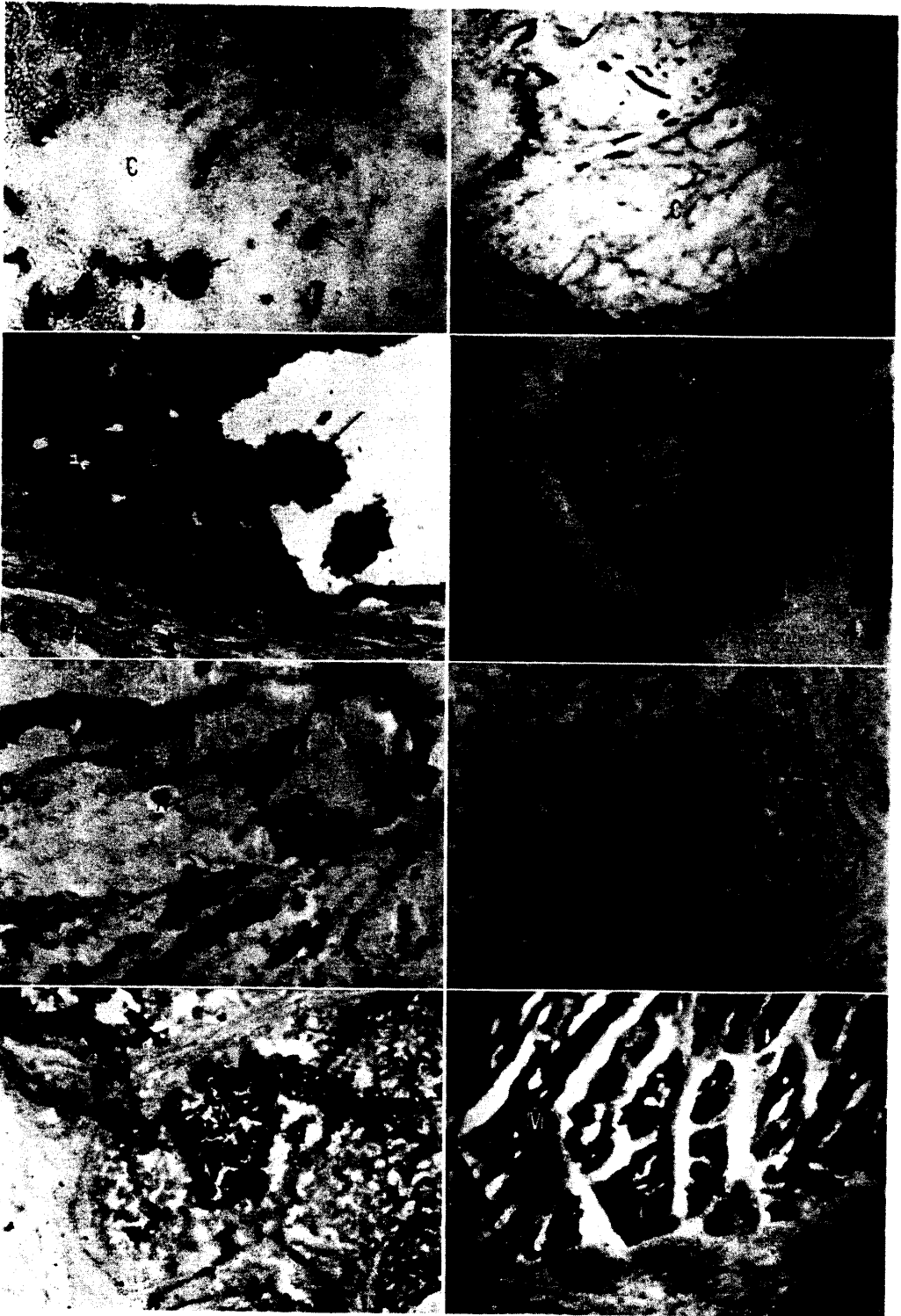
The cellular atypism is arranged from the first to the third group (I, II, III); well differentiated, moderately differentiated and undifferentiated types in cell morphology. The structural atypism is also divided into three subgroups (1, 2, 3); well organized, moderately organized and disorganized types in tissue structure.

The grade of carcinoma cell infiltration was indicated by α, β, γ , (α) represents the one in which the infiltration is hardly observed around of the tumor, the second grade (β) is the carcinoma with a moderate infiltration and the third grade (γ) is the one with a marked infiltration.

RESULTS

As the staining reactions were often not so uniform even in the same specimen in most of the tumors, average staining intensities of them were recorded. The reaction of these five enzymes varied in the carcinomatous tissues compared with that in normal epithelial cells of the gastric mucous membrane except in early carcinomas and in actively growing portions, and the reaction of stromal elements was very similar to the elements in normal tissue. Even if two different types of carcinomas were observed morphologically in the same specimen, the stainability of these five enzymes mostly resembled each other (about 60%). When the carcinomas with two different patterns exhibited different enzyme reactions, the stainability of the poorly differentiated cells was weaker than that of the well differentiated ones. Generally, the stainability of these enzymes in poorly differentiated patterns was weaker than that in well differentiated parts (25%) but a reverse tendency was observed in 15 per cent. In addition, in the same specimens the reaction for most of the enzymes was more intense in papillary area than that in the area with tubular pattern. Mucosal carcinomas (6 cases) were so small that the enzyme reactions of all tumor cells in the carcinomas were uniformly stained, and a remarkable finding in this instance was a considerable reaction of beta-glucuronidase. There was hardly any difference between the reaction of these enzymes of the carcinoma and that of adenomatous polyp as shown in Table 2.

Alkaline phosphatase reaction was almost negative in the carcinoma as in Table 2. The staining intensity did not depend on the cellular and structural atypism and the grade of carcinoma infiltration, but was sometimes faintly positive in mucinous carcinoma muconodulare et mucocellulare. These positive specimens were occasionally found in scirrhous carcinomas and very rarely in medullary ones. Most of the positive specimens which were evaluated as feeble (\pm) showed partly reacted patterns; for example, reacted in few foci or peripheral marginal parts in spite of little morphological change. Necrosis of the carcinoma tissue had generally no reaction and rarely positive. In the fibrous stroma alkaline phosphatase was generally absent, but in exceptional cases it was reactive in the stroma adjacent to the proliferating margin of carcinoma. Collagen fibers often exhibited a weak reaction. When lymphoid cell infiltration in carcinoma tissue was copious, the infiltrating cells and lymph follicles dis-



played a slight staining intensity. In capillary wall and endothel of blood vessel of the neoplasma and the gastric mucosa, this enzymatic reaction was strong, and it was negative in the adjacent mucous epithelium under any conditions except in intestinal metaplasia revealing varying degrees of the color intensity.

Staining intensities of acid phosphatase were slight or moderate in about half of the tumors, and negative, faint or intense at times. However, almost all cases of polyp and mucinous carcinoma showed a positive response of this enzyme. As a whole, gastric carcinomas had a slightly increasing tendency of the reaction for poor differentiation of the tumor cell and structure, and no tendency for grade of the carcinoma cell infiltration. No significant difference of the stainability between medullary type and scirrhous type was noticed. Proliferating areas such as invading margin, infiltrating part, peripheral layer of the neoplastic foci and isolated small carcinoma cell nestle were occasionally reactive for the enzyme than the other part of the tumor. Necrotic area showed a stronger response. Sometimes carcinoma cells in blood and lymph vessel were observed. They showed a similar or a stronger acid phosphatase reaction than the reaction of the carcinoma tissue out of the vessels except for necrosis. Generally, fibrous element, in which strongly stained cells considered fibroblasts and histiocytes were sparsely observed, was weakly reactive. Lateral inflammatory hyperplastic glands stained more intense than inflammatory glands without hypertrophy in several cases, and intestinal metaplasia was strongly reactive for the enzyme.

Beta-esterase staining intensity differed from negative to strong in stomach carcinoma. The poorer the cellular and structural differentiation, the weaker was the staining reaction of the carcinomas, and the higher the grade of the neoplastic cell infiltration, the weaker was the staining intensity. Beta-esterase reaction of medullary carcinoma was generally weaker than that of scirrhous

1. Alkaline phosphatase stain, $\times 50$, adenocarcinoma: The activity is generally negative in carcinoma (C) except for capillary walls but rarely positive in carcinoma cells in lymph vessels (\uparrow).
2. Alkaline phosphatase stain, $\times 50$, adenocarcinoma: Adjacent stroma shows rarely a positive reaction (\uparrow).
3. Acid phosphatase stain, $\times 50$, adenocarcinoma: The reaction of actively proliferating portions (\uparrow) of carcinoma is often stronger than of the other part.
4. Acid phosphatase stain, $\times 50$, simple carcinoma: The activity is increased at the peripheral part of the carcinoma cell nestle (C).
5. Acid phosphatase stain, $\times 50$: Both adenocarcinoma (A) and simple carcinoma (S) shows different activities in the same specimen.
6. Beta-esterase stain, $\times 20$, adenocarcinoma: In the vicinity of stroma the tumor cells reveal a positive activity.
7. Beta-esterase stain, $\times 50$, adenocarcinoma: Necrotic part (N) reveals a strong activity.
8. Beta-esterase stain, $\times 20$, simple carcinoma: Neighboring muscle (M) is positively reacted.

one. Rarely, the enzyme reaction was stronger in actively proliferating areas such as small carcinoma cell aggregates, invading margin of the tumor and isolated cell group, etc. than in the other part of carcinoma, and also free carci-

Table 2

	Cases	Alkaline phosphatase	Cases	Acid phosphatase	Cases	Beta-esterase	Cases	Glucuronidase	Cases	Leucine aminopeptidase
CAT	I 11h	18	18	19	19	15	15	15	15	15
	II 47	50	50	47	44	44	44	44	44	44
	III 65	65	65	67	62	62	62	62	62	62
c.s.s.	I									
	II									
	III 39	39	39	37	36	36	36	36	36	36
SAT	1 18	18	18	19	18	18	16	16	16	16
	2 54	64	64	62	53	53	56	56	56	56
	3 69	70	70	71	66	66	65	65	65	65
c.s.s.	1									
	2									
	3 42	43	43	42	36	36	36	36	36	36
INF	α 16	18	18	19	18	18	17	17	17	17
	β 50	51	51	50	47	47	51	51	51	51
	γ 69	71	71	68	64	64	64	64	64	64
c.s.s.	α									
	β									
	γ 38	39	39	40	33	33	35	35	35	35
Mucinous c.	24	25	25	27	19	19	24	24	24	24
Scirrhous c.	60	61	61	59	55	55	56	56	56	56
Medullar c.	26	27	27	28	16	16	17	17	17	17
Polyp	12	14	14	12	11	11	12	12	12	12
Early c.	6	6	6	6	6	6	6	6	6	6

Table 3

	Cases	Alkaline phosphatase	Cases	Acid phosphatase	Cases	Beta-esterase	Cases	Glucuronidase	Cases	Leucine aminopeptidase
CAT	I 7	5	5	6	6	5	5	5	5	5
	II 17	16	16	17	17	17	17	17	17	17
	III 37	34	34	35	37	37	34	34	34	34
c.s.s.	I									
	II									
	III 20	19	19	17	15	15	15	15	15	15
SAT	1 7	5	5	6	6	5	5	5	5	5
	2 17	16	16	17	17	17	17	17	17	17
	3 37	34	34	35	37	37	34	34	34	34
c.s.s.	1									
	2									
	3 20	19	19	17	15	15	15	15	15	15
INF	α 6	6	6	5	6	6	7	7	7	7
	β 26	27	27	27	23	23	23	23	23	23
	γ 25	22	22	23	24	24	24	24	24	24
c.s.s.	α									
	β									
	γ 21	19	19	16	15	15	17	17	17	17
Scirrhous	26	26	26	24	20	20	22	22	22	22

□ -, □ ±, ▨ +, ▩ ++, ■ +++.

Tables 2,3 The enzyme activities of specimens stained histochemically were graded microscopically on the basis of color reaction in - to +++ by inspection of all cells; complete negative -, faint +, slight +, moderate ++, and intense +++.

noma cells revealed a strong reaction in blood and lymph vessels. In necrotic area the color intensity was strong. The staining intensity of muscle tissue near the carcinoma in most cases and that of gastric gland near the carcinoma in some cases were moderately increased. This enzyme was usually absent in fibrous stroma, but in rare cases it was strongly positive at the adjacent zone of fibrous stroma to carcinoma cell nestle, and histiocytes proliferated in the stroma stained strongly.

Gastric carcinoma had no leucine aminopeptidase in over half of cases (106 cases), and sometimes this enzyme partially showed the positive response in the other cases despite no morphological change (29 cases) and diffusely positive in the rest (39 cases). The reaction had almost no relation to cellular atypism, structural atypism and degree of carcinoma cell infiltration, but generally strong positive cases were more in mucinous carcinoma, carcinoma muconodulare et mucocellulare, than in the other carcinomas. The stainability was positive in necrotic part at times. Positive reactions of leucine aminopeptidase were rarely noticed in the stroma, especially in the vicinity of the tumor. In 39 specimens the neoplasma was accompanied with intestinal metaplasia. But in half of the intestinal metaplasia the leucine aminopeptidase reaction was negative in the tumor irrespective of cases with the positivity in the adjacent intestinal metaplasia (15%), while the reaction of the tumor was similar to that of intestinal metaplasia in the other cases (16.5%). On the other hand, positive reaction of the tumor was observed in the 34 specimens without neighboring metaplastic area (24%). When their specimens were wider and larger, the rate of neighboring intestinal metaplasia would be increased.

The stainability of beta-glucuronidase was mainly slight or moderate in the carcinoma. and the more marked the carcinoma cell infiltration, the stronger was the staining intensity. No strong reaction was noticed in carcinoma muconodulare et mucocellulare. In general, the enzyme reaction was increased in the peripheral layer of carcinoma foci and the other growing portions. Necrotic area showed a marked staining intensity, and free carcinoma cells invaded into vessels and nearby tissues showed an increased reaction in some cases. Lateral hyperplastic glands stained stronger than glands in normal state. The fibrous stromal element exhibited weak reaction of the enzyme, and most of wandering cells infiltrated were light in color.

In summarizing the statistical histochemical estimation of gastric carcinomas is shown in Table 2, and that of fibrous stroma in Table 3.

DISCUSSION

Histochemical demonstrations of alkaline phosphatase on the mucous mem-

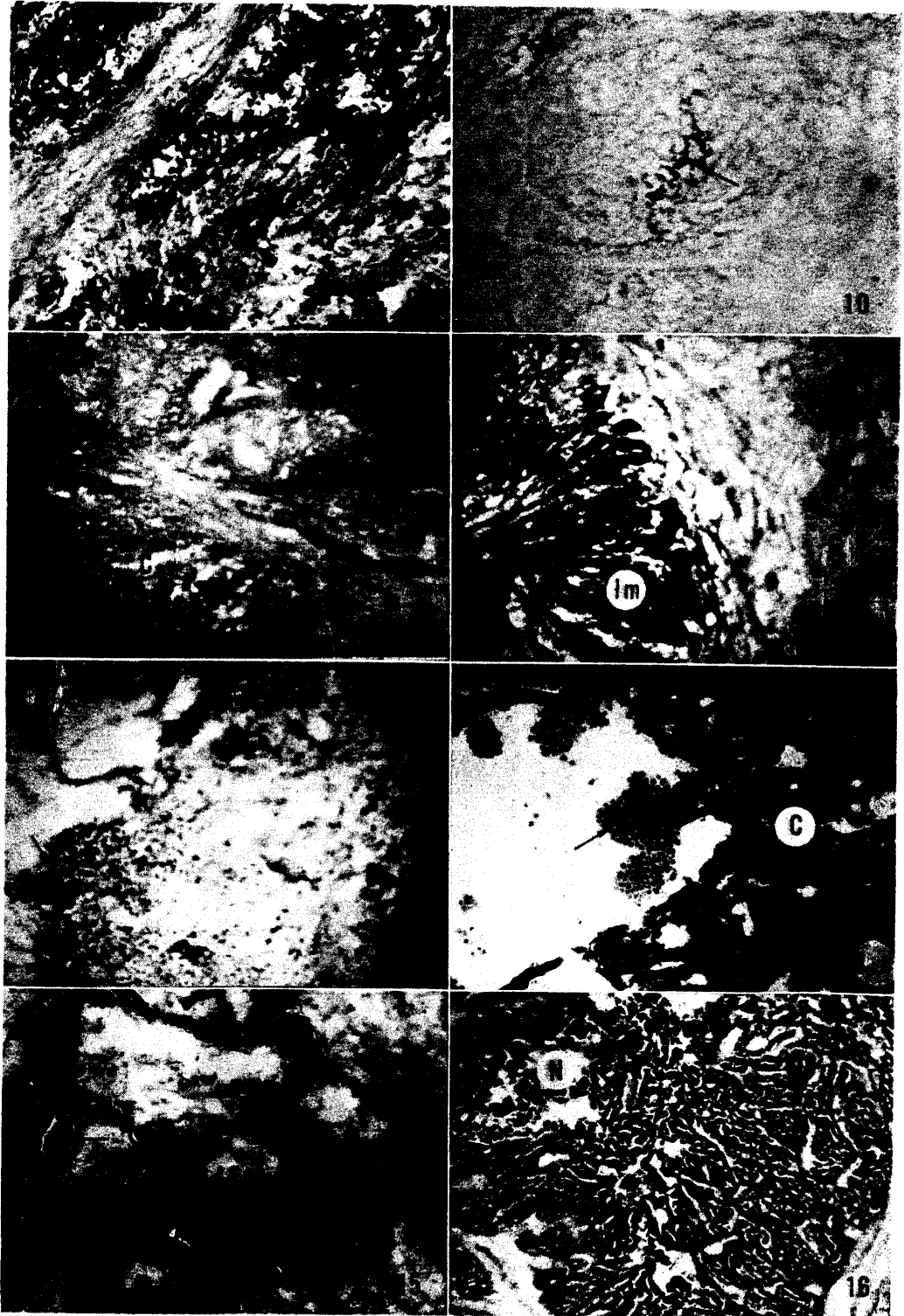
brane and carcinoma of the human stomach have been made by several investigators. They¹⁻¹¹ reported the negative reaction in most of the normal mucosa and carcinomas except for small blood vessels and some connective tissues, and FODDEN¹², MADDEN *et al.*¹³, PLANTEYDT *et al.*⁸ and YOSHITOSHI *et al.*¹⁴ mentioned certain activities in intestinal metaplasia. But SCHOLL *et al.*¹⁵ stated that there was no activity in scirrhous carcinoma and some focal nuclear staining in medullary carcinoma. Further, TAKAMATSU¹⁶, MANHEIMER *et al.*¹⁷, MITOMI¹⁸, TAKASE¹⁹ and TANAKA *et al.*¹¹ observed positive activities in some carcinomas of the stomach. In the present investigation the majority of specimens showed negative activity of the enzyme as the gastric mucosa did and the rest reacted weakly, but about half of the specimens of carcinoma muconodulare et mucocellulare showed the positive reactions though it was weak. This result indicated a certain relationship between the alkaline phosphatase activity and the mucin producing function. In Golgi complex of epithelial cell related to mucin which produces a lower level of alkaline phosphatase has been verified chemically²⁰, and in that and in plasma membrane of intestinal mucosa alkaline phosphatase was found to be present histochemically with electron microscope^{21, 22}. According to MANHEIMER *et al.*¹⁷ a papillary tumor showed a strong reaction, but in the present study no specimen with strong staining intensity was found except for necrotic area. KABAT *et al.*², AOKI *et al.*⁴, RUTENBERG *et al.*⁶ and the others reported positive reactions in the stroma of several gastric carcinomas. Similarly, stromal staining reactions observed in the vicinity of the growing part of the tumor in exceptional cases of the present investigation. In these exceptional cases the identical part of stroma often showed positive reactions for leucine aminopeptidase staining as well as for alkaline phosphatase. A strong reaction in capillary walls is observed in neoplastic tissues and mucous membrane of the stomach. Several workers^{33, 34} have suggested that alkaline phosphatase is concerned mainly with the "active transport" of chemical substances across the cell membranes.

The acid phosphatase activity of the mucous membrane has been studied by many workers and it is found remarkably strong in metaplastic area. GOMORI⁵ stated nine out of 11 carcinomas of the stomach to reveal positive activity, and AOKI *et al.*⁴, REINER *et al.*²⁶ and FANGER *et al.*²⁷ demonstrated also positive activity in the tumor. ROSEMAN *et al.*²⁸, REINER *et al.*²⁶ and RUTENBERG *et al.*⁶ reported a variable staining reaction unrelated to histologic grading in the carcinoma. Most of them described the activity of the carcinomas were weaker than that of adjacent glands except for REINER's two cases, but in the present paper the activity was very variable with each specimen. The tumor had a slight increasing tendency of the enzyme activity for poor differentiation of the cell and structure. Therefore, it is considered that acid phosphatase related

to digestion, secretion, excretion and pigment formation in the cellular metabolism is not directly essential for the growth of gastric carcinoma. However, it must be borne in mind that the activity is increased in some actively growing portions. In mucinous carcinoma the majority of cases were reactive for this enzyme. The acid phosphatase reaction has been found in lysosome, lipofuscin and endoplasmic reticulum with electron microscope. Increasing reaction of acid phosphatase in necrotic part according to GOMORI and this paper implies an increasing function of lysosomes which probably provide the enzymes for the digestion of the cell's own cytoplasm^{20,21}.

The beta-esterase activity was estimated in normal mucosa of the stomach by DAWSON *et al.*⁹, MALATY *et al.*³⁰, CORRETA *et al.*³¹, PLANTEDYDT *et al.*⁸, TANAKA *et al.*¹⁰ and KAWASHIMA *et al.*¹¹. In the metaplastic area, its activity was very strong. WACHSTEIN *et al.*³² reported that in seven carcinomas of the stomach, this enzyme activity was demonstrable in tumor cells and the staining reaction was strongest in the more mature tumors, only slight in anaplastic carcinomas, and stromal cells slight. In the present investigation similar results were obtained. The other characteristic finding was the fact that this enzyme was more weakly reactive in scirrhous carcinomas than in medullary one. According to NOVIKOFF²¹ and FREEMAN⁰ the beta-esterase is located in endoplasmic reticulum and lipofuscin of some tissue, but the beta-esterase activity stained by the present method is diffuse in the cytoplasm of carcinoma cells under the light microscope. Histiocytes of the stroma in the present study revealed a strong cytoplasmic reaction as was reported by HOSODA *et al.*³³ etc. . According to MONIS *et al.*³⁴ the esterase activity of macrophages seems to be related to their phagocytic and metabolic activities. This enzyme is as active as acid phosphatase and glucuronidase in the necrotic portions.

Leucine aminopeptidase is absent in normal gastric mucosa, but HANABUSA *et al.*⁵ reported a positive reaction in adenocarcinomas of the stomach in proliferating and degenerated areas. WILLIGHAGEN *et al.*³⁶ found all four gastric carcinomas which they studied to be positive, and WATTENBERG³⁷ observed an intense activity in seven and weak activity in three instances among the 20 carcinomas of the stomach, although the amount of stainable enzyme varied in various microscopic fields. On the other hand, MONIS *et al.*³⁸, GLENNER *et al.*³⁹, FISHER *et al.*⁴⁰ stated that gastric carcinoma and the other tumors were rarely reactive for the enzyme in their observations of most neoplasms. Some of them emphasized that the connective tissue in stroma, adjacent to the tumor often had positive activities, which was characteristic of fibroblastic activity and was not related to the presence of malignant cells. While on the positive activity of stroma, SYLVEN *et al.*⁴¹ considered the possibility that the secretion of proteolytic enzymes by growing tumor cells might contribute to their ability to invade



adjacent tissues. In the present paper most cases showed negative or almost negative reaction and only about 35% of all was reactive in the tumor cells, and rarely showed positive reaction in the stroma, mostly at the vicinity of the tumor. From the present observations stromal reactions were positive in young fibrocytes and fibroblasts at the growing part of the connective tissue and probably at the part of mesenchymolysis in the vicinity of the neoplasma. Two-thirds of mucinous carcinomas revealed positive activity of leucine aminopeptidase as in the case of alkaline phosphatase and acid phosphatase and all of strong positive cases were carcinoma solidum simplex, but there is no significant difference between scirrhous type and medullary type. Necrotic areas are occasionally reactive as reported by TANAKA *et al.*¹⁰ and the others. WATTENBERG³⁷ and the others reported that chronic gastritis with intestinal metaplasia often show an increased staining intensity of leucine aminopeptidase. PLENTEY *et al.*⁸ and WATTENBERG³⁷ state that there is a relationship between intestinal metaplasia and carcinoma of the stomach. This opinion is supported by the present results; namely, 16.5% of the carcinoma with intestinal metaplasia showed leucine aminopeptidase positive response while 24% of the tumor without any metaplastic area was positive. According to TANAKA *et al.*¹⁰ and KAWASHIMA *et al.*¹¹, glucuronidase is reactive strongly in parietal cells and intestinal metaplasia weakly or moderately in the other epithelial elements as well as succinic dehydrogenase and other NAD-dependent dehydrogenases. MONIS *et al.*⁴² found among 14 carcinomas, four with intense reaction, three with moderate activity, and three weakly active and the tumor cells did not react uniformly. Similarly the glucuronidase activity varied in the present study, and the activity of the specimens with high grade of carcinoma cell infiltration displayed stronger than low grade ones as reported by TANAKA *et al.* CAMPBELL⁴³

9. Leucine aminopeptidase stain, $\times 50$, scirrhous mucinous carcinoma: In mucinous carcinoma the activity is intense and weak in its stroma at times.
10. Leucine aminopeptidase stain, $\times 50$, adenocarcinoma: Positive reaction is found in the central necrosis (\uparrow).
11. Leucine aminopeptidase stain, $\times 20$, adenocarcinoma: Rarely positive response is found in both the carcinoma (C) and stroma (St).
12. Leucine aminopeptidase stain, $\times 20$, simple carcinoma: Leucine aminopeptidase negative carcinoma with strongly reacting metaplastic area (Im) is observed at times.
13. Beta-glucuronidase stain, $\times 20$, simple carcinoma: Sometimes the growing marginal part (\uparrow) shows increased activity.
14. Beta-glucuronidase stain, $\times 50$, adenocarcinoma: On the contrary, decreasing activity is rarely noticed in the actively proliferating portion (\uparrow).
15. Beta-glucuronidase stain, $\times 50$, adenocarcinoma: Note the intense reaction of carcinoma cells in the lymph vessels (\uparrow).
16. Beta-glucuronidase stain, $\times 50$, adenocarcinoma: Necrotic area (N) is strongly reactive for the enzyme.

demonstrated that the enzymatic activity was generally highest in the most actively growing portions of carcinomas of mouse, and a strong reaction at the cell membrane. Similar findings were noticed in the present study; namely, glucuronidase was localized in lysosomes observed in electronmicroscope, and necrotic area showed strong activity. According to WACHSTEIN *et al.*³² the activity is usually concentrated in the luminal aspect of colonic neoplastic cells. Some of specimens of the stomach exhibited similar staining features in the present study but in the stomach, no characteristic finding was observed.

SUMMARY

The activities of five hydrolytic enzymes, alkaline and acid phosphatase, beta-esterase, leucine aminopeptidase and beta-glucuronidase, of human gastric carcinomas from 180 patients were investigated histochemically. Alkaline phosphatase activity was almost negative in the carcinoma but was weakly positive in this tumor at times (about 10 to 20 per cent). Acid phosphatase activity which displayed a slightly increasing tendency of the reaction in poorly differentiated tumor was variegated and mainly from feeble to moderate in activity. Beta-esterase reaction was in varying degrees with each case, but more malignant the carcinomas, the weaker was the activity. Leucine aminopeptidase was positive in about 30 to 60 per cent of the specimens observed but the reaction was founded to be localized often in some areas and generally similar to alkaline phosphatase reaction. The activities of leucine aminopeptidase, alkaline phosphatase and beta-esterase were positive at a higher rate in mucinous carcinomas than in non-mucin producing one. Beta-glucuronidase activity was slight or moderate in general but rather strong in the early stage of carcinomas.

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