Histochemical studies of hydrolytic and oxidative enzymes in the human intestinal tumors

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Abstract

The distribution and activities of five hydrolytic and eight oxidative enzymes were histochemically studied in 60 different tumors of the human intestines. Benign polyp showed similar activities of most enzymes as those in normal crypt cells of large intestine with exception of higher activity of succinic dehydrogenase in benign polyp than in crypt cells. Malignant polyp had higher activities of most oxidative enzymes. Reticulo-sarcoma had weak activities of all enzymes. Carcinoid had strong activities of glucose-6-phosphate dehydrogenase and isocitric dehydrogenase while very weak of succinic dehydrogenase. Carcinoma showed varying degrees of the activity of all enzymes. Alkaline phosphatase and aminopeptidase were almost negative in all cells but in the stromal elements their weak activities were sporadically observed. Most enzymes were decreased in the central area of the carcinoma cell nestle, while in the infiltrating area or in the margin of cell nestle they were not decreased and sometimes increased.

HISTOCHEMICAL STUDIES OF HYDROLYTIC AND OXIDATIVE ENZYMES IN THE HUMAN INTESTINAL TUMORS

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There are few brief reports about enzyme histochemical study of intestinal tumors (TOMITA, 1957[26], MONIS *et al.*, 1959b[10], WACHSTEIN, 1962[27]) and a systematic study is presented by WATTENBRG (1959[29]) about five oxidative enzymes. Biochemically many observations are seen about enzyme activities of carcinomas and other tumors (AKAHORI and OKINAKA, 1964[1]) since WARBURG's hypothesis in 1928 (quoted from Science, 1956[28]). Systematical enzyme histochemical studies about tumors are now in progress, such as KITAMURA (1963[8]) about gastric carcinoma, NOBUTO (1965[16]) about breast tumor and OGATA *et al.* (1962[18]) about gastric carcinoma.

This report deals with the distribution and activities of five hydrolytic and of eight oxidative enzymes in 60 intestinal tumors removed surgically. It was intended to see metabolic features of intestinal tumors, metabolic differences in various tumors, how the enzyme activity changes in the central region or marginal area of the tumor, and whether there is any relation of the enzymatic pattern among normal intestinal epithelium, polyp, early carcinoma and carcinoma.

MATERIALS AND METHODS

All materials used were surgically removed human intestinal tumors, comprising 2 reticulum cell sascomas of the large and small intestines, 4 benign polyps of the large intestine, 1 carcinoid of the caecum, 1 malignant polyp of the large intestine, 47 carcinomas of the large intestine, 3 metastatic carcinomas of the small and large intestines and 2 squamous cell carcinomas of the rectum. Two specimens were obtained from distant part of the carcinoid tumor.

Pathological diagnosis was made by the hematoxylin-eosin staining from the serial sections. Carcinomas were divided into three groups according to their cell atypism and structual atypism; namely, well differentiated, moderately differentiated and poorly differentiated carcinomas.

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Specimens were immediately brought and stored in a deep freezer at $-30°C$. The serial sections were cut at 15—30μ in a $-20°C$ cryostat. The mounted fresh sections were dried at room temperature for 15 minutes. Some of them were fixed in 10% formalin and stained with hematoxylin- eosin.

For the histochemical demonstration of hydrolytic enzymes, the sections were fixed in 10% formalin for 10 minutes and rinsed in distilled water, and immediately incubated with the following media.

Alkaline phosphatase : 10 mg of sodium α-naphthyl acid phosphate were dissolved in 20 ml of Clark and Lub's buffer at pH 9.2 and 20 mg of diazo blue B added. They were incubated for 30 minutes at $37°C$, dehydrated and mounted on balsam.

Acid phosphatase : 10 mg of sodium α-naphthyl acid phosphate were dissolved in 20 ml of $0.1\,M$ Michaelis buffer at pH 5.8 and 20 mg of diazo blue B added. Incubation was one hour at $37°C$. Sections were dehydrated and mounted on balsam.

β-esterase : 10 mg of β-naphthyl acetate were dissolved in 1 ml of acetone, and 20 ml of $0.1\,M$ Michaelis buffer at pH 7.2 and 20 mg of diazo blue B added. Incubation was at $20°C$ for 30 minutes. The sections were mounted on glycerol.

β-glucuronidase : The method of Seligman *et al.* (1954[25]) was applied in which 6-bromo-2-naphthy-β-D-glucuronide was a substrate.

Aminopeptidase : The method of Nachlas *et al.* (1957[15]) was used.

For the histochemical demonstration of oxidative enzymes, fresh frozen sections cut at 15—30μ in a cryostat, were dried in room temperature, and mounted with the following substrate solutions.

Succinic dehydrogenase : The incubation medium was consisted of 5 ml of $0.2\,N$ sodium succinate, 5 ml of $0.1\,M$ phosphate buffer at pH 7.6 and 10 ml of 5 mg/3 ml Nitro blue tetrazolium (BT). The sections were incubated at $37°C$ for 30 minutes, fixed in 10% formalin and mounted on balsam.

Lactic dehydrogenase : The incubation solution contained 4 ml of $1/2\,M$ sodium lactate solution, 3 ml of Nitro BT solution (5 mg/3 ml), 11 ml of $0.1\,M$ phosphate buffer at pH 7.5, 2.5 mg of NAD (100%), 2 ml of $0.1\,M$ KCN (adjusted to pH 7.5 with $0.5\,M$ HCl).

Malic dehydrogenase : The incubation medium was consisted of 5 ml of $1\,M$ sodium malate, 3 ml of Nitro BT solution (5 mg/3 ml), 10 ml of $0.1\,M$ phosphate buffer at pH 7.4, 2.5 mg of NAD, 2 ml of $0.1\,M$ KCN (adjusted to pH 7.6 with 0.5 M HCl).

Glutamic, α-glycerophosphate and β-hydroxybutyric dehydrogenase : The incubation solutions contained 4 ml of $1\,M$ specific substrate solution, 3 ml of Nitro BT solutions (5 mg/3 ml), 11 ml of $0.1\,M$ phosphate buffer at pH 7.6,

2.5 mg of NAD (100%), 2 ml of 0.1 M KCN (adjusted to pH 7.6 with 0.5 M HCl).

Glucose-6-phosphate dehydrogenase: The incubation medium was consisted of 6 ml of 0.05 M disodium glucose-6-phosphate, 4—5 ml of Nitro BT solution (5 mg/3 ml), 17 ml of 0.1 M Veronal buffer at pH 7.4. 3 ml each of 0.01 M MgCl$_2$ and 0.5 M MnCl$_2$ and 7 mg of NADP.

Isocitic dehydrogenase: The incubation medium was consisted of 4 ml of 0.1 M sodium isocitrate, 3 ml of Nitro BT solution (5 mg/3 ml), 11 ml of 0.1 M Veronal acetate buffer at pH 7.4, 2 ml each of 0.01 M MgCl$_2$ and 0.5 M MnCl$_2$ and 2.5 mg of NADP.

Monoamine oxidase: Incubation medium was consisted of 25 mg of tryptamine hydrochloride, 4 mg of sodium sulfate, 4 mg of Nitro BT, 5 ml of 0.1 M phosphate buffer at pH 7.6 and distilled water 15 ml.

For lactic and malic dehydrogenases, incubation was carried out at 37°C for 30 minutes, and for other dehydrogenases for one hour.

These enzyme activities were compared with those in normal intestinal epithelium.

RESULTS

The distribution and activity of each enzyme were divers in tumor cells. Sometimes even the adjoining cells showed different activity. Therefore, it is difficult to classify accurately the grade of enzyme activity. Considering this diversity, each enzyme activity in tumors was classified according to the average activity of the majority of tumor cells and was summarized in Tables 1 and 2.

Alkaline phosphatase: The activity was negative in all the tumors examined except a weak reaction was sporadically demonstrated in two cases of reticulosarcoma. It was strongly positive only in the capillary walls, which were most abundant in the benign polyp especially near its surface but less abundant in the malignant polyp. A weak alkaline phosphatase activity sporadically appeared in the stromal elements of carcinoma. But no special relations were observed between the positive site and the tumor area such as necrosis or infiltration.

Acid phosphatase: Benign polyp showed a weak to moderate activity similar to that of the crypt cells of normal large intestine. However, in malignant polyp its activity markedly decreased. It was moderate in carcinoid but very weak in reticulosarcoma. Its activity was diverse in carcinoma. Generally the poorly differentiated carcinoma showed a less activity than the well differentiated one except the undifferentiated mucinous carcinoma which showed a strong activity. The infiltrating area at the periphery of carcinoma showed an

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Table 1   The Activity of Hydrolytic Enzymes in Human Intestinal

Enzyme	AlP −	±	+	⧺	⧻	AcP −	±	+	⧺	⧻	β-Eat −	±	+	⧺	⧻	AmP −	±	+	⧺	⧻
Normal large intestine (8 cases)																				
Surface epithelium	6	2						6	2				1	2	5	6	1	1		
Crypt	8						1	6	1				1	4	3	6	2			
Bottom of crypt	8						1	6	1				1	4	3	6	2			
Reticulum cell sarcoma (2 cases)	2							2			2					1	1			
Carcinoid (2 cases)	2								2		2					2				
Benign polyp (4 cases)	4							3	1					3	1	4				
Malignant polyp (1 case)	1					1								1		1				
Well differentiated carcinoma (8 cases)	7	1				4	3	1			1	1	3	3		6	2			
Moderately differentiated carcinoma (23 cases)	13	3				5	2	7	2		2	7	3	3	1	12	3			1
Poorly differentiated carcinoma (23 cases)	22					4	8	10		1	4	7	9	2	1	18	1	3	1	
Metastatic carcinoma of small intestine (3 cases)	3					3					1	2				3				
Squamous cell carcinoma of rectum (2 cases)	2					2					1	1				2				

Key: − = no staining; ± = faint staining; + = slight staining; ⧺ = moderate staining; ⧻ = strong staining.

Table 2   The Activity of Oxidative Enzymes in

Enzyme	SDH −	±	+	⧺	⧻	LDH −	±	+	⧺	⧻	MDH −	±	+	⧺	⧻	α-GDH −	±	+	⧺	⧻
Normal large intestine (8 cases)																				
Surface epithelium			1	3	4			2	3	3			1	4	3			2	4	2
Crypt		2	5	1				3	5				3	4	1				4	4
Bottom of crypt			1	3	4			2	5	1			2	3	3			1	4	3
Reticulum cell sarcoma (2 cases)	1	1				1	1				2					2				
Carcinoid (2 cases)		1	1							2				1	1					2
Benign polyp (4 cases)			2	2					3	1			3	1					1	3
Malignant polyp (1 case)				1				1							1					1
Well differentiated carcinoma (8 cases)		1	4	3					7	1			1	5	2				5	3
Moderately differentiated carcinoma (16 cases)		10	4	2				5	8	3			5	10	1			6	9	1
Poorly differentiated carcinoma (23 cases)	2	11	8	2				2	16	5			11	10	2				16	7
Metastatic carcinoma of small intestine (3 cases)			1	2					1	2				3					1	2
Squamous cell carcinoma of rectum (2 cases)			1	1		1	1				1	1							2	

increased activity in certain cases. The stromal elements of carcinoma had a weak activity.

β-Esterase : The activity slightly increased in benign polyp compared with the normal crypt cells of large intestine. But malignant polyp showed a weaker activity than the crypt cells. It was negative in carcinoid and reticulosarcoma. Carcinoma showed various activities from weak to strong even in the same specimen, but most of them had less activity than that of normal epithelium of rectum. A fairly strong activity was sometimes observed in the central necrotic area of carcinoma nestle. It was weak in the stromal elements of all tumors.

Aminopeptidase : The activity was negative in the polyp and the carcinoid. In reticulosarcoma weakly positive areas partially appeared. Carcinomas showed almost negative reaction, but some of them showed a weak to moderate activity sporadically. The sporadical appearance of this enzyme reaction was observed more often in the poorly differentiated carcinoma than in the well differentiated carcinoma. However, no special

Tumors

β-Gl				
−	+	±	++	+++
	2	4	2	
	4	4		
	4	4		
2				
			2	
	1	3		
			1	
	2	6		
	9	7		
1	1	9	12	
	1	2		
	1	1		

Human Intestinal Tumors

GDH					β-HDH					G6PDH					ICDH					MAO				
−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++	−	±	+	++	+++
		5	3				6	2		1		5	2		1		5	2						
		7	1			3	4	1			3	4	1			3	5							
		3	3			1	4	2	1		3	4	1			3	5							
2					2					2					2									
	1		1			2							2					2			2			
	3		1				3	1				3	1				4						2	
	1						1						1				1						1	
1		3	4			4	2	2			2			6			2	5	1			1	2	1
	10	5	1		1	1	10	4		1		10	5			4	10	2			1	3	2	
4	10	9				1	17	5		2		10	11			2	16	5			2	6	1	
		1	2				3					1	2		1			2						
		1	1			1	1					1	1		1		1							

correlations were observed between the positive site and the tumor area such as the nectrotic area or the infiltration. It was usually negative in most of the stromal elements, but sometimes a weak or slight reaction appeared at random.

β-Glucuronidase : The polyp had a similar activity as that of the normal crypt cells. Carcinoid and reticulosarcoma showed a weak reaction. Generally carcinoma had a weaker activity than that of normal epithelium but in the infiltrating area, sometimes in the scirrhously invading region and the central necrotic area this enzyme activity increased. The stromal elements of carcinoma had a very weak activity.

Succinic dehydrogenase : The activity was strong in both the benign and the malignant polyps. Especially a very strong activity was observed at the adenomatously proliferating area near the surface of the benign polyp. Carcinoid showed a markedly decreased activity as compared to normal epithelium. It was very weak in sarcoma. Carcinoma had varying activities, but most of them showed a less activity than normal crypt cells. However, the infiltrating area at the periphery of tumor did not show any decrease in the activity but sometimes an increased activity. This tendency was especially prominent in the well differentiated carcinoma, in the scirrhously invading carcinoma cells, and in the carcinoma cell nestles surrounded by much stromal defence zone. The activity was very weak in the stromal elements.

Lactic dehydrogenase : The polyp showed a moderate activity as normal epithelium. Carcinoid showed a slightly increased activity than that of normal epithelium. This enzyme activity was weak to slight in reticulo-sarcoma but still stronger than other dehydrogenases. Generally, carcinoma showed a moderate activity. Mucinous carcinoma, the scirrhously invading region, and sometimes the central area of tumor showed a higher activity than normal crypt cells. Stromal elements had slight activity.

Malic dehydrogenase : The activity was similar in normal epithelium, benign polyp and carcinoid, but stronger in malignant polyp. Reticulo-sarcoma had a weak activity. The activity in carcinoma was almost similar or slightly decreased compared with that in normal epithelium. The invading carcinoma cells at the periphery of cell nestle showed a stronger activity than the central ones, but this tendency was not so prominent as in succinic dehydrogenase specimens. The stromal elements showed a slight activity.

α-Glycerophosphate dehydrogenase : The distribution and activity of this enzyme was similar to those of succinic dehydrogenase in polyp, carcinoma and reticulo-sarcoma. Carcinoid showed a moderate activity.

Glutamic and β-hydroxybutyric dehydrogenases : Activities of both of these enzymes were similar in polyp but in carcinoma they were a little decreased comparing with normal crypt cells. Reticulo-sarcoma showed a very

weak to almost negative activity. In carcinoma, the distribution of both enzymes was similar to that of succinic dehydrogenase. The stromal elements had a very weak activity.

Glucose-6-phosphate dehydrogenase : The activity in the benign polyp did not differ from that in normal epithelium, while it increased slightly in malignant polyp. This enzyme activity increased considerably in carcinoid compared with normal epithelium contrary to that of succinic dehydrogenase. The reticulosarcoma showed a faint activity. In the majority of carcinoma the activity was stronger than in normal epithelium.

Isocitric dehydrogenase: The polyp had the activity similar to the normal epithelium. The distribution of this enzyme in carcinoid and in carcinoma was similar to that of glucose-6-phosphate dehydrogenase.

Monoamine oxidase : Only 19 cases were examined of this enzyme. Its actitivity was slight in carcinoid, but moderate in polyp and in carcinoma.

The well-differentiated carcinoma showed a stronger activity than the poorly-differentiated one.

DISCUSSION

It is generally accepted that the polyp of intestine has an intimate correlation with carcinoma. The predilection site and age are very similar (FISHER, 1952)[6]. The activity of the hydrolytic enzymes in benign polyp is almost the same as in normal epithelium (SEITO, 1965a)[22]. There is hardly any specific relation between the distribution of hydrolytic enzymes and the histological features of benign polyp. On the other hand, oxidative enzyme activities generally are increased in the benign polyp as compared with normal epithelium, and this tendency is most prominent in the proliferating place. As mentioned in the previous report, most enzymes, especially succinic dehydrogenase, showed a strong activity in epithelial cells at the basal part of normal crypt. By using radioautography SEITO (1965b)[24] demonstrated that the incorporation of thymidine-H^3 into these cells was prominent. COLE (1963)[4] proved that the human large intestine synthesized DNA on the surface and this part coincided with the proliferating area of the epithelial cells. WATTENBERG (1959)[29] reported that the polyp with cell atypism showed the strong activities of succinic, α-glycerophosphate dehydrogenases and monoamine oxidase, while in the polyp obviously being carcinoma, these activities decreased but NAD-, and NADP-diaphorases revealed more activities.

The enzyme activity in malignant polyp differed from that in benign polyp on the following points: acid phosphatase decreased, succinic, malic, and α-glycerophosphate dehydrogenases increased and lactic dehydrogenase was un-

changed.   Tomita (1957)[26] reported that the mitochondria in epithelial cells of early stage polyp did not differ from that of the normal epithelium of human intestine.   He also stated that as atypism of the cell progressed, mitochondria increased in number and size, but as the cell malignancy advanced, mitochondria differentiated irregularly and generally decreased in number.   In the present study the enzymes contained in mitochondria showed a tendency nearly parallel to Tomita's finding.

The activity of each enzyme in cancer tissue was diverse, sometimes even the adjoining cells showed the somewhat different activity.   Therefore, it is not easy to make stepwise classification of the staining intensity of enzyme reaction.

Alkaline phosphatase was generally absent in the large intestinal epithelium and carcinoma cells, but it was occasionally demonstrated in the stromal elements.   Monis et al. (1960)[11] reported that no correlation was observed between alkaline phosphatase activity and the infiltration of carcinoma.   The results of the present study agree with theirs.

Acid phosphatase reacted positively in the normal epithelium of large intestine (Seito, 1965a)[22].   Ogawa et al. (1962)[19] showed that acid phosphatase had a connection with Paneth cells and mucinous cells.   In our study mucinous carcinoma showed rather strong activity.   Willighagen (1960)[80] reported that acid phosphatase became less active as carcinoma grew less differentiated, while Reiner et al. (1957)[21] found that the acid phosphatase activity and the extent of the differentiation of carcinoma showed no relation.   In our study acid phosphatase activity generally decreased in the central area of cell nestle, but it did not decrease or sometimes increased in the proliferating area.

Cohen et al. (1951)[3] stated that β-esterase showed weak or almost negative response in the tumor tissue except the malignant and observed no relation between the activity and the grade of the differentiation of carcinoma.   On the other hand, Wachstein (1962)[27] reported that the carcinoma of stomach and large intestine decreased in the activity as the differentiation became poorer.   The result of our study coincide with the finding of Wachstein.

Leucine aminopeptidase was almost negative in human large intestine (Seito, 1965a[22]), but in carcinoma the positive reaction appeared sporadically.   According to Monis et al. (1950 a)[9], leucine aminopeptidase activity was positive in the fibroblast of connective tissue.   However, Glenner et al. (1957)[7] reported that aminopeptidase activity showed the proteolytic activity around the tumor.   Concerning the latter fact, Braun-Falco (1957)[2] stated that aminopeptidase activity was one of the indications of the invading activity of tumor.   By Nachlas, Crawfold and Seligman's method, Okamoto et al. (1961)[20] observed positive aminopeptidase activity both in the proteolytic process and in the fibrous proliferating tissues.

β-Glucuronidase is believed to be localized in lysosomes, and its activity increases when the cell is injured (DE DUVE *et al.*, 1955)[5]. In our study the increase of β-glucuronidase was observed in the central necrotic area of cell nestle. This tendency was also reported in breast cancer (NOBUTO, 1965)[16], in stomach cancer (OGATA, *et al.*, 1962)[18] and in esophageal cancer (SEITO, *et al.*, 1966)[23].

Oxidative enzymes showed varying intensities even in the same specimen. Generally succinic dehydrogenase activity decreased in malignant tumors. In carcinoma the activity is higher in the well-differentiated one than in the poorly-differentiated (WATTENBERG, 1959[29], MONIS *et al.*, 1959b)[10]. A similar tendency was also observed in the intestinal tumors in our study. In addition, increased activity was observed in the proliferating area of carcinoma cell nestle and scirrhously invading region of the poorly-differentiated carcinoma.

Lactic dehydrogenase activity was strong in most cases of carcinoma. Lactic dehydrogenase was increased in the patients with carcinoma, cardiac disease and liver disturbance, and its activity in serum was useful in determining the effect of therapy and the extent of the spread of carcinoma (AKAHORI and OKINAKA, 1964)[9].

In general malic dehydrogenase activity was weak to moderate in carcinoma.

MORI *et al.* (1963a)[12] reported that α-glycerophosphate dehydrogenase activity was rather strong in carcinoma. In our study α-glycerophosphate dehydrogenase showed a distribution pattern similar to that of succinic dehydrogenase, namely, its activity decreased in the central area but sometimes rather increased in the proliferating area and the invading margin.

Glutamic dehydrogenase also showed the distribution similar to the succinic dehydrogenase. MONIS *et al.* (1959b)[10] examined the enzyme distribution of glutamic and β-hydroxybutyric dehydrogenase in various tumors and reported that the former activity was rather strong and the latter was the strongest in colonic carcinoma although the activities of both of them were weak in other tumors.

Glucose-6-phosphate dehydrogenase is one of the enzymes of the pentose-phosphate cycle. As reported by WARBURG (1956)[28], this cycle was very active in carcinoma. KITAMURA (1963)[8] suggested that the pentose cycle is highly active in gastric carcinoma from his histochemical observation of the high NADP-diaphorase activity in it. From the biochemical study, WARBURG (1956)[28] suggested that the metabolic features in carcinoma cells are highly active in anaerobic glycolysis and low in the aerobic. In our study, the poorly-differentiated carcinoma and the central area of cell nestle of well-differentiated carcinoma showed the tendency similar to Warburg's hypothesis, because they had a low activity

of succinic dehydrogenase and a high activity of glucose-6-phosphate dehydro-
genase. However, the different finding was observed at the proliferating area
in the periphery of cell nestle, because it showed a high activity of both succinic
dehydrogenase and glucose-6-phosphate dehydrogenase. This discrepancy might
be attributed to the differences of analytical methods in both experiments, be-
cause in biochemical study the tissue is analysed as a whole.

The distribution of isocitric dehydrogenase resembled that of glucose-6-
phosphate dehydrogenase (MORI *et al.*, 1963b[13], 1964[14]). Further study will be
necessary to clarify why these two enzymes, belonging to the different metabolic
cycle, show a similar distribution in carcinoma.

Monoamine oxidase was histochemically demonstrated in mitochondria by
WACHSTEIN (1962)[27]. WATTENBERG (1959)[29] reported that this enzyme activity
was higher in well-differentiated carcinoma than in poorly-differentiated one.
Our results are in accord with his finding.

Generally, the stromal elements of carcinoma showed lower activities in all
enzymes. It is interesting to note that alkaline phosphatase and aminopeptidase
demonstrated sporadic positive reaction. Further analysis will be necessary to
determine the cause of irregular appearances of these enzymes in tumors. Reti-
culo-sarcoma showed weak activities of all the enzymes examined. NOBUTO *et
al*[16]. also demonstrated a similar tendency in lymph-, fibro- and neurofibro-
sarcomas. Carcinoid showed a weak activity in succinic dehydrogenase and β-
esterase, and had a strong activity in lactic and NADP-dependent dehydrogenase.

SUMMARY

The distribution and activities of five hydrolytic and eight oxidative enzymes
were histochemically studied in 60 different tumors of the human intestines.
Benign polyp showed similar activities of most enzymes as those in normal crypt
cells of large intestine with exception of higher activity of succinic dehydrogenase
in benign polyp than in crypt cells. Malignant polyp had higher activities of
most oxidative enzymes. Reticulo-sarcoma had weak activities of all enzymes.
Carcinoid had strong activities of glucose-6-phosphate dehydrogenase and isocitric
dehydrogenase while very weak of succinic dehydrogenase.

Carcinoma showed varying degrees of the activity of all enzymes. Alkaline
phosphatase and aminopeptidase were almost negative in all cells but in the
stromal elements their weak activities were sporadically observed. Most enzymes
were decreased in the central area of the carcinoma cell nestle, while in the
infiltrating area or in the margin of cell nestle they were not decreased and some-
times increased.

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EXPLANATION OF FIGURES

Letters in figures show the following meaning; T: tumor, C: central area of tumor, N: necrotic area of tumor, P: peripheral part of tumor, M: muscle layer, E: almost normal epithelium.

Figs. 1—4 Well-differentiated adenocarcinoma of the rectum. Note the activities of acid phosphatase (Fig. 1), sucinic dehydrogenase (Fig. 2) and α-glycerophosphate dehydrogenase (Fig. 3) is decreased in the central area but not decreased or sometimes even increased in the advancing periphery. Lactic dehydrogenase (Fig. 4) shows rather strong activity in the central area of the carcinoma comparing the normal epithelium. ×4

Fig. 5 Adenocarcinoma of the colon, lactic dehydrogenase, showing the increased activity in the carcinoma. ×4

Figs. 6—7 Carcinoid of the cecum. Glucose-6-phosphate dehydrogenase (Fig. 6) activity is stronger in the tumor tissue than in the normal epithelium, while succinic dehydrogenase (Fig. 7) activity appeared *vice varsa.* ×4

Fig. 8 Carcinoid of the cecum, glucose-6-phosphate dehydrogenase, showing the same specimen as Fig. 6. ×40
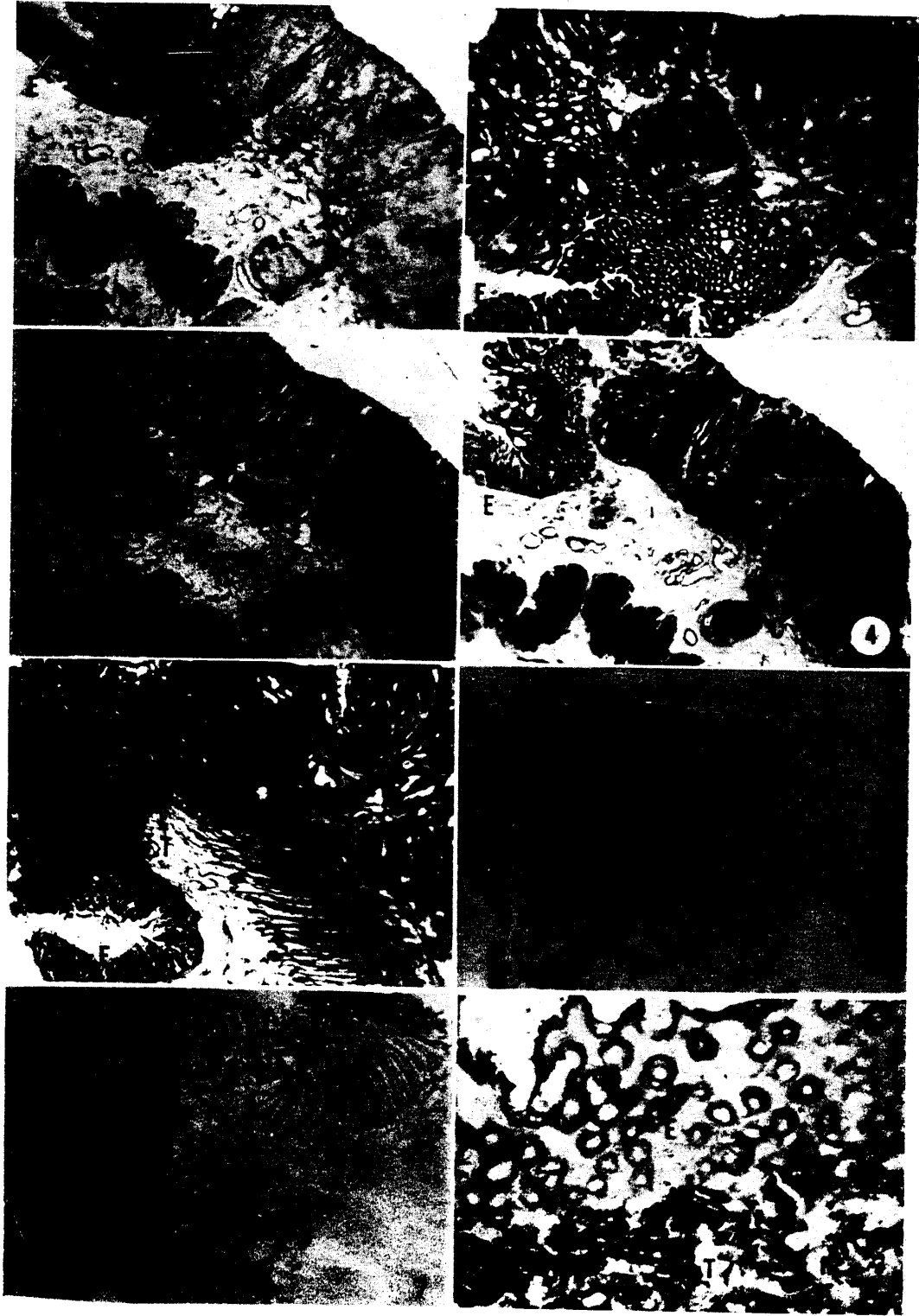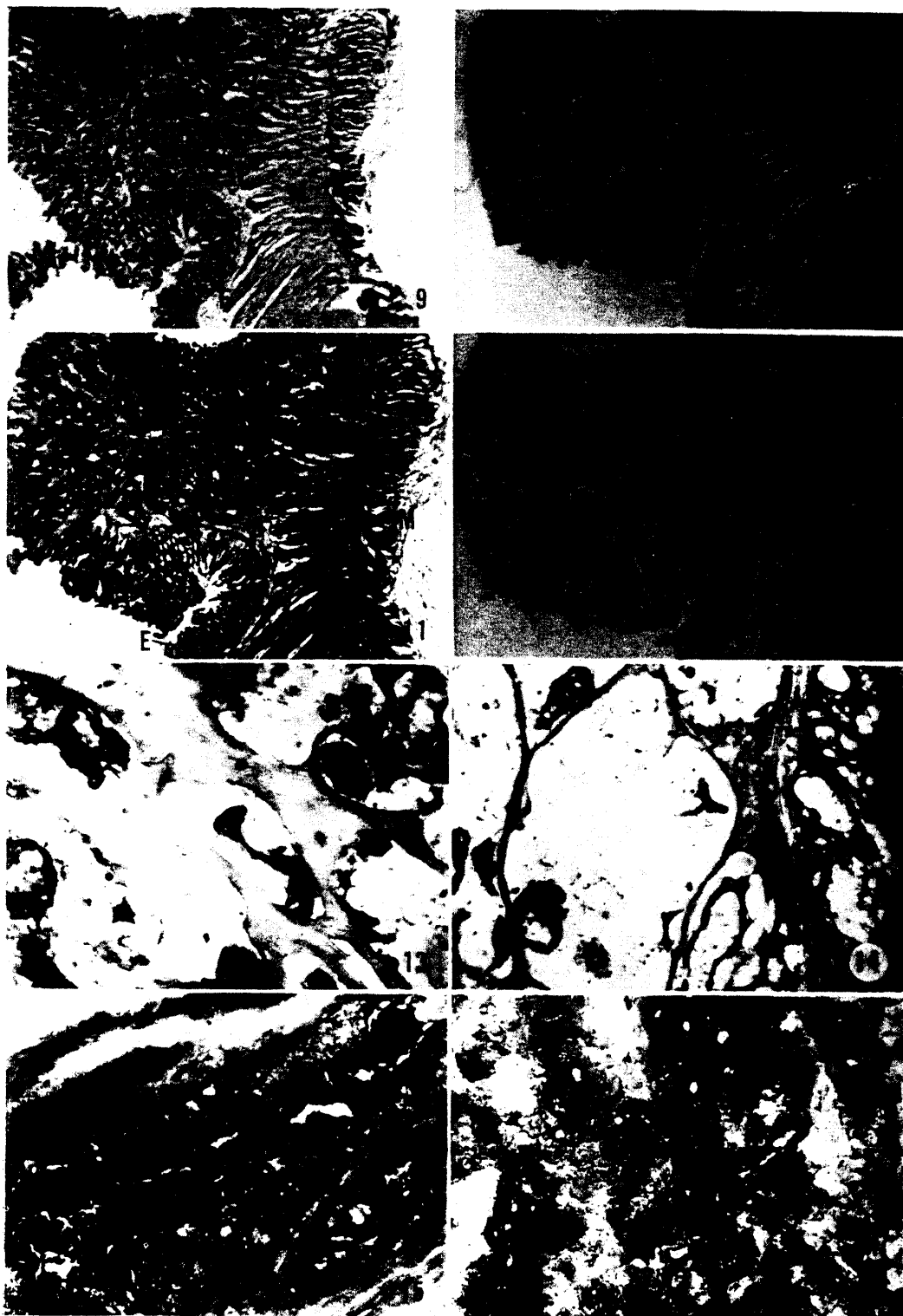
Figs. 9—12 Serial sections of poorly-differentiated adenocarcinoma of the sigma, showing the advancing area of the carcinoma. Note high activities of succinic dehydrogenase (Fig. 9), β-glucuronidase (Fig. 10), malic dehydrogenase (Fig. 11) and isocitric dehydrogenase (Fig. 12) in the scirrhously invading area to the muscle layer. $\times 4$

Figs. 13—14 Mucinous carcinoma of the rectum, showing the strong activity of acid phosphatase (Fig. 13) and lactic dehydrogenase (Fig. 14). $\times 40$

Fig. 15 Adenocarcinoma of the rectum, β-glucuronidase, showing the increased activity in the central necrotic region. $\times 40$

Fig. 16 Adenocarcinoma of the rectum, aminopepitdase, showing the positive reaction in the carcinoma. $\times 40$