

HISTOCHEMICAL STUDIES ON GASTRIC CANCER AND OTHER GASTRIC DISEASES: WITH SPECIAL REFERENCE TO THE GENESIS OF GASTRIC CANCER AND THE INTERSTITIAL REACTION OF GASTRIC CANCER TISSUE

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Introduction

The trials to give morphological evidences for the pathogenesis and pathophysiology of the several gastric diseases by performing a histochemical examination in addition to the pathological one, were attempted by many authors¹⁻⁷⁾ and some knowledge has already been obtained. We have carried out histochemical examinations of the

chronic gastric diseases involving chronic gastritis, the gastric ulcer, the gastric polyp and gastric cancer, and discussed the development of cancer from the above-mentioned chronic gastric diseases and the growth of the cancer, based on the results of the examinations.

Materials and Methods

There were 42 gastric cancer cases consisting of 16 ulcer cancer, 18 gastritis cancer and 8 polyp cancer cases. Besides, 16 gastric ulcer, 12 chronic gastritis and 3 gastric polyp cases were examined in contrast with the above cancer cases, respectively.

All the cases were admitted to our clinic and gastric resections were performed. The specimens were fixed immediately after the operation. The following stainings were performed.

For routine histological examination: Hematoxylin-eosin staining.

For alkaline and acid phosphatase: Gomori's modified technique.⁸⁾

For RNA: Kurnick's pyronin-methylgreen staining.

For DNA: Feulgen's reaction.¹⁰⁾

For polysaccharide: PAS reaction.

For lipase: Takeuchi's method.¹¹⁾

Results

I. Histochemical findings of the specimens of the gastric ulcer, the chronic gastritis and the gastric polyp.

1) Findings of the specimens of the gastric ulcer.

In the gastric ulcer specimens, an

increase of the alkaline phosphatase activity was often seen in the superficial layer and the glandular cells of the mucosa at the edge of the ulcer, and similar findings were observed in the stroma of the mucosa. The acid phosphatase activity increased in the glandular tissue and the stroma of the mucosa at the edge of the ulcer. RNA and DNA reactions also increased in the glandular cells of the

mucosa in many cases. In the callous tissue of the ulcer, the alkaline phosphatase activity increased in an overwhelming majority, increasing in the vascular walls, the perivascular tissues and even in the serosa around the bottom of the ulcer. The acid phosphatase activity showed no definite tendency, and RNA and DNA reactions increased in some cases (Table 1, Fig. 1,2).

Table 1. Histochemical changes of the gastric ulcer cases

	Phosphatase						RNA			DNA		
	Alkaline			Acid								
	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged
Mucous epithelium at the edge of the ulcer	10	2	4	11	2	3	12	2	2	10	2	4
Connective tissue of the ulcer bottom	13	1	2	5	3	8	8	4	4	9	4	3

(The table indicates the number of cases)

2) Findings of the specimens of chronic gastritis.

The chronic gastritis cases were divided into two groups; the group of hypertrophic gastritis having proliferation and thickening of the gastric mucosa, and the group of atrophic gastritis showing thinning of the gastric wall and atrophy of the proper gastric gland. The alkaline and acid phosphatase activity had a strong tendency to decrease in the superficial layer of the glandular cells and the stroma of both groups of gastritis. It decreased markedly

in the group of atrophic gastritis. RNA reactions increased slightly in some cases of both groups, while DNA reactions showed a decrease in all the cases (Table 2, Fig. 3).

3) Findings of the specimens of the gastric polyps.

Though the increase of the RNA and DNA reactions was observed in the glandular epithelium with remarkable proliferation in the gastric polyp case the activation of the alkaline and acid phosphatases was not conspicuous (Table 3).

Table 2. Histochemical changes in the mucous epithelium of the chronic gastritis cases

	Phosphatase						RNA			DNA		
	Alkaline			Acid								
	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged
Gastritis hypertrophicans	1	2	0	0	3	0	2	1	0	0	2	1
Gastritis atrophicans	0	5	4	1	6	2	6	0	3	1	5	3

Table 3. Histochemical changes of the gastric polyp cases

	Phosphatase						RNA			DNA		
	Alkaline			Acid								
	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged
Glandular epithelium	1	0	2	1	0	2	2	0	1	2	0	1
Interstitial connective tissue	1	1	1	1	0	2	1	0	2	0	0	3

II. Histochemical findings of the specimens of gastric cancer.

1) Findings on the ulcer cancer.

In the gastric cancer cases regarded histologically to be developed from the gastric ulcer, many of them showed an increase of alkaline phosphatase activity in the superficial layer and the glandular cells of the mucosa adjacent to the cancer tissues. There was a strong tendency to activation of the alkaline phosphatase also in the cancer cells and in the

interstitial connective tissues of the cancerous tissue (Fig. 4). When the alkaline phosphatase activity was intense in the mucosa or the stroma adjacent to the tumor, the cancer cells in the vicinity also showed strong activity of the alkaline phosphatase. On the other hand, when the alkaline phosphatase activity was not lively in the mucosa and the interstitial connective tissue, the cancer cells also showed low activity of the alkaline phosphatase. The acid phosphatase activity

was a little low in general compared with the alkaline phosphatase activity, and in either case of increase or decrease, the acid phosphatase activity showed the same tendency in the mucosa, in the interstitial connective tissue, and in the cancer cells as the change of the alkaline phosphatase. The positive substance of PAS reaction

increased a little in the cancer cells of the cases showing the increased phosphatase activity (Fig. 5). RNA and DNA reactions had a strong tendency to increase in the mucosa adjacent to the cancer tissues, in the cancer cells and in the interstitial connective tissues of the cancerous tissues (Table 4).

Table 4. Histochemical changes of the cases of the ulcer cancer

	Phosphatase						RNA			DNA		
	Alkaline			Acid								
	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged
Cancer cells	9	3	4	8	4	4	13	0	3	11	2	2
Mucous epithelium adjacent to the cancerous tissue	10	2	4	7	4	5	11	1	4	10	3	3
Interstitial connective tissue	11	2	3	7	3	6	13	2	1	13	0	3

In particular, the regenerated mucous epithelium showing the atypia or the malignant changes at the edge of the gastric ulcer had a remarkable increase of phosphatase activity, PAS positive substances, and RNA and DNA reactions compared with those of the glandular tissues in the remote areas (Fig. 6,7,8).

2) Findings on gastritis cancer.

The alkaline and acid phosphatase activity showed a normal or slightly lower level in the mucosa adjacent to the cancer tissue, the cancer cells and the interstitial connective tissue in many cases of gastritis

cancer. But there were a few cases showing an increase of activity. RNA and DNA reactions had a strong tendency to increase in the mucosa, interstitial connective tissue and cancer cells (Table 5, Fig. 9,10).

3) Findings on polyp cancer.

The phosphatase activity in the tissues and in the cancer cells of the polyp cancer was indefinite, while DNA and RNA reactions showed a tendency to increase in the tissues as well as in the cancer cells (Table 6, Fig. 11).

Table 5. Histochemical changes of the cases of the gastritis cancer

	Phosphatase						RNA			DNA		
	Alkaline			Acid								
	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged
Cancer cells	4	9	5	4	4	10	16	0	2	14	0	4
Mucous epithelium adjacent to the cancerous tissue	3	3	12	3	2	13	15	1	2	13	0	5
Interstitial connective tissue	3	8	7	4	5	9	13	2	3	13	1	4

Table 6. Histochemical changes of the cases of the polyp cancer

	Phosphatase						RNA			DNA		
	Alkaline			Acid								
	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged	Increase	Decrease	Unchanged
Cancer cells	3	2	3	3	2	3	7	0	1	6	1	1
Mucous epithelium adjacent to the cancerous tissue	2	1	5	1	3	4	2	2	4	3	2	3
Interstitial connective tissue	2	3	3	1	3	4	5	2	1	6	2	0

III. Relation between the interstitial reaction of gastric cancer and the histochemical findings.

In order to show the growing pattern of cancer Imai¹²⁾ made the following classification based on the interstitial reaction at the tip of the cancerous invasion. (1) Cirrhotic type (C-type):

This type shows no outburst-like growth of the cancer cells but remarkable proliferation of the interstitial connective tissues around the tip of the invasion.

(2) Progressive type (P-type): This type shows no proliferation of the interstitial connective tissue at the tip of the growth of the cancer and shows the vigorous

invasion and proliferation of the cancer cells into the interstice of the tissue. The P-type appears often with the C-type. The P-type was classified as P_I, P_{II}, and P_{III}, according to degree. (3) Lymphatic and blood vessel permeation type (L-type); This type shows where the tumor proliferates, the cancer cells filling the relatively gross lymphatic canals and the blood vessels. The L-type was also divided into L_I, L_{II}, L_{III}, L_{IV}, and L_V according to its degree.

The relation between the growing pattern of cancer by Imai's classification and the alkaline phosphatase activity of the tissue was as follows: In the cirrhotic type, the alkaline phosphatase activity of the cancer cells was strongly positive in many cases. The increase of the alkaline phosphatase activity was remarkable at the proliferating portion of the interstitial connective tissue which was accompanied by the infiltration of the lymphatic cells

and the exudation of the fibrocytes and fibroblasts (Fig. 12). Furthermore, even in the cases showing the cirrhotic type, there were a few cases where the alkaline phosphatase activity decreased. In these cases, the alkaline phosphatase activity was decreased in both the cancer cells and the interstitial connective tissues. In cases of the P_{III}-type, the severe progressive type where the reaction of the connective tissue was slight in the interstitium of the cancer tissues, the alkaline phosphatase activity decreased in the connective tissue and in the cancer cells in many cases (Fig. 13). In cases of the P-type where the invasion of cancer cells made its way into the lymphatic canals and the blood vessels and was accompanied by the reactive proliferation of the connective tissue around the vessels, increasing activity was seen in the alkaline phosphatase (Table 7).

Table 7. The interstitial reaction and alkaline phosphatase

		Increase	Decrease	Unchanged
Mucous epithelium adjacent to the cancer tissue	Cirrhotic type	9	2	13
	Progressive type	6	4	8
Cancer cells	Cirrhotic type	12	4	8
	Progressive type	4	10	4
Interstitial connective tissue	Cirrhotic type	13	3	8
	Progressive type	3	10	5

The acid phosphatase activity increased slightly in the cases accompanied by proliferation of the interstitial connective tissue. But the changes of the acid

phosphatase activity showed no characteristic pattern compared with those of the alkaline phosphatase activity.

The characteristics of the histochemical

findings in the above mentioned 3 chronic gastric diseases and gastric cancers, were summarized as follows: In the gastric ulcer cases, the alkaline as well as acid phosphatase activity increased in the superficial layer of mucosa, the glandular cells and their stroma at the edge of the ulcer. RNA and DNA reactions also increased in the superficial layer of the mucosa and the glandular cells. The increase of the alkaline phosphatase activity was seen in the callous tissues of the ulcer bottom. In the chronic gastritis cases, the decrease of the alkaline and acid phosphatase activity and the decrease of the DNA reaction were observed in the superficial layer of the mucosa, the glandular cells and the interstitial connective tissues in both hypertrophic and atrophic gastritis. In the gastric polyp cases, an increase of the RNA and DNA reactions was seen in the glandular epithelium.

In the ulcer cancer cases, the increase of the alkaline phosphatase activity, RNA and DNA reactions, and PAS positive substances was seen in the superficial layer of the mucosa, the glandular cells

adjacent to the cancerous tissues, the cancer cells and the interstitial connective tissue of the cancerous tissues. Particularly, the regenerated mucous epithelium at the edge of the ulcer which had a tendency to be transformed in the atypia or the malignant change, showed a remarkable increase of phosphatase activity, PAS positive substances and RNA and DNA reactions. In gastritis cancer, a tendency to decrease in alkaline and acid phosphatase activity was generally seen and a tendency to increase was observed in the RNA and DNA reactions. In polyp cancer, an increase of the RNA and DNA reactions was recognized in all the tissues.

In the cirrhotic type of gastric cancer observed by Imai, the increase of the alkaline phosphatase activity was noticed in the interstitial connective tissue as well as in the cancer cells. On the other hand, in the permeation type of gastric cancer, a tendency toward decrease of the alkaline phosphatase activity was seen in its stroma and its cancer cells. The changes of the acid phosphatase activity showed no definite tendency.

Discussion

Phosphatase is the enzyme catalyzing the hydrolysis and the synthesis of the phosphorous ester of the aliphatic series and aromatic compounds, and the phosphatase activity is believed to increase when these metabolisms are accelerated. Pearson and Novikoff¹³⁾ observed the increase of the alkaline phosphatase activity in the necrotic regions of the cancerous tissues of the rat which had hepatic cancer produced by DAB administration. According to the present authors' examinations, in the gastric ulcer the increase of the alkaline phosphatase activity was seen in the superficial layer

of the mucosa and the glandular cells at the edge of the ulcer, and also in the callous tissue of the ulcer bottom. The necrotic changes, infiltration of the inflammatory cells, proliferation of the fibrin and formation of the granulation tissues are seen as the histological findings in the gastric ulcer. These findings show the destruction of the tissues as well as their repair mechanism. These tissue reactions of the gastric ulcer are thought to have a close relation to the increase of the alkaline phosphatase activity. It is generally accepted that in the edge of the ulcer the necrosis and destruction as

well as the regeneration of the mucosa are repeated, the metaplasia or the error of regeneration occurs in this process, and the invasive proliferation of the epithelial cells results in the cancerogenesis. It is very interesting that according to our examinations of the regions showing atypic proliferation or the malignant changes of the regenerated epithelium at the edge of the gastric ulcer, the increase of the RNA and DNA reactions was seen in the atypic cells and the increase of the alkaline phosphatase activity was markedly observed in the atypic cells and in the stroma.

The increase of the alkaline phosphatase activity was seen in the cancer cells, their interstitial connective tissue and the surrounding mucosa in the case of ulcer cancer. This fact means that the tissue of ulcer cancer is receiving information of the histological characteristics from the gastric ulcer, and that the metabolisms of the ulcer cancer tissue are influenced by those of the gastric ulcer tissue.

The decrease of alkaline phosphatase activity was seen in the cancer cells, their interstitial connective tissue and the surrounding mucosa in the case of gastritis cancer. The decrease of alkaline phosphatase activity was also recognized in the mucosa, as well as in the interstitial connective tissue of chronic gastritis. Thus, a similarity in alkaline phosphatase activity is observed between gastritis cancer and its developmental basis from chronic gastritis.

Kawahara ¹⁴⁾ discussed the interstitial reaction of gastric cancer and alkaline phosphatase activity. He described the high alkaline phosphatase activity of the cancer cells in the reactive outburst stage showing the vigorous interstitial

reaction, and the low alkaline phosphatase activity in the non-reactive outburst stage showing the weak interstitial reaction.

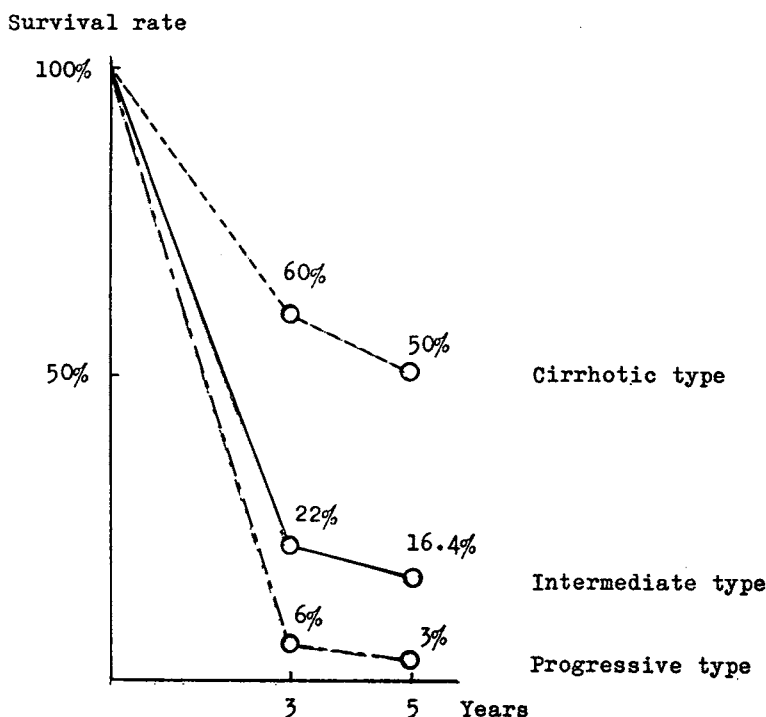
According to our examination, many cases of the ulcer showed an increase of alkaline phosphatase activity in the tissues, but a few showed a decrease. Many of the gastritis cancer cases showed a decrease of alkaline phosphatase activity in the tissues, but a few showed an increase. Therefore, in some ulcer cancer cases, where the ulcer bottom turned into scar tissue completely and the old callous tissue was formed, lacking a fresh interstitial reaction like the infiltration of the inflammatory cells and the exudation of the fibroblasts, the enzym activity became poor in the callous tissue and in the cancer cells, though invading and growing. On the other hand, in some gastritis cancer cases where profuse granulation was seen as the interstitial reaction, an increase of alkaline phosphatase activity was shown. The fact generally observed that the enzym activity was strong in the tissues of the gastric ulcer and ulcer cancer and was weak in the tissues of chronic gastritis and gastritis cancer, would be based on the vigorous reaction of the granulation in the interstitium in the former and the poor reaction in the latter.

The interstitial reaction of cancer is regarded as either a result of the accompanying inflammatory reaction, or as a kind of defensive reaction of the host to the cancer. The present authors examined 138 cases of the radical operation for gastric cancer and observed the relation between the degrees of interstitial reaction of the cancer tissue and the survival rate of the patients operated upon. The 3 years survival rate was 60 percent and the 5 years survival rate was 50 percent in the cases

of the cirrhotic type showing proliferation of the interstitial connective tissue. In contrast, the 3 years survival rate was 5 percent and the 5 years survival rate was only 3 percent in the cases of the progressive type showing almost no in-

terstitial reaction. In fact, the poor growth of cancer in the cases having strong interstitial reactions is a result of the defensive response of the host to the tumor (Fig. 14).

Fig. 14. Interstitial reaction and survival rate



Further, it is assumed that the cancer cells became necrotic and the tissue reaction there caused an increase of

alkaline phosphatase activity in the region of the inflammatory proliferation of the interstitial connective tissues.

Conclusion

In 16 gastric ulcer, 12 chronic gastritis, 3 gastric polyp, 16 ulcer cancer, 18 gastritis cancer and 8 polyp cancer cases, the alkaline and acid phosphatase activity, RNA and DNA reaction, PAS reaction, and lipase activity were examined in the tissues. The results obtained were as follows:

In the gastric ulcer and ulcer cancer, the increase of alkaline phosphatase activity was recognized in the superficial layer of the mucosa and glandular cells at the edge of the ulcer, the callous tissue, the interstitial connective tissue and the cancer cells. On the other hand, in chronic gastritis and gastritis cancer, the

decrease of alkaline phosphatase activity was observed in the superficial layer and glandular cells of the gastric mucosa, the interstitial connective tissue and the cancer cells. The decrease was especially noticeable in atrophic gastritis. In all the tissues of ulcer cancer, gastritis cancer and polyp cancer, the increase of DNA and RNA reactions was clearly seen in the adjacent mucosa, the interstitial connective tissue and the cancer cells. It was worthy of notice that the phosphatase activity, DNA and RNA reactions, and PAS reaction

increased conspicuously in the region where the atypia or the malignant changes of the regenerated epithelium were shown at the edge of the gastric ulcer. On the other hand, in the cirrhotic type of gastric cancer accompanied by the strong interstitial reaction, the increase of alkaline phosphatase activity was observed in the interstitial connective tissue as well as in the cancer cells. It was interesting that this phenomenon might be regarded as a defensive reaction of the host to the cancer.

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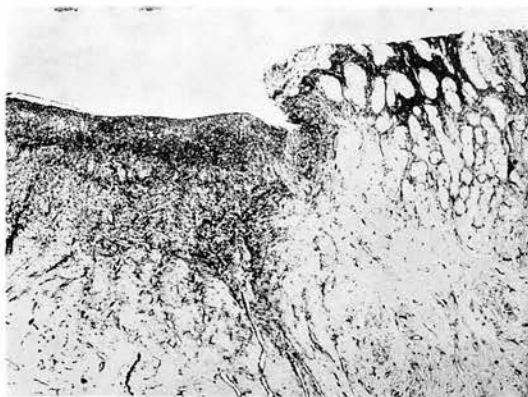


Fig. 1. Gastric ulcer, alkaline phosphatase staining, $\times 22$. The activity of alkaline phosphatase is increased in the mucous gland and the interstitial tissue around the ulcer and in the ulcer bottom.

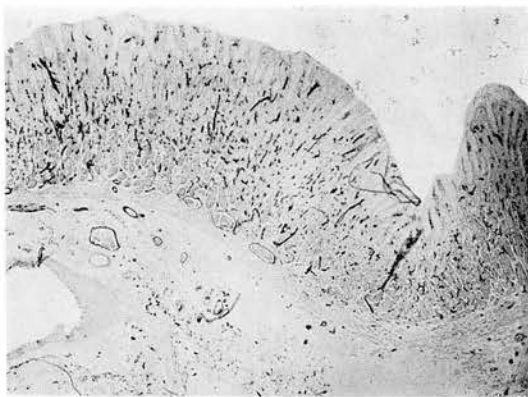


Fig. 3. Gastritis chronica, alkaline phosphatase staining. $\times 22$. The activity of alkaline phosphatase is shown decreasing in the superficial layer of the mucous membrane, the glandular cells and also the interstitial tissue.



Fig. 2. Gastric ulcer, Brachet's staining⁹⁾ for RNA, $\times 22$. RNA reaction is intensified in the mucous glandular cells.



Fig. 4. The ulcer cancer of the stomach, alkaline phosphatase staining, $\times 19$. The activity of alkaline phosphatase is increased in the cancer cells and the stroma.

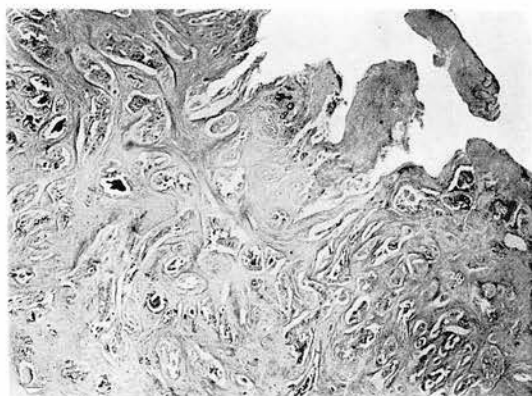


Fig. 5. The ulcer cancer of the stomach, PAS staining, $\times 22$. The positive PAS reaction is seen in the cancer cells and the stroma.

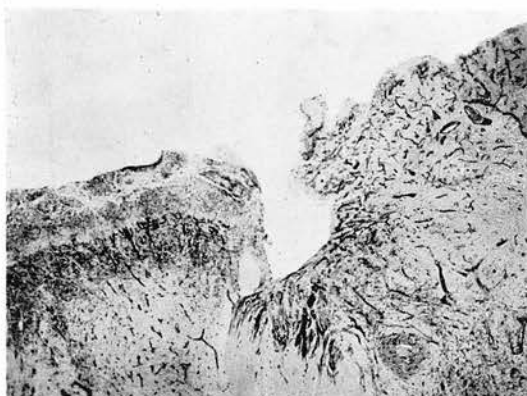


Fig. 7. The early ulcer cancer of the stomach, acid phosphatase staining, $\times 19$. The activity of acid phosphatase is increased in the cell group malignantly changed at the edge of the ulcer.

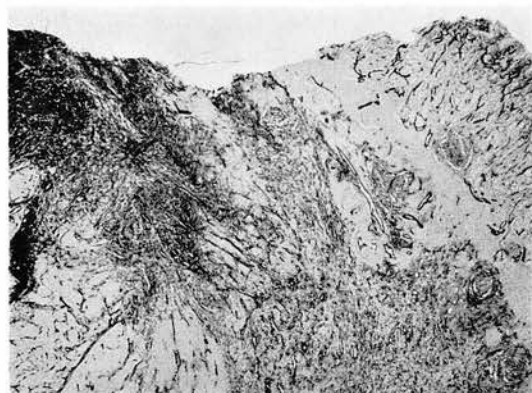


Fig. 6. The minute ulcer cancer of the stomach, alkaline phosphatase staining, $\times 19$. The activity of alkaline phosphatase is intensified in a group of atypical regenerated cells at the edge of the ulcer.

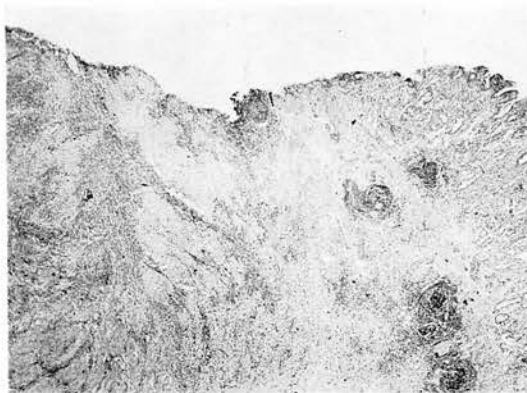


Fig. 8. The initial ulcer cancer of the stomach, Brachet's staining for RNA, $\times 19$. RNA reaction is intensified in the malignant cells at the edge of the ulcer.



Fig. 9. The gastritis cancer, alkaline phosphatase staining, $\times 19$. The activity of alkaline phosphatase is weakened in the cancerous tissues and the mucous membrane adjacent to the cancer.



Fig. 11. The polyp cancer of the stomach, Brachet's staining for RNA, $\times 34$. RNA reaction is markedly intensified in the cancer cells.

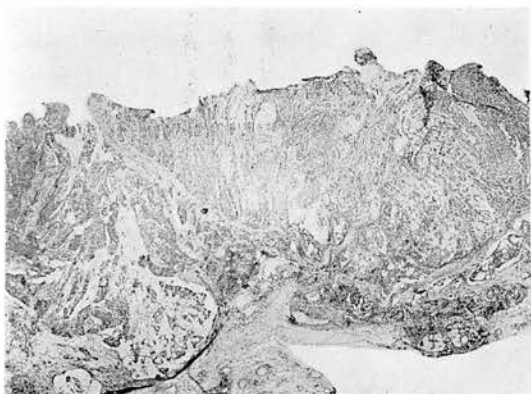


Fig. 10. The gastritis cancer, Feulgen's staining for DNA, $\times 19$. DNA reaction is intensified in the cancer cells.

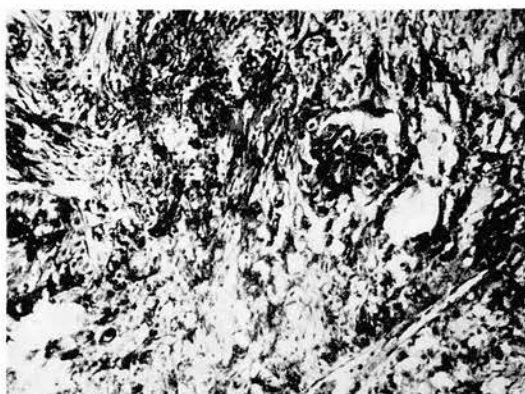


Fig. 12. The ulcer cancer of the stomach, alkaline phosphatase staining, $\times 150$. The activity of alkaline phosphatase is increased in the cancer cells and the stroma, where the interstitial connective tissue is markedly proliferated.

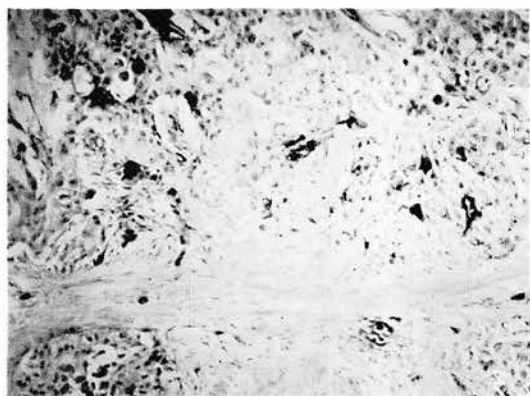


Fig. 13. The gastritis cancer, alkaline phosphatase staining, $\times 150$. The activity of alkaline phosphatase is weakened in the cancer cells and the stroma of the gastritis cancer where the interstitial connective tissue is scarcely developed.