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STUDIES ON SUCCINIC DEHYDROGENASE ACTIVITY OF GASTRIC MUCOSA IN MICE

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SUMMARY

Succinic dehydrogenase is considered to be one of the most important enzymes that demonstrate the functional state of the parietal cell of gastric mucosa. Succinic dehydrogenase of the gastric mucosa was first done histochemically by Padykula with the parietal cell in rat. Thereafter many authors have described on the study of succinic dehydrogenase of the gastric mucosa and the relationship between the acid secretion and the enzyme activity of parietal cells.

The present work deals with histochemical and biochemical studies of the relationship between the acid secretion and the enzyme activity of parietal cells in mice administered histamine and atropine.

Mice fasted for 24 hours before use were injected subcutaneously 0.01, 0.1, 1 or 10 mg per kg of histamine and atropine. Histochemically, sections were cut at 15 μ in a cryostat and stained by the method of Nachlas *et al.* using nitrobluetetrazolium. The stained preparation was examined by our own method. Biochemically, samples were prepared by homogenating entire gastric wall with a glass homogenizer and incubated in a medium containing nitro-bluetetrazolium.

Succinic dehydrogenase activity concentrated predominantly in the parietal cells of the gastric mucosa in mouse. Other gastric cells showed lower activities than the parietal cell.

When the acid secretion of the stomach in mouse was stimulated by histamine, the succinic dehydrogenase activity was increased, both biochemically and histochemically, and the activity was decreased when the acid secretion was inhibited by atropine. Opposite results were obtained with the gastric pH.

The effects of histamine stimulation and atropine inhibition to acid secretion were observed predominantly in the cases administered large doses of the drugs. Similar effects were observed on the succinic dehydrogenase activity.

The increase or decrease of the activity was determined by fine or coarse production of the blue granules in the parietal cells.

The present method of histochemical judgement for the succinic dehydrogenase activity was very convenient for investigating the relationship between the enzyme activity and acid secretion. The advantage of this method was verified by applying the biochemical determinations in addition to the histochemical ones.

INTRODUCTION

The histochemical investigation of succinic dehydrogenase of the gastric

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mucosa was first done by Padykula[1] with the parietal cell in rat. He showed that succinic dehydrogenase activity of the gastric glands was much more intense in the parietal cells than either in the chief or mucous cells.

Using biochemical and histochemical methods, Villareal and Burgos[2] reported that succinic dehydrogenase activity increased when acid secretion of the stomach in rat was stimulated by mecholyl, but when the acid secretion was inhibited by an organic mercurial compound (mersalyl), the activity decreased.

With normal human gastric mucosa obtained by means of the suction biopsy, Niemi[3] found a strong succinic dehydrogenase activity in the parietal cells, no activity in the mucous cells and a very weak one in the chief cells.

On the relationship between the gastric acidity and succinic dehydrogenase activity of parietal cells, Miyoshi[4] has shown histochemically that succinic dehydrogenase activity increases in case of hyperacidity and decreases in case of hypoacidity or anacidity.

The present paper deals with histochemical and biochemical studies of the relationship between acid secretion and succinic dehydrogenase activity of parietal cells in mice administered histamine or atropine.

MATERIALS AND METHODS

The relationship between acid secretion (gastric pH) and succinic dehydrogenase activity was studied by histochemical and biochemical methods using mouse, weighing about 25 g, injected histamine chloride and atropine sulphate.

The groups used were as follows. Histamine was injected subcutaneously to four groups of mice in doses of 0.01, 0.1, 1 and 10 mg per kg. The enzyme activity was measured at 15, 30, 45 and 60 min. after stimulation. Atropine groups were treated in the same manner as the histamine groups. The control group was injected physiological saline. All experiments were consisted of five mice.

Histochemical Method

Killing of mouse and removal of stomach: Mouse was fasted for 24 hours before use. After killing by decapitation, the stomach was immediately removed and opened along the greater curvature. The pH of the gastric contents was determined near the mucosal surface by a pH paper, the gastric wall was washed thoroughly with physiological saline, stretched on a filter paper and rolled 3-4 mm wide from the cardiac to the pyloric portion along the lesser curvature as illustrated in Fig. 1.

Preparation of the sections: The material was immediately placed on a previously well cooled mineral plate without fixation and transferred into a cryostat ($-20°C$). After freezing the material adequately, it was cut into sections of 15 μ. Each section was immediately mounted on a slide glass in

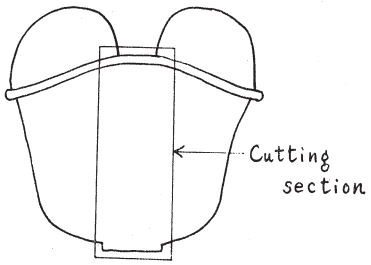

FIG. 1. Preparation of the sample

the cryostat. Then. the section was quickly removed from the cryostat and thawed at room temperature by placing a finger under the slide.

Preparation of the substrate solution: Incubation of the sections was carried out with a medium consisting of following solutions; 10 ml of 0.1% aqueous ditetrazolium chloride solution (nitro-BT), 5 ml of 0.2 M aqueous sodium succinate solution and 5 ml of 0.1 M sodium phosphate buffer (pH 7.6).

Condition of incubation: Incubation was carried out at 37°C for 20 min. After incubation, the sections were washed with physiological saline, fixed with 10% neutral formalin for 10 min, immersed into 15% ethanol for 5 min. and mounted in glycerogel.

Control preparation: The control medium was a mixture of the tetrazolium chloride and the phosphate buffer. The use of a control was necessary for evaluating the possible presence of endogenous substrates.

Morphological orientation: For morphological orientation, fresh frozen sections of 15 μ in thickness were used and stained with hematoxylin and eosin.

Judgement: The reaction for the succinic dehydrogenase system with nitro-BT resulted in a formation of rich formazan which was deposited on mitochondria in the form of fine dark purple or blue granules.

The degree of succinic dehydrogenase activity of parietal cells was represented by +, ++ and +++ according to the amount of granules precipitated in the cell cytoplasm. The activity was graded as + when blue granules were coarse in the cytoplasm of parietal cells. The activity was designated as ++ when blue granules were dense in the cytoplasm, but the cell nuclei were visible through the mass of blue granules, while +++ was employed when the nuclei were not visible. Judgement of the enzyme activity was made at five sites. One of them was in pyloric antrum and the others in gastric body as illustrated in Fig. 2.

For convenience, the ratio in thickness of the mucosal layer demonstrating

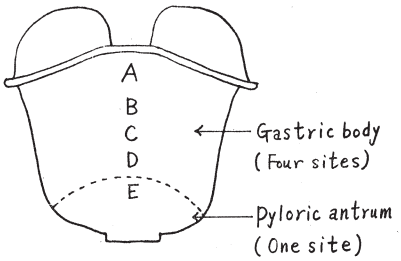

FIG. 2. Sites of the judgement

each degree of succinic dehydrogenase activity to the total mucosa was expressed as x: 10. The succinic dehydrogenase activity in a part of the site was conveniently represented by the product of the degree of the activity and a tenth of x. Thus, when the degree of the activity was + and x was 2, the succinic dehydrogenase activity was $1 \times 2/10$. The enzyme activity of each site was represented by the sum of each activity of the parts. In this manner, the sum of the activities of the five sites shows the index of the activity of a given gastric mucosa.

Biochemical Method

Sample for the biochemical determination was prepared by homogenating 100 mg of entire gastric wall in 2 ml of water with a glass homogenizer for 3 minutes. Incubation medium consisted of 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.6), 1 ml of 0.1% aqueous nitro-BT and 0.5 ml of 0.2 M aqueous sodium succinate. To this medium was added 1 ml of the stomach homogenate containing approximately 50 mg wet tissue. For control medium, 0.5 ml of distilled water was added instead of sodium succinate. Incubation was carried out at 37°C in a water-bath for 30 minutes. The formazan derivative produced by the reduction was extracted with ethanol-acetone and the absorption rate was measured spectrophotometrically at 550 mμ.

RESULTS

Normal gastric mucosa: The mouse stomach consists of two parts, a cardiac portion which is non-glandular and lined with stratified squamous epithelium and a pyloric portion which contains the gastric glands.

A weak activity of succinic dehydrogenase was observed in the basal layer of the squamous epithelium in the cardiac portion, a higher activity in the gastric glands where the parietal cells were intensely stained. In the gastric glands nitro-BT was intensely reduced by the parietal cells which were located principally in the middle third of each gland. The succinic dehydrogenase activity was much more pronounced in parietal cell than in the other cells of gastric mucosa. The superficial layers of the mucosa reacted very lightly or

not at all, most of the epithelial cells of the gland were positive and stained dark purple or blue. Neck cells also stained deeply. Mucous cells were colored very lightly. No enzymatic activity was demonstrated in walls of blood vessels, muscle layers, or connective tissue.

The succinic dehydrogenase activity was observed only in the cytoplasm, never in the nuclei and indicated by the intracellular deposition of a granular blue pigment. When the incubation time was prolonged, or when the acid secretion was stimulated by large doses of histamine, the nuclei of the parietal cells were overloaded with the blue granules. In the pyloric portion containing parietal cells, the mucosal layers were thicker in body than in antrum. It is supported by these facts that the acid secretion and succinic dehydrogenase activity of the parietal cells were predominant in body than in antrum.

In control mouse the average value of pH in gastric secretion near the mucosal surface was 2.5 and the average grade of the succinic dehydrogenase activity was found to be 6.0 (Table 1, Fig. 3).

TABLE 1. Average Value of Succinic Dehydrogenase Activity (Histochemical) —Histamine Groups—

Time after administration	15 min		30 min		45 min		60 min	
Dose	Gastric pH	Activity	Gastric pH	Activity	Gastric pH	Activity	Gastric pH	Activity
0.01 mg/kg	2.5	6.3	2.5	6.2	2.6	5.6	2.6	5.7
0.1 mg/kg	2.5	6.3	2.4	6.9	2.4	7.1	2.3	8.0
1 mg/kg	2.3	8.5	2.4	7.9	2.1	7.8	2.2	8.2
10 mg/kg	2.2	8.2	1.8	8.5	1.6	8.4	2.0	8 2
Control	Gastric pH 2.5 Activity 6.0							

Gastric mucosa stimulated by histamine: Under histochemical observation, the succinic dehydrogenase activity was generally increased by histamine (Figs. 4, 5). Almost no effect was observed in the group administered the dose of 0.01 mg per kg of body weight. A slight increase of the enzyme activity appeared in the 0.1 mg per kg group 30 min after stimulation and the gastric pH showed a decrease. In the 1 mg per kg group, an intense activity was observed 15 min after stimulation and it lasted for a considerably long time. With the 10 mg per kg group, a similar tendency was observed and the gastric pH was significantly decreased 15 min after stimulation.

A marked increase of the succinic dehydrogenase activity and decrease of gastric pH was observed in the group of large dose of 1 or 10 mg per kg.

Higher magnification revealed an increase in the succinic dehydrogenase activity as that of blue granules within the parietal cell.

Biochemical studies also showed almost the same tendency on the succinic dehydrogenase activity and acid secretion as histochemical ones (Table 2).

TABLE 2. Average Value of Succinic Dehydrogenase Activity (Biochemical) —Histamine Groups—

Time after administration	15 min		30 min		45 min		60 min	
Dose	Gastric pH	Absorption Rate of light	Gastric pH	Absorption Rate of light	Gastric pH	Absorption Rate of light	Gastric pH	Absorption Rate of light
0.01 mg/kg	2.3	0.365	2.2	0.370	2.2	0.398	2.5	0.344
0.1 mg/kg	2.2	0.400	2.4	0.352	2.2	0.326	2.4	0.342
1 mg/kg	2.2	0.395	2.1	0.440	2.0	0.455	2.2	0.410
10 mg/kg	2.0	0.412	1.6	0.490	1.4	0.510	2.0	0.440
Control	Gastric pH 2.5 Absorption Rate of light 0.330							

Gastric mucosa inhibited by atropine: The succinic dehydrogenase activity of the gastric mucosa in mouse was generally decreased by atropine (Figs. 6, 7, 8). There was a slight decrease of the enzyme activity 30 min after atropine injection with the 0.01 mg per kg group. In such cases, the gastric pH tended to be increased slightly 30 min after injection. In the 0.1 mg per kg group, a more pronounced decrease of the succinic dehydrogenase activity observed from 15 min to 60 min. In the 1 mg per kg group, a decrease of the activity began to appear 15 min later and became much more intense as time passed. In the 10 mg per kg group, a significant increase of the gastric pH was observed 15 min after injection. A marked difference between the control and the atropine group was shown in the group of large dose of 1 mg or 10 mg per kg (Table 3).

TABLE 3. Average Value of Succinic Dehydrogenase Activity (Histochemical) —Atropine Groups—

Time after administration	15 min		30 min		45 min		60 min	
Dose	Gastric pH	Activity	Gastric pH	Activity	Gastric pH	Activity	Gastric pH	Activity
0.01 mg/kg	2.6	6.3	3.0	4.7	3 2	5.1	2.9	5.9
0.1 mg/kg	2.7	4.4	3.1	5.0	3.2	4.8	3.3	4.9
1 mg/kg	2.8	4.2	3.3	4.0	3.6	3.8	4.0	3.6
10 mg/kg	4.0	3.9	4.2	3.8	4.1	4.0	4.0	4.0
Control	Gastric pH 2.5 Activity 6.0							

It was demonstrated under higher magnification that blue granules of diformazan were decreased within the parietal cell by atropine administration. This effect was shown not only histochemically but also biochemically (Table 4).

Relationship between dose of drug and gastric pH: Gastric pH was decreased by histamine stimulation and the decrease was marked with larger doses. In atropine groups, the results were contrary to histamine groups;

TABLE 4. Average Value of Succinic Dehydrogenase Activity (Biochemical) —Atropine Groups—

Time after administration	15 min		30 min		45 min		60 min	
Dose	Gastric pH	Absorption Rate of light	Gastric pH	Absorption Rate of light	Gastric pH	Absorption Rate of light	Gastric pH	Absorption Rate of light
0.01 mg/kg	2.7	0.329	2.8	0.284	2.9	0.283	2.8	0.322
0.1 mg/kg	3.1	0.303	3.0	0.243	3.6	0.304	3.2	0.325
1 mg/kg	4.2	0.226	3.5	0.173	3.7	0.177	3.2	0.173
10 mg/kg	4.4	0.214	4.0	0.192	4.1	0.176	4.0	0.227
Control	Gastric pH 8.5 Absorption Rate of light 0.330							

namely the gastric pH was increased and became higher in cases given larger doses of the drug. The large dose of these drugs gave always significant effects on the acid secretion of the stomach (Table 5).

TABLE 5. Relationship between Dose of Drug and Gastric pH

Drug→	Histamine					Atropine				
Gastric pH ↓	10 mg/kg	1 mg/kg	0.1 mg/kg	0.01 mg/kg	Control	0.01 mg/kg	0.1 mg/kg	1 mg/kg	10 mg/kg	Total
1.1~1.5										
1.6~2.0	31	10	2	2						45
2.1~2.5	9	30	36	25	8	6	1	1		116
2.6~3.0			2	13	2	24	19	6		66
3.1~3.5						7	12	14		33
3.6~4.0						2	7	12	19	40
4.1~4.5						1	1	4	19	25
4.6~5.0								2	1	3
5.1~5.5									1	1
5.6~6.0								1		1
Total	40	40	40	40	10	40	40	40	40	330

TABLE 6. Relationship between Dose of Drug and Succinic Dehydrogenase Activity (Histochemical)

Drug→	Histamine					Atropine				
Activity ↓	10 mg/kg	1 mg/kg	0.1 mg/kg	0.01 mg/kg	Control	0.01 mg/kg	0.1 mg/kg	1 mg/kg	10 mg/kg	Total
8.1~9.0	16	12	2							30
7.1~8.0	4	7	7	2		1	1			22
6.1~7.0		1	9	4	2	7	1			24
5.1~6.0			2	13	3	6	5	1		30
4.1~5.0				1		3	10	6	7	27
3.1~4.0						3	3	11	13	30
2.1~3.0								2		2
Total	20	20	20	20	5	20	20	20	20	165

Relationship between dose of drug and succinic dehydrogenase activity: From histochemical point of view, the succinic dehydrogenase activity was increased by histamine stimulation. The more the dose of histamine, the

higher the activity.

On the contrary, the enzyme activity was decreased by atropine administration. The decrease of the activity became more pronounced with larger dose of the drug (Table 6). The large dose of these drugs gave significant effects on the succinic dehydrogenase of gastric mucosa in all the cases. Biochemically, almost the same tendency was observed (Table 7).

TABLE 7. Relationship between Dose of Drug and Succinic Dehydrogenase Activity (Biochemical)

Drug→ Absorp. Rate of light ↓	Histamine				Control	Atropine				Total
	10 mg kg	1 mg/kg	0.1 mg/kg	0.01 mg/kg		0.01 mg/kg	0.1 mg/kg	1 mg/kg	10 mg/kg	
0.501~	5									5
0.401~0.500	11	14	4	5						34
0.301~0.400	4	6	15	15	4	8	8			60
0.201~0.300			1		1	12	11	6	9	40
0.101~0.200							1	14	11	26
Total	20	20	20	20	5	20	20	20	20	165

Relationship between gastric pH and succinic dehydrogenase activity: In the histamine group, the increased succinic dehydrogenase activity was accompanied by a decreased pH of the gastric contents, whereas in the atropine group the decreased activity was associated with an elevated gastric pH. In rare cases, histamine group and atropine group showed the same pH value of gastric content. In such cases the succinic dehydrogenase activity of gastric mucosa was more intense in histamine group than in atropine group (Tables 8, 9). This fact was also demonstrated biochemically (Tables 10, 11). This discrepancy may be due to the fact that the gastric pH was measured on the gastric contents collected throughout the examination period, while the determination of the succinic dehydrogenase activity was accomplished instantaneously.

In these cases, any significant difference was not observed between the controls and the groups administered small or moderate dose of histamine and

TABLE 8. Relationship between Gastric pH and Succinic Dehydrogenase Activity (Histochemical)
—Histamine Groups—

Gastric pH→ Activity ↓	1.1~1.5	1.6~2.0	2.1~2.5	2.6~3.0	Total
8.1~9.0	2	12	16		30
7.1~8.0		3	17		20
6.1~7.0			13	1	14
5.1~6.0			6	9	15
4.1~5.0				1	1
Total	2	15	52	11	80

TABLE 9. Relationship between Gastric pH and Succinic Dehydrogenase Activity (Histochemical)
—Atropine Groups—

Gastric pH→ Activity ↓	2.1~2.5	5.6~3.0	3.1~3.5	3.6~4.0	4.1~4.5	4.6~5.0	Total
7.1~8.0	1	1					2
6.1~7.0	3	4	1				8
5.1~6.0	2	8	2				12
4.1~5.0		7	9	8	2		26
3.1~4.0		5	3	10	11	1	30
2.1~3.0				1		1	2
Total	6	25	15	19	13	2	80

TABLE 10. Relationship between Gastric pH and Succinic Dehydrogenase Activity (Biochemical)
—Histamine Groups—

Gastric pH→ Absorp. Rate of light ↓	1.1~1.5	1.6~2.0	2.1~2.5	2.6~3.0	Total
0.501~	5				5
0.401~0.500	1	19	14		34
0.301~0.400		3	34	3	40
0.201~0.300			1		1
Total	6	22	49	3	80

TABLE 11. Relationship between Gastric pH and Succinic Dehydrogenase Activity (Biochemicel)
—Atropine Groups—

Gastric pH→ Absorp. Rate of light ↓	2.1~2.5	2.6~3.0	3.1~3.5	3.6~4.0	4.1~4.5	4.6~5.0	5.1~	Total
0.301~0.400	2	11	2	1				16
0.201~0.300		14	9	12	3			38
0.101~0.200			6	8	9	1	2	26
Total	2	25	17	21	12	1	2	80

atropine. However, in the group of large dose, a marked increase or decrease of the enzyme activity was observed. In some cases of the both (histamine and atropine) groups, unexpected results were observed in the succinic dehydrogenase activity and the gastric pH. Such unexpected results may be due to difficulties in technical measurement and instability of the enzyme activity.

DISCUSSION

Hally[5] reported that abundant large ovoid mitochondria with numerous cristae and dense ground substance are characteristic of the parietal cell. Lazarow and Cooperstein[6] related this fact to the presence of the oxidative enzymes localized within the mitochondria. Green, Lester and Ziegler[7] showed

that structually intact cristae are necessary for oxidative phosphorylation. Hally[5] also reported that the parietal cell is a specific efficient cell having a greater oxygen consumption rate than any other mammalian cell. Davies[8] reported that only 10 per cent of the volume and only 5 per cent of the dry weight of the stomach is parietal cells and 10,000 small calories are required to secrete one gram mole of hydrochloric acid. He thought that the energy was produced by glycolysis in the parietal cell and most of the energy was consumed for the concentration of the hydrogen ions.

Hollander[9] suggested that the various basic metabolic reactions of parietal cell appeared to be essentially of the same character as those in tissue cells generally, reactions of the Krebs cycle, those involved in the coupling of oxidative processes and the production of high energy phosphate bonds, etc.

According to Davenport[10], it is reasonable to suppose that parietal cells are capable of drawing energy from glycolysis for the performance of their secretory function. In 1953, he[11] described that the inhibition of acid secretion by fluoroacetate is a proof that the tricarboxylic acid cycle might be included in the acid secretory mechanism, for fluoroacetate inhibited the cycle and thereby broke one of the main pathways of glucose and pyruvate oxidation.

Furthermore, it has been suggested by Conway[12], Davies[13] and others that the basic process of acid secretion is an oxidation-reduction system. In 1957, Davenport[14] showed that the parietal cell has a greater oxygen consumption rate than any other mammalian cell.

Histamine is the most potent stimulus of gastric secretion and effective when given parenterally or when applied locally to the gastric mucosa. Presumably it acts directly on the cells of the gastric glands though mucosal vasodilatation may be an added factor. The stimulating action is somewhat reduced by vagotomy and atropinization but is not affected by the antihistamic drugs. The resulting secretion is copious in volume and high in acid content. True achlorhydria might be defined by Bockus[15] as a condition in which gastric pH remains above 6.0 after maximal histamine stimulation (0.04 mg per kg body weight).

Davenport[10][11] showed that the rate of glycolysis is increased by stimulating the cells with histamine. Davies[8] considered that there was an increase in the rate of oxygen uptake when acid secretion was stimulated by histamine and that whatever the mechanism of stimulation, the final effector at the cellular level was histamine itself.

More recently Marks[16] reported that the acid secretory response to intra venous or subcutaneous histamine stimulation increases in a characteristic manner as the dosage is increased and that the duration of the plateau level of maximal secretion is about 15 minutes with the small dosage and tends to be progressively longer duration with the larger doses of histamine. Moreover, he observed in replicate experiments variations due to a large extent to the

inevitable variations in the functional state of the parietal cells. This concept was supported by the fact that day to day variations of similar magnitude are also observed in the responses to the same dose of histamine administered by intravenous infusion where the question of resorption does not arise. Besides histamine itself, such variations may be due to one or more objective signs such as an increased concentration of pepsin, increased peristaltic activity and bile regurgitation into the stomach. Whatever the explanation, the functional state of the parietal cell accompanies such variations of acid secretion. This fact also explains the functional variations in mouse tissues.

Atropine and many synthetic anticholinergic drugs exert an inhibitory action against vagal stimulation and hence reduce gastric secretion. These drugs appear to exert their effects by blocking the action of the parasympathetic mediator, acetylcholine, at both the neuro-effector site of glandular cells and the ganglia of the parasympathetic nervous system.

Succinic dehydrogenase is an enzyme that oxidizes succinic acid to fumaric acid and forms a part of an important step in the oxidation of pyruvic acid through the tricarboxylic acid cycle. Its localization may, therefore, be taken as an indicator of the activity of the energy pool which produces hydrochloric acid. It is known that the activity of a great number of enzymes, such as various dehydrogenases and oxidases, is dependent upon the active form of their -SH groups[17]. Maintenance of this group is therefore of great importance.

Succinic dehydrogenase plays a vital role in respiratory processes of most living cells and forms a link in the chain of reactions concerned with the oxidation of lipids, carbohydrates and proteins[18].

Recently Nitro-BT has been used generally as a tetrazolium salt to for demonstrating succinic dehydrogenase histochemically. According to Nachlas *et al.*[19], it is a more sensitive indicator of dehydrogenase activity than any other tetrazolium, competing successfully with oxygen for electrons even in very thin frozen sections.

Since it was shown by Baldwin[20] that succinic dehydrogenase is firmly attached within the cell probably to the mitochondria, many investigators[1,21~27] have demonstrated the enzyme not in the nuclei, but within the mitochondria of the cell cytoplasm.

According to Bourne and Malaty[22], an examination of the cytological distribution of the reaction showed that the nuclei give either a negative or a very faint reaction. In the cytoplasm the result of the reaction is a deposit of blue granules some of which, in some cells, are arranged in a peri-nuclear fashion. However, in bird erythrocytes this enzyme has been demonstrated in the nuclei, this according to Brachet[28] might be due to absence of mitochondria in bird erythrocytes. Chatterjèe[29] demonstrated nucleolar localization of succinic dehydrogenase in normal and malignant cells from epidermoid carcinomatous tissue of human cervix. This was a new finding apart from its

well established cytoplasmic counterpart.

In mouse stomach, the succinic dehydrogenase activity is much more intense in parietal cell than in the other cell of gastric mucosa, but mucous and chief cells are stained very slightly. On the contrary, Niemi[3] showed that the DPN diaphorase and succinic and lactic dehydrogenase activities localize solely in the parietal cells of the human gastric mucosa. This finding differs considerably from what has been reported on the stomach of the mouse.

Dawson[30] observed faint perinuclear staining of surface mucosal cells, none of mucous cells lining crypts; heavy staining of acid secreting cells, moderate staining of enzyme secreting cells in human stomach. All of these histochemical differences may indicate the functional ones existing between the stomachs of mouse and man.

Villareal and Burgos[2] showed that in the rat and frog succinic dehydrogenase activity increases with stimulated acid secretion by mecholyl and histamine and decreases when the acid formation is inhibited. His chemical data also revealed that the enzyme activity increases when gastric secretion is stimulated.

Telkkä and Kuuistos[31] described that atropine and scopolamine did not modify the succinic dehydrogenase activity in the gastric mucosa of rat and mouse within 30 to 120 minutes of their administration. But in this study, the enzyme activity decreased when the acid secretion was inhibited by larger doses of atropine.

In Myren's report[32], a highly significant correlation was found between the number of succinic dehydrogenase active parietal cells per given area of mucosal surface and the values of gastric secretion before and after stimulation with large doses of histamine. Furthermore, a similar degree of correlation was also observed between the values of secretion and the thickness of the mucosal layer with succinic dehydrogenase active parietal cells in the biopsy specimens.

Recently, following maximal stimulation by histamine,—0.04 mg per kg, the dosage which gives the maximal acid secretion to the human stomach according to Kay[33] and Card and Marks[16],—the enzyme activity was shown in a far greater proportion of parietal cells than was observed under basal conditions and, as well, a slightly greater amount of activity in each cell was observed[34]. It appears reasonable to conclude from these findings that the secretory activity of parietal cells is related to the activity of succinic dehydrogenase demonstrated and may reflect the relationship of cellular activity, in this case the secretion of acid, with energy metabolism.

Furthermore, Correia, Filipe and Santos[34] emphasized this relationship by the fact that after histamine injections the number of parietal cells showing DPN diaphorase and succinic dehydrogenase activity is markedly increased, also indicating that under basal conditions only a proportion of the total

number of parietal cells produce acid.

Alley[35] described that histamine inhibits the activity of the chief and mucous cells of the gastric mucosa, but no changes in the enzyme activity of chief cells were observed in the study of Correia *et al.*[34]. Any significant changes were not shown in the succinic dehydrogenase activity of chief cells and mucous cells at least in the present work.

According to Telkkä and Kuusisto[31], the drugs depressing the gastric secretion, in the doses used, did not substantially influence the succinic dehydrogenase activity, whereas the gastric secretion stimulating drugs caused in general an increase in the enzyme activity.

In this work, the effects of histamine stimulation and atropine inhibition to the acid secretion and succinic dehydrogenase activity in mouse stomach were predominantly observed in cases administered the large doses of the drugs. No difference of the enzyme activity in mouse stomach was observed between controls and cases given the small doses usually applied to man.

Succinic dehydrogenase activity has been also found in the metaplastic epithelium. It was demonstrated by Planteydt and Willighagen[36] that the epithelium of intestinal metaplasia is not only morphologically but also histochemically identical with the epithelium of the duodenum.

Mendeloff and Monis[37] referred to the fact that succinic dehydrogenase is less active or reduced in concentration in the inflamed tissues containing ulcerative colitis, but not altered in distribution. Monis *et al.*[24] demonstrated low succinic dehydrogenase activity and high DPN diaphorase activity in the tumor tissues as compared with normal tissues. Both of these enzymes are thought to be localized in the mitochondria. TPN diaphorase in the study showed low activity in tumor tissues. According to Planteydt *et al.*[38], all tumors showed a moderate succinic dehydrogenase activity.

Necrotic tissues were always almost completely depleted of the enzyme activity and in supporting tissues of parenchymatous organs very little activity was demonstrable[39].

Myren and Torgerson[40] reported that a decrease of the succinic dehydrogenase activity is generally associated with morphological signs of degeneration of the parietal cells, while complete absence of activity indicates necrosis of these cells. They also noted that the newly formed epithelium shows a similar low succinic dehydrogenase activity as that found in normal chief and mucous cells.

Variation of the enzyme activity has been frequently experienced. Many conditions influence the enzyme activity of the parietal cells and acid secretion. In the normal gastric glands, staining variation was found in the same specimen and even in the same crypt[41]. Unexpected results in the present study may be caused by difficulties in technical measurement and instability of the enzyme activity. Be the matter what it may, the individual cells may be in a different

physiologic state.

CONCLUSION

The histochemical and biochemical investigations on the activity of succinic dehydrogenase, which is the most important enzyme demonstrating the functional state of the parietal cell, were made in relation to acid secretion.

Succinic dehydrogenase activity was localized predominantly upon the parietal cells of the gastric mucosa in mouse. Other gastric cells showed lower activities as compared with parietal cell.

The succinic dehydrogenase activity was increased, both histochemically and biochemically, when acid secretion of the stomach in mouse was stimulated by histamine, and the activity was decreased when the acid secretion was inhibited by atropine. The contrary results were obtained on the gastric pH.

The increase or decrease of the succinic dehydrogenase activity was determined by fine or course production of the blue granules in the parietal cells.

The effects of histamine stimulation and atropine inhibition to acid secretion were observed predominantly in the cases administered larger doses of the drugs. Similar effects were observed on the succinic dehydrogenase activity.

The present method of histochemical judgement for the succinic dehydrogenase activity was very convenient to investigate the relationship between the succinic dehydrogenase and acid secretion. The advantage of this method was verified by applying the biochemical determinations in addition to the histochmical ones.

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EXPLANATION OF FIGURES

FIG. 3. Succinic dehydrogenase activity in gastric mucosa of a mouse. It was much more pronounced in parietal than in the other cells of gastric mucosa. Gastric pH: 2.5, ×100.

FIG. 4. Succinic dehydrogenase activity in gastric mucosa of a mouse stimulated with 0.1 mg per kg of histamine after 45 min following injection. Gastric pH: 2.4, ×100.

FIG. 5. Succinic dehydrogenase activity in gastric mucosa of a mouse stimulated with 1 mg per kg of histamine after 45 min following injection. Note the great increase of succinic dehydrogenase activity in the parietal cells. Gastric pH: 2.1, ×100.

FIG. 6. Succinic dehydrogenase activity in gastric mucosa of a mouse treated with 0.01 mg per kg of atropine after 45 min following injection. Gastric pH: 3.2, ×100.

FIG. 7. Succinic dehydrogenase activity in gastric mucosa of a mouse treated with 0.1 mg per kg of atropine after 15 min following injection. Gastri cpH: 2.7, ×100.

FIG. 8. Succinic dehydrogenase activity in gastric mucosa of a mouse treated with 1 mg per kg of atropine after 60 min following injection. Note the great decrease of succinic dehydrogenase activity in the parietal cells. Gastric pH: 4.0, ×100.

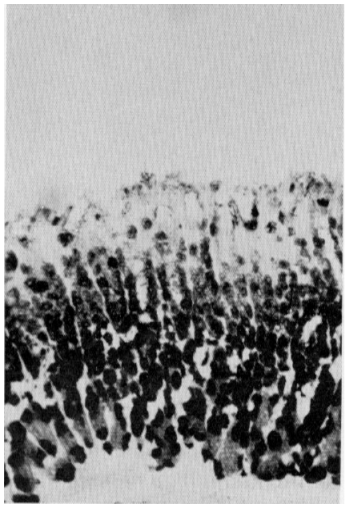

FIG. 3

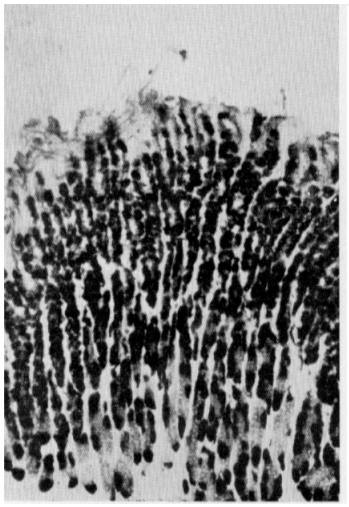

FIG. 4

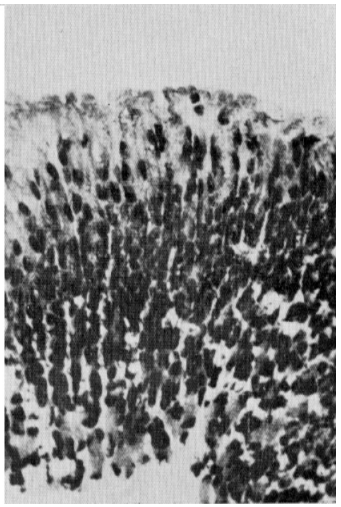

FIG. 5

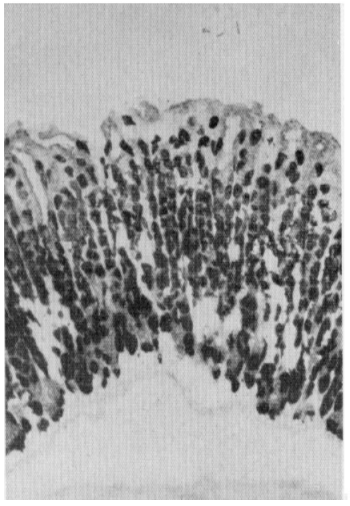

FIG. 6

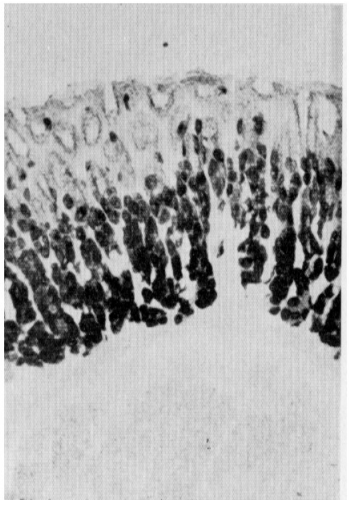

FIG. 7

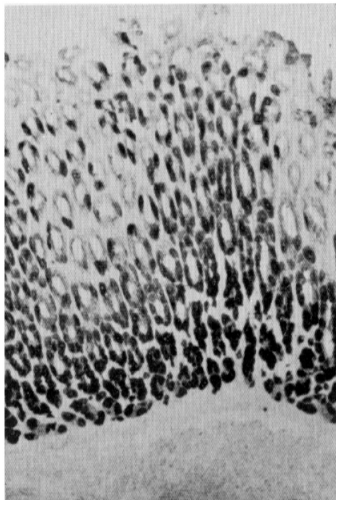

FIG. 8