glands and making with the capillaries a kind of 'plume' close to the surface of the epithelium. Enzyme activity in the capillary walls could also be seen for 5-nucleotidase and more weakly for acid phosphatase. For D.P.N., although very few capillaries presented any activity, there was a weak reaction in almost all cases.

Some cellular elements of the stroma also presented enzyme activity, although weakly, with all the enzymes studied except alkaline phosphatase and T.P.N.

Some collagenous fibres also reacted to A.T.P. Sometimes it was possible to see a moderate reaction of the basal membrane of some glands with 5-nucleotidase and acid phosphatase, and less often with A.T.P.

In some sections in which the muscularis mucosae was present, the fibres reacted intensely with A.T.P. and 5-nucleotidase and weakly with T.P.N.

COMMENTS

The introduction of gastric biopsy has opened new possibilities for the study of the gastric mucosa in vivo, both from the morphological and the histochemical viewpoints, by which the functional and histological pictures can be related. Hitherto, almost all cytochemical studies, and in particular enzyme studies, have been made in animals. Our study confirms the intense activity of D.P.N. in the parietal cells and the weak reactivity of the principal and superficial cells to this enzyme. Niemi et al. (1960) found that the surface epithelial cells were negative, and the principal and some of the mucous cells of the neck weakly positive. They consider that the intense activity of the parietal cells for D.P.N. must be related to the task of these cells in producing hydrochloric acid. For this process a great deal of energy is needed and is provided by the adenosine triphosphate that is made by phosphorylation in the cycle of the tricarboxylic acid. Both D.P.N. and dehydrogenase enter this cycle, and their site and intensity can be regarded as an index of this activity. Hally (1959) has verified with the electron microscope that the parietal cells of the mouse have a great number of mitochondria, in accordance with their wide enzymatic activity; Davenport (1957) has also shown that the parietal cells of the mouse account for a large consumption of oxygen.

In our study the site of esterase in the principal cells has proved to be the same as that described by Chessick (1953) but we have not found any previous references to the sites of non-specific alkaline phosphatase, A.T.P., and 5-nucleotidase in the gastric mucosa. We have verified the site of acid phosphatase in the cytoplasm of principal cells, which had also been observed by Gomori (1956), finding no reaction in parietal cells. Rutenberg and Seligman (1955), however, and Roseman, Simon, and Sleisenger (1959), working with another method for this enzyme, refer to intense activity in the parietal cells.

2 In pathological conditions and in changes induced by histamine

Physiological or pharmacological processes which take place within the cell are often accompanied by enzymatic as well as by structural changes. Such correlations merit study, for the role of different enzyme systems in certain functions of the cell may thus be elucidated. The information can be particularly valuable at the present time when the functional significance of intracellular components is still little understood.

The purpose of this further study was to observe the changes in enzyme activity in the cells of human gastric mucosa, before and after stimulation of gastric secretion induced by histamines in normal subjects. The mucosa was obtained by peroral biopsies and various enzymes were demonstrated by current histochemical techniques. Although somewhat similar studies were carried out with mice and frogs (Villarreal and Burgos, 1955), work on enzyme changes in human gastric mucosa at the time of writing has apparently been confined to homogenate studies (Vitale, Jankelson, Connors, Hegsted, and Zamcheck, 1956).

MATERIAL AND METHODS

Gastric biopsies were obtained by means of the Crosby capsule as described by Crosby and Kugler (1957). Twenty patients were studied and the sex and age distribution is recorded in Table I. The group included patients who previously had had a gastrectomy for

<table>
<thead>
<tr>
<th>TABLE I</th>
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<tbody>
<tr>
<td>MATERIAL STUDIED</td>
</tr>
<tr>
<td>Group</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
</tr>
<tr>
<td>Duodenal and gastric ulcers</td>
</tr>
<tr>
<td>Gastric ulcer</td>
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<tr>
<td>After gastrectomy</td>
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</tbody>
</table>
Histochemistry of the gastric mucosa

duodenal ulcer. Except in two of the nine normal cases, biopsies were taken before and 30 minutes after the injection of histamine, 0.04 mg./kg., the dosage which gives the maximal acid secretion according to Kay (1953) and Card and Marks (1960).

RESULTS

GASTRIC OR DUODENAL ULCER AND POST-GASTRECTOMY PATIENTS The activity of 5-nucleotidase and the non-specific esterases of principal cells in the mucosal biopsies from patients with duodenal ulcer was slightly but definitely greater than that seen in normal mucosa but no differences in acid phosphatase activity were apparent (Table II). In the same biopsies the D.P.N. activity of individual parietal cells was markedly greater than in normal biopsies. However, not all parietal cells in both normal and abnormal mucosa appeared to exhibit activity as judged by comparisons of the enzyme preparation with sections stained with haemotoxylin and eosin. No significant differences were noted in T.P.N. activity. The parietal cells showed no histochemical activity with the other enzymes studied.

No changes, either in the degree of activity or in the distribution of the enzymes studied, were found in the biopsies from patients with gastric ulcer. In biopsies from post-gastrectomy patients the principal cells exhibited a greater intensity of reaction for acid phosphatase and 5-nucleotidase compared with those in normals and that of the non-specific esterases was decreased. In parietal cells a slight decrease in activity of D.P.N. was apparent. Succinic dehydrogenase activity, studied in three cases only of this group, was confined to parietal cells and no differences between these three cases and normals were apparent. However, as noted with D.P.N., not all parietal cells showed activity.

The site and degree of activity of alkaline phosphatase and of A.T.P. were similar to those found in normal cases.

ENZYMATIC ALTERATIONS AFTER HISTAMINE INJECTIONS Two changes were observed in the D.P.N. activity of parietal cells in normal biopsies after histamine stimulation (Figs. 1 and 2), namely, a slightly greater activity of D.P.N. in each cell and, more striking, a greater number of cells showing that activity (Table III). A similar situation was found in T.P.N. activity in two cases. Thus the percentage of parietal cells showing D.P.N. and T.P.N. activity increased greatly over that observed in resting states, particularly in those cells in the superficial layers of the mucosa.

TABLE II

<table>
<thead>
<tr>
<th>Cases</th>
<th>Alkaline Phosphatase in Blood Vessels</th>
<th>A.T.P. in Blood Vessels</th>
<th>5-Nucleotidase in Principal Cells</th>
<th>Acid Phosphatase in Principal Cells</th>
<th>Esterase in Principal Cells</th>
<th>D.P.N. in Parietal Cells</th>
<th>T.P.N. in Parietal Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+(+)</td>
<td>+ (+)</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Duodenal and gastric ulcers</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+(+)</td>
<td>+++</td>
<td>+</td>
<td>+ or +(+)</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+(+)</td>
<td>+</td>
<td>+</td>
<td>+(+)</td>
</tr>
<tr>
<td>Gastrectomy</td>
<td>+++</td>
<td>+++</td>
<td>+ + or +(+)</td>
<td>+ (+)</td>
<td>+</td>
<td>+ (+)</td>
<td>+</td>
</tr>
</tbody>
</table>

*For each enzyme the most representative structure has been chosen.

TABLE III

<table>
<thead>
<tr>
<th>Cases</th>
<th>5-Nucleotidase in Principal Cells</th>
<th>Acid Phosphatase in Principal Cells</th>
<th>D.P.N. in Parietal Cells</th>
<th>Succinic Dehydrogenase in Parietal Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Histamine</td>
<td>After Histamine</td>
<td>Before Histamine</td>
<td>After Histamine</td>
</tr>
<tr>
<td>Normals</td>
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<td>++</td>
<td>+(+)</td>
<td>+(+)</td>
</tr>
<tr>
<td>Duodenal and gastric ulcer</td>
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<td>++</td>
<td>+(+) or +(+)</td>
<td>+(+) or +(+)</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>++</td>
<td>++</td>
<td>+(+) or +(+)</td>
<td>+(+) or +(+)</td>
</tr>
<tr>
<td>Gastrectomy</td>
<td>++ or +(+)</td>
<td>++ or +(+)</td>
<td>++ (++)</td>
<td>++ (++)</td>
</tr>
</tbody>
</table>

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FIG. 1. Activity of D.P.N. in gastric mucosa before histamine.

FIG. 2. Activity of D.P.N. in gastric mucosa after histamine action. Note that compared with Fig. 1 a much greater number of parietal cells are reacting.

FIG. 3. Activity of D.P.N. in gastric mucosa before histamine action in a case of gastrectomy.

FIG. 4. Activity of D.P.N. in gastric mucosa after histamine action. Compare with Fig. 3.
Almost identical responses in parietal cells were observed in most of the mucosal biopsies from the patients with gastric ulcer and from post-gastrectomy patients (Figs. 3 and 4). In the three biopsies from gastrectomy patients in which succinic dehydrogenase activity was studied, the activity of this enzyme was slightly increased in parietal cells (Figs. 5 and 6) but the much greater number of parietal cells exhibiting this enzyme was particularly striking, again paralleling the changes noted with D.P.N. and T.P.N. The enzyme activities of principal cells, 5'-nucleotidase, acid phosphatase, and the non-specific esterases were unchanged when examined 30 minutes after histamine stimulation. One other finding merits special comment. After histamine injections, the A.T.P. activity near mucosal basement membranes and capillaries was noticeably increased. Alkaline phosphatase, which is somewhat similar in distribution to that of A.T.P. as far as vascular staining is concerned, did not react in this way.

**COMMENT**

Our chief observations may be summarized as follows. Under basal conditions the histochemically demonstrable activity of D.P.N. in the parietal cells of gastric mucosa in patients with duodenal ulcer is greater than was observed in normal biopsies. The activity of this enzyme was less than normal in post-gastrectomy patients. Following 'maximal' stimulation by histamine, the activity of D.P.N. in all three groups, and of succinic dehydrogenase in three patients, 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. Similar but less striking responses were noted in the activity of T.P.N. in the parietal cells. It appears reasonable to conclude from these findings that the secretory activity of parietal cells is related to the activity of the oxidative enzyme demonstrated and may reflect...
the relationship of cellular activity, in this case the secretion of acid, with energy metabolism. Thus, the activity of D.P.N. in parietal cells of patients with duodenal ulcer, who produce more hydrochloric acid than do normal subjects, was greater than that found in normal biopsies. This relationship is emphasized by the fact that after histamine injections the number of parietal cells showing D.P.N. 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. These findings are in accordance with the theoretical considerations of histamine action discussed by Card and Marks (1960).

The restriction of the oxidative enzyme activity to parietal cells is of interest, when one considers that in the rat and other species the mitochondria of parietal cells are more numerous and much larger than those in other cells of the gastric mucosa (see International Review of Cytology, 1961, for review). If the relationship of oxidative enzyme activity to acid secretion in parietal cells is more than coincidental, and the energy derived from oxidative intracellular metabolism is an important factor in the production of acid, it will be of interest to study the state of mitochondria with the electron microscope in patients in whom secretion of acid by the parietal cells is depressed as well as in those with hypersecretion. These points are made with the awareness that any of the differences observed might be due to sampling of different sites of the gastric mucosa in which variations in enzyme pattern might be inherent. It would seem, however, that the findings are not due to such variations because of the similar trends found in the large number of biopsies taken.

From a comparative viewpoint the histochemical studies of mouse and frog gastric mucosa after histamine stimulation by Villarreal and Burgos support our findings. After such treatment, an increase in the number of parietal cells exhibiting oxidative enzyme activities was observed, as well as an increase in the amount of activity in each parietal cell (Villarreal and Burgos, 1955). Using homogenates of gastric mucosa Vitale, Jankelson, Connors, Hegsted, and Zamcheck (1956) found a rise in succinic dehydrogenase activity after histamine stimulation. From the present studies not only can this finding be confirmed but extended in that the increase of activity of this enzyme was confined to parietal cells.

It is not possible to interpret with certainty the increases in A.T.P. activity of mucosal basement membranes or capillaries after histamine injections, but among the many speculations may be included its possible relationship to vasodilatation and transport across cell membranes during phases of increased cell activity.

Alley (1935) was among the first to state that histamine inhibits the activity of the principal and mucous cells of the gastric mucosa. No changes in the enzyme activity of principal cells, however, were observed in this study.

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