# The Mass of Gastric Mucosa Cells Measured by X-Ray Absorption

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It was previously shown (*2*) that the utilization of selected bands in a long wavelength, continuous x-ray spectrum makes it possible to determine the dry weight of single cells and cell structures by absorption measurements. A consideration of the theory of x-ray absorption revealed that the mass of a structure in biological material might be determined with an error of less than 5% even if there were relatively large variations in the proportion of the constituent elements. Calculations showed that a great change in carbon, nitrogen, or oxygen content (in the case of nitrogen up to 60%) produces an error of only 5% in the mass determination. The same error will occur if other elements such as phosphorus, sulfur, chlorine, and calcium are present in very high concentrations; i.e., the concentration of phosphorus would have to be about 5%, sulfur 14%, chlorine 17%, and calcium 9%. Thus only in special instances, e.g., calcified tissues, need a correction for other elements, with the exception of hydrogen, be made. The correction for the absorption of hydrogen is calculated by means of a formula derived for the purpose (*1, 3*), and this correction is less than 10%. Mass can be determined by x-ray absorption technique with an over-all error under 10%.

In the present study the absorption measurements were applied to the determination of the dry weight of the cells of dog gastric mucosa from an animal which had been given a series of intramuscular injections of histamine in beeswax to produce a chronic hyperacidity. One injection of 30 mg was given daily for 45 days, and on the last day 40 mg of nembutal per kg was administered intravenously to kill the dog, and the tissue was removed. The dog was one of a group used for another study by D. J. Ferguson, of the Surgery Department of the University of Minnesota, and we are indebted to him for the material. Small pieces of gastric mucosa from the fundus were quickly dissected, immediately frozen in isopentane cooled by liquid air, placed on a block of previously degassed paraffin in a freezing-drying apparatus and dehydrated at about −80° C and $10^{-5}$ mm mercury pressure for a week. The frozen-dried tissue was infiltrated with paraffin in the apparatus; air was admitted to the system only after the tissue was submerged in the molten parafin.[3] Sections were cut at 4 μ to 7 μ, placed directly over the slits in the brass disks supporting tissue during measurement, and deparaffinized with xylol.

TABLE 1

DRY WEIGHTS OF DOG GASTRIC MUCOSA CELLS

| | Dry wt ($g\ 10^{-12}/\mu^2$) ± standard error of mean | | | Dry wt ($g\ 10^{-12}/\mu^3$) | | | | Mass ratios | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean of whole chief cell area | Mean of epithelial cytoplasm (secretion-free) | Mean of whole parietal cell area | Thickness (μ) | Mean of whole chief cell area | Mean of epithelial cytoplasm (secretion-free) | Mean of whole parietal cell area | Chief | Epithelial | Parietal |
| Frozen-dried section | 1.5 ± < 0.1 | 1.2 ± < 0.1 | 0.6 ± < 0.1 | 4.7 | 0.3 | 0.2–0.3 | 0.1 | 2.5 | 2.0 | 1 |
| " " " | 2.7 ± 0.1 | 2.0 ± < 0.1 | 0.9 ± < 0.1 | .. | .. | ..... | .. | 3.0 | 2.2 | 1 |
| " " " | 2.1 ± 0.1 | 1.5 ± < 0.1 | 0.9 ± < 0.1 | .. | .. | ..... | .. | 2.3 | 1.7 | 1 |
| " " " | 2.2 ± 0.1 | 1.5 ± < 0.1* | 0.9 ± < 0.1 | 7.0 | 0.3 | 0.2 | 0.1 | 2.4 | 1.7 | 1 |
| Formalin-fixed section | 1.7 ± < 0.1 | 1.5 ± < 0.1 | 0.9 ± < 0.1 | 7.6 | 0.2 | 0.2 | 0.1 | 1.9 | 1.7 | 1 |
| " " " | 2.3 ± < 0.1 | 2.0 ± 0.1 | 1.2 ± < 0.1 | 7.6 | 0.3 | 0.2–0.3 | 0.1–0.2 | 1.9 | 1.7 | 1 |

* The secretion in the upper part of the epithelial cells had a mass of $3.3 \pm 0.1 \cdot 10^{-12}\ g/\mu^2$ which corresponds to $0.5 \cdot 10^{-12}\ g/\mu^3$.

In order to observe the effect on cell mass of formalin fixation and alcohol dehydration as applied routinely for histological preparations, the block of embedded frozen-dried tissue left after the sections were removed for absorption measurements was deparaffinized in xylol, hydrated through graded alcohols, and placed in 10% formaldehyde overnight. This was followed by dehydration in alcohols, paraffin infiltration, sectioning, and measurement of x-ray absorption in the same manner as used for the frozen-dried material.

Measurements were made following the technique described (*1–3*). X-rays (3000–4000 volts), filtered through an aluminum foil 9 μ thick, were employed. This gave a beam with maximum intensity at 8–12 A. Lippman photographic film (Gevaert, Antwerp), which has a resolving power of about 1 μ, was used to record the radiation intensities. Each microradiogram also included a simultaneous exposure of a mass reference system consisting of two strips of a thin nitrocellulose film. The microradiogram of the sample and reference system was enlarged 200 to 600 times by photomicrography, using constant illumination. By photometric measurements of small areas (0.5 $mm^2$) in the enlarged images, the x-ray

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[3] We are indebted to Mr. B. Malmstrom for preparing the paraffin blocks.

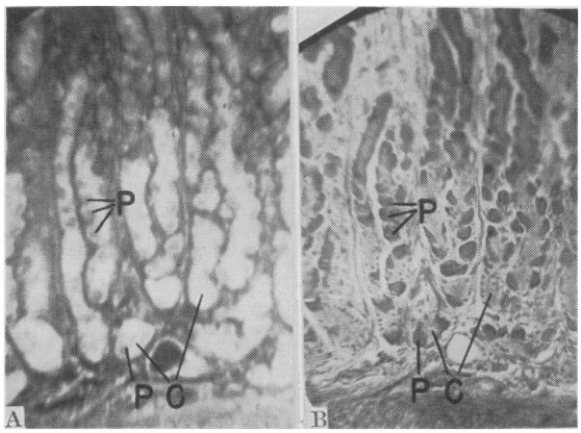


FIG. 1. (A) Microradiogram of section of frozen-dried gastric mucosa, 130×. The lighter the area the greater the absorption of x-rays. (B) Photomicrograph of same section after staining. Parietal cells (P) have least absorption and chief cells (C) the greatest.

absorption in the cells was compared to that in the reference system, and the mass per unit area was calculated. From approximations of the thickness of the sections employed, estimates of mass per unit volume were made.

masses of the various cells may be seen in Figs. 1 and 2. The photometric measurements were made on the secretion-free cytoplasmic area of the epithelial cells in the enlarged microradiogram. The nuclei were too small to permit reliable measurements, but it can be seen by inspection that they show less absorption than the secretion-free cytoplasm, while the secretion itself within the cytoplasm has the greatest absorption. Nuclei in parietal and chief cells could not be distinguished in the photographs, so that measurements were made on whole cells. Usually ten cells of each type in a photomicrogram were measured photometrically, and the means, with their standard deviations, were calculated. Thickness measurements on the sections were made with the micrometer screw of a microscope which was used with oil immersion. Since the thickness could be determined only approximately, the calculations of dry weight per unit volume do not have as great accuracy as those of dry weight per unit area, and accordingly the standard deviation was not estimated for dry weight per unit volume.

It is apparent from these experiments that the mucus- and enzyme-secreting cells have a greater mass than those secreting acid. The greatest variability in mass was found in the chief cells, and this may have been due to the cells' being in different stages of secretory activity.

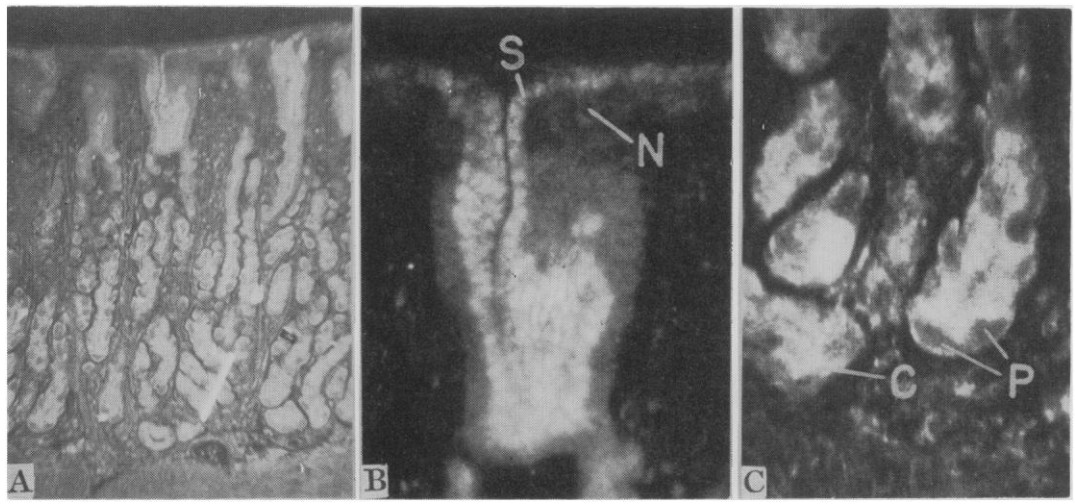


FIG. 2. (A) Microradiogram of section of frozen-dried gastric mucosa, 88×. (B) Microradiogram of epithelial cell area in same section, 330×. Nuclei (N) of epithelial cells have less absorption than the cytoplasm. The secretion (S) above the nucleus has strong absorption. (C) Microradiogram of parietal and chief cell area in the same section. The chief cells have greater absorption than the parietal cells, and nuclei cannot be seen in either.

After the microradiograms were obtained, the same sections were stained with hematoxylin and eosin without removing them from the brass disks, and photomicrographs were taken having the same enlargement as that used for the microradiograms. The photograph of the stained tissue facilitated identification of individual cells in the enlarged radiograms.

Results of the measurements are given in Table 1. The nitrocellulose films employed as mass reference weighed 0.350 mg/cm², and the elementary composition was: C, 46.7%; N, 6.6%; O, 41.4%; H, 5.3%. A factor was calculated as described (*1, 3*) to convert the absorption of substance of this composition to mass. The relative

The formalin fixation and alcohol treatment processes routinely used for histological preparations effected a loss in mass of the chief cells, as shown by the mass ratios in Table 1, indicating that the protein denaturation did not prevent solution of a certain amount of the cellular material.

### References

1. ENGSTRÖM, A., in Butler, J. A. V. and Randall, J. T., (Eds.) *Progress in biophysics and biophysical chemistry.* London: Butterworths Scientific Publications, in press. Vol. I.
2. ENGSTRÖM, A. and LINDSTRÖM, B. *Nature,* Lond., 1949, **163,** 563.
3. ———. *Biochim. Biophys. Acta,* 1950, **4,** 351.