Automated Analysis of Cellular Change in Histological Sections

Abstract. *An image analysis computer has been used to count and measure goblet cells in the epithelium of the bronchiolar tree of rats exposed to sulfur dioxide vapor. A technique was developed which enabled a bioassay of the irritant effects of the gas.*

The "Quantimet" (QTM) image analyzer (*1, 2*) operates on the principle of line scanning for counting and measuring (area) any particle of sufficient contrast in a microscope image (Fig. 1). The QTM has now been adapted for the evaluation of increased mucus production in the lungs of rats exposed to sulfur dioxide vapor. This first success in the application of the machine to the analysis of microscope images of biological material has been achieved by modification of staining techniques involving the elimination of background detail.

The lungs of rats exposed to sulfur dioxide vapor show increased mucus production due to (i) increases in the number of goblet cells in the epithelial layers of the trachea and bronchioli and (ii) increased activity of the glands in the trachea (*3*). These changes resemble those in human chronic bronchitis where they are used to study the disease. The major need is a bioassay of the effects of various air pollutants on the respiratory tract. The QTM has been used to assess quantitatively the increase in goblet cell activity. The rats used were of the SPF Carworth Europe strain which normally show little goblet cell activity in the respiratory tract. Sixteen male rats were divided into two groups of eight each. One group was exposed to sulfur dioxide vapor at a concentration of 300 parts per million for 10 consecutive daily periods of 6 hours; the other group was kept as controls. When not being exposed, all rats were kept in free-run cages and were provided with free access to food and water. At the end of the exposure periods, the rats were left in the cages for 48 hours before being killed by intraperitoneal injection of barbitone sodium. The lungs were fixed by injecting 4 ml of buffered Formalin (10 percent) down the trachea; the lungs were left in the fixative overnight. The left lower lobe bronchus was then exposed, severed at the carina, and processed for sectioning. Sections (5 micron) were cut at intervals (100-micron) throughout the entire specimen and stained with iron periodic acid–Schiff reagent (PAS) without counterstain. Manual counts were made with the × 20 objective with the microscope attached to the QTM and a mask in the eyepiece, thus ensuring that the same size field was counted by both the manual counter and the QTM. One thousand microscope fields of epithelium from each animal were counted both manually and by QTM, counts being made of goblet cells or droplets of PAS-positive material. The QTM was also set simultaneously to accumulate the area covered by the PAS-positive material in the epithelial layer.

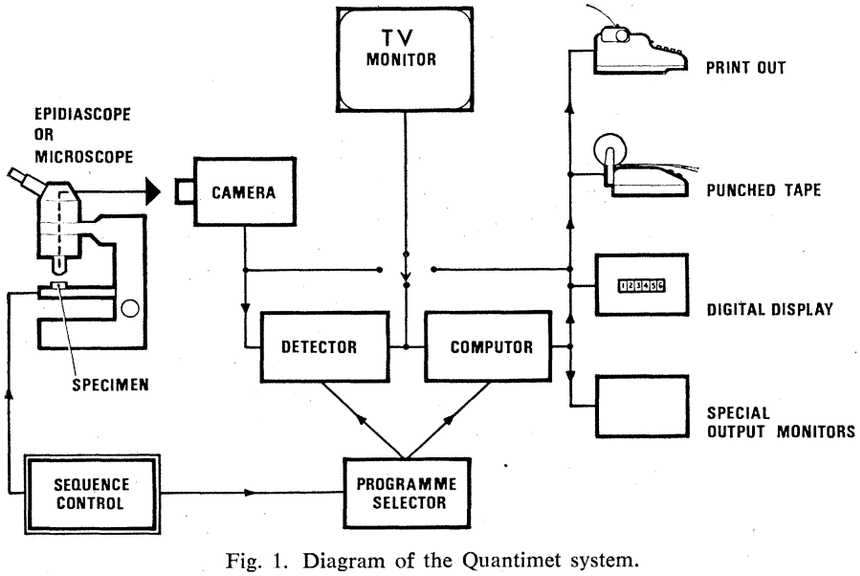

Fig. 1. Diagram of the Quantimet system.

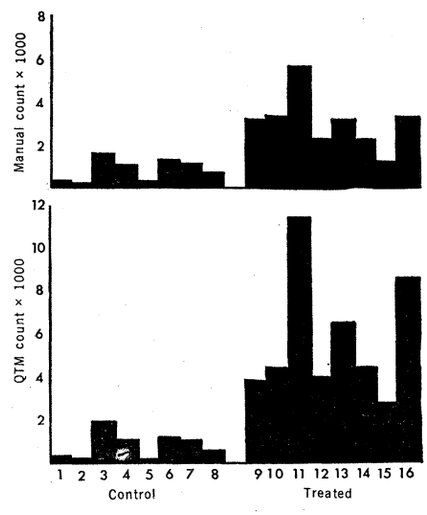

Fig. 2. Comparison of goblet cell counts of 1000 microscope fields of epithelium made both manually and by QTM.

It is apparent that with regard to absolute numbers, the manual counts do not compare directly with the counts provided by the QTM (Fig. 2). This is mainly due to the machine's indiscriminately counting every droplet of PAS-positive material, whereas the manual counter tended to discriminate between odd droplets and those forming goblet cells and counted only the goblet cells. When manual counts were made directly from the television monitor, the correlation between machine and manual counts became closer. Assessment of increase of mucus in the epithelial layer is more accurately shown by measurement of the total area covered by PAS-positive material in this layer. Secretion of mucus into the lumen, where it is not assessed by this technique, can affect the results considerably, but even so, the QTM has facilitated a comparatively accurate quantitative assessment of mucus production in the epithelial layer of the rat bronchiolus.

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References and Notes

1. M. Cole, *Microscope* **15**, 148 (1966); C. Fisher, *Proceedings of the Particle Size Analysis Conference* (Society for Analytical Chemistry, London, 1967), p. 77.
2. The "Quantimet" image analyzer is manufactured by Metals Research Limited, Cambridge, England.
3. L. Reid, *Lect. Sci. Basis Med.* **8**, 235 (1960); *Thorax* **15**, 132 (1960); *Brit. J. Exp. Pathol.* **44**, 437 (1963).

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