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A QUANTITATIVE STUDY OF SOFT TISSUE SPECIMEN SHRINKAGE DURING PREPARATION FOR SCANNING ELECTRON MICROSCOPY

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It appears generally accepted today that the critical point technique of dehydration should be used in the preparation of soft biological tissues for scanning electron microscopy. This technique is said to ensure minimal tissue shrinkage and distortion of surface detail. Boyde and Wood[1] felt that freeze drying is a better technique as there is less tissue shrinkage and distortion. This assessment, as well as that of the critical point technique appear to be based on subjective impressions, as we have found no evidence in available literature of quantitative studies to substantiate this.

In this investigation the shrinkage of three types of tissue were studied. Specimens of Wistar strain albino rat soft palate, tongue and small bowel were used. Five 5 mm specimens were cut, using scalpel blades fixed in parallel, and the tissues fixed in 10 per cent neutral buffered formol saline for at least one week. The specimens were then removed from the fixative, lightly blotted, and photographed using standardized lighting and a Canon F1 35 mm Camera with bellows attachment set at constant magnification, having a 65 mm macro-lens with a mirror attachment, so that the specimens were illuminated by direct lighting, to obviate shadows.

Later the specimens were rephotographed, under the same standard conditions, at the completion of dehydration. An engineer's steel rule was included in each photograph. Prints of X 10 enlargement were then made. A planimeter was used to measure the area of each of the specimens on the photographs and the percentage shrinkage calculated. This procedure was carried out for specimens dehydrated using the following techniques, airdrying; the critical point technique; the camphene technique of Watters and Buck[2]; and three freeze drying techniques. In all a total of 90 specimens were examined.

Following dehydration the specimens were mounted on stubs with colloidal graphite and coated with gold palladium. They were examined in a Cambridge S4 stereoscan operated at 20 kV.

There was considerable variation in shrinkage depending on both the tissue and technique used (Table 1).

TABLE 1 Mean shrinkage values

Percentage shrinkage with the various techniques

Specimen				Freeze Dried		
	Air Dried	Critical Point	Camphene	Edwards Coating Unit	Edwards Pearse	Gallenkamp
Tongue	42,5	43,0	42,8	26,0	29,8	36,0
Gut	64,4	65,4	65,8	34,2	29,0	54,5
Soft Palate	35,2	32,8	37,4	19,0	19,8	31,8

Examination of the surface of various specimens with the scanning electron microscope revealed almost no difference between them.

References

1. Boyde, A. and Wood, C. J. Microsc., 90, 221, 1969.
2. Watters, W.B. and Buck, A.C. J. Microsc., 94, 185, 1971.