

the location and shift of the absorbance peak as a function of pressure are the same as those reported by Zahner and Drickamer⁸ for the same material under a presumably uniform pressure. Nevertheless, the absorption bands are considerably sharper and narrower when measured in a microscope spectrophotometer as shown by the dotted line in Fig. 5.

Now since the position of the absorption band is known as a function of pressure, the microscope spectrophotometer has been used to determine accurately, for the first time, the pressure gradient of a material such as nickel dimethylglyoxime in the diamond high pressure cell.⁷ Using the instrument described, spectral measurements corresponding to as many as ten thousand microsections of the sample in the pressure cell can be determined. In practice, it was found that about seventy measurements gave enough information to draw pressure contours maps of the diamond surface (using the position of the absorbance peak vs pressure relation). An extension of the pressure gradient studies into materials of different compressibilities indicates that determinations of the compressibilities of a material as a function of pressure can probably be made using this technique.

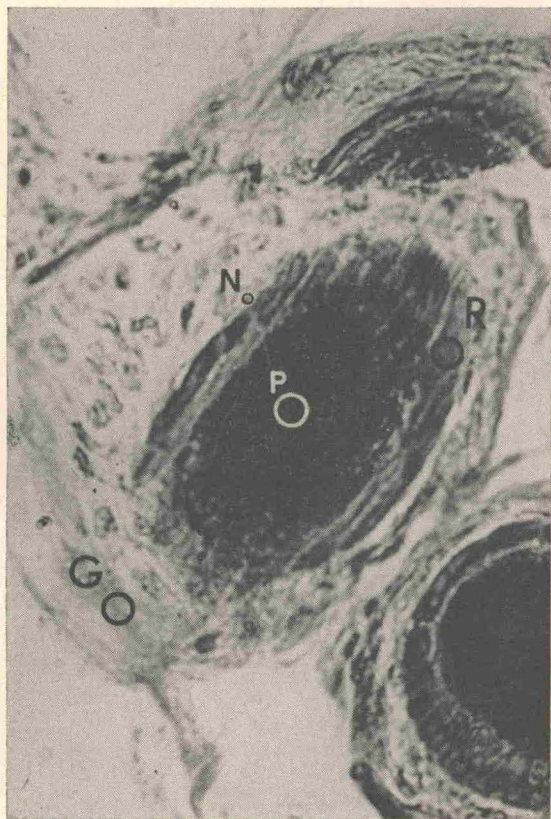


FIG. 7. Photograph of stained thin section of sheep hair follicle.

⁸ J. C. Zahner and H. G. Drickamer, *J. Chem. Phys.* 33, 1625 (1960).

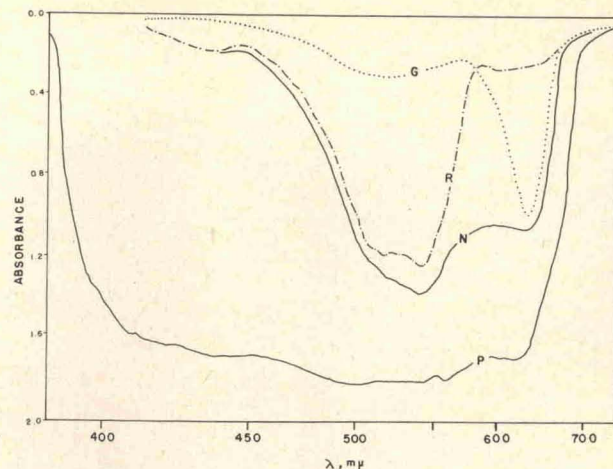


FIG. 8. Spectra of selected regions indicated in Fig. 7 from sheep hair follicles.

The application of the microscope spectrophotometer to conventional microscope specimens may be demonstrated with a stained thin section taken from the hair follicle of a sheep (see Fig. 7). The sample is stained with safranin and fast green dyes, but since the reproduction is not in color here, the color of the areas is appropriately designated. The green area (G) is connective tissue, the sebaceous cells (R) surrounding the hair follicle are red, the follicle tissue (P) is a deep purple color, while the nuclei of the tissue cells (N) are reddish purple. The areas designated were selected for the spectral determinations given in Fig. 8. The scan area for all measurements was $10 \mu^2$ except an area of $1 \mu^2$ is used for the determination of spectrum of the nuclei. These spectra were taken using a $100\times$ achromatic objective.

Other applications of microscope spectrophotometry discussed by Caspersson¹ and Wolken and Strother² include studies in pigment chemistry, photoreceptors in living cells, and the metabolism of living cells. The use of micro-analytical spectroscopic techniques has been discussed by them as well as by Mason.⁹ It is expected that the present instrument equipped with the proper optics may also be satisfactorily used for these applications.

In general, we can say that the microscope spectrophotometer is an aid to spectroscopic analytical problems where very small quantities of material (as small as 10^{-9} g) are available or where the sample is nonhomogeneous. In addition, the instrument described herein can be used for ultraviolet, visible, and near-infrared spectral determinations on samples which require a working distance of up to 15 mm. In this respect, the instrument is adaptable to spectral observations of materials under pressure as well as in a vacuum, and at very low as well as at elevated temperatures.

⁹ W. B. Mason in *Conference on Submicrogram Experimentation, Arlington, Virginia, 1960*, edited by N. D. Cheronis (Interscience Publishers, New York, 1960), pp. 293-310.