ON THE REFRACTIVE INDEX OF HISTOLOGICAL TISSUE SECTIONS IN VISIBLE AND IN ULTRAVIOLET LIGHT

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In the course of our work on the distribution of ultraviolet absorbing substances in thin tissue sections it was found desirable to use methacrylate sections, from which the embedding medium has not been removed. In order to be able to assess the usefulness of media other than those hitherto customary in ultraviolet microscopy (e.g. pure glycerol [1], glycerol saturated with chloralhydrate or zinc chloride [3]), we have measured the refractive indices at various wavelengths in both the visible and ultraviolet ranges of fixed tissue sections and of a number of potential imbibition media including polymerized butylmethacrylate (with an admixture of 5 per cent methylmethacrylate).

Method.—The measurement of the refractive indices of the tissue has been performed by first determining the dispersion curves of a number of imbibition fluids and next by finding the best match at a few selected wavelengths of these fluids with the tissue section.

The refractive indices of the liquids used have been measured in a simple refractometer (see Fig. 1), consisting of a quartz slide (S) and two optically polished quartz slips (C_1 and C_2). The coverslips are attached to a brass ring so as to enclose between them and the slide a shallow cell consisting of two prismatic spaces with a common refracting rib. The liquid to be measured is retained in these spaces by capillary force. This cell, when introduced in the light path of a mirror yielding an image of the exit slit of a monochromator, causes the slit image to be doubled. From the geometry of the apparatus and the mutual distance of the two slit images, the refractive index of the liquid is computed. With a refracting angle of the prismatic spaces of 5°, the dispersion curve (from 578 mμ to 248 mμ inclusive) of distilled water could thus be measured to an accuracy of one unit of the third decimal place. The dispersion curve of polymerized methacrylate has been determined in the same refractometer. To this end, a drop of prepolymerized methacrylate was allowed to polymerize *in situ*. Furthermore, measurements have been made on a thin wedge of methacrylate embedded in water and in glycerol.

The matching of the refractive indices of the medium and the tissue section has been checked by microphotography under conditions of dark field illumination. A reflecting objective[1] has been used, in connection with a reflecting condenser, the maximum aperture of which was just below the aperture of the central stop in the objective. Though under these conditions the dark field picture is not as easily inter-

[1] This objective (num. apert. = 0.85) was built a few years ago by the optical firm "Oude Delft", Delft, The Netherlands. It is a quartz objective with spherical mirrors. The linear obscuring ratio is 22 per cent.

preted as when the condenser aperture exceeds that of the objective, the main features of the section are easily recognizable and any slight mismatch in the refractive indices of fluid and section shows up very brightly. Apart from the brightness of the dark ground pictures, the frequently striking differences between the pictures obtained with too low or too high refractive indices respectively of the immersion medium are helpful in finding the correct match.

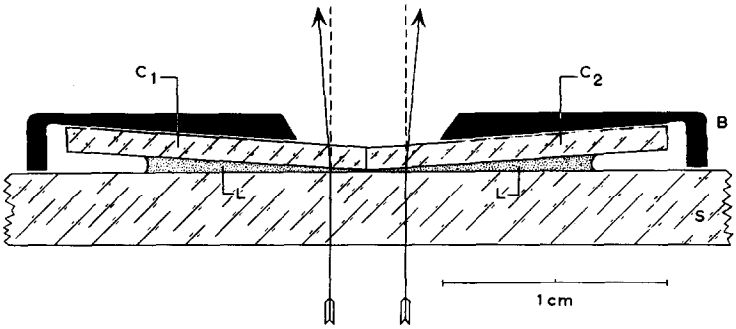

Fig. 1.—Diagram of the refractometer cell used for determining the refractive indices of the imbibition fluids. *S*, quartz slide; C_1 and C_2, quartz coverslips; *B*, brass ring; *L*, sample to be measured. The light path has been indicated by arrows.

Both the refractometer and the microscope derived their illumination from a mercury arc, the following spectral lines of which were isolated by a monochromator: 578, 546, 436, 405, 366, 334, 312, 302, 289, 280 and 265 mμ.

In order to facilitate the renewal of the imbibition medium of an object during the observation, a simple perfusion chamber of minimal depth has been constructed (see Fig. 2). In a quartz slide *S*, which in our microscope is to be used in a vertical plane, two depressions (D_1 and D_2) have been ground. The coverslip *C*, onto which the section is stuck, is firmly clamped onto the slide, so as to leave the upper parts of the depressions uncovered. Through two finely drawn out glass tubes dipping into these depressions, a constant flow of the liquid across the section can be effected. Though the distance between the slide and the coverslip does not exceed the thickness of the section, the complete renewal of the fluid within a small section is a matter of minutes.

Results.—The results of a number of measurements on rat liver sections fixed in either Bouin's, Zenker's or Carnoy's fluid, embedded and cut in paraffin and deparaffinized in xylene, along with the dispersion curves of a few media, are presented in the graph of Fig. 3. The data on the tissue have been obtained with mixtures of benzene and cyclohexane at the wavelengths 265 and 280 mμ. For the range 289 mμ to 334 mμ inclusive, mixtures of methyl-benzoate and decaline were found to be suitable, whereas at longer wavelengths including the visible range media containing α-chloronaphthalene and butylcarbitol were used. Within single sections different parts (notably erythrocytes, nucleoli) presented slightly different matches. Similarly, the dispersion curves of differently fixed tissues and of sections with different histo-

ries did not coincide exactly. We did not, however, further investigate these differences, which did not exceed a few units of the second decimal place, as they seem to be unimportant as causes of serious diffraction effects.

Discussion.—Whereas data as to the refractive index of tissue sections in the visible light are fairly numerous (e.g. [2, 4, 6]) and well within the range of our results, we are aware of only one report [5] on the determination of the ultraviolet

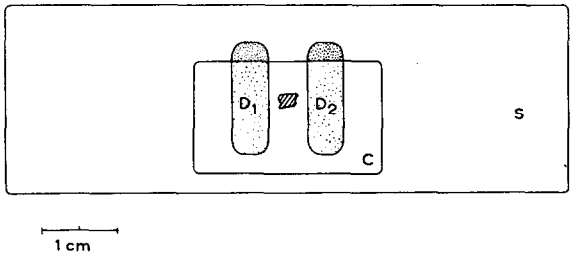

Fig. 2.—Quartz perfusion chamber for thin sections. S, quartz slide; D_1 and D_2, depressions, ground in S; C, coverslip onto which the section is stuck. Clamps and glass tubes omitted.

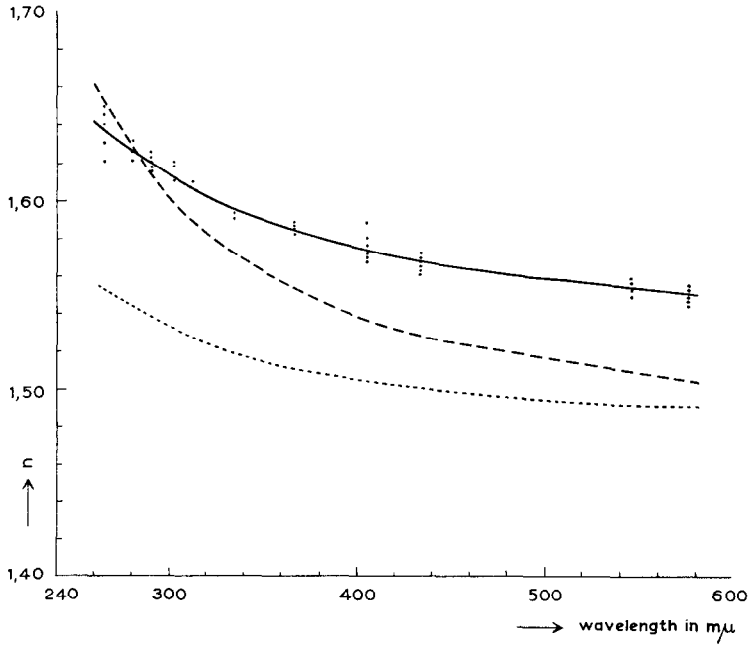

Fig. 3.—Dispersion curves of histological sections (———), benzene (– – – –) and of polymerized butylmethacrylate, mixed with 5 per cent methylmethacrylate (· · · · · ·). The latter curve almost coincides with that of a concentrated solution of chloralhydrate in glycerol (not drawn). Actual measurements on tissue sections are represented by dots.

refractive index of fixed tissue. Our results confirm the prediction of Rudkin and Corlette to the effect that the ultraviolet refractive index of fixed cell material should be somewhat higher than the computed $n = 1.60$ of their most refractive glycerol-zinc-chloride mixture.

Whether polymerized butylmethacrylate can be considered a suitable medium for ultraviolet photography depends on the nature of the investigation. Judging by its dispersion curve it is equivalent to glycerol-chloralhydrate mixtures. If, however, there is no objection to the methacrylate being removed from the section, the use of a medium with a higher refractive index, e.g. benzene, which in thin layers is sufficiently transparent down to 265 mμ, seems to be safer. A number of other fluids has been tested, but we have been unable to find a medium with approximately the same dispersion curve as fixed tissue sections.

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REFERENCES

1. Caspersson, T., *Skand. Arch. Physiol.* **73**, suppl. 8 (1936).
2. Hancox, N. M. and Kruszinsky, J., *Exptl. Cell Research* **11**, 327 (1956).
3. Köhler, A., *Z. wiss. Mikroskop.* **21**, 129 (1904).
4. Ruch, F., *Physical techniques in biological research* **3**, 149 (1956).
5. Rudkin, G. T. and Corlette, S. L., *J. Biophys. Biochem. Cytol.* **3**, 821 (1957).
6. Swift, H. and Rasch, E., *Physical techniques in biological research* **3**, 353 (1956).

A RAPID METHOD FOR THE QUALITATIVE ANALYSIS OF FREE AMINO ACIDS IN ANIMAL TISSUES BY PAPER CHROMATOGRAPHY

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Ever since the first application of chromatographic methods on filter paper by Consden, Gordon and Martin [1] in 1944, tremendous progress has been made in the use of the equipment and the overall improvement of the method by modifying the moving phase and the developing technique, but comparatively less attention has, however, been paid to the preparation of the material for analysis. In the case of the free amino acids in animal tissues it has been customary to prepare extracts of tissues by deproteinising with alcohol, trichloracetic acid or tungstic acid and subject them to analysis after adjusting the pH. In this paper is outlined a rapid procedure which does not require extraction and which makes possible the analysis of free amino acids in small quantities of animal tissues by paper chromatography. A similar method

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