A simple method for observing refractive index gradients in liquids

When proteins are separated by preparative ultracentrifugation, it is desirable to visualize the positions of the proteins in the centrifuge tube so that the various layers may be accurately removed. Previously, this has been done by use of the optical system of the Tiselius apparatus[1]. However, the bath must be lowered to avoid immersing the tubes completely.

The refractive index inflection points (peaks in the Schlieren diagram) may be easily located without auxilliary apparatus, as shown in Fig. 1. A card ruled with parallel lines set at 45° is viewed through the centrifuge tube, which is immersed in water in a parallel-sided vessel (a heat filter from a microscope lamp is satisfactory). The tube shown in Fig. 1b is of lusteroid and is the size used in the Spinco swinging bucket No. SW39 L rotor. It contains 2% bovine serum albumin overlayed with distilled water. The pattern is reminiscent of that seen with the Philpott Svensson cylindrical lens system. When the boundary between similar solutions is observed in a parallel-sided vessel such as a 1-cm light path cuvette (Fig. 1a), a slightly different but equally usable type of pattern is seen. As in the scale method of Lamm[2], the extent of the deflections may be varied by changing the distance between the tubes and the diagonal line chart. In the illustration shown, the chart consisted of lines 1 inch apart and was 38 inches behind the tubes. Since the deflection is also proportional to the cotangent of the angle made by the diagonal lines and the horizontal, the deflection can be increased by decreasing this angle.

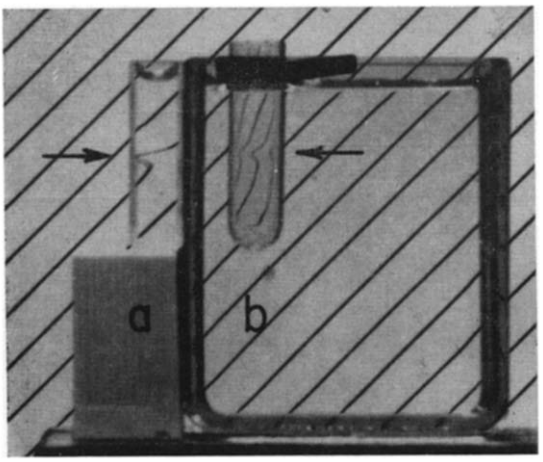

Fig. 1

By this method, the fluid above a "peak" may be gently removed with a fine pipette. Two component systems are easily distinguished, but more complex systems often are difficult to resolve.

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An X-ray study of crystalline cytochrome c

Cytochrome *c* from various sources has recently been obtained in crystalline form[1,2,3,4]. An attempt has now been made to obtain crystals large enough for X-ray analysis. The method of crystallization was the same as the one described earlier[1], using a 1–2% solution of purified reduced cytochrome, extracted from the pectoral muscle of the king penguin or the heart muscle of horse. The king-penguin cytochrome was easily crystallized as thin plates or long prisms[1]; however, the crystals were too small for X-ray work. Horse cytochrome, on the other hand, at first failed to crystallize, but after a year one of the samples was found to contain some large crystals of cytochrome, together with amorphous material. These crystals were prismatic needles, about 0.2 mm long and 0.03 mm wide at the most, and similar to the rod-like crystals of king-penguin cytochrome obtained earlier[1]. They showed a ferri-cytochrome spectrum and could be reduced by addition of dithionite. The crystals were birefringent and dichroic, the directions of high refractive index and of high absorption being parallel to the needle axis. X-ray analysis

proved that the crystals were tetragonal and that the needle axis was the tetrad axis. On the basis of the X-ray pictures the prism faces could be assigned to the form {110}; the indices of the terminal faces were uncertain, but were probably of the form {221}.

The wet crystals gave good X-ray pictures with sharp reflections extending to spacings of less than 3 Å. In order to determine the unit cell dimensions and space group, precession pictures were taken of the *hk*o and *ho*l zones. The cell dimensions of the wet crystals were $a = 72.6$ Å and $c = 38.9$ Å. *h*oo reflections were absent for *h* odd, and oo*l* reflections were all absent except for oo8. This made it difficult to be sure of the space group, but it is probably $P4_12_12$, which has 8 general positions, so that the unit cell would be expected to contain 8 molecules of cytochrome *c*. Owing to the few crystals available, I was unable to measure either their density or the dimensions of the dry unit cell. Fortunately the density and liquid content of most kinds of protein crystals are of the same order. In saturated ammonium sulphate solution the density is usually 1.24 and the liquid content between 30 and 55 vol. %. Assuming a liquid content of 40% and 8 molecules in the unit cell, the molecular weight is calculated as 12,000, in fair agreement with the accepted value. On account of its stability and low molecular weight, and especially in view of its outstanding biological importance, cytochrome *c* would be an interesting protein for a detailed structure analysis by X-rays. It would be an advantage for this purpose, however, to find a form with fewer molecules in the unit cell.

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Stoffwechsel von embryonalen Zellen in Gewebekulturen

LESLIE, FULTON UND SINCLAIR[1] berichten in dieser Zeitschrift, dass embryonale Zellen unter den Bedingungen der Gewebekultur aerob gären und bestätigen damit eine frühere Arbeit[2] aus unserm Institut. Anders jedoch als wir ziehen sie aus ihren Versuchen den Schluss, dass embryonale Zellen auch unter physiologischen Lebensbedingungen aerob gären; während wir gefunden haben[3] dass embryonale Zellen unter physiologischen Lebensbedingungen, im lebenden Tier, einen reinen Oxydationsstoffwechsel haben. Würden die embryonalen Zellen im Körper aerob gären, so wie die Krebszellen, so würde ein Embryo pro Stunde etwa 15% seines Trockengewichts an Milchsäure producieren, eine Menge, die sicherlich bereits JUSTUS VON LIEBIG gefunden haben würde. Tatsächlich hat nichts den Fortschritt der Krebsforschung mehr verzögert, als die unrichtige Behauptung, dass nicht nur die Krebszellen, sondern auch die embryonalen Zellen aerob gären. Denn durch diese Behauptung wird der tiefgreifende biochemische Unterschied zwischen Krebszellen und embryonalen Zellen – die partielle Anaerobiose – geleugnet.

Es sei mir erlaubt zu fragen, warum man sich die Mühe macht, embryonale Zellen für Stoffwechselmessungen in Gewebekultur zu züchten, obwohl doch unbegrenzte Mengen an embryonalen Zellen in lebenden graviden Tieren für derartige Untersuchungen zur Verfügung stehen; und obwohl von allen Methoden die Gewebekultur die ungeeignetste ist um zu untersuchen, ob die embryonalen Zellen partielle Anaerobier sind, wie die Krebszellen. Denn seit Jahren ist es bekannt[4], dass die Gewebekultur carcinogen ist.

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