Molecular Orientation and Light Absorption

RECENT discussions by several authors¹⁻⁵ have stressed the importance of considering the orientation of molecules in the measurement of light absorption in cytological material.

In a system of molecules having parallel orientation (anisotropic system), the incident vibration is decomposed into two perpendicular components. These components are differently absorbed (dichroism). In many cases, however, the light absorption of such systems is measured with unpolarized light. If the transmission (I/I_0) of one component is a and the other b , the total transmission for unpolarized light can be shown to be $(a + b)/2$. If the same molecules are randomly orientated in the same space, the transmission will be $\sqrt{a.b}$. Thus, by measuring the light absorption of an anisotropic system using unpolarized light, a systematic error will be introduced, depending on the ratio of a to b . The magnitude of this error must be investigated experimentally for each special case.

The following summarizes some results for nucleic acids. The preparation used was deoxyribose nucleic acid made according to Hammarsten. Two types of experiments were carried out.

In the first, nucleic acid molecules in solution were orientated by streaming in the apparatus designed by Signer⁷ (1,100 r.p.m.; radius of rotating cylinder, 24.75 mm.; distance between rotating and fixed cylinder, 0.5 mm.; light-absorbing layer, 50 mm.). As a light-source for 260 m μ a Beckman spectrophotometer (band-width 1 m μ) was used. The intensities were measured by a 1P28 photomultiplier. The amplified photocurrent was recorded by an Esterline Angus meter (0.5 m.amp.). An interchangeable Glan prism was inserted between the streaming apparatus and the photomultiplier tube. The dichroism and the absorption of unpolarized light of orientated and unorientated molecules in the same solution were measured. The results of a typical experiment are presented in the accompanying table.

The second type of experiment was made with an apparatus especially devised for microscopic specimens. The constructional details of this apparatus will be published elsewhere⁶. The molecular orientation was brought about by stretching a nucleic acid gel into microscopic fibres or strips. Some results of measurements similar to the above are summarized in the table.

It is evident from the first type of experiment that, with moderate degrees of orientation, giving a dichroism (E_a/E_b) of 1.3, the error lies within 2 per cent at a transmission of 50 per cent at 260 m μ (in the direction of higher transmission values).

In the second type of experiment it is interesting to note the high values of dichroism in freshly made

fibres. The deviation or error calculated from these values will be 6 per cent at 50 per cent transmission. At lower transmission values, however, the deviation will be considerable.

In cytological material (with few exceptions, for example, sperm heads), the dichroism of nucleic acid generally is very low¹, detectable only with specially designed instruments⁶. Thus, it seems clear that the effect of molecular orientation on the cellular nucleic acid absorption of unpolarized light does not introduce any serious error, such as has been assumed^{2,3}.

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¹ Caspersson, T., *Chromosoma*, **1**, 605 (1940).

² Commoner, B., and Lipkin, D., *Science*, **110**, 41 (1949).

³ Commoner, B., *Science*, **110**, 31 (1949).

⁴ Pollister, A. W., and Swift, H. H., *Science*, **111**, 68 (1950).

⁵ Wilkins, M. H. F., Faraday Society, September meeting, 1950.

⁶ Ruch, F. (to be published).

⁷ Signer, R., *Trans. Farad. Soc.*, **32**, 296 (1936).

Applications of Multiple-Beam Interferometry

Two recent communications on applications of multiple-beam interferometry, one by Bruce¹, the other by Bruce, Macinante and Kelly², call for critical comment.

Bruce has described two applications of transmission-like multiple-beam interference fringes in reflexion. These are applied to (a) the method I have already described³ for measuring the thickness of thin films, and (b) the interferometric method for measuring oscillations described by Tolansky and Bardsley in 1948^{4a}.

Doubtless this modification has some advantages in very limited cases; but it will be of interest to find whether the method used (which, incidentally, is not fully described) can really be extensively employed in the examination of the complex topographies now so frequently under examination with multiple-beam interferometry. Although the application is of interest, the principle of the method of converting reflexion into transmission-like fringes by cutting out beams is not at all new. It was, of course, first discussed in detail by Otto Lummer fifty years ago⁵. I have long been familiar with this, having taught it to a succession of students for more than fifteen years, and having used it in interferometrical studies occasionally, though not to very much advantage. In simple low-magnification cases it is not difficult to cut out individual beams; but with the higher-power microscopic observations now often used the

Experiment	Orientated molecules					Unorientated molecules. Transmission (v) of unpolarized light (260 $m\mu$)		Per cent deviation ($\frac{v-u}{v} \cdot 100$)
	Dichroism Transmission (260 $m\mu$)		Extinction quotient	Transmission (u) of unpolarized light (260 $m\mu$)				
	$a = \frac{I_a}{I_0}$	$b = \frac{I_b}{I_0}$		measured	calc.	measured	calc.	
I (streaming)	0.11	0.06	1.3	0.09	0.082*	0.09	0.078*	5
II (fibres)	0.72	0.36	3.2	0.53	0.54	—	0.51	6
„	0.34	0.03	3.2	—	0.18	—	0.11	64

* Calculations give approximate values, as the molecules are not completely orientated by this method.