SUMMARY

1. Oral administration of a pure vitamin A preparation at a dose 400 times the normal requirement has been shown to produce effects such as reduced food intake, loss of weight, skeletal fractures and haemorrhages in young albino rats. The degree of toxicity is approximately proportional to the total quantity of vitamin A administered.

2. The effects of administration of large doses of vitamin A on the metabolism of calcium, phosphorus and nitrogen have also been studied. A negative balance for calcium, phosphorus and nitrogen sets in and continues for a considerable period after the administration of large doses of vitamin A has ceased.

3. No changes were detected in the levels of calcium and inorganic phosphorus of the blood.

4. Very little change in the relative mineral composition of the bones was observed. Increased excretion of calcium and phosphorus is therefore probably due to thinning of the bones.

5. Estimation of vitamin A in the liver suggests that administration of amounts of vitamin A, several times greater than the normal requirements, reduces the absorption of vitamin A from the intestine.

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An Improved Method for Measuring Degree of Polarization

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Measurements of the depolarization of fluorescence have recently been used for determining the rotational relaxation times of protein molecules (Weber, 1952*a, b*), and they provide a technique of considerable flexibility and usefulness. Such work requires a high degree of accuracy in the measurement of percentage polarization, which is

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not readily achieved by the methods hitherto described (Perrin, 1929; Lewis, Lipkin & Magel, 1941; Lewis & Bigeleisen, 1943). In connexion with a recent study (Harrington, Johnson & Ottewill, 1956), an improved apparatus has been devised, based upon a method first proposed by Lyot (1929) and later improved by Wright (1934). Its advantages depend upon the principle of matching the intensity and position of a fringe system in two halves of a divided field, rather than judging the

Fig. 1. Diagrammatic representation of polariscope.

complete disappearance of the fringes. Subjectivity and eye strain have thus been drastically reduced, yet the apparatus remains simple and much less expensive than alternative electronic methods (Wille, 1949).

APPARATUS

The apparatus was constructed of three tubular, optically blacked, brass sections, T_1 , T_2 and T_3 , with dimensions as given in Fig. 1. The plano-convex collimating lens L_1 was of focal length 5 cm. and was fixed with respect to T_1 . It was followed immediately by a stop 1 cm. in diameter. The centre section T_2 contained the two compensators C_1 and C_2 , mounted on thin annular brass rings, with respective internal diameters of 2.5 and 2.3 cm. Microscope coverslips of Chance glass were cemented to both surfaces of C_1 , which could be rotated about a vertical axis (in the plane of the diagram) by means of a Muirhead slow-motion dial graduated from 0° to 270° . A vernier allowed angular positions to be read to 0.1° . Only one coverslip was mounted on C_2 , whose axis of rotation, provided with a 'click-stop' at the zero position, was at 45° to that of C_1 . The biplate Q was constructed from two quartz sections, one laevorotary and the other dextrorotary, cut normal to the optic axis and cemented side by side to give a divided photometric field, in which the straight junction line was fine and sharp. It was mounted between C_1 and C_2 , with its junction line perpendicular to the axis of C_2 and in a plane perpendicular to the axis of T_2 . The thickness of the quartz plates was chosen to be 1.76 mm. in order to give rotations for the Hg green line (λ 5461 Å) of $+45^\circ$ and -45° . Lens L_2 , of focal length 3 cm., was mounted in an extension tube T_3 . A Savart plate S , of such dimensions (1 cm.²) that it gave an angular separation of its interference fringes of 1.2' of arc (Wright, 1934), was mounted (at the end of a rocking arm) directly behind L_2 , and was followed by a Nicol prism N (of square cross-section, 7 mm. side). The tube T_3 could be adjusted inside T_2 , and in turn the Nicol prism could be rotated inside T_3 . The three-dimensional disposition of components may be seen in Fig. 2.

In setting the Nicol prism with respect to the Savart plate, a distant plane-polarized source was viewed through T_3 , from which L_2 had been removed. The tube T_3 was then rotated about its axis (and if necessary N with respect to S) until fringes were obtained. Finally, N was rotated inside T_3 until the best contrast was achieved. L_2 was then replaced in T_3 and the latter adjusted in T_2 until focused on the nearer surface of the biquartz plate. With vertically

Fig. 2. Photograph illustrating three-dimensional disposition of components.

polarized incident light on stop B and compensators C_1 and C_2 in the zero position, T_3 was then rotated about its axis until the fringes were clear and perpendicular to the junction line of the biquartz.

Optical principles

Assume O is a source of partially plane-polarized light, with the electric vector vertical. By means of stop B and the lens L_1 a parallel beam is obtained through the compensator C_1 . The difference in amplitude of the two perpendicular vector components in the beam could therefore be accurately compensated by rotation of C_1 (which preferentially reflects the vertically vibrating component). The angular setting of the compensator necessary to achieve unpolarized light depends on the percentage of plane polarized light in the incident beam and requires calibration (see below).

In the absence of the biquartz Q and compensator C_2 [as in the apparatus of Weber (1952*a, b*)] the passage of plane-

polarized light through C_1 is indicated by the observation of typical Savart interference bands, continuous across the whole field. As C_1 is adjusted towards complete compensation (i.e. giving unpolarized transmitted light), the fringes gradually disappear. Such an end point is not in general satisfactory; by introducing the biquartz and compensator C_2 it is replaced by one involving the matching of illuminated halves of the field.

If now C_2 be moved from its zero position (C_1 still giving complete compensation), it imparts a certain small degree of polarization over the whole field, and the fringes in the two halves match in both intensity and position. On the other hand, if C_1 has not given complete compensation, the plane-polarized vector will be rotated $+45^\circ$ in one section of the biquartz and -45° in the other (λ 5461 Å). In passing through C_2 , the proportion of polarized component in one half of the field will then be increased, and in the other half decreased, so that the fringes observed through T_2 will no longer match in intensity (because of the difference in degree of polarization) or position (because of the different vibration directions). The compensator C_1 must then be adjusted until matching is complete, and this is facilitated when the fringes are perpendicular to the dividing line of the biquartz. Since the eye is most discriminating when the level of illumination is low, high sensitivity is largely dependent on the introduction of a very small amount of polarization by C_2 .

CALIBRATION OF THE APPARATUS

The instrument was calibrated by means of a Watts Standard spectrometer. A 125w mercury arc source was employed, the light of which was diffused by a plate of opal glass, and then made monochromatic by means of an Ilford filter no. 807 (λ 5461 Å). The light through the collimating tube was stopped down at the exit end with a 6 mm. circular stop, giving an unpolarized parallel beam across the spectrometer table. A single glass plate mounted vertically and symmetrically on the spectrometer table was used to obtain transmission polarization. The vertical placing of the glass plate was checked by reflecting the source slit into the spectrometer telescope at various angles. The angle of the plate with respect to the line of the collimator and telescope tube was determined by means of the usual spectrometer techniques. The telescope lenses were then removed and the polarization apparatus was fixed rigidly and carefully as a continuation of the telescope tube. It was then rotated about its own axis until the axis of the first compensator C_1 was perpendicular to that of the glass plate on the spectrometer table. By rotating the spectrometer table through various angles, a variable amount of polarization was introduced into the light beam transmitted through the glass plate. The percentage of plane-polarized light transmitted by the plate was computed from the Fresnel formulae, multiple reflexions being taken into account (Wright, 1934).

The use of undeviated transmitted light from a single glass plate for carrying out the calibration instead of reflected light from the front face of a prism was advantageous, since in the latter case rotation of the polarization apparatus was required. It can be seen from the application of the Fresnel formulae that for a single glass plate a change in polarization for transmitted light of from 0 to 27% involves an angular change of the plate from 0 to 70° . For reflected light from a prism, the angular change for the same polarization range is from 0 to 10° , and considerable accuracy in angular settings was required.

It was necessary to measure accurately the index of refraction, n_g , of the glass plate in order to compute the percentage polarization by the Fresnel formulae. Although the determination of the refractive index of very thick plates and very thin films is standard procedure, an accurate measurement of n_g for thin plates of approx. 1 mm. is not easily obtained by conventional methods. A method employing a Zeiss interferometer for this measurement was therefore devised.

After calibration of the polariscope, the plate which was used as a source of polarization was cut, fitted into one compartment of the interferometer cell and mounted vertically, with the plane of the plate normal to the light-path. A series of sucrose solutions of varying concentration was prepared and the index of refraction of each determined with an Abbé refractometer to ± 0.0002 . The fringe displacement was observed for each sucrose solution (both compartments containing the same solution). The equation for the interferometer of the Zeiss type is

$$ln - ln_s = f_0 \lambda, \quad (1)$$

where l is the path length of the double cell, f_0 the number of fringes corresponding to the scale reading for the zero-order band, λ the wavelength of light employed, n the index of refraction of the solution, and n_s that of the solvent.

Fig. 3. Determination of refractive index of polarizing glass plates of different thickness.

With the glass plate in position and the same sucrose solution in both compartments of the interferometer, equation (1) may be written

$$(l-d)n_0 + n_g d - l n_0 = f_0 \lambda,$$

or

$$n_0 = n_g - f_0 \lambda / d, \quad (2)$$

where n_g is the refractive index of the glass plate, $\frac{1}{2}d$ its thickness and n_0 is the refractive index of the sucrose solution. Thus a plot of n_0 against f_0 gives the refractive index of the plate as the intercept on the n_0 axis (Fig. 3).

RESULTS

In Table 1 is presented a typical set of results, obtained with the polariscope, on a sample of bovine serum albumin which had been coupled with the fluorescent dye 1-dimethylaminonaphthalene-5-sulphonyl chloride. The high degree of

reproducibility can be clearly seen, indicating that measurements of the percentage polarization of fluorescence can be made with a standard deviation less than 1% of the percentage polarization. Results with the apparatus have already been published (Harrington *et al.* 1956).

SUMMARY

An improved apparatus for measuring the degree of polarization, and its calibration by means of the light transmitted at varying incidence by a single glass plate, are described.

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Table 1. *Results obtained on bovine serum albumin conjugate with the polariscope* (pH, 7.3; I , 0.05)

Temp.	Polarization (%)			s.d.
		Mean		
4.8°	17.4, 17.1, 17.2, 17.2	17.23	0.11	
11.3	16.9, 17.0, 16.7, 16.9	16.88	0.11	
16.6	16.5, 16.3, 16.2, 16.2	16.30	0.12	
22.9	15.5, 15.4, 15.5, 15.6	15.50	0.07	
27.7	14.5, 14.7, 14.8, 14.6	14.65	0.11	
33.6	14.4, 14.4, 14.5, 14.4	14.43	0.04	
40.2	13.3, 13.4, 13.6, 13.5	13.45	0.11	

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