By this method, full-scale deflection was easily obtained with 0.05 μg adrenaline and 0.2 μg noradrenaline, a sensitivity well below that necessary for determination of the levels expected in plasma. Mixtures of adrenaline and noradrenaline yielded values of fluorescence that were equal to the sums of the individual components.

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Schwarzschild-Villiger Effect in Microspectrophotometry

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It has been suggested by Naora (*1, 2*) and Naora and Sibatani (*3*) that the microspectrophotometric data hitherto published are unreliable. Owing to internal reflections in the magnifying optical system, the light passing through the parts of the field adjacent to the object causes stray light that is added to the light passing through the object, so that the measured value of the transmittance is altered. This effect, described first by Schwarzschild and Villiger (*4*) in the microdensitometry of photographic images of the sun, may be avoided by illuminating only an area restricted to the part of the object to be measured, instead of illuminating the whole field of the instrument.

In the microspectrophotometer devised by Naora, a part of the field 1–5 μ in diameter is illuminated by means of an inverted microscope identical to that used for magnifying; the actual illuminated field is the image of an iris diaphragm of variable diameter (2–10 mm) reduced to 1/2000 by the illuminating microscope. Measurements made with this instrument on Feulgen-stained material yielded values of extinction much higher than those obtained by authors working with other microspectrophotometers. According to Naora, the magnitude of the flare light caused by the Schwarzschild-Villiger (S-V) effect is about 13.5% in the optical system commonly used in microphotometric equipments. As this flare light acts additively in the measurement of the transmittance, the relative error may be extremely high when making measurements on objects of low transmittance, attaining up to 10–200 times the true value. The theoretical bases of the calculations of Naora have been criticized by Ornstein and Pollister (*5*), who showed that the error must be much less than assumed by Naora. However, their paper contains no quantitative data nor experimental evidence.

As the magnitude of the S-V effect may be largely influenced by the construction and the actual working conditions of the instrument, we attempted to check the histophotometer that we constructed some years ago (*6*), and used in a number of investigations (*7–10*). An instrument free of S-V effect must give a transmittance value of zero when measuring wholly opaque objects. A selection was made of test objects satisfying the following conditions: to be opaque, to have the same size and the same optical properties (refractive index, etc.) as the objects used in the former biological investigations (nuclei), and to be distributed in the field with an analogous density. Blood cells (erythrocytes and lymphocytes) heavily overstained with iron hematoxylin were found suitable, either in smears or in paraffin sections; particles of lamp black 1–20 μ in diameter in smears mounted in Canada balsam also proved useful.

The histophotometer was used under the same optical conditions as in previous investigations: Wild's fluorite oil immersion objective 50 ×, N.A. 1.00 compensating ocular 10 ×, total magnification on the screen 2000 ×, condenser N.A. 1.20 used without immersion, giving a working N.A. of approximately 0.90, Köhler illumination, field diaphragm closed to give an illuminated area 150 μ in diameter, aperture diaphragm fully opened. The measurements consistently gave an apparent transmission of 2% for the test objects and even large changes in the conditions of illumination proved unsuccessful to bring out lower values. As the true transmittance of the test object is probably not exactly null, but only very small, it may be concluded that the value of the flare light due to the S-V effect is at most 2%. If I_0 is the intensity of light measured on the blank, I the intensity of light measured after passing through the specimen, and F the amount of flare light which acts additively in each measurement,

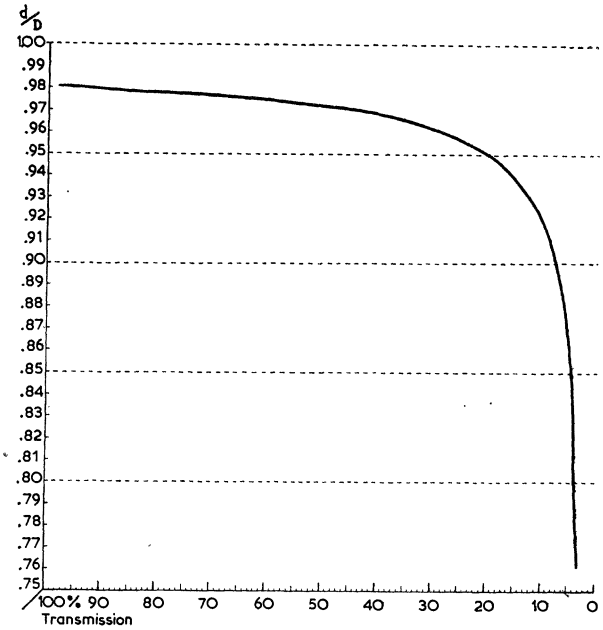

FIG. 1. Ratio d (density found)/D (true density), when measuring with an instrument giving 2% flare light.

the true optical density D of the object is: $D = \log (I_0 - F) - \log (I - F)$.

If the calculation is made in the usual way, taking no account of the flare light, a false value d of the optical density is obtained $d = \log I_0 - \log I$. In the graph, the values of the ratio d/D have been calculated at each apparent transmission value ($T = I/I_0$) for an amount of 2% flare light. It is obvious that the values of d are in any case too low, and that the error increases rapidly with the density of the object (Fig. 1).

When comparing the densities of different objects, the effective error of the ratio of their densities is much lower than the errors of the individual measurements. We have calculated the relative error committed when comparing an object of (apparent) transmission 90% with objects of different transmissions. Up to transmissions of 45%, the relative error is less than 1%; for objects of transmission 40, 30, 20, and 10%, the error is respectively 1.12, 1.82, 2.96, and 5.15%. In previous determinations made with our instrument, most of the transmission readings ranged between 85 and 25%; and readings less than 15% were encountered in few cases only. Under these conditions, the validity of the measurements does not appear to be enhanced by the S-V effect.

It is worth mentioning that other optical systems may give rise to much greater S-V effects. Two apochromatic oil immersion objectives 100×, N.A. 1.30 and 1.40 (not used in actual histophotometric measurements) tested under the same conditions showed about 5% of flare light. The use of such objectives needs correction for flare light when measuring objects of low transmittance; the calculations may be done with the above formula.

In experiments made with his illuminating system, Naora observed a progressive decrease in the transmission values of Feulgen-stained nuclei when the field diaphragm was reduced from 650 to about 1 μ. Naora admitted that the true value for the transmission is the lowest one. This statement may not be true. A field diaphragm acts as such only when the conditions of Köhler illumination are strictly adhered to, i.e., first, the light source must be imaged in the back focal plane of the objective; second, the lamp condenser must be imaged in the plane of the object; third, the plane of the field diaphragm must coincide with the plane passing through the optical center of the condenser lens system; fourth, the lamp condenser must be aplanatic. If those conditions are not met, the field diaphragm acts also as an aperture diaphragm. When an aperture diaphragm is closed, the phase changes in the object are partially rendered[1] as amplitude changes. As most stained biological objects are known to be really a mixture of phase and amplitude details (*11*), it is doubtful whether the transmittance measured in the Naora instrument with the diaphragm fully closed is the true transmittance.

[1] For this reason, we use a condenser aperture just a little under the objective aperture. Some authors (Swift, Ornstein, and Pollister) prescribe small condenser apertures for histophotometric measurements in order to reduce *flare*. Although the magnitude of the error committed in reducing the aperture of the condenser is not known, it seems probable that a moderate reduction (up to 2/3 of the objective aperture) is not objectionable. However, in our test experiments, closing the aperture diaphragm of the condenser to this value did not reduce appreciably the magnitude of the S-V effect.

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Excretion of Heptachlor Epoxide in Milk of a Dairy Cow Fed Heptachlor

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It has been reported that dogs and rats fed heptachlor (1 or 3a,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene), a new insecticide and also one of the components of technical chlordane, store in their fat a metabolically altered epoxide derivative of this compound (*1, 2*). Because of these findings it was considered desirable to determine whether heptachlor or the heptachlor epoxide is excreted in the milk of a cow following oral administration of heptachlor.

Capsules of heptachlor dissolved in corn oil were administered to a cow at the rate of 3 mg/kg/day, divided into two equal doses, for 14 days. Milk yields and samples for chemical analysis consisting of combined aliquots of the morning and evening milking were taken one week before the administration of the heptachlor and at frequent intervals thereafter during its administration, and for 2 months following its discontinuance.

The separation of the butterfat from the milk is based upon the technique of Frawley and Davidow (*3*), the analysis for heptachlor upon a test developed originally for the determination of chlordane (*4*), the analysis of heptachlor epoxide upon the method described by Radomski and Davidow (*2*). The heptachlor epoxide necessary for the preparation of the standard concentration curve was isolated from the fat of a dog fed heptachlor. Since the completion