glaze of mucus lining them. This glaze often effectively prevents the lateral seepage of the liquid latex and confines it to a single system of tunnels.

In contrast, the tunnels of ants have surfaces that are greatly "nibbled". It is apparent, also, that ants may utilize the upper part of an earthworm tunnel shaft as the entrance to their own nests. The mucus lining of earthworm tunnels seems to serve as a culture medium for fungi that attract ants. The upper portions of an earthworm tube become enlarged and roughened by the action of ants.

In addition to making possible the casts of the tunnels of subterranean animals, the latex method has been used to reveal the nature and extent of shrinkage cracks in dry seasons. It has shown in three dimensions the nature of continuous, interconnected voids in soil and the quality of the channels by which soil and water may move from one horizon to another.

It is believed that this technique offers further possibilities for the better understanding of the impacts of animals on soil formation and modification, and of the nature of the larger interconnected voids in the soil (Fig. 1).

Manuscript received June 10, 1953.

The Quantitative Determination of Adrenaline and Noradrenaline in Mixtures[1, 2]

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A method for the determination of "total adrenaline-like substances" in blood has recently been reported (*1*). The method is based on the fluorimetric determination of the reaction product obtained by the condensation of the catechol amine with ethylenediamine (*2*). The 436-mμ line of a mercury arc was used for excitation and the total fluorescence above 500 mμ was measured. By this technique, the two principal sympathetic substances present in the body, adrenaline and noradrenaline, could not be differentiated in mixtures. Each substance, however, formed a separate condensation product with ethylenediamine, the adrenaline derivative fluorescing with five times the intensity of the noradrenaline analog.

The difference in fluorescence emission between these two compounds suggested that the fluorescence spectra of these substances might differ in shape. In order to test this hypothesis, the fluorescence spectrum of each condensation product was determined for 2 exciting frequencies: the 365-mμ and 436-mμ lines of the mercury arc. The apparatus employed was a model DU Beckman spectrophotometer modified to function as a fluorescence spectrophotometer in a fashion described elsewhere (*3*). The results are shown in Fig. 1.

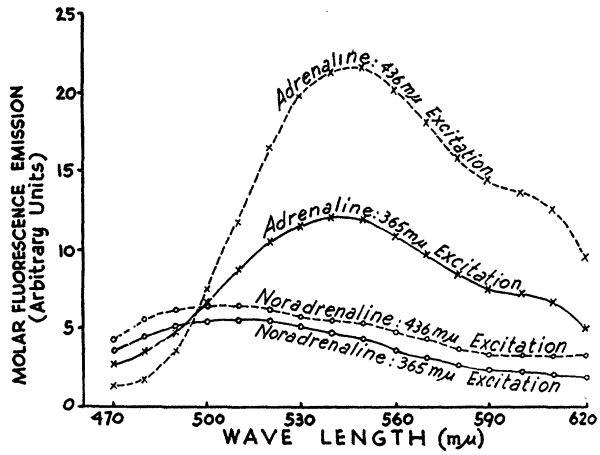

FIG. 1. The fluorescence spectra of the ethylenediamine condensation products of adrenaline and noradrenaline obtained with 2 exciting frequencies. The spectra were obtained with concentrations of 5.5×10^{-9} mole/ml and 21.9×10^{-9} mole/ml of adrenaline and noradrenaline, respectively.

It is seen that the adrenaline peak emission is further toward the red end of the spectrum than the noradrenaline peak by 30 mμ and 50 mμ for the 365-mμ and 436-mμ excitations, respectively. The intensity of fluorescence of the adrenaline derivative at its peak emission is 2.2 and 3.5 times greater than that of the noradrenaline for the 365-mμ and 436-mμ excitations. It is to be noted that the fluorescence emission of the adrenaline derivative on excitation with the 436-mμ line is almost twice that obtained with the 365-mμ line, whereas the noradrenaline analog shows no such increase with change of exciting sources. These differences in spectral characteristics provide a basis for the quantitative differentiation of these two important hormones in biological mixtures.

The technical aspects of the analytical differentiation were simplified by employing a Farrand photoelectric fluorometer. The primary consisted of Corning filters No. 5113 and 3389 which pass the 436-mμ mercury arc line. Two different secondaries were employed, the first consisting of Corning filters No. 5433 and 3384 which pass a narrow band peaking in the region of 510 mμ and the second consisting of Corning filter No. 2418 which cuts off below 600 mμ. These particular filters were employed because they yielded maximum differentiation between the adrenaline and noradrenaline derivatives and minimum blanks. The fluorescence of the mixture was recorded with each secondary filter combination and 2 simultaneous linear equations were solved for the content of adrenaline and noradrenaline in the mixture. The coefficients of the equations were determined from separate measurements of standard solutions of adrenaline and noradrenaline in the same fashion.

[1] This work was supported in part by the Flora Warner Lasker Memorial Research Fund.

[2] We wish to thank Oliver H. Lowry of the Department of Pharmacology, Washington University School of Medicine for allowing us to use his fluorescence spectrophotometer and for advice and assistance in this study.

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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Manuscript received June 8, 1953.

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,

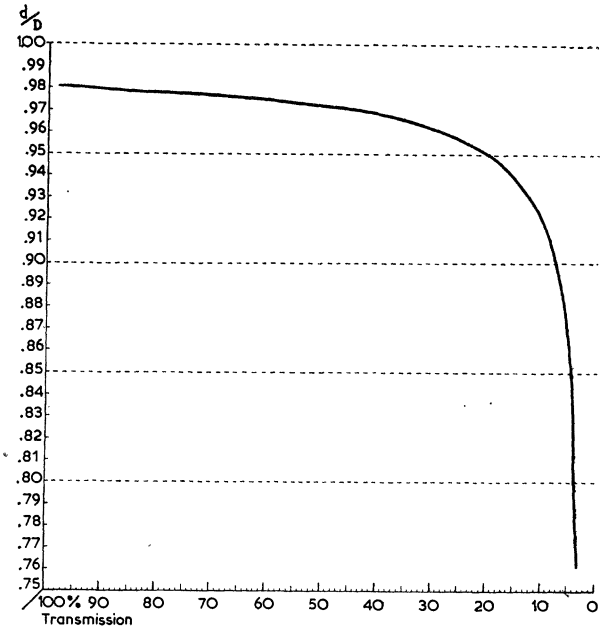

FIG. 1. Ratio d (density found)/D (true density), when measuring with an instrument giving 2% flare light.