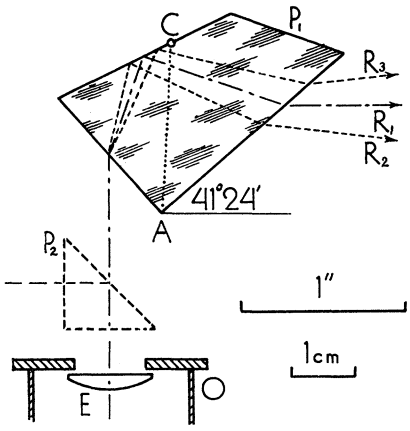

Fig. 1. Arrangement of a postmonochromatizing, right-angle deviation prism above the microscope. *O*, ocular; *E*, eye lens; P_1, quartz Pellin-Broca prism in position for wavelength of $\lambda = 253.6$ mμ; *C*, axis of rotation; *AC*, bisector; R_1, optical axis referred to the "nominal" wavelength; R_2, R_3, rays of longer and shorter wavelengths, respectively, which do not reach the sensitive cathode of the detector; P_2, auxiliary removable prism for visual observation through a willemite screen placed to the left of P_2.

identical monochromator, then one obtains light of 99.96 percent purity at the second exit slit.

At the University of Kansas Medical Center, a singly monochromatized double-beam microspectrophotometer, originally built by Wiseman (*11*) and Stowell (*12*), was converted into a doubly monochromatized one without extensive modification in the general layout of the instrument. The original instrument included a totally reflecting quartz prism mounted above the microscope, to direct the emerging light beam to the photomultiplier.

The necessary right-angled bend in the microscope beam can be obtained by use of a Pellin-Broca prism (*9*) that combines both the constant deviation of the 45° prism and the dispersive properties of a standard 60° prism. Rotating this Pellin-Broca prism about a particular axis brings radiation of any wavelength into the detector (Fig. 1).

Therefore, in our modifications, the original prism was replaced by a quartz prism after the method of Pellin and Broca. Because this was a double-beam system, the control beam, too, had to be doubly monochromatized. It is essential that the two prisms be as similar as possible and that they be rotated through exactly the same angle. To attain this objective, an aluminum angle, carrying both prisms, was mounted above the microscope. It was pivoted on either end, the pivot points representing the axis of rotation of the prisms. Also, the beam splitter was turned so that both the microscope and the control beam follow optical tracks which are parallel to each other.

To control the tilt of the two prisms, an attached lever was fitted with a ball-bearing follower that engaged a rotatable cam. This cam was so constructed that the graduation of the wavelength scale became linear. This arrangement facilitates the eventual coupling of the two monochromator controls.

As a result of these alterations, the control beam multiplier was raised to the level of the microscope beam detector. The two multipliers could thus be housed in a common, evacuable compartment. The wire lead feeding the multiplier outputs to the control grid of the first amplifier stage could thus be made of minimal length. This change made possible the elimination of occasional drifts of the microammeter, which could occur even when the multipliers were not exposed to light. It must be realized that a grounded metal cabinet containing a sufficiently charged lead is, in principle, nothing but an air-filled ionization chamber. The sporadic readings obtained when such a cabinet is used are evidently due to cosmic or other highly penetrating radiations. Deck (*3*) also reported such phenomena, and a number of workers demand evacuated compartments for photocells or multipliers, and for the first amplification stage as well.

The performance curve was taken according to specifications of the National Bureau of Standards (*13*). The measured transmittances of the K_2CrO_4 solution fit the standard curve to within 2 to 3 percent of the full-scale value except at the ends of the useful range (220 to 600 mμ), where the response of the multipliers drops to zero. In addition, toward shorter wavelengths, the transmission of the optical components diminishes because of the rapidly growing opacity of the quartz, the Vycor, and the Corning 9741 glass that makes up the envelopes of the multipliers (*14*).

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References and Notes

1. H. V. Halban and J. Eisenbrand, *Proc. Roy. Soc. (London)* A116, 153 (1927).
2. T. R. Hogness, F. B. Zscheile, A. E. Sidwell, *J. Phys. Chem.* 41, 379 (1937).
3. W. Deck, *Helv. Phys. Acta* 9, 3 (1938).
4. F. P. Zscheile, *J. Phys. & Colloid Chem.* 51, 903 (1947).
5. K. S. Gibson, *Natl. Bur. Standards (U.S.) Circ. No. 484* (1949).
6. T. Caspersson, *Cell Growth and Cell Function* (Norton, New York, 1950).
7. R. L. Sinsheimer, thesis, Massachusetts Institute of Technology (1942).
8. P. M. B. Walker *et al.*, *Photoelec. Spectrometry Group Bull. No. 8* (1955), p. 192; *Discussions Faraday Soc. No. 9* (1950), p. 461.
9. G. R. Harrison, R. C. Lord, J. R. Loofbourow, *Practical Spectroscopy* (Prentice Hall, New York, 1948), pp. 56, 399, 406.
10. G. Kortüm, *Kolorimetrie, Photometrie und Spektrometrie* (Springer, Berlin, 1955), p. 321.
11. G. G. Wiseman, thesis, University of Kansas (1950).
12. R. E. Stowell, *Lab. Invest.* 1, 129 (1952).
13. *Natl. Bur. Standards (U.S.) Circ. No. 1017* (1955).
14. This project was carried out, in part, under Atomic Energy Commission contract AT (11-1)-263. The advice and criticism of Robert E. Stowell are gratefully acknowledged.
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24 December 1957

Interaction between Allithiamine and Metal Enzyme

It is well known that reducing substances such as sulfhydryl compounds convert "allithiamine," a derivative of vitamin B_1, into allyl disulfide and thiaminyl thiol, in vitro as well as in vivo (*1–4*). It has been pointed out recently by Honda (*4*) that ascorbic acid, although it is a strong reducing agent, does not reduce allithiamine.

The reducibility of allithiamine by sulfhydryl compounds has been made the basis for the microdetermination of sulfhydryl groups in the blood of metamorphosing insects (*5*). In the course of this study (*6*) it was found that melanin formation in the blood of silkworms, *Bombyx mori* and *Philosamia cynthia ricini*, was retarded remarkably in the presence of allithiamine. Melanin formation was markedly suppressed when 0.5 ml of the blood of pupating worms was mixed with 3 ml of phosphate buffer (*p*H 5.91) and with 1 ml of allithiamine (250 μg/ml) at room temperature, whereas it occurred immediately in the control in which allithiamine was replaced by distilled water or thiamine (250 μg/ml), as is shown in Fig. 1.

Measurement of oxygen uptake confirmed the visual observation that allithiamine suppresses melanin formation. The degree of retardation by allithiamine of melanin formation seems to correspond to the concentration of sulfhydryl groups in the blood (*5*).

It is known that melanin formation is inhibited by sulfhydryl compounds such as cysteine in vitro as well as in vivo (*7*). But this inhibition in the blood of the present materials was not so strong because melanin formation took place in the control. Therefore, the remarkable suppression of melanin formation observed in the present experiment may be due mainly to the formation of thiaminyl mercaptide by the reaction between cupric tyrosinase and the sulfhydryl group of thiaminyl thiol derived directly from allithiamine.

Coincident with the reduction of allithiamine, two substances will be pro-

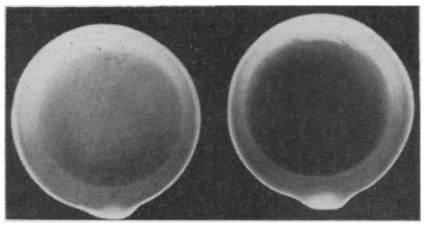

Fig. 1. Melanin formation in blood of *Philosamia cynthia ricini*. (Left) Retarded melanin formation by allithiamine; (right) normal melanin formation.

duced: allyl mercapto radical (CH_2=CH—CH_2—S·) and thiaminyl thiol [(B_1)—SH]. The former is well known to combine with sulfhydryl (SH) groups in vivo (*2, 4, 8*), so that it is probably the latter which will combine with cupric tyrosinase according to the following equation:

$$(B_1)\text{-S-S-}CH_2\text{-CH=}CH_2 + R\text{-SH} \xrightarrow{\text{(metal enzyme)}}$$

allithiamine; SH group in blood

$$R\text{-S-S-}CH_2\text{-CH=}CH_2 + (\text{Metal enzyme})\text{-S-}(B_1)$$

allyl disulfide; thiaminyl mercaptide

This interpretation of the above results may be applicable to the reaction between allithiamine and other metal enzymes in vitro as well as in vivo (*9*).

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References and Notes

1. M. Fujiwara, H. Watanabe, K. Matsui, *J. Biochem. (Tokyo)* **41**, 29 (1954); M. Fujiwara *et al., ibid.* **41**, 273 (1954); M. Fujiwara, M. Yoshimura, S. Tsuno, *ibid.* **42**, 591 (1955).
2. Y. Sahashi and H. Shibasaki, *J. Agr. Chem. Soc. (Japan)* **25**, 57 (1951).
3. T. Tamaki, *Vitamins* (Japan) **7**, 352 (1954); H. Nanjo, *ibid.* **9**, 290 (1955).
4. M. Honda, *Japan. J. Hyg.* **11**, 38 (1956).
5. M. Kato, unpublished results.
6. This work was aided by a grant from the Ministry of Education of Japan. I wish to thank Dr. M. Ichikawa, Dr. Z. Yoshida, and Dr. M. Fujiwara for their assistance.
7. A. B. Lerner, *Advances in Enzymol.* **14** (1953).
8. M. Fujiwara and T. Matsukawa, *Vitamins (Japan)* **6**, 857 (1953).
9. E. Martell and M. Calvin, *Chemistry of the Metal Chelate Compounds* (Prentice-Hall, New York, 1952); M. Kato and Z. Yoshida, in preparation.

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Effect of Light on Fluorescence of Ethylenediamine Derivatives of Epinephrine and Norepinephrine

In 1953 Weil-Malherbe and Bone (*1*) described a procedure for the differential estimation of epinephrine and norepinephrine in mixtures. They found that, after incubation with ethylenediamine, the fluorescence of these two compounds differed enough so that at 500 mμ the ratio of the fluorescence of epinephrine to norepinephrine was 0.98 whereas at 550 mμ it was 4.4. The solutions appeared to be stable for 24 hours at room temperature under ordinary light conditions. Persky and Roston (*2*) published curves representing the emission spectra of these substances which were in substantial agreement with these findings.

Studies conducted by Aronow and Howard (*3*) and studies made in our own laboratory (*4*) suggested that entirely different ratios were obtained when the procedure was carried out in a room illuminated by red or orange light. The difference appeared to be due to changes in the intensity and distribution of the norepinephrine fluorescence. Crude experiments performed at that time indicated that about 80 percent of the fluorescence of the norepinephrine was lost if the reaction was carried out in daylight. When daylight was excluded, the ratio of epinephrine to norepinephrine at 485 mμ was approximately 0.2 and at wavelengths above 550 it was about 3.2, when light at a wavelength of 436 mμ was used to activate the solutions. The effect of blue light was also evidenced by the decay during activation at 436 mμ in the Farrand photoelectric fluorometer, as noted by Valk and Price (*5*) and Mangan and Mason (*6*).

Mangan and Mason (*6*) have recently published curves for the fluorescent products of the above reaction which differ from those of Persky and Roston (*2*). It is the purpose of this report (*7*) to suggest that the differences described may be related to quantitative or qualitative differences, or both, in the exposure to light and, furthermore, that the exclusion of blue and ultraviolet light results in an emission spectrum differing from the previously published findings.

Measurements were made with the Aminco-Bowman spectrophotofluorometer. In order to obtain the entire emission spectrum between 400 and 600 mμ without interference from scatter by the source of activating light, high concentrations of the amines were employed. However, experiments with the concentrations employed by Mangan and Mason produced similar results except for the presence of a significant blank at the lower concentrations.

Duplicate samples of epinephrine and norepinephrine (*8*) were prepared in alumina-treated acetic acid. One of the samples of each amine was incubated with ethylenediamine and ethylenediamine dihydrochloride for 40 minutes at 50°C. After saturation with sodium chloride, the solutions were extracted with isobutanol. The final extract contained the equivalent of 12 μg of epinephrine or norepinephrine per milliliter. The entire procedure was carried out in a room illuminated by daylight. The duplicates were carried through the same procedure in a room illuminated only by a 25-watt ruby darkroom lamp.

Samples were read at intervals of 5 mμ on the spectrophotofluorometer, and the galvanometer readings are illustrated in Fig. 1. The spectra were obtained by irradiating the samples at the wavelengths producing maximum activation; this area was found to lie between 420 and 425 mμ for norepinephrine and between 430 and 435 mμ for epinephrine. When both solutions were activated at 436 mμ, the relationship illustrated was slightly altered inasmuch as the relative fluorescence of norepinephrine was reduced by 7 percent. After the initial reading was taken of those solutions that were prepared in the darkroom, the cuvettes were exposed to daylight for 5 minutes in order to reduce the fluorescence of the norepinephrine sample to approximately 50 percent. The results of these experiments are illustrated in Fig. 1.

In Fig. 1, the upper left-hand curves were obtained from the solutions carried through the procedure in the dark. The middle curves on the left were obtained by rereading the same solutions after exposing them to daylight for 5 minutes. The lower curves on the left represent the galvanometer readings on the duplicates of the above samples which were carried through the procedure without excluding daylight. The similarity of the curves obtained from the second and third experiments to those of Mangan and Mason and of Persky and Roston is apparent. It can also be seen that daylight, in addition to causing a loss of fluorescence of norepinephrine, causes a small shift in the fluorescent maxima.

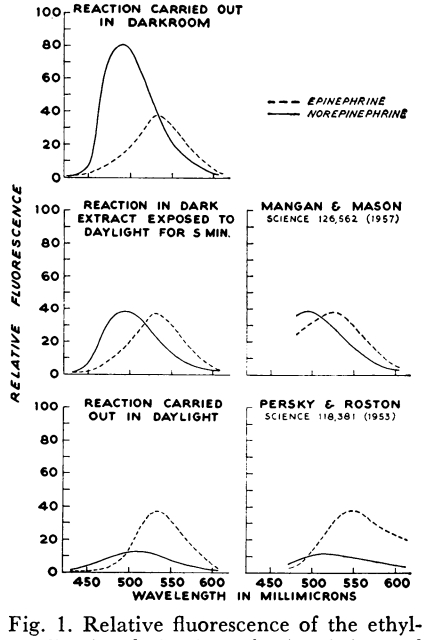

Fig. 1. Relative fluorescence of the ethylenediamine derivatives of epinephrine and norepinephrine.