Table 1. Oxygen consumption of *Habrobracon* exposed to 30 lb of oxygen.

Age (hr)	Stage of development	Oxygen consumed/μl hr 25 wasps		Oxygen consumed (percentage of amt consumed by controls)
		Air-exposed controls	Oxygen-treated	
80	Larva in cocoon	98 ± 6	92 ± 2	94
96	Larva in cocoon	98 ± 5	97 ± 5	99
112	Prepupa	55 ± 3	56 ± 2	102
116	Prepupa and white pupa	58 ± 2	39 ± 4	67
120	White pupa	53 ± 3	24 ± 7	45
144	White pupa	42 ± 4	4 ± 1	10
168	Pigmented pupa	37 ± 1	10 ± 1	27
192	Pigmented pupa	62 ± 6	47 ± 4	76

used routinely in our laboratory in order to arrest the development of pupae so that they can be used for various physiological studies. Although both a decrease in oxygen consumption and arrested development result from exposure of pupae to 30 lb of oxygen, it is not clear at present in what way, if at all, these two types of injuries are related. It appears from these data that injury from exposure to oxygen occurs with the onset of the white pupal stage and that there is a greater oxygen-sensitivity in older white pupae and in younger pigmented pupae than in wasps in the other stages of development.

These studies show that individuals of *Habrobracon* in certain stages of development are injured by oxygen while those in other stages apparently are unaffected. The reason for this stage-influenced sensitivity is not known and is a point of interest in continuing investigation.

It seems clear, however, that this difference in sensitivity is not due simply to a difference in the rate of metabolism. Although larvae are more active and also more oxygen-resistant than pupae, pupae whose rate of metabolism has been lowered by exposure to lower temperature (10°C) are more oxygen-resistant than pupae whose rate of metabolism is higher due to exposure to temperature of 26°C before treatment with oxygen (*2*). It is commonly known that organisms are more sensitive to oxygen when in a higher metabolic state than when their metabolism has been lowered. Adults of *Drosophila azteca* whose metabolic rate had been raised by elevation in temperature were more sensitive to oxygen poisoning than flies of lower metabolism (*4*). In rats, the lowering of metabolism by removal of the adrenal cortex reduces oxygen sensitivity, while the injection of adrenal cortex extract into adrenalectomized rats increases their oxygen-sensitivity (*5*).

If some basic cellular process is involved, it seems probable that the difference in sensitivity between larvae and pupae would be of a quantitative nature but that both would be affected. Since pupae are injured but larvae or prepupae are not, it appears that some oxygen-sensitizing process occurs in the pupal stage but not in the larval or prepupal stages. A comparison of metabolism at these various stages of development is required to determine the processes involved.

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2 January 1958

Double Monochromation in Ultraviolet Microspectrophotometry

Ultraviolet microspectrophotometers include, besides a light source and a detector (photocell or multiplier), a monochromator and a suitable ultraviolet microscope. To avoid excessive irradiation of the object, the monochromator is usually placed between the light source and the microscope.

Several difficulties had to be met in constructing a workable microspectrophotometer. Chromaticity of the optical system, such as is encountered in Zeiss monochromats, has been overcome by the use of catoptric or catadioptric objectives, as in the systems designed by the American Optical Company and by Bausch & Lomb. Fluctuations of the light source that produce absorptions or transmissions not related to the object have been minimized, either by stabilizing the sources or by introducing double-beam systems. Nonlinearity of the detectors has been corrected by using null methods—for example, by inserting a rotating variable sector into the light beam passing through the microscope. It is the reading of the sector aperture which gives the transmissivity in this case, and the detectors monitor constant intensities only.

And yet, even arrangements employing all these features work well only if certain additional conditions are fulfilled. The apparatus ought to be used in a spectral region where the output of the lamp is high and the detector exhibits a high sensitivity to the incident signal. These restrictions are necessary because of the stray light present when a single monochromator is used. At the exit slit, not only light of the "nominal" wavelength but also spurious scattered and reflected light, embracing the entire spectrum, is present. Such over-all random light may amount to several percent of the total radiation.

The random light results in a significant number of false readings in spectrophotometry and microspectrophotometry, especially when transmission measurements are made in a spectral region where there is little emission from the light source or when the response of the detector is weak, or when both these conditions exist. (It is immaterial whether this decrease in response is due to the characteristic of the sensitive surface of the detector or to a strong absorption in the optical train, including the envelope of the detector.) The situation is still further complicated if the specimen strongly absorbs at the nominal wavelength setting of the monochromator. Coincidence of some or of all of the factors listed necessarily results in serious errors when stray light, at or near the peak sensitivity of the detector, is not absorbed by the optical system, including the specimen and cell envelope. Under such circumstances Halban and Eisenbrand (*1*) recorded errors of up to 75 percent.

Hogness and his coworkers (*2*), Deck (*3*), Zscheile (*4*), Gibson (*5*), and others have established the conditions for precise spectrophotometry. Caspersson (*6*), Sinsheimer (*7*), and Walker and others (*8*) have established those of microspectrophotometry (see also Harrison *et al.*, *9*, and Kortüm, *10*). It is now generally acknowledged that double monochromation is essential for exact transmission measurements. If initially monochromatized light, containing 2 percent of stray light, is fed into a second,

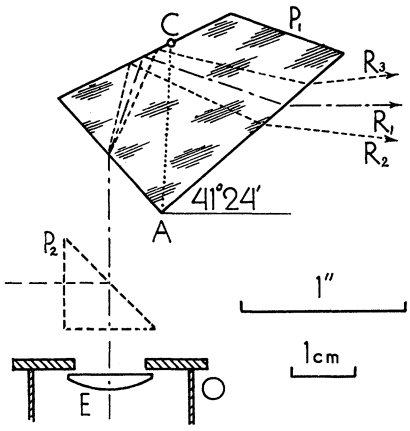

Fig. 1. Arrangement of a postmonochromatizing, right-angle deviation prism above the microscope. *O*, ocular; *E*, eye lens; P_1, quartz Pellin-Broca prism in position for wavelength of $\lambda = 253.6$ mμ; *C*, axis of rotation; *AC*, bisector; R_1, optical axis referred to the "nominal" wavelength; 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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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-