tion (*5*). This result strongly supports our conclusion that hydrogen peroxide is bound to catalase in the respiring cell.

Many other data support our identification of complex I; these include the gradation of the activity toward nitrite, formate, and ethanol, and the formation of the distinctive azide-catalase-peroxide complex.

By adding various formate concentrations to the respiring bacteria we find that roughly 2 mM formate halves the steady-state concentration of complex I. For this case, we have already shown that the steady-state hydrogen peroxide concentration can be computed (*6*). Our result for respiring *M. lysodeikticus* is about 10^{-8} *M* H_2O_2.

At such low peroxide concentrations, the "catalatic" decomposition of hydrogen peroxide into water and oxygen proceeds relatively slowly[3] because of the

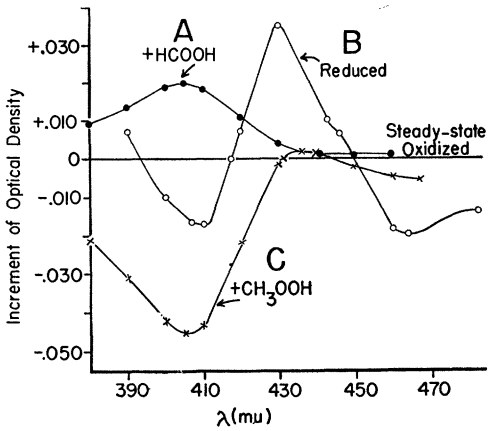

FIG. 2. Spectra representing changes in the optical density of a respiring suspension of *M. lysodeikticus*: (*A*) when 1.7 mM formate is added, (*B*) when the cytochromes are reduced by anaerobiosis, (*C*) when 67 μM methyl hydrogen peroxide is added. (Expt 924.)

peculiar mechanism of catalase action (*6, 8*). The addition of a suitable donor such as formate or nitrite causes catalase to act "peroxidatically" and thereby reduces the steady-state peroxide concentration. Thus the endogenous peroxide concentration may be regulated by the exogenous donor concentration.

On the basis of our *in vitro* studies of catalase action, Cole, Bond, and Fishler (*9*) have postulated that nitrite protects mice from x-radiation by reaction with complex I. And Hollaender has also shown the protection of bacterial cells from x-rays by formate, ethanol, and glycols (*10*). Our data on the *in vivo* interactions of catalase, hydrogen peroxide, and donor substances now give direct evidence in favor of the mechanism by which catalase may participate in the control of hydrogen peroxide and perhaps in protection from ionizing radiations.

[3] We estimate that about four times as much peroxide is decomposed "catalatically" as "peroxidatically" in the respiring cell (*6*).

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Manuscript received February 25, 1952.

Schwarzschild-Villiger Effect in Microspectrophotometry

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Since at least 1903 (*1*), the experienced microscopist has been aware of the cause of and cures for lenticular glare (flare). This fault of refracting optical systems has been one of the main enemies of the cytologist in his constant battle for microscopic contrast and resolution. Cellular microspectrophotometry has been a natural outgrowth of cytology (*2, 3*). When the cytologist turns to this new technique in cellular studies, he naturally brings with him his thorough background in microscopical practice, which includes the routine rules for the control of lenticular glare.

These are (*4–7*): (*a*) the smaller the cone of illuminating light (i.e., the smaller the condenser aperture), the less the glare; (*b*) the smaller the area of the object illuminated, the less the glare.

The effect of glare in microdensitometry (in terms of [*b*]) came to the attention of the astrophysicists Schwarzschild and Villiger (*8*) in a study on the intensity distributions in the image of the solar disk. Naora has based a recent critique (*9*) of present methods of cellular microspectrophotometry on his familiarity with the Schwarzschild-Villiger effect. Although giving considerable attention to the dependence of glare on the magnitude of the area illuminated, he has apparently neglected the fact that, as the condenser aperture is reduced, glare is reduced and he thereby overestimates the magnitude of the glare error in the current technique.

Thus far, in most microspectrophotometric studies, small condenser apertures have been used, and for convenience of searching and maintaining adequate alignment the illuminated field has been kept as large as 30 μ in diameter. For example, Swift has explicitly prescribed such conditions for the expressed purpose of reducing glare to a low level (*10*). With clean optics, and with the refractive index of the specimen matched by the mounting medium (*3*), the above precautions limit the total *measured* glare intensity in

the plane of the image from *all* sources (scattering by lens inclusions, reflections from lens mounts and the inside of the "blackened" microscope tube and stops, lens aberrations, and the multiple reflections between the lens surfaces) to considerably less than 3% of the focused illuminating intensity for most oil immersion systems. For transmittances which fall in the range of those of the *quantitative* studies already published (viz., *10–15*), this error has been negligible beside the other known errors of the method. It cannot have any relation to the validity of the conclusions these authors have drawn from their data.

Naora has used the expression $\frac{(1-\bar{r})}{1+(m-1)\bar{r}}$ as representative of their total flux, imaging, $(1-\bar{r})^m$, plus glare flux through a lens system with m air-glass interfaces of average reflectance $\bar{r}$ (*9*). He has assumed that equal fractions of the total glare flux and imaging flux fall on a given area of the image. (It is important to note that this equation applies only to the case of m plane air-glass interfaces, parallel to one another, and for the case of perfectly normal incidence.)

There are at least two ways in which the above theoretical approximation deviates seriously from the actual situation in microscopical systems. First, very few surfaces in such an optical train are plane elements perpendicular to the optical axis. As a result, all off-axis flux contributes glare diverging from the element at which it "originates" in such a way that the following element usually intercepts less of the solid angle of glare flux than of the focused imaging flux. (As an extreme example, between an objective and an ocular, up to 99% of the glare flux may be lost outside the aperture of the ocular, whereas all the imaging flux is passed if the field of illumination is set equal to the field limited by the ocular field diaphragm in accord with standard microscopical practice. The same holds for glare developed in the ocular with reference to the photocell entrance pupil or a photographic plate [*7*]). Thus, for small but finite[1] condenser apertures, Naora's calculations represent a large overestimate. As noted above, most microspectrophotometric work is carried on with a small condenser aperture. Second, if a large condenser aperture is chosen for microphotometric studies, as in Naora's case (condenser N.A. equal to objective N.A., in the range of 1.25 [*9, 16*]), most rays in the system meet the glass-air interfaces at angles very different from the normal. Under these conditions, $\bar{r}$ is increased (4), and therefore the glare flux is also increased. This, plus the increase in aberration glare as the illuminating aperture is increased, may account for the large amount of glare demonstrated experimentally by Naora's system with even small illuminated fields.

When specimens with high extinctions are studied, additional precautions will be in order, as Naora has indicated. His approach (that of Schwarzschild and Villiger) of limiting the illumination entirely to the minute area measured is the simplest solution when the convenience of larger illuminated fields of view may be abandoned. Special precautions will be even more important, however, in terms of distributional error and the aberration glare of the lens system. The curve-correction method for distributional error (*17*) adequately corrects for both glare and inhomogeneous distribution of chromophore.

[1] Not so small as to constitute a close approach to a condition where all the flux can be considered to be contained in an infinitesmal on-axis pencil. As this latter condition is approached, glare again increases toward Naora's computed values.

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Manuscript received March 18, 1952.

Study of Irritants Related to Piperine[1]

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The piperidine nucleus joined by the amide linkage to an unbroken nine-carbon chain produces a peppery pungency taste in compounds of quite different composition, such as pelargonylpiperidide (*1*), 2-phenylthiophene-5-carboxy-piperidide (*2*), and the piperine of black pepper, *Piper nigrum*. However, the pleasant bite of this spice has been duplicated only by the piperidides of β-cinnamenyl-acrylic acid, the 5-phenylpentenoic acid, and 5-phenyl-*n*-valeric acid, of which 5-phenyl-*n*-valeroyl piperidide has the most pungent taste (*3*).

It is known that the pharmacologic activity of certain compounds containing piperidine is increased by substitution in the piperidine ring. It was of interest,

[1] Thanks are due to Donald K. Tressler, scientific director, the Quartermaster Food and Container Institute of the Armed Forces, Chicago, Ill., for his interest in this work; to David Peryam, of the same institute, for outlining the taste-testing procedure used; and to the Evans Research and Development Corporation, New York, for carrying out the panel testing.