concentrations of sucrose, on the other hand, the disruption of the globes is sufficiently retarded to permit the demonstration of the sequential phases of the lysis.

An elucidation of the primary action of penicillin on bacterial cell walls may well provide one of the clues to the mechanism of action of the drug. Park and Strominger (*8*) have provided a definitive chemical basis for an explanation of the primary action of penicillin.

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References and Notes

1. K. Liebermeister and E. Kellenberger, *Z. Naturforsch.* 11b, 200 (1956).
2. J. Lederberg, *Proc. Natl. Acad. Sci. U.S.* 42, 574 (1956).
3. H. Eagle and A. K. Saz, *Ann. Rev. Microbiol.* 9, 173 (1955).
4. *Escherichia coli* strain B was obtained from Mark Adams.
5. H. E. Hopps *et al., J. Bacteriol.* 72, 561 (1956).
6. C. Weibull, *ibid.* 66, 688 (1953).
7. R. L. Lester, *J. Am. Chem. Soc.* 75, 5448 (1953); E. A. Grula and S. E. Hartsell, *J. Bacteriol.* 68, 171 (1954).
8. J. T. Park and J. L. Strominger, *Science*, this issue.

31 October 1956

Effect of Digoxin on Myokinase Activity

In a study of "energetic-dynamic cardiac insufficiency," Munchinger (*1*) reported that strophanthin and digilanid enhanced the adenosine triphosphatase (ATPase) activity of rat-heart homogenate. Attempts to repeat this observation using actomyosin prepared from heart gave results that varied from 40 percent to zero activation, depending on the relative purity (by reprecipitation) of the actomyosin preparation. The loss of digoxin enhancement on reprecipitation indicated that a component other than actomyosin was sensitive to digoxin. The present report suggests that this component may be myokinase (*2*).

Actomyosin (myosin B) was prepared from dog heart by the method of Szent-Gyorgi (*3*). Adenosine triphosphatase activity was measured by incubating myosin B and adenosine triphosphate (ATP) in KCl ($0.15M$)- veronal buffer ($0.02M$) at *p*H 7.3 At the end of the incubation time, 20-percent trichloroacetic acid was added, the mixture was centrifuged, and phosphorus was determined on an aliquot of the supernatant by the method of Fiske and Subbarow (*4*).

Myokinase was prepared according to Kalckar's method (*5*). The final trichloroacetic acid precipitation was omitted. The myokinase solution was dialyzed against distilled water and finally centrifuged at 18,000 *g* for 30 minutes. The final product contained 0.28 mg of N per milliliter and showed only one major peak when subjected to electrophoresis for 90 minutes in $0.1M$ veronal buffer at *p*H 8.5.

Myokinase activity was determined following the procedure of Bendall (*6*). A myosin B preparation was used as a specific ATPase hydrolyzing only the terminal phosphate group of ATP. Because the myosin B was in sufficient excess to hydrolyze 45 to 46 percent of the 10-minute acid-labile phosphate of ATP in 2 minutes, the extra phosphate liberated in 10 minutes was a function of the myokinase concentration and was taken as myokinase activity. The myokinase preparations, when present in optimum concentration, liberated 100 percent of the 10-minute labile phosphate.

We found that myosin B from rat heart was difficult to free from myokinase activity, whereas that from dog heart was relatively easy to free. Myosin B from dog heart was therefore chosen for the test system. Figure 1 shows the effect of digoxin on myokinase activity. The myosin B and ATP concentrations are constant throughout. Bars 1 through 6 denote increasing quantities of myokinase added to the test system. The activating effect of digoxin appears to be confined to the systems in which myokinase is a limiting factor. At high myokinase concentrations (bar 6), the total activity was inhibited, and digoxin had no influence on the reaction. This decrease of the over-all reaction was apparently the result of inhibition of ATPase activity of myosin B rather than to an effect on myokinase activity. At a lower myokinase concentration (bar 5), which appears to be the optimum myokinase concentration in our test system, digoxin was without effect. When the myokinase concentration was decreased below the optimum level (bars 2, 3, and 4), digoxin activated the myokinase reaction, bringing the reaction to the level obtained with optimum concentrations. With no added myokinase, digoxin had no effect on the ATPase activity of myosin B.

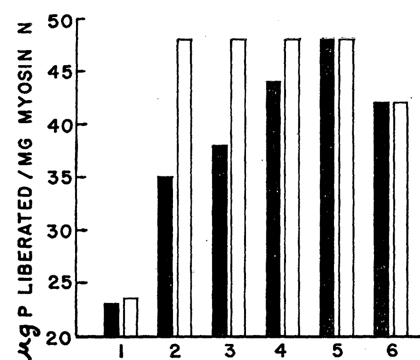

Fig. 1. Effect of digoxin on the activity of myokinase. The incubation tubes contained the following: KCl, $0.15M$; veronal buffer, $0.02M$ at *p*H 7.3; $CaCl_2$, $0.01M$; ATP, $3.75 \times 10^{-4}M$, myosin B (0.46 mg N/ml, 0.2 ml; and digoxin, 10 µg/ml. The total volume was 2.0 ml. Black bars, controls; open bars, with digoxin. Myokinase (0.28 mg N/ml) was added as follows: bar 1, none; bar 2, 0.025 ml; bar 3, 0.05 ml; bar 4, 0.1 ml; bar 5, 0.2 ml; bar 6, 0.5 ml.

Hasselbach and Weber (*7*) have shown that the Marsh (*8*) and Bendall (*9*) relaxing factor of muscle inhibits the ATPase activity of myosin by extending substrate inhibition to physiological concentrations. The demonstration of Bendall (*6*) that the Marsh-Bendall factor exhibited all the characteristics of myokinase prompted us to include in the present study the effect of digoxin on the ATPase-inhibiting characteristics of myokinase. Table 1 shows the effect of digoxin on the myokinase inhibition of ATPase activity of myosin B at two concentrations of ATP. At lower ATP concentrations ($0.005M$), the myokinase inhibition is slight (32 percent) but the ability of digoxin to reverse myokinase inhibition is evident. At higher ATP concentrations ($0.01M$), inhibition approaches 73 to 74 percent, and the ability of digoxin to reverse the myokinase effect is obvious.

Table 1. Effect of digoxin on the myokinase inhibition of ATPase activity of myosin B at two concentrations of ATP. The figures are based on 6 individual runs. Three runs were made on one myosin and myokinase preparation and three runs were made on a different myosin and myokinase preparation. The tubes contained the following: KCl, $0.15M$; veronal buffer, $0.02M$ at *p*H 7.3; $MgCl_2$, $0.005M$; myosin B (0.46 mg N/ml), 0.15 ml; myokinase (0.28 mg N/ml), 0.5 ml; and digoxin, 10 µg/ml. The total volume was 2.0 ml.

ATP Concn. (*M*)	Phosphorus liberated (µg/mg of myosin N hr)			
	Control	Digoxin	Myokinase	Myokinase and digoxin
0.005	1842 ± 24	1868 ± 28	1270 ± 36	1884 ± 32
0.01	1520 ± 58	1610 ± 32	402 ± 27	1458 ± 60

According to Weber (*10*), contraction of muscle is associated with ATP breakdown and lasts as long as ATP continues to be hydrolyzed. Relaxation sets in as soon as ATP breakdown is prevented. Physiologically induced relaxation is based on an inhibition of ATP hydrolysis by the Marsh-Bendall factor (myokin-

ase). It would appear that activity within the area of myokinase might allow digoxin to enhance the contractile response of muscle at the expense of relaxation by (i) activating the myokinase reaction (ADP → ATP) and (ii) by suppressing myokinase inhibition of myosin ATPase.

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References and Notes

1. R. Munchinger, *Cardiology* 22, 145 (1953).
2. This work was supported by a grant from the American Heart Association.
3. A. Szent-Györgyi, *Chemistry of Muscle Contraction* (Academic, New York, 1951), p. 151.
4. C. H. Fiske and Y. Subbarow, *J. Biol. Chem.* 66, 375 (1925).
5. H. M. Kalckar, *ibid.* 148, 127 (1943).
6. J. R. Bendall, *Proc. Roy. Soc.* (*London*) B142, 409 (1954).
7. W. Hasselbach and H. H. Weber, *Biochim. et Biophys. Acta* 11, 160 (1953).
8. B. B. Marsh, *Nature* 167, 1065 (1951); *Biochim. et Biophys. Acta* 9, 247 (1952).
9. J. R. Bendall, *Nature* 170, 1058 (1952).
10. H. H. Weber, *Biochim et Biophys. Acta* 12, 150 (1953).

6 December 1956

Spectral Reflectance Applied to the Study of Heme Pigments

It is a common practice to make qualitative and rough quantitative estimates of the content of pigmented substances in materials by the intensity and spectral distribution of color. It is rather surprising, then, that more use has not been made of spectral reflectance in qualitative and quantitative analytic chemistry. It is the intention of this report (*1*) to point out the potentialities of the method, particularly in biochemical analysis, and to illustrate its application in the investigation of certain heme pigments.

The use of spectral reflectance curves in the specification of color by the physicist is well known. Lermond and Rogers (*2*) have recently pointed out the possible wider utility of spectral reflectance measurements in chemical analysis and have reviewed the limited amount of work in this field. Applications in biochemistry seem to be particularly rare.

In the course of research on pigment systems in fish flesh, we investigated the use of spectral reflection measurements. A standard reflection attachment to the Beckman DU spectrophotometer was used. Samples, either 90-mesh, nonabsorbing powders with absorbing solutions or solids added, or tissue forced through a 16-mesh, stainless-steel screen, were packed into 1¼ by 1/16-in. aluminum planchets and covered with glass plates. Comparison was made with a standard consisting of the nonabsorbing diluent powder or with a disk of high-fired alumina.

Adherence to Beer's law was tested by adding different amounts of standard copper sulfate solution to the crystalline alumina diluent and measuring the absorbancy at 620-mμ wavelength. The law seems to be applicable at this wavelength and for the range of concentrations indicated (Fig. 1). These results are at variance with those that Winslow and Liebhafsky (*3*) reported for reflectance from known concentrations of metals spot-tested on paper. The discrepancy may be due to the difference in the thickness of the supporting media for the samples used in the two experimental situations. Measurements were attempted with whole blood dispersed on 90-mesh crystalline alumina. In this case, adherence to Beer's law was found at low concentrations, but oxidative changes caused deviation in the higher range.

In transparent, internally absorbing systems (dielectrics), it can be assumed that reflection takes place at phase interfaces and by diffuse scattering from large molecules. Adherence to Beer's law would signify that, for systems of similar opacity, the average path length of the incident and emergent beam is the same and would also justify the use of absorbancy (optical density) in the plotting of spectral reflectance data. Thus spectral reflectance can presumably be used for the comparative quantitative analysis of stable systems of dielectric materials of similar general composition. Such measurements have been applied in the present work.

The use of the method for the characterization of pigments by their reflectance curves is illustrated in Fig. 2. Various derivatives of respiratory pigments can be identified in the reflection spectra of tuna flesh that is exposed to a variety of oxidative environments. An advantage afforded by this technique is the possibility of evaluating the spectral absorbance of opaque concentrated systems without recourse to the special cells, or to dilution in solution with its attendant possibility of alterations, that are employed in transmission methods. For example, the spectrum of whole human blood can be directly determined using an inert crystalline alumina diluent (Fig. 2*c*).

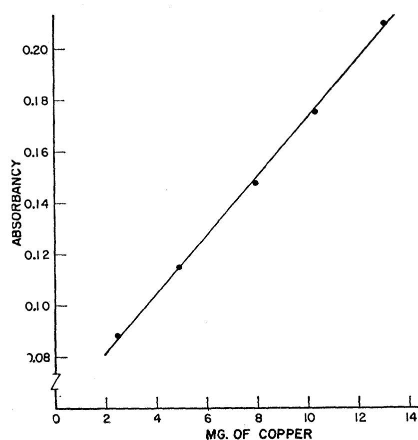

Fig. 1. Beer's law plot for copper on 90-mesh crystalline alumina.

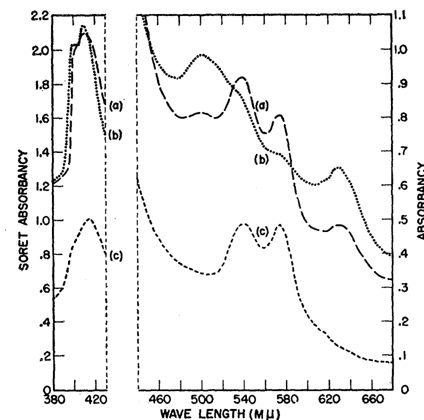

Fig. 2. Spectral reflectance curves *a*, mixed oxymyoglobin and metmyoglobin in tuna flesh; *b*, metmyoglobin in tuna flesh; *c*, oxyhemoglobin in whole blood.

Spectral reflectance is particularly suited to *in situ* studies on pigment systems where extractive procedures are difficult or impossible or where such procedures would cause undesirable changes. It is characteristically a simple technique in that a minimum of preparative and extractive operations is involved. Specifically, absorbance studies on coagulated proteins were possible; in addition, the examination of residues after the extraction of certain components gave a more complete picture of extraction efficiency and relative solubilities. Furthermore, one is able to follow the course of induced or natural chemical reactions in such systems. For the heme protein systems studied, the wavelengths of absorption maxima in reflection corresponded exactly to those found in transmission, and complete interchangeability of data was noted. Thus the large amount of data accrued from measurements in transmission would be available to workers in the field of reflectance in systems where this interrelationship is found to be true.

The measurement of spectral reflectance offers a method for the study of dielectric materials that absorb and reflect internally, as opposed to surface reflectors such as metals. It is felt that this investigative tool merits the further attention of the biological and analytic worker.

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