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 $\rightarrow$ ATP) and (ii) by suppressing myokinase inhibition of myosin ATPase.

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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*).

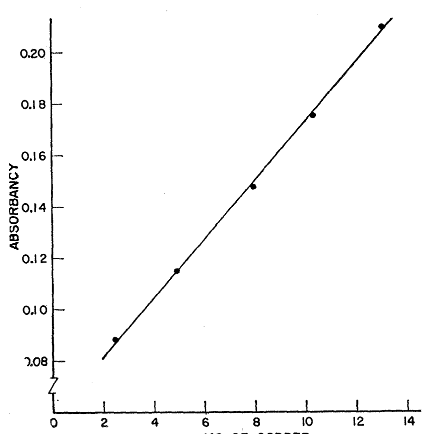

Fig. 1. Beer's law plot for copper on 90-mesh crystalline alumina.

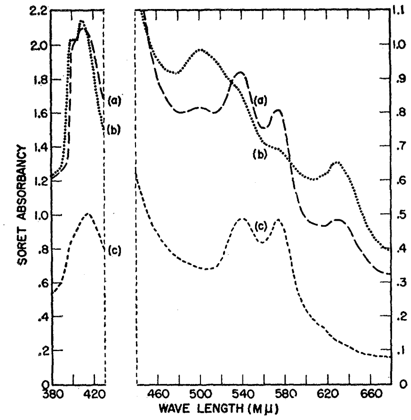

Fig. 2. Spectral reflectance curves *a*, mixed oxymyoglobin and metmyoglobin in tuna flesh; *b*, metmyoglobin in tuna flesh; *c*, oxyhemoglobin in whole blood.

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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References and Notes

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25 October 1956

Reactions of Honey Bees in the Hive to Simple Sounds

Beekeepers have known since before Aristotle (*1*) that honey bees (*Apis mellifera*) produce characteristic sounds while engaged in certain activities. The possible significance of these sounds for the bees has been a matter of debate (*2, 3*). Indeed, honey bees seem to be insensitive to air-borne sounds, although they are able to receive vibrations through the legs (*3, 4*). Hansson (*3*) has reported that bees in hives stopped normal activities when they were subjected to pure tones at frequencies of 100 to 1500 cy/sec at rather high intensities (audible at distances up to 250 m). The insects stopped moving when the sounds were turned on, but, if the sounds continued, began to move slowly within a few seconds.

We have confirmed and extended these observations by finding that continuous irradiation of hives with sounds of certain frequencies and of sufficient intensities caused an almost total cessation of movement of workers and drones in the hives for up to 20 minutes (*5*). The quiescence of the bees was so complete that a beekeeper could safely open the hive and carry out routine servicing without the usual treatment with smoke.

Sounds of known frequencies were produced by an audio oscillator that activated through an amplifier either a loudspeaker (for frequencies below 400 cy/sec) or a microphone (for higher frequencies) (*6*). The behavior of the honey bees, all of the Italian race, was observed in an ordinary glass-sided observation hive. The speakers were usually placed about 0.5 to 1 m from the hive, but tests were also made with the speakers in contact with the hive. The results are given in Table 1.

Table 1. Effects of sounds on honey bees in an observation hive: 0, no observable effects; +, bees move more slowly; ++, the majority of the bees stop but some still move slowly; +++, almost all bees stop and remain motionless as long as the sound is on. The sound pressure for frequencies with +++ effect are minima needed to induce the effect; other sound pressures are the highest obtainable with the equipment.

Frequency (cy/sec)	Sound pressure (db)	Effect
50	106	0
80	115	0
100	120	+
150	125	++
200	122	++
300	119	+++
400	118	+++
500	116	+++
600	107	+++
800	108	+++
1000	113	+++
1500	124	++
2000	128	+
3000	127	0
4000	117	0
6000	112	0
8000	113	0
10000	102	0

With sounds of sufficient intensity at frequencies of 300 to 1000 cy/sec, the bees stopped moving almost entirely as long as the sounds continued. The most effective frequencies were between 500 and 800 cy/sec. Below 300 and above 1000 cy/sec, the bees either showed reduced activity or were not affected, even with intensities higher than those that sufficed at the proper frequencies. The bees returned almost immediately to normal activities when the sound was discontinued. There were no observable reactions to these sounds by bees at the entrance to the hive or by workers in the field. These observations support the idea that the sounds are received by the bees through the legs after the hive was caused to vibrate by absorption of the air-borne sound. The most effective frequencies in this case, however, were not those found by Autrum and Schneider (*4*) to be most effective in stimulating the subgenual organs in the legs of the honey bee.

The results are like those of Hansson (*3*), except that cessation of activity at the intensities we used was almost complete and persisted as long as the sound continued. It is impossible to determine from Hansson's report the actual intensities he used, but they were probably lower than those we used.

Bees in standard beehives were tested with similar results. With the sounds on, the covers and supers of the hives were removed and the frames lifted out. The bees on the frames remained still as long as sounds of the proper frequencies and intensities continued. It was possible, therefore, to work in hives using only sound. This was done for about 2 months with three hives, using sound at a frequency of 600 cy/sec at about 120 db, which was projected from a speaker alongside the hive. There was no sign of habituation of bees to this sound.

Certainly the equipment used to produce these sounds is much more expensive than that needed for smoking hives. It is possible, however, that inexpensive vibrators attached to hives could be used. The high intensities of these sounds make some form of ear protection necessary, but free use of both hands in working in the hive is possible and there is no need for ventilation of the hive by the bees, as there is with smoke. Sound may thus, under special circumstances, have some use in apiculture.

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5. The work reported in this paper, which is No. 2102 in the journal series of the Pennsylvania Agricultural Experiment Station, was supported in part by research grant No. E-802 from the National Microbiological Institute, National Institutes of Health, U.S. Public Health Service. We are happy to express our appreciation to E. J. Anderson of Pennsylvania State University for his expert help and advice.
6. Hewlett-Packard audio oscillator model 200-A; Stromberg-Carlson amplifier model AU42; University loud speaker model PA-30; Altec microphone model 633A. Sound pressures in decibels re 10^{-16} watt/cm^2 were measured at 1 m from the speakers with a calibrated Scott sound-level meter type 410-B.

29 November 1956