THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 69:296–298, 1977

Vol. 69, No. 3
Printed in U.S.A.

STRATUM CORNEUM STUDIES WITH PHOTOACOUSTIC SPECTROSCOPY

ALLAN ROSENCWAIG, PH.D., AND ELI PINES, PH.D.

Bell Laboratories, Murray Hill, New Jersey, and Department of Skin Biology, Johnson & Johnson Research, New Brunswick, New Jersey, U. S. A.

Conventional spectroscopic studies on opaque membranes are difficult to pursue because of excessive light scattering and complications arising from the specimen's surface. Recently, a new spectroscopic technique, photoacoustic spectroscopy, unlike conventional optical spectroscopy, has been demonstrated to be an informative technique amenable to spectroscopic studies of solids and membrane-like samples.

We have applied photoacoustic spectroscopy to the study of hydration and maturation of newborn rat stratum corneum, and have obtained clean spectra in the 220 to 450 nm region indicative of a change in thermal diffusivity with increased hydration, and biochemical changes associated with the initial maturation period.

In biology, one must often deal with materials which, in their intact state, are generally quite difficult to study spectroscopically, primarily because of excessive light scattering [1]. Recently a new spectroscopic technique has been developed [2–4] that has been found to be eminently suitable for highly light-scattering systems. In this technique, called photoacoustic spectroscopy, the sample to be studied is placed inside a closed chamber, a photoacoustic cell. This cell is filled with a gas, such as air at room temperature and pressure, and in addition contains a very sensitive microphone. The sample is then illuminated with monochromatic light, which is chopped at some frequency within the range of 50–5000 Hz. If the sample absorbs any of the incident radiation, some energy level in the sample is excited, and the energy level must subsequently de-excite, usually by means of a nonradiative or heating mode of de-excitation. The periodic input of light thus results in a periodic heating of the sample, with a subsequent periodic heat flow from the sample to the surrounding gas. The gas near the sample surface responds to this periodic heat flow with an oscillating motion that produces a periodic pressure change in the cell. The microphone detects this pressure change as an acoustic signal.

Typically, we illuminate the sample with less than 1 milliwatt/cm^2 of light, which results in millidegree changes in the sample temperature, and in periodic cell pressures of less than a microbar (10^{-6} atmospheres). Since the strength of the acoustic signal in the photoacoustic cell is closely related to the amount of light absorbed by the sample, a plot of the acoustic signal as a function of photon wavelength, that is a photoacoustic spectrum, bears a close resemblance to a true optical absorption spectrum. Furthermore, since only absorbed light can produce an acoustic signal, scattered light, which presents such a serious problem in transmission spectroscopy, presents no serious problem in photoacoustic spectroscopy.

The theory and mathematics of the photoacoustic effect in solids have been published elsewhere [5, 6]. With regard to the sample, there are three important parameters. These are the sample thickness, l; the optical absorption length, $1/\beta$, where β is the absorption coefficient in cm^{-1}; and the thermal diffusion length, l_t, which determines how far a periodic heat wave can travel in a solid before dissipating. The thermal diffusion length $l_t = \sqrt{\frac{2\alpha}{\omega}}$, where α is the thermal diffusivity ($\alpha = \frac{k}{\rho C}$; k = thermal conductivity, ρ = density, C = specific heat), and ω is the frequency of the heat wave (rad/sec), and in our case is simply the frequency at which the light beam is being chopped. In general, the photoacoustic signal is a complicated function of these parameters [6].

There are two unique features of photoacoustic spectroscopy that might play an important role in in vivo studies of mammalian tisue. The first feature involves depth studies of absorption. At a high chopping frequency, the thermal diffusion length in a material is quite small, and thus absorption of light only within this small thermal diffusion layer near the surface can be studied. As the chopping frequency is lowered, the thermal diffusion length increases, and thus light absorption deeper within the material can be studied.

The second feature involves opaque materials, such as entire mammalian bodies or limbs. At low chopping frequencies, the thermal diffusion length will generally be larger than the opitcal absorption length $1/\beta$ for all wavelengths of interest. Under this condition, the photoacoustic spectrum contains no information about the intrinsic absorption characteristics of the sample. However, if one increases the chopping frequency until $l_t < 1/$

Reprint requests to: Dr. E. Pines, Department of Skin Biology, Johnson & Johnson Research, New Brunswick, New Jersey 08903.

β, then the photoacoustic spectrum will be representative of the true optical absorption spectrum. Since it is possible to construct an open-ended photoacoustic cell, it is therefore possible to obtain absorption spectra on opaque objects, as in in vivo studies of mammals, via the photoacoustic effect. Such a capability may prove extremely useful in medical research and diagnostics.

In this communication, we will describe some photoacoustic experiments on intact excised stratum corneum, in which spectral changes attributable to maturation effects and changes in hydration were observed.

MATERIALS AND METHODS

Newborn rat stratum corneum specimens were harvested using Vinson et al's procedure [7] 24 and 0–60 hr post partum for the hydration and maturation studies, respectively. For the maturation study, the stratum corneum was kept at ambient conditions for 2 days, subsequently placed in the photoacoustic cell, and its acoustic spectrum measured over the 220 to 450 nm region.

For the hydration study, the stratum corneum was cut into 12 equal samples and placed in a dry box for 48 hr. The moisture content was then increased to the desired levels by exposing dry duplicate samples for 24 hr to atmospheres of differing relative humidities. The water content was determined gravimetrically from one of the samples and the other was placed in the photoacoustic cell and its acoustic signal measured at 285 nm.

RESULTS

A representative ultraviolet photoacoustic spectrum of a translucent newborn rat stratum corneum membrane is shown in Figure 1. The spectrum displays an increasing signal at 375 nm, a broad maximum in the 284 to 288 nm region, a minimum at 257 nm, and a rapid increase in signal beyond 225 nm.

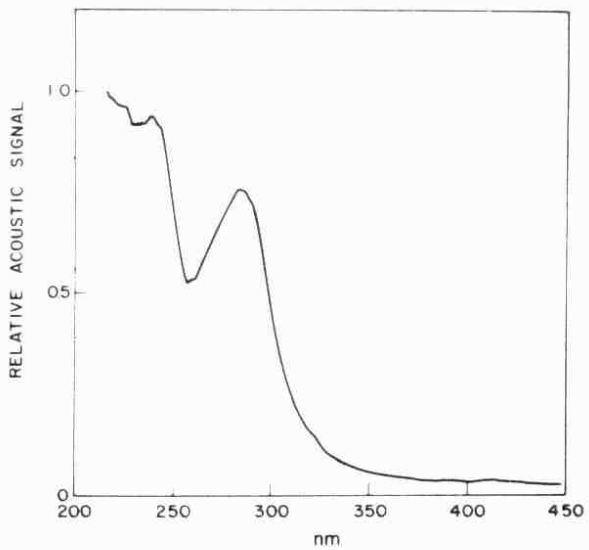

FIG. 1. Representative photoacoustic spectrum of newborn rat stratum corneum.

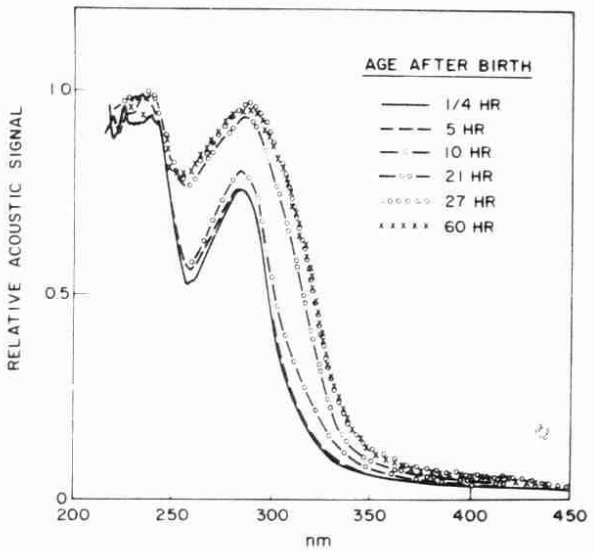

FIG. 2. Photoacoustic spectra of newborn rat stratum corneum during the maturation period.

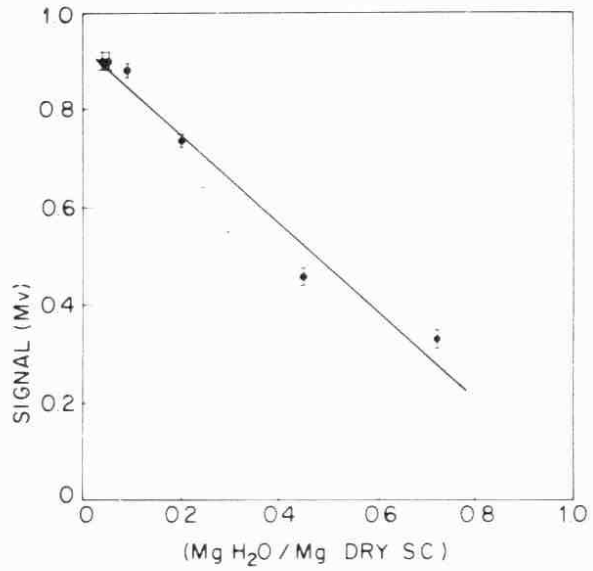

FIG. 3. Photoacoustic signal at 285 nm as a function of water content for newborn rat stratum corneum. *S.C.* = stratum corneum.

All of the amino acids absorb to a greater or lesser extent in the ultraviolet region, but of the 20 or so amino acids which commonly occur in protein, only 4 possess a characteristic absorption band in the 220 to 300 nm region: the 3 aromatic amino acids – tryptophan, tyrosine, and to a lesser extent phenylalanine – and the disulfide amino acid cystine [8]. The newborn rat stratum corneum spectrum is principally due to these, with the bulk being attributable to tryptophan, tyrosine, and/or other substituted indole or phenolic chromophores [9].

Figure 2 shows the photoacoustic spectra obtained from newborn rat stratum corneum during the initial 60-hr maturation period. It is important to note that the following spectral changes ob-

served during this initial period are quite pronounced: (1) an increase in peak intensity of 33% and 17% at the 257 and 285 nm regions, respectively, (2) a 5-nm spectral shift to higher wavelength in the position of the maximum with increasing age, and (3) a much broader band shape, resulting in an increase by a factor of 3 in the spectral area.

Figure 3 shows the photoacoustic signal vs the gravimetrically determined water content for newborn rat stratum corneum. The theory of the photoacoustic effect in solids [6] and its dependence on the thermal diffusivity, which is, in turn, a function of water content, shows that the observed change in the photoacoustic signal is to be expected.

DISCUSSION

Major and rapid biochemical and structural changes are to be expected during the initial maturation period when the stratum corneum matrix is altered to develop its "barrier" functions, and adapt to its post-partum environment.

Recent mechanical and differential-scanning-calorimetry studies (E. Pines, unpublished data) indicate the occurrence of significant changes within the stratum corneum matrix during the initial maturation period. In a subsequent publication we will discuss in more detail, our photoacoustic results and their possible correlations to the observed changes in the stratum corneum. For now, however, we attribute the pronounced changes in the photoacoustic spectra to changes in the number and types of bonds, changes in the electrostatic environment, and also some possible changes in chemical composition. All of these changes can occur as a result of the major biochemical and structural changes occurring in the stratum corneum during the maturation period.

The dependence of the photoacoustic signal on water content within the stratum corneum (Fig. 3) is readily explainable in terms of the changes in the thermal properties of the stratum corneum with changes in the water content. The in vitro demonstration of the applicability of photoacoustic studies on mammalian tissues, and the fact that photoacoustic spectroscopy can be used for in vivo measurements as well (A. Rosencwaig and E. Pines, unpublished data) not only in the ultraviolet but also in the visible and infrared wavelength regions, indicates the potential of the technique for in vitro and noninvasive in vivo studies of diseased states, moisture content, substantivity, and percutaneous absorption in mammalian tissues.

REFERENCES

1. Everett MA, Yeargers E, Sayre RM, Olson RL: Penetration of epidermis by ultraviolet rays. Photochem Photobiol 5:533–542, 1966
2. Rosencwaig A: Photoacoustic spectroscopy of biological materials. Science 181:657–658, 1973
3. Rosencwaig A: Photoacoustic spectroscopy. Anal Chem 47:592A–604A, 1975
4. Rosencwaig A: Photoacoustic spectroscopy of solids. Physics Today 28:23–30, 1975
5. Rosencwaig A, Gerhso A: Photoacoustic effect with solids: a theoretical treatment. Science 190:556–557, 1975
6. Rosencwaig A, Gerhso A: Theory of the photoacoustic effect with solids. J Appl Physics 47:64–69, 1976
7. Vinson LJ, Koehler WR, Lehman MD, Masurat T, Singer EJ (eds): Basic studies in percutaneous absorption, Semi-Annual Report No. 7 to the Army Chemical Center, Edgewood, Md, Jan–Jun Sec 3, 1964
8. Handbook of Chemistry. Second edition. Edited by HA Sober. Cleveland, Ohio, CRC Press, 1973 pp B72–B74
9. Beaver GH, Holiday ER: Ultraviolet absorption spectra of proteins and amino acids. Adv Protein Chem 7:319–386, 1952