EDGEWOOD ARSENAL
TECHNICAL REPORT

EATR 4499

CHEMICAL REACTIONS AND MOLECULAR ASSOCIATION EFFECTS IN SKIN

I. TECHNIQUES OF MEASURING ELECTRONIC ABSORPTION SPECTRA

by

Edward J. Poziomek
Millard M. Mershon
Thaddeus J. Novak

March 1971

DEPARTMENT OF THE ARMY
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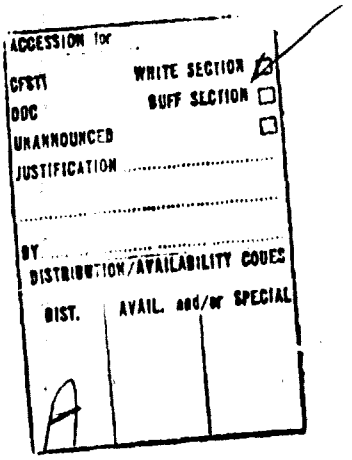

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FOREWORD

The work described in this report was authorized under Task 1T061102B71A02, Life Sciences Basic Research in Support of Materiel. The work was performed between July and November 1970. Data are contained in notebook 7554.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences National Research Council.

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DIGEST

Simple techniques have been developed to measure the ultraviolet and visible electronic absorption spectra of skin. The methods utlize both dorsal thoracic and ear skin of albino rabbits. The procedure with intact skin involves mounting the sample on a cardboard holder placed in a Cary 14 spectrophotometer. Spectra are shown of ear skin untreated and treated with strong tincture of iodine, USP; dorsal thoracic tissue taken from a section that developed erythema after exposure to iodine tincture; and a mixture of ear and back skin scales. Also, a chamber is described for keeping skin samples moist while obtaining spectra.

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I. TECHNIQUES OF MEASURING ELECTRONIC ABSORPTION SPECTRA

I. INTRODUCTION.

There is interest in obtaining spectra of skin for purposes of medical research, pathology, and forensic science studies. Most of the recent work on the use of spectroscopy in skin analysis involves infrared techniques.[1-3] There are also several reports on the ultraviolet and visible spectrophotometry of skin,[4-9] but these were not easily adaptable to studies that we have been performing on molecular association effects and chemical reactions in skin. As an illustration of the techniques we have developed, representative ultraviolet and visible spectra of dorsal thoracic and ear skin tissue of albino rabbits are presented. Both intact samples and scales were used. The transmittance of strong tincture of iodine on skin is illustrated. Also, a simple chamber that can be used when obtaining spectra of moist skin is described.

II. EXPERIMENTATION.

A. Choice of Animal Species.

Absence of pigment and minimum thickness are desirable properties of skin specimens to be used for studies involving light transmission. Skin of albino rabbits is useful because it is free of pigments other than hemoglobin. The average thickness of dorsal thoracic skin is less for rabbits than for any other common laboratory animal, with the exception of the monkey.*

Rabbits are convenient models for either in vivo or in vitro studies of chemical penetration and reaction in skin. Albino rabbits are inexpensive, uniform, of convenient size, and less resistant to skin penetration than most other species. It is easy to compare results of toxicological and in vitro studies by using applications on rabbit skin.

B. Preparation of Samples.

Clipped dorsal thoracic skin of freshly killed albino rabbits was prepared for spectroscopy studies by removing the subcutaneous muscle layer and associated connective tissue. This was done by carefully using a scalpel to cut between skin and muscle layers that were drawn in opposite directions by pressure on pairs of forceps.

Prepared skin was stretched over 1-cm by 2-cm windows cut in cardboard that was shaped to fit the reference and cell compartments of the Cary 14 spectrophotometer. The dimensions of the windows were sufficient to permit good skin support without danger of blocking the light beam. An ordinary desk stapler was used to attach skin to the 1-mm thick cardboard.

Although total thickness of rabbit dorsal thoracic skin averages only about 1.25 mm,* a thinner and more hair-free specimen was desired to decrease light scattering. Better specimens were obtained from the inner, concave portion of the rabbit ear. Such skin is supported by cartilage of the ear, and the dermis is much thinner than elsewhere. Hair in the ear is sparse and fine.

Specimens of ear skin were obtained from freshly killed rabbits. Ears were amputated, then massaged to drain blood vessels before clotting could occur. Scissors were used to trim away portions of the ear that were not flat or that contained hair. The remaining piece was cut into long

*Callahan, J. F. Medical Research Laboratory, Edgewood Arsenal. Unpublished results.

slices at least 2 cm wide. A scalpel was used to separate skin from cartilage at one end of the slice. Enough skin was freed to permit good forceps grip on the free ends. Pressure was then applied to peel the skin away from the cartilage.

Specimens of ear skin are about one-fourth as thick as dorsal thoracic skin. (Samples of ear skin varied in thickness from 0.25 to 0.35 mm, with the average near 0.30 mm.) Samples are easily mounted on cardboard as has been described, but special care is needed to keep them moist.

C. Effect of Moisture.

The samples of stretched skin were kept moist with water-soaked pads of paper tissue on each side of the cardboard holders. Moistening was necessary to maintain the normal structural spacing of skin components until test substances could be permitted to penetrate. In some cases it was desirable to permit the skin to dry overnight at room temperature. The dried specimens are more nearly transparent but much less permeable than the hydrated skin.

Skin samples, especially ear dermis tissues, dry very rapidly when exposed to air. Other than weight loss, there are two simple indicators of the rate of drying; decrease in skin thickness and increase of light transmission. Dry ear skin samples are slightly less than one-half as thick as the moist tissues. Curve B in figure 1 illustrates the rapid rate of light transmission increase (optical density decrease) when a moist ear skin sample is placed in the spectrophotometer cell compartment and allowed to dry. The heavy line at early times represents a movement of the pen because of light scattering effects by the sample. The wavelength was set at 300 nm, which corresponds to an absorption band peak. This band shifts to 287 nm as the skin dries.

The quality of spectra from dry skin was always better than that from moist tissue. In some instances, however, it may be desirable to follow spectral absorption changes using wet skin. Because of this, a sample chamber was designed (figure 2) that allowed spectral measurements without the sample drying out. The chamber is a screw-top metal can with holes drilled for cell windows. Circular calcium fluoride plates were mounted with epoxy cement. The size of the cardboard skin holder was adjusted to fit the can. A simple clamp made from two plates and a magnet placed at the bottom of the can kept the holder in position. Moistened filter paper lined the can, giving an air atmosphere saturated with water vapor. Curve A (figure 1) shows a slight increase in the absorbance of a skin sample placed in the can. This increase is a result of a small amount of water being absorbed to replace that lost during handling of the sample outside the can prior to the spectral measurements.

III. ABSORPTION SPECTRA.

Figure 3 through 5 illustrate spectra of mixed ear and dorsal thoracic skin scales, ear skin treated and untreated with strong iodine tincture, and erythematous dorsal thoracic skin.

A piece of dry ear skin slightly thinner than the average of the samples examined usually was chosen as reference. This allowed absorption to be measured to at least down to 250 nm. If the reference skin was thick, the absorbance curve fell below 0 optical density near 310 nm. If the reference was much thinner than the sample, very strong absorption appeared below 310. Generally, hemoglobin bands were not noted in spectra of ear skin. In several cases (spectra not shown), very weak absorption was noted at 415 nm. This could be caused by hemoglobin because it exhibits a very strong absorption band at 417 nm.[4]

The erythema observed on the skin of clipped rabbits treated with strong iodine tincture (7% I_2, 5% KI) is caused by hemoglobin. The absorption bands (577, 541, 415, and 350 nm) noted

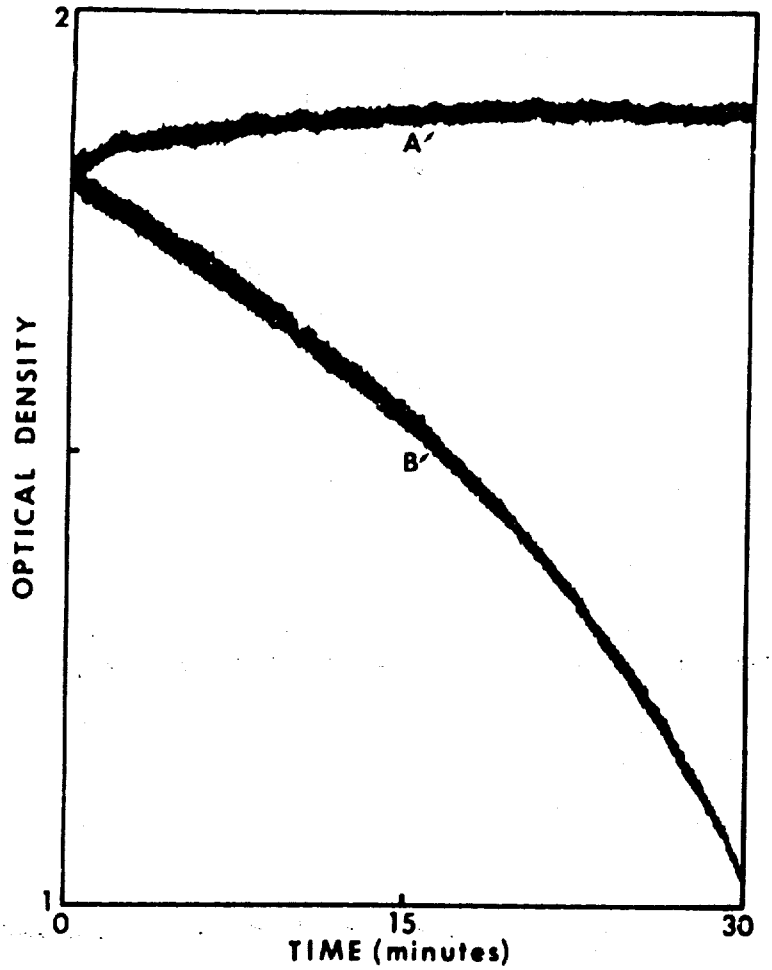

Figure 1. Transparency of Ear Skin to 300-nm Light

Moist skin was placed either in a chamber saturated with water vapor (curve A) or allowed to stand exposed to ambient air (curve B).

in the skin spectrum (figure 5) correspond closely to bands (578, 548, 417, and 350 nm) found for oxyhemoglobin in greatly diluted hemolyzed blood.[4]

Skin samples treated with strong iodine tincture (essentially I_3^-), alcoholic iodine, or iodine vapor give absorption bands corresponding to triiodide ion. An example is given in figure 4. A broad band at 363 nm and increased absorption near 290 nm are evident. Triiodide absorption bands in water appear at 353 and 288 nm.[10]

IV. CONCLUSIONS.

The techniques described in this paper provide a simple way to measure electronic absorption spectra of skin. They incorporate the sophistication inherent in the capabilities of the Cary 14 spectrophotometer. Near infrared measurements also could be made.

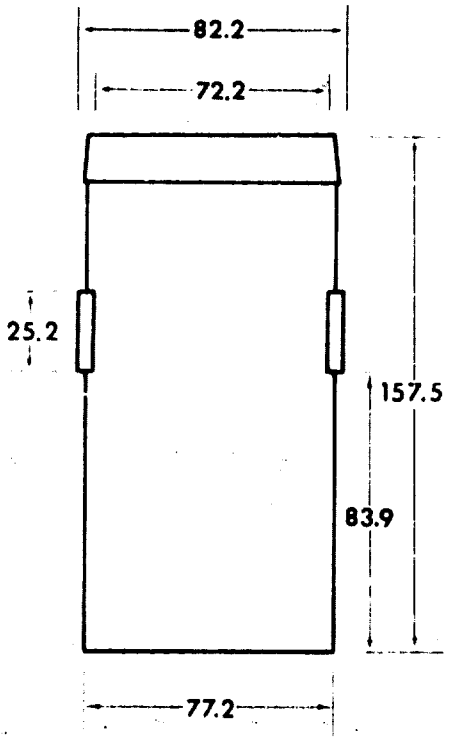

Figure 2. Screw Type Metal Can Used to Keep Skin Samples Moist During Spectral Measurements

The windows are circular CaF_2 plates. All dimensions are in millimeters.

One can use these techniques for studying molecular association effects and chemical reactions in skin medium. The chamber that was developed for keeping skin samples moist during spectral measurements also can be used to study skin effects with chemical vapors.

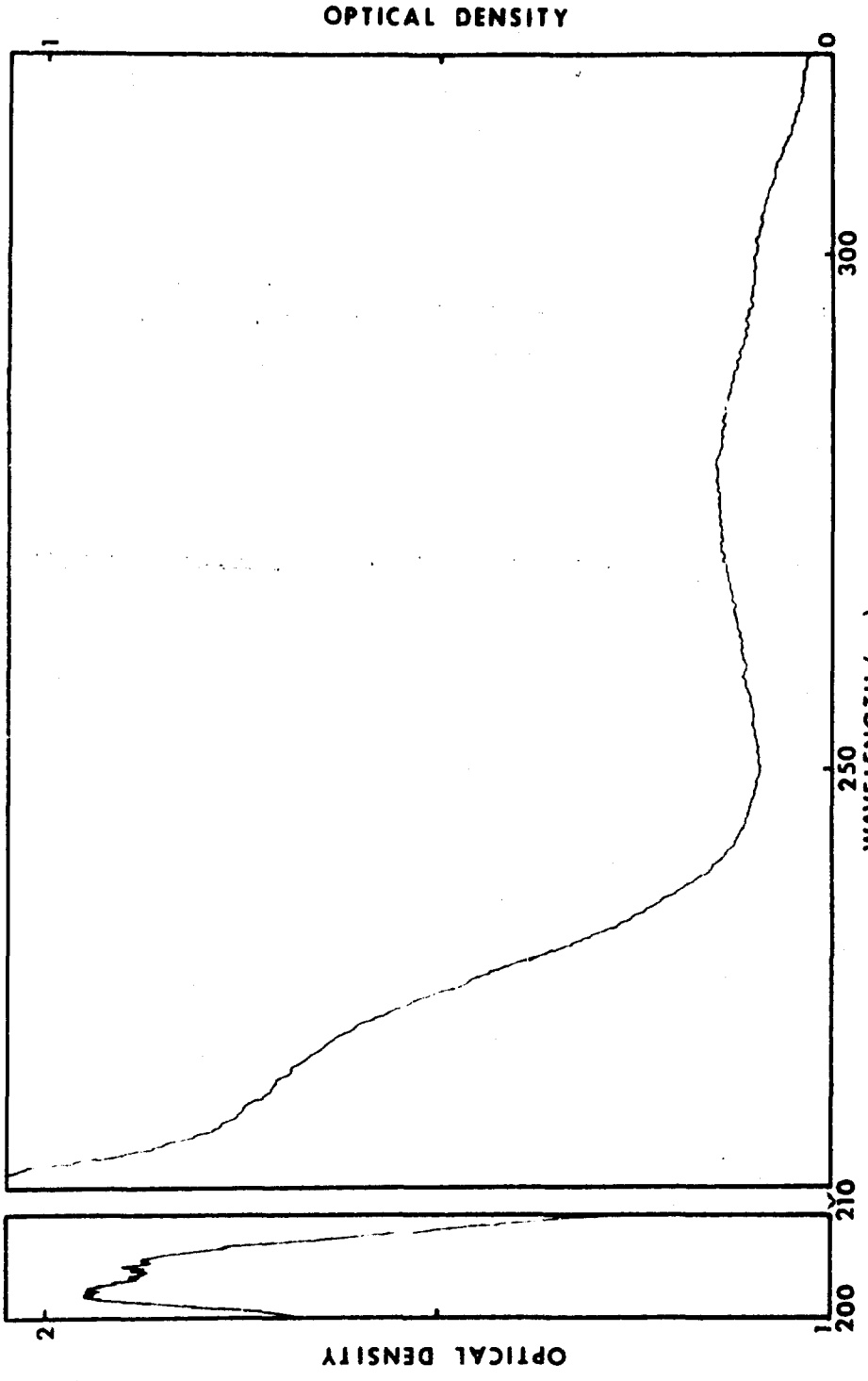

Figure 3. Absorption Spectrum of Dry Ear Skin and Dorsal Thoracic Skin Scales on a Nujol Film Between CaF_2 Plates in the Sample Compartment With Air As Reference

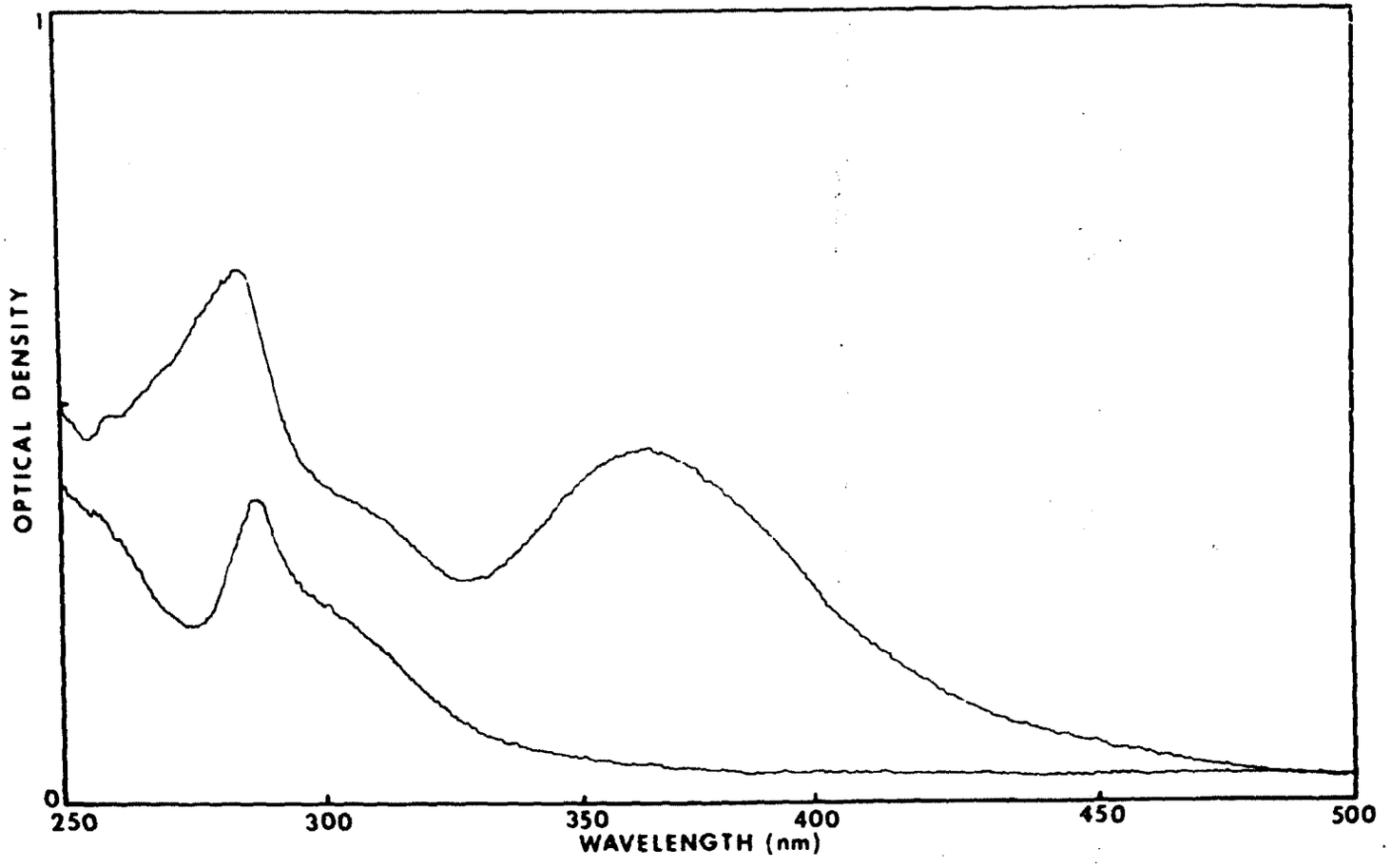

Figure 4. Absorption Spectra of Dry Ear Skin

The lower curve is the absorption of skin versus a slightly thinner skin sample as reference. The upper curve is the absorption of skin treated with iodine tincture and allowed to dry.

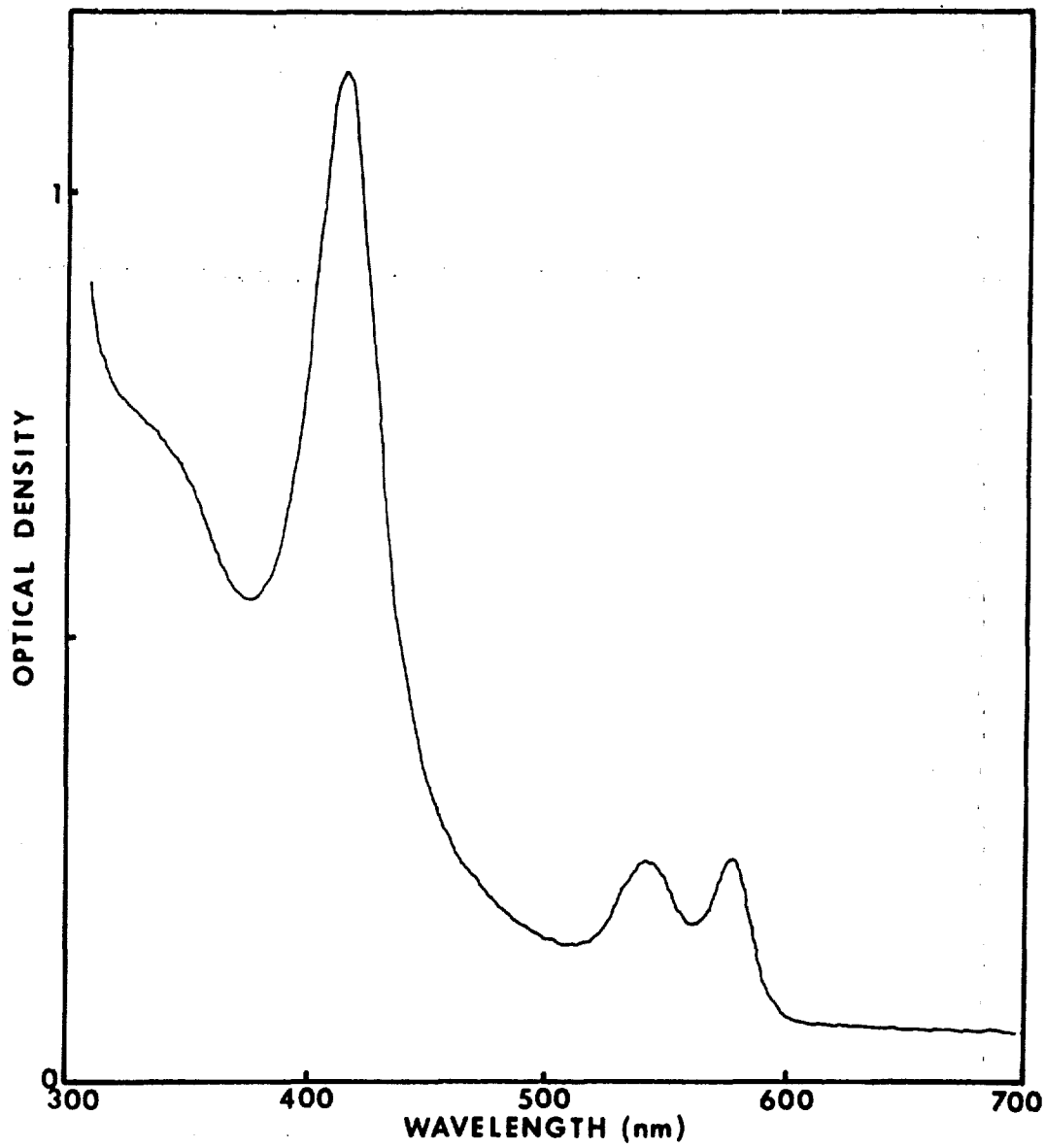

Figure 5. Absorption Spectrum of Dry Dorsal Thoracic Skin That Developed Erythema After Treatment With Strong Iodine Tincture

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14. KEYWORDS

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