## **Preliminary and Short Report**

## STUDIES OF SOME PHYSICAL PROPERTIES OF THE DIHYDROXYACETONE COLOR COMPLEX\*

LEON GOLDMAN, M.D., EVA WITTGENSTEIN, DONALD BLANEY, M.D., PH.D., JOHN GOLDMAN AND FAYE SAWYER

The color developed by dihydroxyacetone in contact with human skin continues to be studied extensively (1, 2). The physical characteristics of the colored products have been reported previously (3, 4, 5).

Thin layers of pure dihydroxyacetone crystals were first studied by microscopy and then with polarized light, phase contrast and fluorescence microscopy. The so-called "pure" crystals were irregularly polyhedral, mostly hexahedrol with total length varying from 240-260  $\mu$ , total width approximately 90  $\mu$ , and the triangular corner faces were approximately 50-60  $\mu$  on the edges. The crystals had a characteristic whitish-blue fluorescence under Wood's Light, 3650Å.

After exposure of dihydroxyacetone to pure ammonia or to air, the crystal size changes to long, irregular yellow crystalline masses, with variable color play under polarizing filters and develops a brownish fluorescence when observed by ultraviolet light filtered through the Wood's filter. The new crystalline masses were large, irregular tubular forms varying in width from 20  $\mu$  to 600  $\mu$  in length. Infrared absorption studies of pure dihydroxyacetone crystals and the yellowish crystalline masses were done by Lial Brewer and Robert Keenan of the Occupational Field Headquarters of the U.S. Public Health Service in Cincinnati. He found no differences in the spectra except in the carbonyl absorption region. The darker the sample, the more acidic was the carbonyl absorption. This was due probably to the further state of oxidation of the product. He observed no NH structure and confirmed our previous (2) findings of no quinone structure of the vellowish crystalline masses.

To study the color produced in living human skin, the skin was examined under stereobinocular microscopy with and without clearing of the skin (6). The color was evident as yellowish streaks in globular masses in the superficial part of the keratin, with accentuation along skin ridges,

Received for publication October 26, 1960.

poral orifices, comedonal area and areas of thickened or heavy skin. Under ultraviolet light filtered through the Wood's filter, reddish-brown fluorescence of these color masses was observed. This fluorescence appeared to be the same as that observed when the yellow crystalline masses were examined. The skin surface was stripped with Scotch tape. These mounts were suspended in water and also in mineral oil, and examined under the microscope with regular light, phase contrast and fluorescence microscopy. The pigment masses were found to be scattered in irregular groups as amorphous material in the superficial keratin. There was no evenness about the distribution patterns.



FIG. 1. Color reflectance curves, forearm, 24 hours after local application (G. E. Reflecting Electrospectrophotometer).

- A. Subject 1-suntan-3 days B. Subject 2-10% dihydroxyacetone and 5% methylanthranilate
  - C. Subject 2-10% dihydroxyacetone

B. Subject 3-90% dihydroxyacetone
E. Subject 3-90% dihydroxyacetone
F. Subject 1-10% dihydroxyacetone and 5% methylanthranilate

<sup>\*</sup> From the Department of Dermatology of the College of Medicine of the University of Cin-cinnati and The Research Foundation, Children's Hospital, Cincinnati, Ohio.

The authors are grateful to Neil Davis, Ph.D., of the Research Division of the Children's Hospital, Cincinnati, for his critical review and suggestions for study.

Additional studies were done in the fixed section to study the distribution of the coloring. The thin sectioning of routine microscopic sections destroyed much of the intensity of color. In deeply colored materials, such as warty growths, the satisfactory study of the stained masses was seen best in sections fixed in formaldehyde, unstained and deparafinized. It has been shown previously that formaldehyde did not disturb the color complex once it was formed, although it could inhibit its formation (2, 3). Preliminary efforts to develop a phosphomolybdic histochemical test for the color complexes were not successful, even though phosphomolybdic acid adsorbed on strips of filter paper does react with the dihydroxyacetone applied to the skin surface. (Evidence of unreacted dihydroxvacetone.)

As an additional study, the skin colored with different concentrations of dihydroxyacetone was examined by the G. E. Reflecting Electrospectrophotometer, under the direction of Robert K. Johnson and Cecil Kenyon, at the Color Division, Hilton-Davis Chemical Company, Cincinnati (Fig. 1). These reflectance curves were compared in one individual to a natural sun tanning on adjacent area of the forearm. As can be seen, the curves are identical for the color produced by dihydroxyacetone and by sun tanning. Also, the curves are uniform for different individuals and with different concentrations of dihydroxyacetone. In general, these curves are somewhat similar to color transmission curves of solutions of dihydroxyacetone-arginine reactions.

## SUMMARY

The color product(s) formed by dihydroxyacetone was studied in its crystalline phase by phase contrast microscopy, polarization, fluorescence, infrared and ultraviolet absorption and by color transmission. In addition, the colored products formed in human skin after application of dihydroxyacetone were studied by surface microscopy, microscopic sections and by color reflectance.

## REFERENCES

- 1. WITTGENSTEIN, EVA AND BERRY, HELEN K.: On the nature of the staining of skin with dihydroxyacetone. Science (in press).
- GOLDMAN, LEON, BARKOFF, JOEL, BLANEY, DONALD, NAKAI, TAKASHI AND SUSKIND, RAYMOND: Investigative studies with dihydroxyacetone and glyoxal. J. Invest. Derm., 35: 161, 1960.
- 3. WITTGENSTEIN, EVA AND BERRY, HELEN K.: Reaction of dihydroxyacetone (DHA) with human skin callus and amino compounds. J. Invest. Derm. (in press).
- 4. GOLDMAN, LEON, BARKOFF, JOEL, BLANEY, DONALD, NAKAI, TAKASHI AND SUSKIND, RAYMOND: The skin coloring agent dihydroxyacetone. GP, 23: 96, 1960.
- GOLDMAN, LEON, BLANEY, DONALD AND GOLD-MAN, JOHN: Topical therapy with dihydroxyacetone. Acta Dermatovener., (in press).
- GOLDMAN, LEON: Clinical studies in microscopy of the skin at moderate magnification. A.M.A. Arch. Derm., 75: 345, 1957.