THE PORPHYRIN CONTENT OF SKIN*

M. A. PATHAK, PH.D. AND J. W. BURNETT, M.D.

The ability to synthesize the tetrapyrrole nucleus of the porphyrins appears to be a common attribute of most living tissues. The tetrapyrrole nucleus, as found in catalase, peroxidase and cytochromes, is essential to biological activity, but the porphyrins, with the probable exception of isomer Type III protoporphyrin, are irreversibly oxidized by-products of heme-biosynthesis and have no recognized physiologic function (1, 2).

Among the many compounds implicated in the induction of cutaneous photosensitization none has aroused more clinical interest than the porphyrins. The presence of excess quantities of these compounds in skin, erythrocytes, plasma and other tissues has been considered to lead to the development of cutaneous photosensitiivty in porphyria. The amounts of porphyrin normally present in the urine, feces, erythrocytes, liver and bone marrow have been accurately determined, but little information about the amount of porphyrin normally present in the skin of man and common laboratory animals has been available. Because such information is essential to an understanding of the pathogenesis of photosensitization in porphyria, the range of porphyrin concentration found in a recent study of the normal skin of human subjects and laboratory animals is outlined briefly below and compared with the range found in the skin of porphyric patients and rats with hexachlorobenzene-induced porphyria.

MATERIALS AND METHODS

For determination of the normal range of porphyrin concentration (see Table I), skin was obtained from four newborn infants at the time of circumcision and by biopsy excision from three adults with atherosclerosis, two adult Sprague-Dawley rats and three adult mongrel dogs. Specimens were also taken within two to four hours after death from eleven adults who had died of atherosclerotic heart disease, diabetes, renal failure or trauma. Both the biopsy specimens and the autopsy specimens came from unexposed, mid-abdominal areas.

The porphyrin concentration in porphyric skin (see Table I) was determinedr fom biopsy specimens taken from one patient with mixed porphyria, one patient with hereditary hepatic porphyria and five Sprague-Dawley rats with hexachlorobenzene-induced porphyria (3).

Mixed skin-muscle specimens were obtained from two 4-month human embryos.

Approximately 0.25–0.6 gram of biopsy skin and 0.75–1.5 grams of autopsy skin were used for each analysis. The porphyrin content of all specimens was determined by the method employed by Schwartz *et al.* (4) for determining the concentraiton of porphyrin in blood. After ascertaining the specific wavelengths of the activation and fluorescence emission of the various porphyrins in each analysis (4), it was possible to determine the amount of uro-, copro-, and proto and deuteroporphyrin in each sample by measuring the intensity of fluorescence emitted at 595–600 mμ when the activating wavelength was 400 mμ.

The specimens were frozen at −20° C. immediately after excision and kept at this temperature until they were analyzed, usually within 6–14 days. Before analysis, each specimen was carefully separated from all adipose and connective tissue, cut into pieces measuring 2 x 3 mm, mixed with broken glass, and thoroughly ground in a mortar. Specimens taken from adults after death were cut into small pieces, frozen in liquid nitrogen (−195° C.), ground in a Wiley mill at −25° C., and passed through a sieve of #40 mesh.

RESULTS

The concentrations of the various porphyrins in embryonic skin, prepuce skin from newborn infants and the skin of adults obtained by biopsy and at autopsy varied within a very narrow range (see Table I). As can be seen, *uroporphyrin* was present in all the specimens analyzed. Its concentration in "normal" adult human skin obtained by biopsy excision (0.09 μg/g) was found to be below that in prepuce skin (0.48 μg/g) and in skin obtained from "normal" subjects at autopsy (0.38 μg/g). This may be due to the small size of the biopsy specimens. The uroporphyrin concentration in the skin of normal dogs (0.5 μg/g) was of the same order of magnitude as that in human skin. In specimens taken from the two patients with porphyria and from the five rats with hexachlorobenzene-induced porphyria, the uroporphyrin concentration was consistently higher than in the normal skin obtained by biopsy excision. *Copro* and *proto-deutero*porphyrin (0.02 μg/g and

* From the Research Laboratories of the Department of Dermatology, Harvard Medical School at the Massachusetts General Hospital, Boston 14, Massachusetts.

Supported by Grant CY-5003 from National Institutes of Health, United States Public Health Service

Received for publication October 30, 1963.

TABLE I

Porphyrin content of the skin of normal and porphyric subjects

Type of Skin	Type of Specimen	Number of Specimens	Average Porphyrin Content (μg/g of wet tissue)		
			Uro	Copro	Proto & deutero
Human					
Infant prepuce	Surgical	4	0.48	0	0
Normal, adult	Biopsy	3	0.09	0.02	0.02
Porphyric, adult	Biopsy	1	0.21	0.02	0.075
	Biopsy	1	0.13	0.07	0.045
Normal, embryonic	Autopsy	1	0.25	0.33	1.1
	Autopsy	1	0.51	0.0	0.0
Normal, adult	Autopsy	11	0.38	0	0
Animal					
Normal, adult, rat	Biopsy	2	1.65	0	0
Porphyric, adult, rat	Biopsy	5	2.87	0	0
Normal, adult, dog	Biopsy	3	0.5	0	0

0.02 μg/g, respectively) were found only in adult human biopsies and in the specimen from one embryo. The presence of these porphyrin fractions in the embryonic specimen may be attributable to contamination with muscle tissue. The uroporphyrin content of normal rat skin was found to be significantly greater than that of human skin. In this respect it may be of interest to note that rodents are known to produce much more porphyrin than other mammals (1).

DISCUSSION

It is interesting that the concentration of porphyrin was found to be similar in the unirradiated skin of dogs, rats, and humans and that it did not vary with the age of the donor. To understand the pathogenesis of cutaneous photosensitization in porphyria, it is necessary to know how the porphyrin metabolism of normal and porphyric subjects varies before, during and after exposure to light. The concentration of porphyrin in the skin may determine whether exposure to the requisite wavelengths (*e.g.*, 400 mμ) and adequate energy will or will not induce the cutaneous manifestations of porphyric photosensitivity (exaggerated erythema, urticaria, bullae, atrophy, etc). Uroporphyrin has been reported by Dankemeyer (5) and Schmid (2) to have the greatest photosensitizing potency of the different fractions of porphyrin, followed in order of decreasing effectiveness by the copro- and protoforms.

SUMMARY

To clarify the pathogenesis of cutaneous photosensitization in porphyria, the amounts of porphyrin normally present in the skin of man and some laboratory animals have been determined and compared with the range of porphyrin concentrations found in the skin of human subjects with porphyria and of rats with hexachlorobenzene-induced porphyria. Uroporphyrin was present in all the specimens of normal skin analyzed.

REFERENCES

1. Goldberg, A. and Rimington, C.: Diseases of Porphyrin Metabolism. Springfield, Ill., Charles C Thomas, 1962.
2. Schmid, R.: The porphyrias. Chapter 30 in The Metabolic Basis of Inherited Disease, pages 939–1012. Edited by J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson. New York, McGraw-Hill Book Company, Inc., 1960.
3. Ockner, R. K. and Schmid, R.: Acquired porphyria in man and rat due to hexachlorobenzene intoxication. Nature, **189:** 499, 1961.
4. Schwartz, S., Berg, M. H., Bossemaier, I. and Dinsmore, H.: Determination of porphyrins in biological materials. In Methods of Biochemical Analysis, Vol. VIII, edited by D. Glick. New York, Interscience Publishers, Inc., 1960.
5. Dankemeyer, W.: Über die photosensibilizierende Wirkung von Porphyrinen. Inaug. Diss., Hamburg. Riefling und Weingärtner, 1930.