Experimental Production of Skin Lesions in Human Cutaneous Porphyria.* (27342)

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The experimental production of skin lesions in porphyric subjects by application of monochromatic light of the Soret wave-band has usually been unsuccessful(1,2), although these subjects obviously suffered from sunlight sensitivity. By strict interpretation of the law of Grotthus-Draper, Blum(1) inferred that there is no direct relationship between skin porphyrins and actinic lesions. Nevertheless, Magnus *et al.*(3,4) have obtained photodynamic action spectra (vesiculation) in porphyric individuals, showing that the maximum effect corresponds with the porphyrin Soret band.

Comparative calculations between the energy levels of artificial light sources and those of sky and sunlight show that $I\lambda$ ($\mu W/cm^2$ per $\Delta\lambda = 10$Å) at *ca.* 4050Å for Beck carbon arcs, high pressure mercury burners and xenon lamps is far higher than that to be expected in sky and sun light(5,6). In the present study it has been shown that doses of U.V. light of the 4050Å range approximately equal to natural energy levels fail to produce skin lesions in the majority of porphyrics even though presumably modest doses of sunlight produce lesions in these cases.

The present report is concerned with the basis of the difference between sunlight and artificial light in production of photodynamic skin lesions. Skin biopsies from the hands and lower abdominal wall corresponding to light exposed and non-exposed skin were studied by fluorescence microscopy. Six cases of cutaneous porphyria have now been studied, including one of erythropoietic and five of hepatic classification, the latter group consisting in type of 3 cutanea tarda (idiosyncratic, or symptomatic), one hereditary cutaneous, one mixed or variegate. In 3 of the 6 patients porphyrin was demonstrable in the skin of the hands, whereas in the skin usually not exposed to sunlight, that of the abdominal wall, the presence of porphyrin was readily demonstrated in all 6 cases. This was of the same distribution and subepithelial localization, but in larger quantities in comparison to the light exposed skin of the hands.

Skin fields adjacent to the sites of biopsies were irradiated with light of 4050Å from an H100-A4 mercury lamp. The energy emission in the Soret band region after monochromatizing ($T_{50} = \pm$ 375Å) exceeded the energy of Minnesota summer sun at noon within this wave length band by a factor of 2. Irradiation with this intensity up to 60 minutes failed to produce any change in the skin of either hand or abdomen. But as soon as modest energy of the near infra-red, *ca.* 26000 Å, was delivered incident with the 4050Å wave band, a significant change was observed. The infra-red intensity, in terms of skin surface temperature increase, never exceeded 41.6°C heating of the field. Within 20-30 minutes after discontinuance of irradiation, erythema, edema and vesiculation appeared at previously completely ineffective dose levels of 4050Å light. The infra-red radiation alone, up to 45 minutes, was followed only by a slowly fading mild erythema. The effect of the combination of both wave bands of light was more consistent in the skin of the abdominal wall, where the porphyrin content was higher. The skin of the hands with notably lower porphyrin content, responded only to much higher dosage levels of the combined light in comparison to the skin of the abdomen.

Change of the sequence of irradiation, employing the 4050Å light first, followed by 26000Å radiation at 50% of the minimum effective dose when given simultaneously, as above, resulted in the same type of lesions. The U.V. light had to be given prior to the infra-red in order to produce erythema and edema. When the infra-red was given first

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TABLE I. Skin Porphyrin Content and Reaction to Irradiation in Cases of Porphyria.

Subject	Diagnosis	Skin porphyrin	4050Å	26000Å	4050Å + 26000Å (simultaneously)	26000Å first 4050Å later	4050Å first 26000Å later
			A. Skin of hands				
RA	PCT (idiosyn.)	+	0	0	EOV	0	EOV
FT	*Idem*	0	0	0	0	0	0
JC	Mixed or variegate	+	0	0	0	0	0
MH	*P. erythropoetica*	0	0	0	0	0	0
EP	PCT (idiosyn.)	0	0	0	0	0	0
EA	Hered. cutan. p.	+	0	0	EOV	0	EOV
			B. Skin of abdomen				
RA	As above	++	0	0	EOV	0	EOV
FT	"	+	0	0	EO	0	EO
JC	"	++	0	0	EOV	0	EOV
MH	"	+	0	0	EO	0	EO
EP	"	++	0	0	EOV	0	EOV
EA	"	++	0	0	EOV	0	EO

Conditions: Dλ eff. abdomen ≤ .5 Dλ eff. hands.
Key: E = erythema; O = edema; V = vesiculation; PCT = porphyria cutanea tarda.

only a transitory erythema appeared, without edema or vesicle formation. The changes which followed the sequence of U.V. then I.R., lasted over several days, whereas the reverse sequence proved ineffective. The observations are brought together in Table I.

Control studies have been carried out in 3 normal subjects and one case of reticuloendotheliosis. In these nothing more than mild transitory erythema was noted after combined irradiation as above described, whether 4050Å and 26000Å simultaneously, or 4050Å followed by 26000Å light.

The reported experiments indicate that the energy required in the laboratory to produce actinic lesions in the skin of porphyrics by light around 4050Å can be brought to the clinically effective dose as received under natural outdoor conditions, as soon as the porphyric skin receives near infra-red radiation as well. It is likely that the production of vesicles in the experiments of Magnus(3,4) relate to a far greater dose of Soret band light than was given in the present experiments. Exact comparison is impossible due to lack of detailed physical data. Nevertheless, the use of a 2 KW xenon arc indicates a much greater spectral intensity than the emission from the 100 W mercury lamp employed.

There is an apparent relationship between dose level response and microscopically detectable porphyrin in the skin. The basis of the relatively low porphyrin concentration in the light exposed skin is as yet not clear. Masking of fluorescence by other substances present in higher concentration than in the unexposed skin, or continuous destruction of porphyrin by the activity of light, are possibilities deserving exploration. In support of the latter is an observation in one of the present cases that the skin biopsy before radiation exhibited considerable porphyrin, while that from a nearby similar area taken after radiation failed to show any porphyrin fluorescence whatever.

It is well known that in cases of cutaneous porphyria, minor, mechanical trauma to light exposed skin, often results in epidermolysis and formation of vesicles or bullae. Whether this form of injury is similar to IR (heat) in the basic character of its effect, remains to be determined.

Summary and conclusions. Light of the porphyrin Soret wave band, *ca.* 4050Å, of intensity comparable to natural energy levels, failed to produce skin lesions in several cases of cutaneous porphyria. Simultaneous, or subsequent infra red irradiation (26000Å) was followed by erythematous, often vesiculobullous lesions. Preliminary application of the same amount of heat of the same wave band followed by the 4050Å light was ineffective. The unexposed skin of the abdomen regularly contained more porphyrin than that of previously light exposed skin of the hands. Lesions were more readily produced by exposure of the abdominal skin to 4050Å and 26000Å wave length energy.

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Histochemistry LXVII. Microscopic Microbiological Assay. Determination of Biotin to 10^{-15} Gram.* (27343)

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Previously, a system for microbiological assay depending on measurement of light absorbance of bacterial suspensions in microcuvettes was described in which 500 to 1000 times less sample than the commonly employed ml-range was used(1). Subsequent developments included elaboration of methods for determination of coenzyme A(2) and biotin(3) in μg-amounts of tissue and their applications to analyses of microtome sections of adrenal(4,5).

The technic has now been extended to determinations with submicroliter volumes of total reaction mixture, as droplets under oil, by measurement of reflectance from suspended bacteria; and, to illustrate its use, analysis of biotin with *Lactobacillus arabinosis* 17-5 (*L. plantarum*, ATCC 8014) in volumes of reaction mixture from 0.6 to 0.01 μl with a sensitivity for the smaller droplets of 10^{-15} g was developed.

Methods. The assembly used for reflectance measurement (Fig. 1) consists of a microscope (Zeiss, standard GFL, with phase contrast condenser, 111Z, having annular diaphragm in position 2 to furnish dark-field illumination—superior to usual dark-field condenser in providing greater intensity of illumination and finer adjustment of area illuminated) with a 2.5 × objective and a microphotographic adapter (Leitz, No. 72.005) containing a shutter, an observation eyepiece, a 12.5 × ocular, and individual cable releases to operate the shutter and a prism which directs the light either to the eyepiece or the photomultiplier tube (Photovolt, Type E) mounted above the adapter. A Photovolt photometer, Model 512, is used. The arrangement of the sample chamber for measurement is shown in Fig. 2.

For each use a clean glass slide (38 × 75 mm) is placed in hot half-conc. nitric acid for 10 min, rinsed well with distilled water, dried in an air stream filtered through cotton, and siliconed on one side by rubbing with clean gauze saturated with 5% Drifilm (General Electric) in chloroform, air-drying, and rinsing with distilled water and draining dry. The surface may be improved by polishing with gauze. The aqueous droplets will rest on the slide with a constant interface diameter if the siliconing is proper.

The sample chamber is made by sealing a Lucite frame (32 × 70 × 8 mm deep) to the siliconed slide by interposing a 2-layer frame of Parafilm M between the Lucite and the slide and applying gentle pressure while heating on a hot plate until the Parafilm loses its milky appearance and becomes transparent. The chamber is filled with about 6 ml of oil (3 vol. *n*-tetradecane, Eastman Kodak No. P2221, plus 1 vol. light liquid petrolatum,

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