# Effect of Rose Bengal and Ultra Violet Light on Porphyrin Excretion in Rabbits.* (17411)

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The photodynamic effect of porphyrins *in vitro* and *in vivo* is well known,[1] as is the relationship of sunlight to so called photosensitive or congenital porphyria. In 10 cases of *Pemphigus foliaceus* characterized by photosensitivity, the author has also found large increases in excretion of urinary coproporphyrin.‡ The question arose as to whether a heightened urinary porphyrin excretion might be the effect of photodynamic activity, rather than, as always hitherto supposed, that the excessive porphyrin was the cause of this activity. The well known occurrence of marked increases of urinary coproporphyrin III in a variety of disease states,[2] *without* photosensitivity, in fact suggested that photodynamic activity in the *Pemphigus foliaceus* cases might be causing the coproporphyrinuria.

Rose Bengal is also known to exert a powerful photodynamic action.[3] In studying the fundamental relationship of light to porphyrin excretion the effect of previous sensitization by Rose Bengal and other photosensitizing compounds was investigated. Some of the results are the basis for the present report.

*Methods.* Adult rabbits of either sex were injected intravenously with a dose of 100 mg of Rose Bengal per kilo of body weight. The dye was dissolved in 10 cc of physiological saline solution.

Unfiltered light from a CH-4 Mercury arc lamp§ of maximum near ultraviolet intensity at 3650 Å, was used for the photosensitization. This wave length corresponds with the maximum absorption of the dye. The hair was shaved from the animal's back and the shaved area was irradiated immediately following the administration of Rose Bengal for 25 minutes at a distance of 12 inches, the approximate focal distance of the lamp.

The concentration of total urinary coproporphyrin was determined with a Lumetron fluorophotometer, in other respects following the technic of Schwartz, Hawkinson, Cohen, and Watson.[4] Their method of isomer analysis[4] was also employed. Crystallization of coproporphyrin methyl ester was done according to methods used previously in this laboratory.[5]

*Results.* The results are given in Table I. The zero value in each instance serves as a control. Rabbits R1-R5 received both Rose Bengal and ultraviolet light at the beginning of day number 1. Control Rabbit No. 1 was injected with Rose Bengal in the same concentration, but the animal was kept in darkness. Control Rabbit No. 2 received ultraviolet irradiation but no Rose Bengal.

Isomer analysis of the coproporphyrin from Rabbit 1 for the first 24 hours after the photosensitization, revealed 96% coproporphyrin type III, indicating that most of the increase was of this isomer. The methyl ester crystallized in typical rosettes melting at 145°, thus confirming the isomer analysis.

The fecal coproporphyrin was determined during the first 4-day periods in 3 rabbits. No increased values were found.

To control the factor of hemolysis, distilled water was injected intravenously in sufficient

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1 Blum, H. F., Photodynamic actions and disease caused by light, Reinhold Pub. Corp., 1941.

‡ Unpublished.

2 Watson, C. J., and Larson, E., *Physiol. Rev.*, 1947, **27**, 478.

3 Fiessinger, N., Albeaux Fernet, M., Aussanaire, M., *Bull. Soc. Franc. Derm. et Syph.*, 1937, **44**, 1768.

§ Black Light Products, Inc., Chicago, Ill., Model No. B1-2.

4 Schwartz, S., Hawkinson, V., Cohen, S., and Watson, C. J., *J. Biol. Chem.*, 1947, **168**, 133.

5 Grinstein, M., Schwartz, S., and Watson, C. J., *J. Biol. Chem.*, 1945, **157**, 323.

TABLE I.
Effect of Rose Bengal and U.V. Light Alone and in Combination on Urinary Coproporphyrin Excretion in Rabbits (γ per 24 hr).

| Day | R1 | R2 | R3 | R4 | R5 | Control 1 | Control 2 |
|---|---|---|---|---|---|---|---|
| 0 | 7.0 | 6.2 | 6.4 | 9.7 | 5.8 | 7.8 | 6.6 |
| | | | Rose Bengal and U.V. light | | | | |
| 1 | 300.0 | 110.0 | 120.0 | 150.0 | 135.0 | 8.6 | 5.2 |
| 2 | 80.0 | 96.6 | 53.2 | 55.0 | 76.8 | 14.0 | 6.3 |
| 3 | 70.0 | 70.0 | 25.0 | 38.6 | 62.8 | 9.0 | 7.2 |
| 4 | 30.0 | 12.0 | 15.0 | 38.7 | 15.7 | 6.8 | 7.4 |
| 5 | 7.2 | 10.0 | 10.0 | 7.7 | 13.8 | 9.4 | 6.8 |
| 6 | 9.4 | 5.0 | — | 15.3 | 9.7 | 8.6 | 8.1 |
| 7 | 10.0 | 6.3 | 15.6 | 21.3 | 9.8 | 6.5 | 7.3 |
| 8 | 10.4 | 7.8 | 12.0 | 16.7 | 10.1 | 5.8 | 6.5 |
| 9 | 10.1 | 9.4 | 11.0 | 16.5 | 8.7 | 7.6 | 7.3 |

TABLE II.
Effect of Hemolysis Produced by Distilled Water or Phenylhydrazine Hydrochloride on Urinary Coproporphyrin Excretion in Rabbits.

| | Urinary Coproporphyrin in γ in 24 hr | | |
|---|---|---|---|
| Day | R6 | R7 | R8 |
| 0 | 7.3 | 9.7 | 10.0 |
| | Distilled water | Phenylhydrazine alone | Phenylhydrazine with U.V. light |
| 1 | 4.1 | 10.0 | 9.9 |
| 2 | 9.7 | 12.0 | 10.0 |
| 3 | 11.0 | 16.0 | 4.5 |
| 4 | 10.8 | 27.0 | 10.9 |
| 5 | 9.7 | 28.0 | 28.0 |
| 6 | 8.4 | 26.1 | 42.0 |
| 7 | | 40.7 | 24.0 |

quantity to cause a rapid and intense hemolysis in one rabbit. As seen in Table II, there was little if any effect on the coproporphyrin excretion.

For the same purpose 30 mg of phenylhydrazine hydrochloride per kg were injected subcutaneously into 2 rabbits. One of these animals (R8) was exposed to U.V. light for 25 minutes immediately after phenylhydrazine administration.

The mild delayed increase noted in the phenylhydrazine experiments in Table II is probably related to increased erythropoietic activity, as previously found by Schwartz and co-workers.[6]

*Summary and conclusions.* The administration of Rose Bengal and ultraviolet irradiation in rabbits results in rapid and marked increase in the excretion of urinary coproporphyrin Type III to values 17 to 40 times those in the controls, returning to normal by the 5th day. Neither the dye alone nor the irradiation alone had any effect.

Simple hemolysis by distilled water had no effect upon the urinary coproporphyrin excretion. Phenylhydrazine produced a slight delayed increase of coproporphyrin excretion believed to be due largely to an increase of coproporphyrin Type I, as a result of increased erythropoiesis.

Additional studies are being undertaken in an attempt to discover the origin of the coproporphyrin excreted and the effects of other photodynamic compounds.

These results constitute the first demonstration that photodynamic activity causes a marked increase in the excretion of coproporphyrin III.

[6] Schwartz, S., Glickman, M., Hunter, R., and Wallace, J., AECD-2109.

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