LXIV. NOTE ON THE EFFECT OF LIGHT UPON UROPORPHYRIN.

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INTRODUCTION.

The following paper gives some account of the phenomena occurring in relation to the action of light upon uroporphyrin, prepared from the urine of a case of haematoporphyrin congenita.

It has been known for some time that uroporphyrin fades upon exposure to daylight, and Schumm [1916] has shown that, as the fading proceeds, the colour of the solution turns from rose-red to brown, and that an additional band appears in the absorption spectrum at 4610–4620 Å.

Apart from this, little seems to be known regarding the photochemistry of uroporphyrin, although a knowledge of it must be of importance in the investigation of the part played by uroporphyrin in the sensitisation to light that is so characteristic of haematoporphyrin congenita.

EXPERIMENTAL.

In the course of some experiments dealing with the factors involved in the process of blistering as observed in the cases of haematoporphyrin congenita, uroporphyrin solutions of different hydrion concentrations were exposed to daylight, and not only did a general fading take place, but there was also some differentiation of fading between the individual solutions.

With the purpose of gaining information concerning the relation between the degree of fading and the hydrion concentration of the solution, two series of experiments were performed, the one with crude uroporphyrin, the other with purified uroporphyrin.

(a) Daylight effects.

Series I. Crude uroporphyrin was prepared by Nebelthau's method [1899] from the urine of the case of haematoporphyrin congenita described by Garrod and Mackey [1921]. This method consists essentially in the precipitation of uroporphyrin and other substances from the urine by acetic acid.

Solutions of the uroporphyrin in phosphate-buffered solutions were made up extending over the range p_H 6.2–8.2. The uroporphyrin was dissolved in $M/15$ Na_2HPO_4 solution in a concentration of 260 mg. per litre. To this was added, as shown in Table I, the necessary amounts of $M/1.5$ KH_2PO_4 solution. In this way undue dilution of the uroporphyrin solution was avoided. (This

latter point was of importance, since colorimetric estimations had to be made, and economy had to be exercised as regards the amount of uroporphyrin used.

The p_H of each solution was confirmed by electrometric determinations.

Table I.

Solution No. ...	1	2	3	4	5
	cc.	cc.	cc.	cc.	cc.
$M/15 \text{ Na}_2\text{HPO}_4 + \text{Up.}$...	4	4	4	4	4
$M/1.5 \text{ KH}_2\text{PO}_4$...	1.6	.4	.1	.02	—
Water ...	.4	1.6	1.9	1.98	2
$p_H (\pm 0.1)$...	6.2	6.8	7.3	7.7	8.2

The solutions were in every case made up in a dark room where the stock solution of uroporphyrin in Na_2HPO_4 was kept. Before exposure to light the solutions were of a bright rose-red colour.

For experiment, the solutions were exposed in sets of five in Pyrex glass tubes to daylight of varying intensity and for different times. (An ordinary photographic exposure meter was used in some cases for comparing the intensities of the different lights, but its use was afterwards discontinued, since the light rarely remained of constant intensity for more than a few minutes at a time.)

After exposure the degree of fading of the solutions was estimated colorimetrically.

Upon exposure to light a general fading always took place throughout the series, the rose colour tending to turn brownish and lighter. This fading was not due to precipitation of the uroporphyrin. Some difficulty was experienced in obtaining trustworthy colorimetric readings after exposure, as the "browning" referred to above made the colour matches with the control solutions very bad and hard to get with any great degree of accuracy. In every case, however, it was found that the solutions at p_H 6.8–7.3 differed most in colour from the control tube.

Series II. In order to make certain upon this point, a further amount of uroporphyrin was prepared from the same source by the method of Fischer and Zerwecke [1924]. By this method uroporphyrin is obtained free from traces of coproporphyrin, and much less "browning" is observed during and after exposure, the final colour being much purer. The above experiments were then repeated exactly in detail with the purified uroporphyrin, when much more constant results were obtained, and the colour matches were far better. Table II illustrates a typical experiment.

Results obtained from an experiment using purified uroporphyrin exposed for 1 day to north light in Pyrex tubes:

Table II.

Solution No. ...	1	2	3	4	5	Control
	cc.	cc.	cc.	cc.	cc.	cc.
Absolute colorimetric reading	18.1	18.4	18.9	18.4	18.2	15
Reading in % increase over control ...	20.6	22.6	26.0	22.6	21.3	—
$p_H (\pm 0.1)$...	6.2	6.8	7.3	7.7	8.2	—

(b) Effects of ultraviolet light.

Upon exposure of solutions, made up as above, to ultraviolet light (Cooper-Hewitt lamp) in quartz tubes, a general fading, irrespective of p_H , was obtained.

No evidence of differentiation was found with either the crude or the purified uroporphyrin.

(c) Effects of exposure to light in absence of oxygen.

In order to determine whether these effects might be due to oxidation, sets of solutions were sealed off in nitrogen, the technique used being similar to that described by Ellis and Newton [1925]. They were then exposed to light. After exposure gas samples from each tube were taken, and the oxygen content determined. The percentage of oxygen found was never greater than 0.1.

Under these circumstances a difference was noted between the behaviour of crude and purified uroporphyrin.

Pure uroporphyrin solutions faded with occurrence of the usual differentiation, and no difference was detected between their behaviour and that of controls set up in tubes open to the atmosphere. It would therefore appear that oxidation plays little or no part in the phenomena of fading described. Crude uroporphyrin solutions, on the other hand, fade slowly in absence of oxygen, but quickly if oxygen be present, at a much greater rate than do solutions of pure uroporphyrin. It is evident that oxidation plays some part in the fading, and it is possible that there are two reactions going on, one a purely photochemical reaction, which occurs with both purified and crude uroporphyrin, and does not involve oxidation, and the other an oxidative reaction, occurring only with the crude uroporphyrin solution.

(d) Spectral effects.

As the solutions fade there is little or no change in the position of the absorption-bands (of which there are four—in the solution of the purified uroporphyrin at 496–513, 533–547, 557–573, 607–618 $\mu\mu$, and in the crude uroporphyrin at 498–525, 537–558, 565–575, 607–618 $\mu\mu$), at least as far as could be determined with a small direct-vision spectrometer.

The bands simply fade away as the solution fades. Within the range of p_H used the appearance of the "Kalilichtreaktion" band described by Schumm at 461 $\mu\mu$ could not be detected.

Conclusion.

It will be seen from the above results that the degree of fading does not increase or decrease progressively with the change in reaction of the solution. It has constantly been found that there is some definite difference in the degree of fading at p_H 7.2–7.6. Though this is not great as compared with the general fading, it has been observed too constantly to be attributable to experimental error.

Whether it has any significance cannot as yet be said, but it is interesting that it should occur at a reaction which approximates to the physiological reaction. It may have some bearing upon the fact that the teeth of the patient, from whose urine the uroporphyrin was obtained, were in March 1918 (when he was two years old) of a pink colour hardly to be distinguished from that of the gums, but three years later had undergone a colour change to brownish-pink.

So far it has not been possible to obtain results in confirmation of the above by using solutions with other buffers.

SUMMARY.

The fading of solutions of uroporphyrin prepared from a case of haematoporphyrin congenita has been investigated within the p_H range 6.2–8.2.

1. Over this range there is a differential fading, which reaches a maximum at p_H 7.3 approximately.

2. The rate of the general fading of uroporphyrin solutions is greater in the case of those containing crude uroporphyrin than in those containing purified uroporphyrin.

3. In the case of crude uroporphyrin, the fading is in part associated with the presence of oxygen; for the fading of pure uroporphyrin the presence of oxygen is not necessary.

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REFERENCES.

- Ellis and Newton (1925). *Amer. J. Physiol.* **63**, 530.
Fischer and Zerwecke (1924). *Z. physiol. Chem.* **137**, 176.
Garrod and Mackey (1921). *Quart. J. Med.* **15**, 319.
Nebelthau (1899). *Z. physiol. Chem.* **24**, 324.
Schumm (1916). *Z. physiol. Chem.* **98**, 123.