LIX. ANIMAL QUINOIDINE.

PRELIMINARY PAPER.

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It is a well-known fact that the nails, skin, teeth and bones fluoresce blue when exposed to a source of ultra-violet radiation. This is best seen by interposing between the source of radiation and the irradiated object a sheet of material which excludes the visible rays. While examining the properties of some of the yeast concentrates obtained in the course of another research, it was observed that these also showed a blue fluorescence. Specimens of normal urine are also known to behave similarly. The coincidence of these facts led to this work. We have found the following literature upon the subject of blue fluorescence in ultra-violet light in the animal organism.

HISTORICAL.

Brucke [1845] stated that the lens of the human eye absorbed blue rays. Helmholtz [1855] found the human retina to be feebly fluorescent. Regnaud [1858] using sunlight described a blue fluorescence in the ultra-violet in the lens of the sheep, dog, cat and rabbit, the central part of the lens retaining this property upon keeping. This did not hold for aquatic vertebrates or mollusca. In the case of the human eye, Setchenow [1859] found that the lens fluoresced strongly like quinine, the blue tone predominating. The cornea on the contrary was only feebly fluorescent and the aqueous humour did not fluoresce at all. The most interesting paper is one by Bence Jones [1866]. In a lecture to the Royal Institution, he described the presence of a substance in animal tissues to which he gave the name of "animal quinoidine." In the course of a research upon the rate at which quinine was distributed to the tissues after administration, he found that all animal tissues and organs contained a substance which fluoresced blue when excited by ultra-violet rays. Its behaviour resembled that of quinine in that the fluorescence was diminished by addition of salt or strong acid, being resistant to treatment with permanganate in acid solution. By comparison with the fluorescence of known concentrations of quinine salts he estimated the amount of this substance in various tissues.

Other work upon the nature of blue fluorescent substances seems to be scanty. Schliess and Lowenfeld [1863] described the blue fluorescence of urine in sunlight. Jaffé [1869] mentioned the blue fluorescence of urine, but the paper in question deals mainly with the properties of urobilin and the green fluorescence of bile salts. In modern times Schanz [1921] investigated the fluorescence of the lens and Langecker [1922] stated that most urines show a blue fluorescence and some a yellow. Familiar urinary constituents such as urochrome, uroerythrin, urobilin, and the porphyrins were not considered to be responsible for the blue fluorescence. In the case of the porphyrins, he prepared a series of salts of haematoporphyrin by the Milroy technique most of which gave a yellow or red fluorescence, the only exceptions to this being the copper salt which fluoresced violet in amyl alcohol, and the lead salt which fluoresced green.

EXPERIMENTAL.

The present paper gives an account of certain observations which we have made upon the nature and distribution of substances which fluoresce blue in ultra-violet light ($\text{\AA}$. 3000–4000). Our observations are qualitative only so far as this paper is concerned, and may be grouped for convenience under the following headings:

- A. Substances of known constitution, and certain commercial preparations.
- B. Animal products: (1) (a) Guano, (b) Urine, (c) Bile.
(2) Other animal extracts.
(3) (a) Gelatin, (b) Skin, (c) Lens.
- C. Plant products: (1) Grass.
(2) Oils.
(3) Other products.

Throughout we have employed as a source of ultra-violet radiation a mercury vapour quartz lamp made by the Hewittic Company, and have used as a screen for the exclusion of the visible rays a plate of glass supplied by Messrs Chance.

According to the assertion of the makers, this screen lets through light of wave length $\text{\AA}$. 3100–3900, the maximum being at a wave length of 3650, at which there is a transmission of some 50 %. Solutions have been examined for fluorescence in thin walled test tubes 13×75 mm., having a thickness of glass of some 0.5 mm. Controls in quartz tubes have not shown different behaviour as regards the colour of the fluorescence.

A. *Substances of known constitution, and certain commercial preparations.*

Table I (a) is a list of substances of known constitution which have shown no fluorescence in solution. In the case of the amino acids care was taken to examine the behaviour in alkaline and acid as well as in neutral solution. Alcohol was added to obtain solution where necessary.

Table I (b) shows certain other substances found to fluoresce a faint blue.

Table I (a). *Substances showing no blue fluorescence in solution.*

Tryptophan, cystine, leucine, histidine, tyrosine, glycine.

Allantoin, caffeine, hypoxanthine, uric acid, creatine, creatinine, xanthine, alloxan, parabanic acid, guanine, urea, guanidine, hippuric acid.

Cholesterol, cholesterol acetate, cholic acid, cholalic acid, laevulose, arabinose, cane sugar (purified by alcohol treatment), mannitol, sodium propionate, sodium maleate, salol, salicin, amygdalin, calcium lactate, starch, crotonic acid, tripalmitin, sodium glycerophosphate, turpentine, histamine, triphenylguanidine, choline, ox blood plasma and serum¹, egg albumin.

Table I (b). *Substances showing blue fluorescence.*

Guanine (commercial specimen; due to impurity), yeast nucleic acid (comm. specimen), lecithin (comm. specimen; very faint), crude lanolin, sodium glycocholate and taurocholate (laboratory specimens, faint blue): in the case of the glycocholate, it was found that the fluorescence could be removed by shaking with chloroform.

Gelatin, Nelson's "gold label" (commercial).

Gelatin from ossein

Gelatin from Coignet "gold label" } flocculated in an electric field.

Gelatin from horn

(In the flocculated gelatin, the fluorescence was much less decided than in the commercial gelatin.)

B. *Animal products.*

1. (a) *Guano.*

We were led to investigate guano extracts from the observation that a commercial specimen of guanine fluoresced a blue colour. Fluorescent extracts can be obtained from guano in several ways of which the following two are typical examples:

Method 1. Guano is extracted with warm 50 % alcohol containing 1 % HCl. From the cooled and filtered extract, the addition of either ether or chloroform extracts the bulk of the blue fluorescent substance, some however always remains behind in the aqueous phase.

Method 2. Guano is extracted with warm acetone. From the cooled and filtered extract practically all the blue fluorescent matter passes into the ethereal layer, upon addition of ether and water, which, upon evaporation, yields a reddish brown residue fluorescing almost a quinine blue when diluted with alcohol containing water to a faint yellow colour. It is relatively insoluble in water in acid solution, but dissolves in quantity upon the addition of alkali. It has not been possible so far to separate the blue fluorescing substance from the pigmented matter. Weight for weight the crude fraction is at least five times more powerful than quinine sulphate as a source of blue fluorescence. Porphyrin is present in this fraction, and can be extracted from it by the method described by Fischer [1923] for the extraction of Kammerer's porphyrin, after solution in ether containing acetic acid. After removal of the porphyrin there are no bands seen in the extracts of fluorescent material, merely a vague absorption up to λ 490.

By treatment of the aqueous phase fractions obtained in method 1 with alcoholic potash, and subsequent shaking with ether, an ethereal extract is obtained which fluoresces green, changing to blue on neutralisation.

¹ Dr Howitt mentioned to us that he has found blue fluorescence in human serum. We therefore examined rabbit serum and found fluorescence. The matter is being further investigated.

(b) *Urine.*

All the urines examined fluoresced a decided blue though this tint is slightly modified by the green fluorescence of urobilin. The urochrome fraction prepared by the Garrod technique [1894] fluoresces blue when diluted to a pale yellow.

As in guano, there are present two classes of fluorescent fractions, distinguished by their behaviour on fractionation from a water-alcohol-ether mixture. There is relatively more of the aqueous fraction present in the urines examined by us than in the guano extracts. The behaviour of the aqueous fraction on saponification with alcoholic potash is similar to that of the guano fraction.

(c) *Bile.*

Bile was treated with acetone and the precipitate removed. When this was shaken with ether in successive portions a deep violet blue fluorescence appeared in the ether phase quite different from the blue fluorescence previously mentioned. We obtained some evidence that there was also a "blue fluorescent substance" present in the aqueous layer, but it is difficult to see, as it is much obscured by bile pigment. It will be noted from Table I that specimens of cholalic and cholic acids showed no fluorescence. Laboratory specimens of Na taurocholate and glycocholate gave a slight blue fluorescence, quantitatively of a different order from the guano extracts. This appeared to be due to an impurity, as, in the glycocholate, the blue fluorescence passed readily into a chloroform layer upon shaking the aqueous solution with chloroform.

2. *Other animal extracts.*

We have obtained chloroform extracts fluorescing blue from sheep's liver, milk, and white hen feathers by extraction with acid alcohol followed by treatment with chloroform, and also from caseinogen by extraction with 95 % alcohol. Blood plasma and serum when diluted showed no fluorescence.

The extracts obtained under (1) and (2) are very similar in type. We have obtained evidence of a different behaviour in the case of (3).

3. (a) *Gelatin.*

Commercial leaf gelatin in the solid state and when dissolved in water fluoresces blue. The fluorescence of the solid gelatin is intensified by soaking in acetone. The blue fluorescence is hardly affected by boiling with dilute acids, but disappears in large part after treatment with alcoholic potash. We have not been able to obtain by any means an ether-alcohol extract from gelatin, which fluoresces blue. Further samples of flocculated gelatin kindly supplied by Prof. Schryver fluoresced a faint blue, but much less intensely than the commercial gelatin.

(b) Skin.

The skin used came from the calf and was freed from subcutaneous tissue as far as possible. Pieces of skin some 12 inches square were used for the experiments. The skin fluoresced an intense quinine blue when examined in the ultra-violet light.

Exp. 1. Skin was heated with 50 % alcohol containing 1 % HCl. None of the fluorescing material was extracted. After standing for some days the alcoholic extract was removed and the skin treated with alcoholic potash for 15 minutes. The mixture was neutralised with acetic acid and HCl. By extraction of the neutral extract with ether, blue fluorescence was seen in the ether layer, but the greater part remained in the aqueous phase.

Exp. 2. Skin was hydrolysed with alcoholic potash as a first step. The hydrolysate was evaporated nearly to dryness on the water bath. An aqueous solution of the residue fluoresced blue. When the alkaline solution was shaken with ether, there was seen a yellowish blue fluorescence in the ether layer. After neutralisation of the solution with acetic acid, the ethereal layer fluoresced blue.

Exps. 1 and 2 indicate that though there is present in skin some blue fluorescent substance which will pass into ether, there is also a large amount of material which will not pass into an ether layer. Exp. 3 shows that this substance can be extracted with dilute acid.

Exp. 3. Skin was heated with dilute aqueous HCl in an attempt to follow the technique of Bence Jones. In this way an extract of a gelatin type was obtained which fluoresced an intense blue, and was readily thrown out of solution by alcohol. The acid extract was concentrated and treated with acetone to dry it. It was then extracted under the reflux condenser with boiling absolute alcohol and ether successively. Neither of these extracts fluoresced. After removal of the ether, the fluorescent residue was hydrolysed with 5 % HCl for some hours. After this the fluorescence was still present though it seemed to be diminished in intensity. Upon neutralisation, again no fluorescent material could be extracted by ether. The acid hydrolysate was neutralised and treated with alcoholic potash, after which treatment again no fluorescent ethereal extract could be obtained.

From these experiments we conclude that though there are present traces of substances in the skin which will yield "blue fluorescent" ether extracts, the main part of the blue fluorescence of skin is due to some other constituent in which fluorescence is associated with a gelatin-like substance.

(c) Lens.

The substance causing blue fluorescence of the lens of the sheep and ox could not be extracted with acid 50 % alcohol, and behaved similarly to the acid aqueous extract of skin in that it could not be extracted by ether, and was largely destroyed upon saponification with alcoholic potash.

C. *Plant Products.*(1) *Grass.*

Grass leaves were treated as described by Plimmer according to the Willstätter technique for the preparation of chlorophyll, xanthophyll and carotene. None of these extracts fluoresced blue; chlorophyll was purplish red, xanthophyll yellow and carotene greenish yellow when examined under our experimental conditions. An extract of carotene prepared from carrots behaved similarly. We found, however, that the first acetone extract of the leaves which is usually discarded in the preparation of chlorophyll, after thorough extraction with light petroleum to remove chlorophyll, fluoresced a quinine blue.

(2) *Oils.*

Certain oils were examined and were found to fluoresce blue. Cod liver oil gave a greenish yellow fluorescence. Table II shows an approximate comparison of the strength of blue fluorescence found in oils purchased locally.

Table II.

Oil	Dilution in ether fluorescing blue with intensity of 0.05 % quinine sulphate
Cotton seed	1 in 18
Linseed	1 in 18
Olive	1 in 3
Colza	1 in 5

In the case of the cotton seed oil it was found that the fluorescent constituent was present in the unsaponifiable fraction after removal of the main part of the cholesterol by digitonin. The others were not examined in this respect.

(3) *Other products.*

An extract prepared by Mr Peskett from cane sugar in the course of the preparation of bios-free cane sugar fluoresced blue.

It was found that a blue fluorescent extract could be obtained from sawdust by treatment with acid 50 % alcohol followed by addition of chloroform.

As mentioned in the introduction, yeast concentrates obtained by the charcoal method described elsewhere [Peters, 1924] fluoresced a quinine blue.

*Some remarks upon the nature of the substances involved in the blue
fluorescence.*

In the main we confirm Bence Jones in that most animal tissues contain substances extractable in ether-alcohol mixtures which fluoresce blue under our conditions. We may call these "animal" quinoidine after Bence Jones, of which typical examples are the guano and urochrome fractions. The substance present passes into an alcohol-ether layer either directly or after saponification with alcoholic potash. There is however some difference in the behaviour of the fluorescent substance in gelatin, skin and the lens, which we may designate

quinoidine "S." Fluorescence largely disappears upon saponification with alcoholic potash, and further cannot be made to pass into an ether layer. Whether this constitutes a real difference or depends upon some accidental association is not clear at present. The absence of blue fluorescence from blood plasma and serum¹, and also from egg albumin, shows that this fluorescence is not an inherent property of proteins, and the fact that crude gelatin fluoresces more powerfully than flocculated gelatin suggests that fluorescence of gelatin is due to an impurity.

The quinoidine fractions from urine and guano are very similar in appearance and behaviour. We think that the following arguments prove that blue fluorescence is not due to the presence of porphyrins: (1) As mentioned above, the traces of porphyrin present can be removed from the guano ether extracts without interfering with their blue fluorescence. (2) In the case of urine, porphyrins were shown to be present in normal urine in traces by Garrod [1893]. Owing to the work of H. Fischer and his colleagues we have to consider as possibilities uro-, copro- and Kammerer's porphyrin. We have never found that these porphyrins fluoresce blue. Specimens of uroporphyrin prepared by one of us from the urine of "Levi," a case of haematoporphyrin congenita, by the methyl ester method of Fischer and Zerwecke [1924] fluoresce an intense red. Crude specimens of coproporphyrin show a yellowish red fluorescence. Turacin, believed by Fischer to be the copper salt of uroporphyrin did not fluoresce under our conditions. (3) The plant porphyrin fractions obtained by us showed no blue fluorescence. (4) The blue fluorescence is not due to the interaction of porphyrin and some other constituent because the addition of uroporphyrin to normal urine produces no intensification of the blue fluorescence, merely a change of hue.

Quinoidine extracts from guano have been obtained which gave no trace of Whitby's [1923] reaction for sterols with formaldehyde and sulphuric acid, and no blue colour with sulphuric acid alone, excluding sterols and lipochromes. Cholesterol itself was excluded by direct test of the pure substance (Table I). The Schlesinger test for urobilin was negative, also the Hymans van den Burgh reaction. Positive indican and kynurenic acid tests could not be obtained.

We feel that it is most probable that blue fluorescence may prove to be actually a property of urochrome for the following reasons. Firstly, the uroquinoidine extracts from guano are at least five times more fluorescent than quinine. If the fluorescence be merely due to contamination, there must be present a substance which is extraordinarily powerful as a source of blue fluorescence. Secondly, the behaviour of the aqueous fractions from guano and urine after saponification with alcoholic potash suggests the decomposition of some complex, which may quite possibly be of the glycuronic acid type. This would be consistent with the recent finding of Pollecoff [1924] that glycuronic acid is a constituent of urochrome. Two views have been

¹ See note, p. 406.

taken about the origin of urochrome. The first is that of Roaf [1921] who considers that it is a derivative of chlorophyll, as he found that feeding upon the green parts of plants increases the amount of the urochrome fraction in the urine. The second is the view of Fischer and Zerwecke [1924], who have found evidence for the presence of certain amino acids in urochrome, and consider it to be a breakdown product of haemoglobin. Our evidence, based upon blue fluorescence, would suggest that urochrome comes from the green parts of plants, not from chlorophyll, but from some "blue fluorescent" substance present in the first acetone extract of the leaves.

The fluorescence of "animal quinoidine" is fairly resistant to oxidation, but readily disappears upon treatment with acid solutions of nitrite.

DISCUSSION.

There are two questions involved in this work. Firstly, do these substances fluoresce blue because of the possession of some common constituent? Further work will doubtless settle this point. Secondly, there is the broader biological question. What is the meaning of this widespread blue fluorescence which we find in animal epithelial structures, in the lens of the eye, in yeast, in sawdust and in grass? Whenever ultra-violet light falls upon these parts it is certain that they must be exhibiting this blue fluorescence. Up to the present it has been considered that the presence of fluorescent substances in the skin is dangerous to the animal organism. This view is based upon the experiments of Raab [1900], Tappeiner [1900], Hausmann [1911] and others upon the toxic effect of fluorescent substances, and upon the blistering found to occur in cases of haematoporphyria in sunlight. Are we to believe that, whereas the presence of a red fluorescence is dangerous, the presence of a blue fluorescence is not? In this connection the experiments of Suarez [1916] are of interest, who obtained from maize a substance resembling quinine in fluorescence, and called by Hofmeister "Zeochin." This zeochin sensitised blood corpuscles and organisms to the light, but when fed to animals in large amounts it did not render them light sensitive, thus suggesting that, normally, the animal can deal with such substances.

If "quinoidine" should turn out to be identical with urochrome, it would be of interest in connection with the work of Fischer and Zerwecke [1924]. They found that urochrome protected paramoecia against the toxic action of porphyrins in the light. It is difficult to believe that there is not some real function in this interaction of ultra-violet light with "quinoidine S." Recent experiments by Hess and Weinstock [1925], Steenbock, Black, Nelson, Nelson and Hoffert [1925] and Coward and Drummond [1925] show that irradiated cholesterol has antirachitic effect so that the presence of fluorescence cannot be essential to the formation of antirachitic substance. It may, however, be in some way directive. There is a "blue fluorescent" substance present in milk, sawdust and certain oils. Further, cotton seed and linseed oil are some

six times more fluorescent than olive oil. It may be mere coincidence, but the facts seem to be related to the findings of Hess and Weinstock [1924] and Steenbock and Black [1924] that irradiated cotton seed and linseed oils give good cures whereas olive oil gives inconstant results.

SUMMARY.

1. The blue fluorescence of tissues in ultra-violet light ($\text{\AA}$. 3000–4000) has been investigated qualitatively. Extracts fluorescing blue can be obtained, as was shown originally by Bence Jones who called the substance responsible "animal quinoidine."

2. Quinoidine extracts can be obtained from urine, guano, milk, feathers, liver and caseinogen, also from skin, commercial gelatin and the lens of the eye. The behaviour of the latter group to solvents is different. Whether this difference is the accident of some association is not yet clear.

3. Phytoquinoidines are present in grass leaves and certain oils, especially linseed and cotton seed, in yeast extracts and also in sawdust.

4. No pure substance among a number examined showed a quinine-like fluorescence. The fluorescence of porphyrins, chlorophyll, xanthophyll, and bile derivatives was not blue.

5. Upon fractionation of normal urine the quinoidine passes into the urochrome fraction (Garrod).

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REFERENCES.

- Bence Jones (1866). *Chem. News*. **13**, 197.
 Brucke (1845). Quot. Bence Jones.
 Coward and Drummond (1925). *J. Soc. Chem. Ind.* **44**, 255.
 Fischer and Zerwecke (1924). *Z. physiol. Chem.* **137**, 176.
 Fischer (1923). Oppenheimer's *Handbuch der Biochem.* **1**, 366.
 Garrod (1893). *J. Physiol.* **13**, 619.
 — (1894). *Proc. Roy. Soc. Lond.* **55**, 394.
 Hausmann (1911). *Biochem. Z.* **30**, 276.
 Helmholtz (1855). *Pogg. Ann.* **94**, 205.
 Hess and Weinstock (1924). *J. Biol. Chem.* **62**, 301.
 — (1925). *J. Biol. Chem.* **73**, xxv.
 Jaffé (1869). *Virch. Arch.* **47**, 400.
 Langecker (1922). *Z. physiol. Chem.* **115**, 1.
 Peters (1924). *Biochem. J.* **18**, 858.
 Pollecoff (1924). *Biochem. J.* **18**, 1252.

- Regnauld (1858). Quot. Bence Jones.
Raab (1900). *Z. Biol.* **39**, 524.
Roaf (1921). *Biochem. J.* **15**, 687.
Schanz (1921). *Pflüger's Arch.* **190**, 311.
Schliess and Lowenfeld (1863). *Schmidt. Jahr.* **120**, 10.
Setchenow (1859). *Arch. Ophth.* **5**, 205.
Steenbock and Black (1924). *J. Biol. Chem.* **61**, 405.
Steenbock, Black, Nelson, Nelson and Hoffert (1925). *J. Biol. Chem.* **73**, xxv.
Suarez (1916). *Biochem. Z.* **77**, 16.
Tappeiner (1900). *Munch. med. Woch.* **47**, 5.
Whitby (1923). *Biochem. J.* **17**, 1.