The Fluorescence Spectra of Pterins and their possible Use in the Elucidation of the Antipernicious Anaemia Factor. Part 1

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The chemical nature of the antipernicious anaemia factor stored in the liver is not yet established. But there is some indication that the argentaffine cells in the epithelium of the stomach and intestine are connected with the elaboration of this material, as they are found in those parts of the mucous membrane which are therapeutically active against pernicious anaemia, and their number is reduced in cases of pernicious anaemia. There is evidence that these cells contain a pterin in their cytoplasmic granules (W. Jacobson, 1939). It has also been suggested that pterins may be connected with the formation of normal red blood cells. Thus Tschesche & Wolf (1936, 1937) could cure with xanthopterin an anaemia produced by feeding growing rats on goat's milk; Simmons & Norris (1941) and Norris & Simmons (1945) cured anaemia in fish with xanthopterin; Totter, Shukers, Kolson, Mims & Day (1944) could delay the onset of nutritional anaemia in monkeys kept on a vitamin M-deficient diet by the injection of xanthopterin, and the rate of regeneration of red blood cells and haemoglobin was increased on administration of xanthopterin to dogs previously made anaemic by bleeding (McKibbin, Schaefer, Elvehjem & Hart, 1942). Further, B. M. Jacobson & Subbarow (1937), and also Mazza & Penati (1937-8), have shown that pterin-like substances may occur in liver extracts potent against pernicious anaemia, and xanthopterin itself has been isolated from the liver (Koschara, 1936).

Xanthopterin, leucopterin and other pterins show intense fluorescence when irradiated with ultra-violet light; the argentaffine cells of the stomach and intestine, and liver extracts (active against pernicious anaemia) also fluoresce under such conditions. It was therefore thought interesting to study the fluorescence spectra of synthetic pterins and to compare these spectra with the fluorescence of extracts prepared from the argentaffine cells of the mucous membrane of the stomach and intestine. The mucous membrane of these organs is known to contain the antipernicious anaemia factor. These results will be reported in the present paper. In a second communication the fluorescence spectra of liver preparations active against pernicious anaemia will be discussed and a possible method of assessing the clinical activity of such extracts by means of their fluorescence spectra will be suggested.

Previously (W. Jacobson, 1939) the properties of the substance present in the argentaffine cells have been discussed. The chemical reactions, absorption and fluorescence spectra were described, and all these seemed to suggest that a pterin was present. Since this paper was published, the chemistry of pterins has developed enormously through the work of Wieland, Purrmann, Koschara, Schöpf and their collaborators. The literature includes descriptions of the synthesis of pure leucopterin (Purrmann, 1940*a*) and xanthopterin (Purrmann, 1940*b*; Koschara, 1943; Totter, 1944) as well as an extensive discussion of their constitutions and their relationships to other pterins. The information available, as far as it is relevant to the present work, is conveniently summarized in Table 1.

The molecular formulae of leucopterin and xanthopterin seem to be definitely established as $C_6H_5O_3N_5$ and $C_6H_5O_2N_5$ respectively. Their constitutions appear to be:

or their tautomeric isomers. For this work the actual constitution is not highly relevant, but for convenience in discussion the above structures only will be considered, and are the basis of those shown in Table 1.

EXPERIMENTAL PROCEDURE

The fluorescence spectra were photographed by means of a Hilger wide-aperture glass spectrograph, with a quartz mercury vapour lamp as light source. The visible light was cut off with ultra-violet glass filters (either Chance's or the more efficient Price's glass being used) and with a $\frac{1}{2}$ cm. quartz cell containing 20% CuSO_4 solution.

Solutions were contained in a 2 cm. quartz cell, the light being focused on to it by means of a quartz rod. The solid materials, powders or thin sections, were mounted on black paper with collodion. The light incident on and emerging from these specimens was focused with quartz lenses. Blanks showed that the amount of scattered light produced by the cell, mounts, solvents, etc., was negligible under the conditions of the experiments.

If the slit was sufficiently narrow to separate clearly the components of the mercury yellow doublet, an exposure of

Table 1. *Structure and properties of pterins studied in the present work**

Substance and general reference	Formula	Colour, white Murexide reaction Reduction of AgNO ₃ Diazo reaction	Ultra-violet absorption spectrum	Fluorescence	General properties
<i>Leucopterin</i>	$\begin{array}{c} \text{C}_4\text{H}_4\text{O}_4\text{N}_6 \\ \text{NH}-\text{C}=\text{O} \\ \\ \text{HN}=\text{C}-\text{C}-\text{NH}-\text{CO} \\ \quad \quad \\ \text{NH}-\text{C}=\text{N}-\text{CH}_3 \\ \text{and tautomers} \end{array}$	-ve† -ve† -ve†	0.1N-NaOH solution 3400 Å. log ε = 4.05 2850 Å. log ε = 3.8 2400 Å. log ε = 4.2	0.1N-NaOH solution† 5160 Å. Strong 4625 Å. Fairly strong <i>Solid</i> † 6200 Å. Weak 5350 Å. Strong 4625 Å. Fairly strong 4360 Å. Moderate	<i>Insoluble</i> in dilute acids and water <i>Soluble</i> in dilute alkalis, conc. H ₂ SO ₄ , and ethylene glycol monoethyl ether† Yellow sodium salt
<i>Xanthopterin</i> [Uropterin] 9-Deoxyleucopterin	$\begin{array}{c} \text{C}_4\text{H}_4\text{O}_4\text{N}_6 \\ \text{NH}-\text{C}=\text{O} \\ \\ \text{HN}=\text{C}-\text{C}-\text{N}-\text{CO} \\ \quad \quad \\ \text{NH}-\text{C}=\text{N}-\text{CH}_3 \\ \text{and tautomers} \end{array}$	+ve +ve +ve	(Fromherz & Kotzschmar, 1938; Hüttel & Sprengling, 1943) Confirmed by present work 0.1N-NaOH solution 3750 Å. $E_{1\text{cm}}^{1\%} = 300$ 2550 Å. $E_{1\text{cm}}^{1\%} = 800$ <i>Neutral solution</i> † 3750 Å. 2750 Å. <i>Glacial acetic acid solution</i> 3800 Å. $E_{1\text{cm}}^{1\%} = 200$ 2700 Å. $E_{1\text{cm}}^{1\%} = 650$	0.1N-NaOH solution† 5920 Å. Weak 5160 Å. Fairly strong 4630 Å. Strong <i>Neutral solution</i> † 6200 Å. Weak 5920 Å. Moderate 5160 Å. Fairly strong 4550 Å. Strong 20% H ₂ SO ₄ solution† 6200 Å. Weak 5920 Å. Moderate 5230 Å. Strong 4600 Å. Fairly strong <i>Solid</i> †	Slightly soluble in water <i>Soluble</i> in dilute acids and alkalis, ethylene glycol; and ethylene glycol monoethyl ether† Reduced by HI, not re-oxidized on dilution Yellow sodium and barium salts
<i>Anhydroleucopterin</i> 8-Deoxyleucopterin	$\begin{array}{c} \text{C}_4\text{H}_4\text{O}_4\text{N}_6 \\ \text{NH}-\text{C}=\text{O} \\ \\ \text{HN}=\text{C}-\text{C}-\text{N}-\text{CH}_3 \\ \text{and tautomers} \end{array}$	+ve -ve	(Wieland & Purrmann, 1939; Schöpf, Becker & Reichert, 1939; Totter, 1944) Confirmed by present work 0.1N-NaOH solution 2400 Å. log ε = 3.9 3400 Å. log ε = 3.9	Blue	(Wieland, Tartter & Purrmann, 1940; Hopkins, 1942) Solubility similar to leucopterin Reduced by HI, re-oxidized on dilution Yellow sodium salt
<i>Ichthyopterins</i> (Hüttel & Sprengling, 1943)	$\begin{array}{c} \text{C}_4\text{H}_4\text{O}_4\text{N}_4 \\ \text{Similar to anhydro-} \\ \text{leucopterin} \end{array}$	Colour, white Murexide reaction weak	(Hüttel & Sprengling, 1943)	(Purrmann, 1941) 6900 Å. 4500-4700 Å.	(Wieland <i>et al.</i> 1933; Wieland & & Purrmann, 1939) Slightly soluble in water and dilute HCl Soluble in NaOH Reduced by HI and re-oxidized on dilution

6-Deoxyleucopterin (Purmann, 1940b)	$ \begin{array}{c} \text{C}_6\text{H}_4\text{O}_2\text{N}_6 \\ \text{N}=\text{CH} \\ \\ \text{HN}=\text{C} \quad \text{C}-\text{NH}-\text{CO} \\ \quad \quad \\ \text{NH}-\text{C}=\text{N}-\text{NH}-\text{CO} \end{array} $	Colour, white				Similar to leucopterin in its chemical properties, basicity and solubility
Isoleucopterin (Wieland & Liebig, 1944)	$ \begin{array}{c} \text{C}_6\text{H}_4\text{O}_2\text{N}_6 \\ \text{NH}-\text{C}=\text{NH} \\ \\ \text{O}=\text{C} \quad \text{C}-\text{N}=\text{C}-\text{OH} \\ \quad \quad \\ \text{NH}-\text{C}-\text{N}=\text{C}-\text{OH} \\ \text{and tautomers} \end{array} $	Colour, white				
Deoxyisoleucopterin (Wieland & Liebig, 1944)	$ \begin{array}{c} \text{C}_6\text{H}_4\text{O}_2\text{N}_6 \\ \text{NH}-\text{C}=\text{NH} \\ \\ \text{CH} \quad \text{C}-\text{N}=\text{C}-\text{OH} \\ \quad \quad \\ \text{N}-\text{C}-\text{N}=\text{C}-\text{OH} \\ \text{and tautomers} \end{array} $					
Erythropterin (Schöpf & Becker, 1936)		Colour, orange → red Murexide reaction - ve	Absorption 3000-3200 Å. No definite bands	Violet	Fairly water soluble No salts with acids and alkalis	
Chrysopterin (Schöpf & Becker, 1936)		Colour, yellow Murexide reaction + ve		Blue-violet in 2N-acetic acid	Intermediate in properties between xanthopterin and erythropterin (acidity, absorption and fluorescence spectra) Slight basicity	
Mesopterin (Schöpf & Becker, 1936)		Colour, white				
Argentaffine cells (Prepared as described in text) (W. Jacobson, 1939)		Colour, yellow Murexide reaction + ve Reduction of AgNO ₃ + ve Dialo reaction + ve	2700 Å. Solid	6200 Å. Very weak 5920 Å. Fairly strong 5350 Å. Fairly strong 4625 Å. Weak <i>Ethylene glycol mono-ethyl ether extract</i> † 6200 Å. Weak 5920 Å. Moderate 5200 Å. Fairly strong 4550 Å. Strong	Fluorescence stable to acids, alkalis and irradiation, unlike riboflavin	
Normal intestinal mucous membrane (Prepared as argentaffine cells)		Colour, white	No defined bands (W. Jacobson, 1939)	5200 Å. Very weak 4550 Å. Slightly stronger		

* For a review of the general properties of pterins see Schöpf & Becker (1936). Guanopterin has since been shown to be isoguanine (Purmann, 1940a).

† Results of present work.

from 30 sec. to 10 min. was generally adequate for the solutions, but was increased to $\frac{1}{2}$ hr. or longer in some cases. The solids and sections needed at least 6 hr. and more usually 8 or 9 hr. for good photographs to be obtained.

The absorption spectra were photographed by Dr Allsopp at the Strangeways Research Laboratory, using the standard Hilger Spekker-photometer apparatus. Dr Brück of the Solar Physics Observatory very kindly undertook to do photomicrometer tracings of the plates of all the interesting fluorescence spectra. The authors are grateful for this helpful collaboration.

Pterins. Hopkins (1895) was the first to investigate the pterins. The synthesis of leucopterin and xanthopterin was effected by Purrmann (1940 *a, b*). It is interesting to note that already in 1908 Sachs & Meyerheim had prepared a number of compounds containing the same pterin ring system which they called azinpurin, long before the structures of the pterins themselves were known.

The following pterin preparations were available; the authors would like to express their gratitude to those persons who so kindly synthesized or extracted the specimens:

Leucopterin, synthetic (Purrmann, 1940*a*). Dr Quibell, Department of Chemistry, Manchester University.

Crude xanthopterin, from butterfly wings (*Gonepteryx rhamni*). Dr Lythgoe, Chemical Laboratory, Cambridge University.

Xanthopterin, synthetic (Purrmann, 1940*b*; four different preparations using various condensing agents). Dr Quibell; Dr F. G. Mann, Chemical Laboratory, Cambridge University.

Rhodopterin (Hopkins, 1941–2). Prof. Sir F. G. Hopkins, School of Biochemistry, Cambridge University.

Leucopterin is a soft white powder, insoluble in water or dilute acid, but readily soluble in dilute alkali (0.1*N*-NaHCO₃ or 0.1*N*-NaOH), giving a very faintly yellow solution.

In discussing the purity of the xanthopterin specimens, it should be remembered that this substance may be partially oxidized to leucopterin, even by atmospheric oxygen under suitable conditions, and that on treatment with dilute hydrochloric or sulphuric acid it tends to be changed into the red rhodopterin (Hopkins, 1941–2). The different xanthopterin specimens varied in colour from lemon yellow to dull red-brown, those having the lighter colour being considered the purer. They were soluble in water, dilute acid (0.1*N*-HCl) and dilute alkali (0.1*N*-NaHCO₃ or 0.1*N*-NaOH), giving yellow solutions. On shaking with dilute hydrochloric acid some reddish residue always remained, which seems to be identical with the rhodopterin described by Hopkins. Probably all the specimens are to some extent contaminated with a little leucopterin; in addition, the darker coloured ones also contain rhodopterin, and possibly other substances.

FLUORESCENCE AND ULTRA-VIOLET ABSORPTION SPECTRA OF LEUCOPTERIN AND XANTHOPTERIN

The absorption spectra of the above specimens were photographed, so that these could be checked against the previously published data. The results do not give absolute extinction coefficients, and so are not reproduced here, but the relative intensities

and positions of the maxima of absorption are discussed below.

Ultra-violet absorption of leucopterin. Leucopterin in 0.1*N*-NaOH solution shows absorption maxima as follows:

Fromherz & Kotzschmar (1938)	3450 A. (1)	2850 A. (0.50)
Hüttel & Sprengling (1943)	3350 A. (1)	2870 A. (0.56) 2400 (1.30)
Present investigations	3400 A. (1)	2850 A. (0.45)

The figures in brackets represent the intensity of absorption relative to that of the ~ 3400 A. band. This method of comparison is necessary since all authors do not use the standard terminology for their extinction coefficients, nor are these always defined. The agreement is adequate; the third band did not show up in all cases because the absorption was not investigated at sufficiently short wavelengths. Leucopterin has therefore distinctive bands at ~ 3400 , 2850 and 2400 A. One point should be stressed, the band at 2850 A. falls at the same wavelength as that of the most intense absorption of uric acid. It is quite conceivable that all the leucopterin specimens so far investigated contained this substance, and this possibility should always be taken into consideration.

Fluorescence spectrum of leucopterin (Pls. 1, 2). The fluorescence spectrum of leucopterin was investigated in alkaline solution and in the solid state. As observed directly, the fluorescing solution looks bright blue, and the solid bluish white. Attempts to get leucopterin into neutral solution failed, and no fluorescence was observed when the suspension in water at pH 7 was filtered and centrifuged to remove the finer particles. Uric acid in alkaline solution gives only a very faint blue fluorescence, and if present as an impurity in the leucopterin would not appreciably alter the character of the spectrum.

The positions and relative intensities of the bands are shown in Table 1. In alkaline solution there are bands at 5160 (mod.) and 4625 A. (strong). The solid gives bands at 6200 (weak), 5350 (strong), 4625 (mod.) and 4360 A. (mod.). As all the bands are broad, the method of measuring the wave-lengths of their maxima is somewhat approximate, but there seems to be a genuine shift in the position and intensity of the most prominent band in the alkaline medium, possibly because salt formation occurs. In solution the weaker maxima are overlaid by the more intense scattering of the visible mercury lines.

Leucopterin has been prepared from the wings of *Pieris brassicae* (Schöpf & Becker, 1933). It seemed of interest therefore to photograph their fluorescence under the same conditions as that of solid leucopterin. Using a very long exposure and a rather wide slit, the spectrum obtained was similar

Photometric curves of fluorescence spectra. A. Leucopterin, in 0.1N-NaOH. B. Xanthopterin, in 0.1N-NaOH. C. Xanthopterin, in neutral. D. Xanthopterin, in 20% H_2SO_4 . E. Ethylene glycol monoethyl ether extract of the argentaffine cells of a carcinoid tumour.

Photometric curves of fluorescence spectra. A. Leucopterin, solid. B. Argentaffine cells of a carcinoid tumour (original specimen). C. Argentaffine cells of a carcinoid tumour (new specimen). D. Intestinal mucous membrane, normal tissue.

to that of the solid leucopterin except that the band at about 4600 Å. was relatively more intense.

Ultra-violet absorption of xanthopterin. The ultra-violet absorption spectra of several of the xanthopterin specimens were investigated in neutral, dilute acid and dilute alkaline solution; the results are collected in Table 1. In alkaline solution there is a broad band at ~ 2550 Å., and a well-defined one at ~ 3750 Å., the ratio of the intensities being about 2.6 : 1. In neutral solution the absorption maxima are at 2750 and 3750 Å., the first band is much sharper, and the ratio of intensities is about 3.0 : 1. The curves for the acid solutions are similar to this. If the xanthopterin is contaminated with a little leucopterin, the latter being insoluble in neutral solution will not affect the absorption spectrum of the xanthopterin at pH 7, which may therefore be taken as standard. In alkaline solution, however, the very strong leucopterin band at 2400 Å. will broaden and enhance that of xanthopterin at 2750 Å., an effect which is actually observed.

The absorption spectrum of xanthopterin in alkaline (0.1 N-NaOH) and acetic acid solution has been published by Totter (1944). His curves show maxima at 2550 and 3750 Å. (relative intensity 2.67) for the former and 2750 and 3800 Å. (relative intensity 3.2) for the latter. Wieland & Purrmann (1939) state that the pterin absorbs between 3950 and 3750 Å. and Schöpf, Becker & Reichert (1939) give a band at 2840–2750 Å. The present results are in adequate agreement with the published data.

Fluorescence spectrum of xanthopterin (Pl. 1). The fluorescence spectra of the different specimens of xanthopterin were photographed in the solid state, in neutral solution, in acid solution (20 % H_2SO_4 , N and 0.1 N-HCl, and 2N-acetic acid), and in alkaline solution (0.1 N-NaOH). A solution in formaldehyde was also investigated, as the argentaffine cell preparations had been fixed in formaldehyde, and it was thought advisable to make sure that this reagent had not modified the fluorescence. The solid xanthopterin shows no fluorescence at all, even when very long exposures are employed. The solutions all fluoresce bright blue; this shows up best if the material is examined by ultra-violet light. If visible light is not excluded, the yellow colour of the solution, particularly when acid is present, modifies the apparent colour of the fluorescence; this may account for the variety of tints mentioned in the literature.

The fluorescence spectra of the neutral, weak acid (2N-acetic acid, 0.1 N-HCl) and formaldehyde solutions show a strong band at 4550 Å., and progressively weaker ones to longer wave-lengths at 5160, 5920 and 6200 Å., the last being ill-defined. In strongly acid solution (N-HCl, 20 % H_2SO_4) there are still four bands at 4600, 5230, 5920, and 6200 Å., but now 5230 is the strongest, and 5920 and 6200 Å.

are enhanced. When the solution is strongly acid, the position of the two shorter wave-length bands is markedly shifted, and there is a very obvious alteration in their relative intensities. In alkaline solution, the two most intense bands are at 5160 and 4630 Å. The latter corresponds to that observed in leucopterin under these conditions; presumably the leucopterin is showing up, as in the ultra-violet spectrum.

The intensities discussed in the preceding paragraph are those observed when the paler coloured specimens of xanthopterin are used. But in neutral solutions of the reddish preparations the weak maxima at 5920 and 6200 Å. are suppressed. In strong acid solution the band at 5920 Å. is much stronger than those at 5230 and 4600 Å., possibly because the solutions now appear red, and some light absorption in the yellow and blue is taking place.

It has already been suggested that the reddish specimens of xanthopterin may contain rhodopterin. This is quite possible as the methods of preparation involve the use of hydrochloric acid which favours its formation. The red residue left after shaking xanthopterin in dilute acid was therefore dissolved in 20 % sulphuric acid, in which rhodopterin is also soluble, and the absorption and fluorescence spectra of this solution were examined. The fluorescence spectrum was similar to that of a reddish xanthopterin in strong acid solution, with bands at 4570 (strong), 5230 (mod.), 5920 (fairly strong), and 6200 Å. (weak), the weakening of the 5230 Å. band being due to the pink colour of the solution. Absorption bands were qualitatively observed at 5050 and 5470 Å. For comparison a solution of rhodopterin in 20 % sulphuric acid was similarly investigated. This gives absorption bands at 5040 and 5480 Å., but shows no fluorescence at all. It seems probable, therefore, that the red residue consists mainly of rhodopterin, showing up in the absorption, but that it still contains appreciable amounts of xanthopterin, detectable in the fluorescence. It follows that the original reddish xanthopterin was probably contaminated with rhodopterin, which would not affect the fluorescence, except that the pink colour of the solution would alter the relative intensities of the bands.

Although no absolutely pure specimen of xanthopterin is available, the results listed in Table 1 for its absorption and fluorescence in neutral and acid solution are probably reasonably correct, as they are based on observations made on three different synthetic samples.

The fluorescence shown by wings of the butterfly *Gonepteryx rhamni*, from which xanthopterin has been prepared, was also photographed. Although solid xanthopterin itself does not fluoresce, it was found that these wings produced a spectrum similar

to that of xanthopterin in strong acid solution, with the long wave-length bands prominent, and the yellow and blue ones weaker. This result is significant in the discussion of the results obtained with the argentaffine cells now to be described.

ABSORPTION AND FLUORESCENCE SPECTRA OF THE ARGENTAFFINE CELLS IN THE GASTRO-INTESTINAL TRACT (Pl. 2)

The argentaffine cells in the mucous membrane of the stomach and the intestine are widely separated from each other, and represent only an exceedingly small proportion of the normal tissue. In these investigations small nodules about 4–5 mm. in diameter, so-called 'carcinoid' tumours formed by argentaffine cells were used, since they provided a material consisting mainly of these cells. Two specimens of such nodules were available, one of which had been used in the previous investigations; both had been fixed in formaldehyde. Both had been treated for periods ranging from 24 hr. to several days with water, aqueous and absolute ethanol, benzene, hexane, cyclohexane, and dioxan. These solvents did not affect the specific granules of the argentaffine cells, but removed other material which might interfere with their fluorescence.

The fluorescence spectra of both preparations were photographed, as well as that of the adjacent normal tissue of intestinal mucous membrane. The latter merely shows very weak bands at 4550 and 5200 Å. Both specimens of the nodules of argentaffine cells show a weak band at 4625 Å., moderately strong bands at 5350 and 5920 Å., and some indication of a less well-defined band at 6200 Å. Hopkins (1941–2) mentions the ready solubility of xanthopterin in ethylene glycol. The more recently acquired specimen was therefore extracted with ethylene glycol monoethyl ether in the hope that xanthopterin if present would dissolve, and the fluorescence spectrum of the extract was photographed. It shows bands as follows: 4550 (strong), 5200 (fairly strong), 5920 (mod.) and 6200 Å. (weak). All these results are included in Table 1.

This extract of the nodule of argentaffine cells shows a spectrum almost identical with that of xanthopterin in neutral solution. The tissue itself shows a distribution of intensity such as might be obtained by superimposing the leucopterin and xanthopterin bands, with an enhancement of the 5920 Å. xanthopterin band, as in acid media. The general appearance of the spectrum is very similar to that of the wings of the yellow butterfly from which xanthopterin has actually been isolated. On the other hand, it should be remembered that solid xanthopterin gives no fluorescence, although this is shown by solid leucopterin. Evidence discussed previously (W. Jacobson, 1939) suggested that the

pterin is associated with a deoxy-sugar in the argentaffine cells. Under such conditions a xanthopterin complex might fluoresce, just as it appears to do in the wings of the yellow butterfly.

The ultra-violet absorption spectrum of the nodules of argentaffine cells shows a band at 2700 Å. similar to that of xanthopterin at 2750 Å.

The possible presence of riboflavin in the argentaffine cells has already been discussed (W. Jacobson, 1939). The detailed chemical evidence given there seems to leave little doubt that the yellow substance responsible for the characteristic reactions of the argentaffine cells cannot be a flavin, as its range of stability in the presence of acids and irradiation is far too wide. Although the fluorescence spectrum of riboflavin has already been published (e.g. Dhéré, 1937, p. 243) it was rephotographed so that a more exact comparison could be made with that of xanthopterin and the argentaffine cells. It shows a single broad band extending from 5800 to 5200 Å. (maximum at 5300 Å.). This band, although it lies in the same region of the spectrum as one of the xanthopterin bands, has a quite distinctive appearance which is easily recognized. Moreover, in contradistinction to xanthopterin, the fluorescence of riboflavin is readily suppressed in N-acid solution. The fluorescence bands of the argentaffine cells do not show any of the characteristics of riboflavin emission, so that its absence may be inferred, in agreement with the previous evidence.

The previously published photomicrometer tracings of the fluorescence spectrum of the original nodule of argentaffine cells (W. Jacobson, 1939) showed the presence of two bands at 5550 and 6100 Å., with an 8 hr. exposure and cooling of the tissue by means of a copper spiral the ends of which were placed in liquid air. The discrepancy between these results and those reported here, which are based on observations on two nodules of argentaffine cells (the old one used for the previous investigation and a second one), is difficult to explain. The background scattering in the older photograph is very extensive and may possibly have shifted the apparent position of the maximum. In the new series of investigations the stray light has been almost completely eliminated.

As the argentaffine cells are normally widely scattered in the mucous membrane of the stomach and intestine the specific fluorescing material can be found in only low concentration in extracts of these tissues. A 0.1N-NaHCO₃ extract from the gastric mucosa shows the same fluorescence spectrum, though very much weaker, as that of the nodules of argentaffine cells. Also the 50% ethanol soluble fraction prepared from that layer of the mucous membrane of the small intestine which contains the argentaffine cells, i.e. the layer of the crypts of Lieberkühn, gives the same fluorescence

spectrum as that recorded above. Its intensity is considerably greater than that of the gastric mucosa, as the argentaffine cells in the crypts of Lieberkühn of the small intestine after removal of the villi and separation from the other muscle coats, are much more numerous per unit area of tissue than in the gastric mucosa. Both gastric and intestinal mucosa contain the antipernicious anaemia factor (W. Jacobson & Williams, 1945).

The chemical reactions and the ultra-violet absorption and the fluorescence spectrum all indicate the presence of xanthopterin, probably as a component of a complex, in the argentaffine cells; leucopterin, obtained from it by oxidation, is also probably present, as its characteristic bands are present in the fluorescence spectrum.

SUMMARY

1. The fluorescence and ultra-violet absorption spectra of xanthopterin and leucopterin have been investigated.

2. These spectra have been employed to investigate the nature of the specific material present in the cytoplasmic granules of the argentaffine cells of the mucous membrane of the stomach and intestine.

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The Fluorescence Spectra of Pterins and their possible Use in the Elucidation of the Antipernicious Anaemia Factor. Part 2

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In the preceding paper (Jacobson & Simpson, 1946) the fluorescence spectra of xanthopterin and leucopterin have been described. The fluorescence spectra of extracts of the mucous membrane of the stomach and intestine and of the argentaffine cells in these tissues were found to resemble, if not to be identical with, that given by xanthopterin.

The present investigations were undertaken in an endeavour, first to identify the fluorescing material in tissue extracts, particularly liver extracts, which show antipernicious anaemia activity, and secondly to find whether there is any connexion between the intensity of the fluorescence and the haemopoietic activity. If the fluorescing substance could be iden-