CLXXXV. THE ULTRA-VIOLET ABSORPTION SPECTRA OF URIC ACID AND OF THE ULTRA-FILTRATE OF SERUM.

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SINCE the ultra-violet absorption bands of uric acid are extremely sharp and well defined in as low a concentration as 1 mg. per 100 cc., it was considered necessary to measure accurately its absorption before that of the ultra-filtrate of serum.

The absorption spectrum of uric acid dissolved in water has been investigated by Dhéré [1909], who found two distinct bands at wave-lengths of Å 2920 and Å 2350. He was unable to measure the extinction coefficient, since at that time no suitable apparatus was available. He does not appear to have observed the change in the spectrum when the acid is dissolved in an alkaline solution.

Reinhard [1927] measured the absorption spectrum of the acid in water, but failed to observe the second absorption band. The present writer has measured the absorption spectrum of uric acid dissolved in water and in an alkaline solution, and has noted a marked difference in its absorption in each case.

METHOD.

A Hilger quarter-plate spectrophotometer and rotating sector were used to measure the absorption. A condensed spark between tungsten steel electrodes was used as the source of radiation.

EXPERIMENTAL.

The concentration of uric acid employed was 0.0026 %; this was chosen as a convenient strength to ensure that the extinction coefficient lay within the limits provided for by the rotating sector. The uric acid was dissolved in (1) distilled water, (2) a phosphate buffer solution at a p_H of 7.4, (3) a 0.1 % solution of sodium carbonate, (4) a 0.1 % solution of lithium carbonate. These solvents were employed in the comparison tube of the rotating sector.

The curves thus obtained are shown in Fig. 1. Their wave-lengths and extinction coefficients at the head and foot of the curve are given in Table I.

Table I.

		Wave-length (Å)		Extinction coefficient	
		Head	Foot	Head	Foot
Band α	In water and at p_H 7.4	2920	2600	1.46	0.28
	In carbonate	2950	2630	1.54	0.35
Band β	In water and at p_H 7.4	2350	2210	1.2	0.66
	In carbonate	—	—	—	—

When dissolved in distilled water or in the buffer solution, the acid shows two distinct bands; when, however, it is dissolved in carbonate the second band disappears. At the same time, band α moves towards the red, and the value of the extinction coefficient at the head and foot of the curve is slightly increased (*v.* Table I). The disappearance of the band is of interest in two connections, namely, (1) the possible variation in the structural formula of uric acid when dissolved in water and in carbonate; (2) the absorption spectrum of serum ultra-filtrate; this will be discussed below.

With regard to the first point, the difference in the absorption spectrum probably indicates a tautomeric change of the molecule.

Fig. 1.

Interrupted line—uric acid dissolved in carbonate.

Continuous line—uric acid dissolved in water or phosphate buffer.

Serum ultra-filtrates.

To the best of the writer's knowledge, the ultra-violet absorption spectra of serum ultra-filtrates have not been investigated. The method of filtration has been described by him elsewhere [1928]. Three essential conditions must be observed, namely, (1) the filtration must be rapid, as it is difficult to keep serum filtrate sterile; the ultra-filtration of 10 cc. undiluted serum should not take more than 2 to 3 hours; (2) as large an area of membrane as possible

should be used; (3) the serum should be kept moving on the surface of the membrane. The pressure used need not be greater than 60 mm. Hg.

From 10 cc. of serum, 4–5 cc. of protein-free filtrate should be obtainable. The filtrate was not used unless the sulphosalicylic acid test was negative.

The results obtained in 14 cases (13 normal and 1 pathological) are tabulated below.

Table II.

Case no.	Wave-length (Å)		Extinction coefficient		Concentration of uric acid estimated from extinction coefficient (mg. per 100 cc.)	Remarks
	Head	Foot	Head	Foot		
1	α 2920	2700	2.2	1.32	3.9	
	β 2350	—	3.4	—	—	
2	α 2920	2650	3.0	0.9	5.3	Fasting 14 hours
	β 2300	—	3.0	—	—	
3	α 2920	2650	2.3	1.08	4.1	2–3 hours after breakfast
	β 2300	—	3.0	—	—	
4	2920	2600	1.5	0.68	2.5	
5	2920	2620	2.4	0.92	4.3	
6	2910	2610	1.56	0.76	2.5	
7	2900	2700	2.4	1.32	4.3	2 hours after lunch. Well marked lipaemia
8	2910	2620	2.24	1.16	4.0	
9	2900	2620	1.54	0.92	2.7	
10	2900	2680	1.06	0.64	1.9	Fasting 13 hours
11	2920	2690	1.3	1.08	2.3	4 hours after large protein meal
12	2920	2770	1.08	0.96	1.9	4 hours after breakfast
13	2920	2600	2.4	0.8	4.3	
14	2950	2750	1.24	1.5	2.2	Advanced syphilis and carcinoma of liver

As an example of the results obtained, the curves of cases 3 and 14 are plotted in Fig. 2.

It is reasonable to suppose that the selective absorption shown is due to uric acid. As will be seen from the table, the time elapsing between the taking of a meal and the withdrawal of blood appears to have no effect.

If the concentration be estimated from the extinction coefficient at the head of the band α (see Fig. 2), the average result agrees very fairly with the figures obtained by chemical analysis of normal blood. At this wave-length it may be assumed that little interference is caused by substances other than uric acid. As the wave-length becomes shorter, the absorption due to other substances becomes greater. As a result, the second band is of varying amplitude; for example, in cases 1, 2 and 3, of Table II it is definite, whereas in other cases, although always visible, it appears only as an inflexion at an approximate wave-length of Å 2350.

The absorption spectrum of uric acid present in the ultra-filtrate of blood-serum is similar to that of uric acid dissolved in water or in the buffer solution, and not to that of the acid dissolved in carbonate.

Work is now in progress to ascertain whether pathological conditions have any marked effect on the normal curve. No. 14 in Table II was obtained from a case presenting advanced syphilis and carcinoma of the liver. Band α is seen as an inflexion and band β has disappeared (Fig. 2). This shows a wide deviation from the normal, which is evidently due to some abnormal substances present in the blood. The method of spectrophotometry may prove of value in the detection of such substances; further work in this connection is in progress.

Fig. 2.

1. Normal curve.
2. Curve from pathological filtrate.

SUMMARY.

1. The ultra-violet absorption spectra of uric acid and of the ultra-filtrate of normal serum have been measured.
2. Uric acid, when dissolved in water, shows two sharp bands: when dissolved in carbonate, only one is visible. This possibly indicates a tautomeric change in the molecule.
3. It is reasonable to assume that the selective absorption shown by serum ultra-filtrates is due to uric acid.
4. The values given for the concentration of uric acid, as estimated by the amount of absorption, are in very fair agreement with those obtained by chemical methods.

5. A filtrate obtained from a pathological case has been investigated; it shows marked general absorption, thus differing widely from the filtrates of normal sera.

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