a disproportionate increase of d will result directly from that of r.

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Paper Partition Chromatography of Riboflavine Decomposition Products

THE photolysis of riboflavine has always been considered[1] to be a complex process, but as a rule only the final products, lumichrome (6 : 7 dimethyl alloxazine) and lumiflavine (6 : 7 : 9 trimethyl *iso*-alloxazine), have been isolated.

Using Crammer's[2] method and subjecting irradiated samples of riboflavine solution to chromatography, we were able to demonstrate several distinct spots with a very bright fluorescence in ultra-violet light. For the runs, either Whatman No. 1 or Schleicher–Schüll No. 237 filter paper and either the descending or ascending arrangement[3] was used with practically the same results. The ascending arrangement showed more irregularities of flow. 0·08–2·5 μgm. of riboflavine gave satisfactory spots, smaller samples giving too faint a spot and larger ones causing some tailing between the start and position R_F 0·35. Fluorescence was observed in filtered ultra-violet light (Phillora WPH—125 W.). The fluorescence was strong enough to allow the assessment of the fluorescence emission maxima of some spots when irradiating the chromatograms by ultra-violet light and observing the spectrum by means of a Hilger prism ; there was some difficulty due to the weak white fluorescence of the paper background. Good separation was observed using the upper layers of mixtures of butanol, acetic acid, and water (4 : 1 : 5) or butanol, pyridine and water (3 : 4 : 7) as the mobile phase.

Riboflavine was identified by comparing with a carefully prepared fresh solution of riboflavine in sodium salicylate (our R_F was higher than that reported by Crammer[2], who found 0·3 in butanol–acetic acid–water). Lumichrome was compared with a sample prepared according to Karrer *et al.*[4]. Its spot interferes with that of the salicylate in butanol–pyridine–water. Several weaker unidentified spots with R_F less than that of riboflavine in butanol–acetic acid–water were observed, the most constant of them (*B*) having R_F 0·3. The possibility that some of the spots are due to more than one substance cannot be excluded until a check by two-dimensional chromatograms is made.

The stability of the substances during chromatography in darkness was tested using the two-dimensional principle, but developing with the same solvent pair (butanol–acetic acid) for the first as well as for the second run. The spots then occupied the diagonal position. As would be expected[5], sodium hydroxide solution changes the fluorescence of lumichrome spot to yellow and extinguishes those of the other photolytic products.

After exposure to hydrogen peroxide in alkaline solution, the spots of riboflavine and lumichrome were accompanied by three more spots, two with blue and one with orange fluorescence, although riboflavine has generally been reported to be stable to oxidizing agents[1]. Heating in alkali resulted in the disappearance of the other spots and the appearance of a new one with a bright ultramarine fluorescence and R_F 0·51 in butanol–acetic acid. The results are summarized in the accompanying table.

	Spot due to	R_F in BuAc	R_F in BuPy	Colour	Fluorescence in u.-v. light
after illumination	(*A*) not identified (adsorbate on paper ?)	0·0	0·0	yellow	yellow
	(*B*) not identified	0·30			yellow
	(*C*) riboflavine	0·37	0·57	yellow	yellow
	(*D*) lumiflavine ?	0·49	0·54	yellow	yellow
	(*E*) lumichrome	0·70	0·76	φ	greenish-blue (max. 490 mμ)
Salicylate (when used as hydrotropic agent)		0·92	0·76	φ or pink	blue
Action of hydrogen peroxide on alkaline solution of riboflavine		0·08 0·22	0·23 0·52		blue
		0·16	0·37		orange

Commercial injection solutions were analysed by this method, which seems suitable for following the influence of various conditions on the rate and nature of photolysis. The nature and conditions of formation of the unidentified substance *B* are under investigation.

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A Source of Error in the Colorimetric Estimation of Uranium

FOR several years I have obtained consistent results with the hydrogen peroxide method[1] for the estimation of uranium, the intensity of the peruranate colour being measured with a Pulfrich photometer with filter *S*.43 (effective filter gravity, 434 mμ). In the hope of achieving greater accuracy and reproducibility, a modern type of spectrophotometer was recently substituted for the visual photometer, the colours being measured at 360 mμ (where the extinction for a given uranium content is nearly three times greater than at 434 mμ). It was found, however, that results were frequently low by as much as 30 per cent, although the same solutions gave the correct result when the absorption was measured with the Pulfrich photometer.

The source of error was eventually traced to the presence of sodium bicarbonate, which is formed in varying amounts when the acid solutions from ether extractions are treated with an excess of sodium