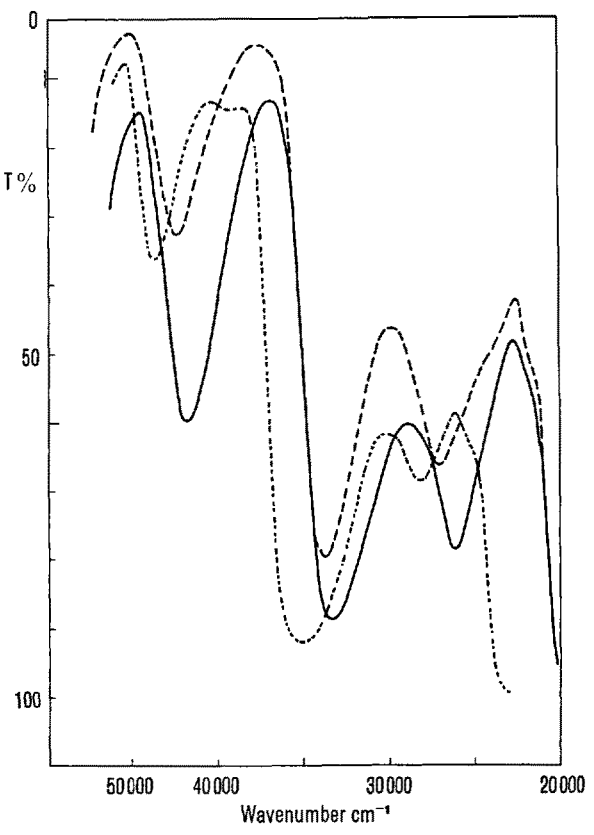
Brèves communications – Kurze Mitteilungen – Brevi comunicazioni – Brief Reports

Absorption Spectra of Riboflavin, Lumiflavin, and Lumichrome in Organic Solvents

Using organic solvent-water mixtures for elution of adsorbed riboflavin on a phenol-formaldehyde cation exchanger, a stage included in the method of riboflavin determination in foodstuffs[1,2], considerable changes in fluorescence intensity and shape of absorption spectra of riboflavin were stated[3]. In the course of extensive investigations on organic solvent effect on fluorescence emission and absorption spectra of riboflavin and lumiflavin[4] it was found that riboflavin in absolute ethanol, acetone, and 1,4-dioxane mixtures with water, and also lumiflavin in the above-mentioned pure solvents, are several times more rapidly photodecomposed when illuminated with visible light than in aqueous solutions. By means of paper partition chromatography in *n*-butanol-acetic acid-water (4:1:5) and spectrophotometry, lumichrome was found as a main product of photodecomposition. It can be considered from the shape of absorption spectra of these three compounds in solvents investigated that the photolability increase is a result of decrease of dissociation and association degree of riboflavin, lumiflavin, and lumichrome with solvent molecules. In comparison with spectra of these compounds in aqueous solutions, significant shifts of near UV-maximum, as the result of solvent polarity, and for riboflavin and lumiflavin decrease of absorbancy in this maximum as the result of solvent association degree, were observed. In addition, in lumichrome spectrum a new maximum appears at approximately 248 mμ band and increases absorbancy in the longest wavelength maximum.



Light absorption spectra of riboflavin (——) in 98% dioxane mixture with water (v/v), lumiflavin (– – –), and lumichrome (- - - -) in pure dioxan. Concentrations of all compounds are 10 μg/ml. Spectra were taken with Unicam Model SP-700 recording spectrophotometer, 1 cm silica absorption cells.

It can be assumed that obtained absorption spectra of riboflavin in 98% dioxane, lumiflavin, and lumichrome in pure dioxane, which has the lowest polarity among solvents investigated (none of these compounds dissolves in inert solvents), are almost the true spectra of these compounds nearly undissociated and minimally associated with solvent molecules. Recently KARREMANN[5] published data dealing with successful use of energies of highest filled and lowest empty molecular orbitals computed for riboflavin and its radicals for calculating positions of their longest wavelength absorption maxima, using a factor given by ISENBERG and GYÖRGY[6] equal to $\beta = 3.26$ eV = 75,000 cal/mol. These calculated positions of maxima correspond closely to the experimentally observed wavelength.

Concerning all precautions and limitations for such estimations, it seems quite probable that wavelengths observed in our investigation for shifted near UV-maxima of all three compounds can be proved by comparing them with values calculated in an analogical way. As the only probable values were obtained when taking into account energy of the second filled and lowest empty orbitals[7] (Table), it seems to be possible that the near UV-maxima

Positions of near UV and the longest wavelength maxima of riboflavin, lumiflavin, and lumichrome (second UV maximum) experimentally measured and calculated

Compound and solvent	Positions of maxima in mμ			
	Near UV		Longest wavelength	
	Measured	Calculated	Measured	Calculated
Riboflavin in 98% dioxan	344	337	440	454
Lumiflavin in pure dioxan	333	337	440	454
Lumichrome in pure dioxan	329	313	382	376

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of riboflavin, lumiflavin and second UV-maximum of lumichrome reflect transitions between these orbitals. The fact that the calculated value for the position near UV-maximum of lumiflavin is of lower frequency than measured experimentally is most probably caused by using riboflavin molecular orbital energies (these two compounds have not exactly the same spectra). An opposite irregularity, however in higher degree, is observed for lumichrome second UV-maximum. In both cases, the effect of specific interaction with dioxane cannot be excluded. In addition, positions of considered maxima of both compounds are slightly over the limits permitted for such calculations[6]. In the solvent used, measured positions of longest wavelength maxima of two of the compounds considered are shifted a little towards shorter wavelengths. Positions of this maximum calculated from energies of highest filled and lowest empty molecular orbitals are not exactly the same (Table).

Zusammenfassung. Der Einfluss organischer Lösungsmittel auf die Erhöhung der Lichtempfindlichkeit und auf die Formen der Absorptionskurven von Riboflavin, Lumiflavin und Lumichrom wurde untersucht; dabei wird eine deutliche Übereinstimmung der zwei längstwelligen Maxima-Positionen in Dioxanlösung und der theoretisch berechneten Positionen festgestellt.

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Influence of Ionizing Radiation on Protein Production by HeLa Cells in Culture

The production of proteins by HeLa human carcinoma cells in culture has been studied by radiochemical methods. The cells were incubated in a medium containing ^{14}C-leucine, the soluble proteins formed were separated into fractions by physico-chemical and immunological methods, these fractions were burned, and their radioactivity estimated with the gas counter[1-3].

We have now examined, by the radiochemical method, the influence of ionizing radiation on the synthesis of (soluble and insoluble) proteins by HeLa. The question of this influence is of interest, among other reasons, in view of the well-known inhibition of antibody formation by irradiation of whole animals. Additional information on the mechanisms responsible on the level of the cell is desirable.

The cells were grown in flat-bottomed flasks as monolayers in 10 ml of a medium consisting of Gey solution, human cord serum and hydrolyzed lactalbumin. 0.5 μc D,L-radioleucine were added as a precursor of protein. For irradiation, the medium in the flasks contained various quantities of tritiated water. Radioincubation lasted either 24 or 48 h. Thereafter, the cells were taken off the wall of the flask with complexon, and an aliquot part of the cells was counted in the hemocytometer. The cell suspension was then used to determine the newly synthesized protein, i.e. the protein containing radiocarbon. In a number of parallel flasks, cell counts were made for the time of the start of the irradiation.

To isolate the soluble proteins, cells and medium were centrifuged at low speed (giving supernatant I), the cells broken by freezing, and the fragments separated by centrifugation (giving supernatant II) and washed with NaCl. The supernatants I and II and the wash solutions were combined, inactive leucine was introduced as a hold-back carrier, trichloroacetic acid (TCA) was added, the precipitate freed from the solution by centrifugation and thorough washing with TCA, burned, and the radioactivity of the CO_2 measured with the gas counter[4,5]. The cell debris, containing the insoluble proteins, were dissolved in warm *n* NaOH, aliquots of these solutions were neutralized with HCl, burned, and measured independently. All values in the Tables are mean values from 2 or 3 flasks; the errors are standard errors.

Table I. Cell counts per flask ($\cdot 10^{-6}$)

Activity of tritium (mc/10 ml)	Start	After 24 h	After 48 h
0	3.25 ± 0.15	4.61 ± 0.40	5.94 ± 0.39
0.5	3.25 ± 0.15	4.28 ± 0.47	6.06 ± 0.54
5	3.25 ± 0.15	3.88 ± 0.23	5.01 ± 0.53
50	3.25 ± 0.15	3.17 ± 0.31	2.61 ± 0.37

Table II. Protein production per flask

Activity of tritium (mc/10 ml)	Activity of protein (^{14}C) (dpm)			
	After 24 h		After 48 h	
	soluble	insoluble	soluble	insoluble
0	9680 ± 570	11620 ± 840	17140 ± 1280	20300 ± 570
0.5	9130 ± 1160	10980 ± 850	15390 ± 1020	20680 ± 2020
5	9430 ± 790	11720 ± 1080	15900 ± 1110	20670 ± 1050
50	8340 ± 780	11510 ± 1000	13890 ± 1860	19680 ± 2950

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