mit dem des EtOAc-Auszugs vereinigt. Der EtOAc-Auszug wurde mit verd. HCl, dann mit H_2O gewaschen, getrocknet und eingedampft. Der Rückstand wurde zusammen mit (A) aus Aceton zweimal umkristallisiert und im Vakuum von 5 mm und bei 160～180° (Badtemp.) sublimiert. Nach zweimaliger Wiederholung dieser Behandlung und anschliessendem Umlösen aus Aceton erhielt man 1.5 mg farblose Nadeln vom Schmp. 253～256°. Diese Substanz zeigt beim Mischen mit der authentischen 2,5-Dimethoxyterephthalsäure keine Depression. Sie fluoresziert stark in EtOH-Lösung unter ultravioletten Strahlen ebenso wie die letztere.

Zusammenfassung

Das durch die Kondensation von Tetrahydrogeraniumsäure mit Toluhydrochinon erhaltene *d,l*-2-Methyl-5-(3,7-dimethyloctanoyl)hydrochinon lieferte bei der Clemmensen'schen Reduktion das *rac*-2-Methyl-5-(3,7-dimethyloctyl)hydrochinon, welches sich mit dem Tetrahydropirolagenin aus Pirolatin als identisch erweist. Dabei wurden auch *l*-2-Methyl-5-(3,7-dimethyloctanoyl)hydrochinon und sein Reduktionsprodukt, *l*-2-Methyl-5-(3,7-dimethyloctyl)hydrochinon hergestellt.

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131. Kunio Yagi and Jun Okuda: Determination of Fluorescence of Flavins on Paper Strips.

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Yagi[1] reported a separatory determination of flavins using paper chromatography. The method consisted of separation of flavins on a paper chromatogram, elution, and followed by determination by the lumiflavin fluorescence method. For a large amount of flavins, electrophoresis[2,3] was applied, where the same elution procedure was also used. If the direct determination of the fluorescence of flavins on a paper strip is available, these procedures will be simplified.

To determine several flavin nucleotides on a paper strip, it is necessary to examine the fluorescence spectrum of each flavin on paper strip for the choice of a fluorescence-selective filter and to know the range where the amount of flavin is parallel to its fluorescence energy. Moreover, if the measurement has to be made by using free riboflavin as a measuring comparison, it is necessary to check the relative value of fluorescence energy of each flavin to the others.

The present work represents examinations of the above-mentioned problems and is an attempt to broaden the utility of direct determination of flavin nucleotides on paper strips.

Apparatus and Materials

For the measurements of fluorescence spectra of flavins on paper strips, the apparatus deviced by Yagi, *et al.*[4] was used.

* Tsurumai-cho, Showa-ku, Nagoya (八木國夫, 奥田 潤).

1) K. Yagi: J. Biochem. (Tokyo), **38**, 161 (1951).
2) K. Yagi, Y. Matsuoka: *Ibid.*, **42**, 757 (1955).
3) P. Cerletti, N. Siliprandi: Biochem. J., **61**, 324 (1955).
4) K. Yagi, T. Tabata, E. Kotaki, T. Arakawa: Vitamins (Kyoto), **7**, 878 (1954).
5) K. Yagi, T. Tabata: J. Japan. Biochem. Soc., **27**, 779 (1956).

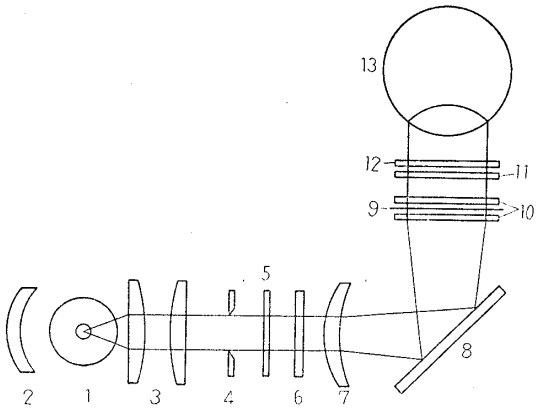

Fig. 1. Optical System of the Apparatus for Determination of the Fluorescence on Paper Strip

1. High-pressure mercury lamp (Mazda SHL-100 UV)
2. Concave mirror
3. Condenser
4. Inlet slit
5. Shutter
6. Ultraviolet-ray selective filter
7. Concave lens
8. Reflecting surface mirror
9. Paper strip
10. Nonfluorescent glass plate
11. Ultraviolet-ray cutoff filter
12. Fluorescence-selective filter
13. Photo-tube

For the estimation of fluorescence energy on paper strips, an apparatus perfected by Yagi, *et al.*[5] was adopted. In this apparatus, as shown in Fig. 1, an ultraviolet-ray screened by a glass filter (Mazda UV-D1: 365 mμ Hg line) is irradiated on a paper strip. The fluorescence energy of flavin passing through this paper is received by the photo-tube through an ultraviolet-ray cutoff filter and a fluorescence-selective filter. For the measurement of fluorescence energy, the electrical system reported before[6] was used. By using this apparatus, the fluorescence energy can be read quickly after the shutter is opened so that the photodecomposition of a fluorescent substance by an ultraviolet-ray may be eliminated.

Brown and Marsh[7] reported a direct determination of riboflavin on a paper strip. In their apparatus, the paper strip moves in front of a slit of fluorometer and the fluorescence energy is recorded by a self-recorder.

Compared with their apparatus, the present one is simpler than theirs in both construction and operation, though it cannot determine the fluorescence which is spread on a rather wide area of a paper strip.

Semm and Fried[8] also reported a simplified apparatus using a photo-cell for the direct determination of fluorescence on a paper strip. In principle, the two are quite similar to each other, but the present apparatus using photo-tube is more convenient for rapid and sensitive determination.

Free riboflavin and flavin mononucleotide (FMN) were chemically synthesized. Flavin adenine dinucleotide (FAD) was prepared by the method of Yagi, *et al.*[9] The purity of this FAD preparation was over 92% and other flavins, metals, and nucleic acids were not detected in the impurities.

Experimental and Discussion

Fluorescence Spectra of Flavins on Paper Strip—The aqueous solution of a flavin nucleotide was placed on a filter paper (Toyo Roshi No. 51) and dried in a dark room. The fluorescence spectrum was obtained by the same way as reported before[4] and results obtained are shown in Fig. 2. The fluorescence spectra of these flavins were the same and nearly agreed with the spectrum of free riboflavin in aqueous solution.[10] The peaks of the spectra of three flavins were recorded at around 530 mμ. Therefore, an interference filter of λ_{max} 530 mμ can be used for each flavin as a selective filter for the determination described below.

Relation between the Quantity and Fluorescence Energy of Flavins—An aqueous solution of a flavin compound was spotted on a paper strip (10 mm. in diameter) and dried as mentioned before. The whole fluorescence energy was measured at once. Then the reading was checked with a paper strip without the flavin and the value so obtained was subtracted as a blank from the above.

In the case of free riboflavin, the fluorescence energy was parallel to its quantity over the range from 1×10^{-1} γ (2.66×10^{-10} moles) to 2×10^{-3} γ (0.53×10^{-11} moles).

In the case of FMN and FAD, nearly the same results were obtained as shown in Fig. 3. The mole of each flavin on paper strip will be calculated by using a control on which corresponding estimated flavin was placed as a comparison.

The diameter of fluorescence zone on paper strip must be less than 10 mm. under the present ex-

6) K. Yagi, T. Arakawa: Vitamins (Kyoto), **6**, 523 (1953).
7) J. A. Brown, M. M. Marsh: Anal. Chem., **25**, 1865 (1953).
8) K. Semm, R. Fried: Naturwiss., **39**, 326 (1952).
9) K. Yagi, Y. Matsuoka, S. Kuyama, M. Tada: J. Biochem. (Tokyo), **43**, 93 (1956).
10) K. Yagi, T. Tabata, E. Kotaki, T. Arakawa: Vitamins (Kyoto), **8**, 61 (1955).

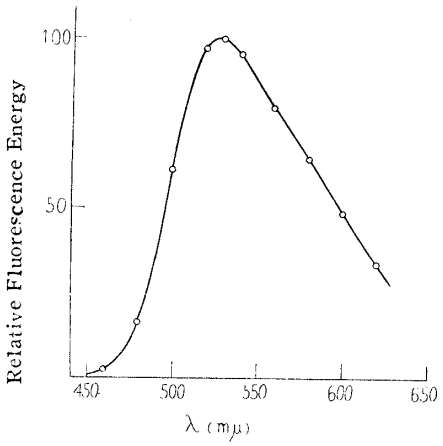

Fig. 2. Fluorescence Spectra of Free Riboflavin, FMN, and FAD

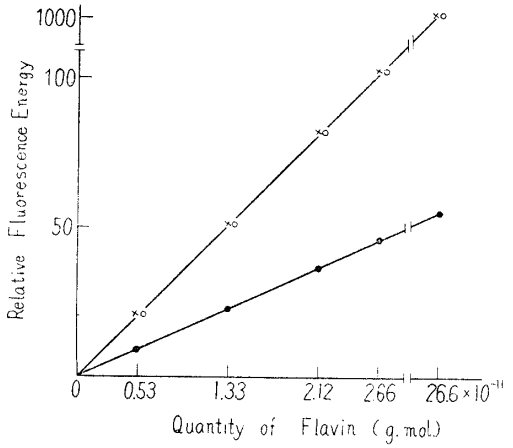

Fig. 3. Relationship between the Quantity of Flavin placed on Paper Strip and Relative Fluorescence Energy
×——× Free riboflavin ○——○ FMN
●——● FAD

perimental conditions. A large fluorescence zone on paper strip and tailing fluorescence zone can not be estimated precisely by using the attachment in this instrument.

Photodecomposition—By the ultraviolet-ray irradiation from a mercury vapor lamp for 10 mins., about 20% of 1.4×10^{-10} moles of free riboflavin or FMN on the paper strip was decomposed to a nonfluorescent substance, while FAD was not easily decomposed. Measurements made within 30 secs. showed a decrease of less than 1%. It is desired that the operation be carried out within a few seconds.

On the contrary, flavins, even free riboflavin and FMN, in aqueous solution were not decomposed to nonfluorescent substances so rapidly by the same irradiation of ultraviolet-ray.

Relative Fluorescence Energy of Each Flavin Nucleotide—When the separatory determination of flavin nucleotides is desired using paper partition chromatography or paper electrophoresis, it is necessary to know the molar ratio of these flavins which are to be separated on the paper strip.

As an examination of the above-mentioned problem, the same volume of equimolar concentrations of flavins was placed on a filter paper and fluorescence energy was estimated within a few seconds. As shown in Fig. 3, the relative values of the fluorescence energy of free riboflavin, FMN, and FAD were 100, 100, and 46, respectively, under the present experimental condition. However, this ratio changes according to the nature of the paper strip, filters, and other factors of the optical system of the instrument. Therefore, it is necessary to examine these factors in each case by using pure free riboflavin, FMN, and FAD.

When the estimated values of the fluorescence of free riboflavin, FMN, and FAD on paper strip are f_1, f_2, and f_3, respectively, and the total flavin in the original solution is estimated as F γ/cc. by the lumiflavin-fluorescence method, the absolute quantity of each flavin in the original solution may be calculated from the following formulae:

$$\text{Free riboflavin } (\gamma/\text{cc.}) = \text{F}\times\frac{f_1}{f_1+f_2+f_3\cdot\dfrac{100}{a}}\times 1$$

$$\text{FMN } (\gamma/\text{cc.}) = \text{F}\times\frac{f_2}{f_1+f_2+f_3\cdot\dfrac{100}{a}}\times 1.21$$

$$\text{FAD } (\gamma/\text{cc.}) = \text{F}\times\frac{f_3\cdot\dfrac{100}{a}}{f_1+f_2+f_3\cdot\dfrac{100}{a}}\times 2.09$$

where a represents the relative fluorescence energy (in %) of FAD to free riboflavin or FMN.

Bessey, *et al.*[11] reported the relative intensity of the fluorescence of free riboflavin, FMN, and FAD to be 100, 100, and 9, respectively, in an aqueous solution at pH 7. The difference of the fluorescence intensities of FAD between that on paper strip and that in an aqueous solution may be attributed to the difference

11) O. A. Bessey, O. H. Lowry, R. H. Love: J. Biol. Chem., **180**, 755 (1949).

in the strength of intermolecular linkage of FAD between flavin and adenine rings in its molecule in the two cases.[12]

Summary

Fluorescence spectra of flavins on paper strip were determined and the peak of the fluorescence spectra of free riboflavin, FMN, and FAD was identically recorded at 530 mμ. The relation between the quantity and fluorescence energy on paper strip was linear over a range of the quantities of flavin from 2.66×10^{-10} moles to 0.53×10^{-11} moles. The relative fluorescence energies of equimolar quantities of free riboflavin, FMN, and FAD on a paper strip were determined under experimental conditions reported herein.

The molar quantity of flavins on a paper strip can be determined directly by fluorometry using a standard concentration of free riboflavin placed on the paper strip as a scale.

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12) G. Weber: Biochem. J., **47**, 114 (1950).

UDC 577.164.12

132. Jun Okuda: Metabolism of Flavin Nucleotides. I. Decomposition of Flavin Nucleotides in Digestive Juice.

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It has already been shown that many coenzymes are derivatives of vitamins and are divided into two classes, i.e. monophosphoric and pyrophosphoric esters.

Vitamin B_2, riboflavin, has both of them, namely, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).[1]

It has been shown that these esters could be synthesized from free riboflavin in living body[2~4] and the enzymes for the phosphorylation of free riboflavin and FMN could be purified.[5,6]

In the case of a rat, it was found that administered free riboflavin was phosphorylated to FMN in the mucous membrane of the small intestines and then it was changed to FAD in the liver and kidney,[7] and the enzymes responsible for these reactions were demonstrated *in vitro* using acetone-dried powder of these organs.[8,9]

However, investigations on the decomposition of these phosphoric esters of riboflavin in the digestive organs have not been reported yet. Results on these subjects might be quite important in understanding the absorption mechanism of flavin nucleotides, which is closely related to nutritional and pharmaceutical fields. They will also give some presumption on the decomposition of other phosphoric esters of vitamins and nucleotides in the digestive organs. The present paper deals with the decomposition of flavin nucleotides in saliva, gastric juice, bile, and pancreatic juice.

* Tsurumai-cho, Showa-ku, Nagoya (奥田 潤).

1) Hereafter following abbreviations will be used in this report: Flavin mononucleotide=FMN, flavin adenine dinucleotide=FAD.
2) H. Rudy: Naturwiss., **23**, 286 (1935).
3) H. Hübner, F. Verzár: Helv. Chim. Acta, **21**, 1006 (1938).
4) J. R. Klein, I. H. Kohn: J. Biol. Chem., **136**, 177 (1940).
5) E. B. Kearney: *Ibid.*, **194**, 747 (1952).
6) A. W. Schrecker, A. Kornberg: *Ibid.*, **182**, 795 (1950).
7) K. Yagi: J. Biochem. (Tokyo), **41**, 757 (1954).
8) K. Yagi: Igaku-to-Seibutsugaku, **19**, 233 (1951).
9) K. Yagi: *Ibid.*, **19**, 305 (1951).