The effects of 3,5-DMI and 3-MIC appear to be indistinguishable from each other. On the other hand, the effects of 5-MIC of plasma FFA were much less intense (50% reduction) and much less prolonged (normal in 2–3 h). As in the isolated adipose tissue system, IDC was ineffective in lowering plasma FFA values.

The lack of any effect of dimethylisoxazole on FFA release in an isolated adipose tissue system is demonstrated in this report and confirms the findings of others[4]. This observation together with the demonstrated effectiveness of 3,5-DMI in reducing plasma FFA levels in intact rats[6] and dogs (Figure) suggests that metabolism to an active agent is required. However, the unexpected finding that 3,5-DMI depresses plasma FFA in the eviscerated rat[6] indicates that the liver is probably not the site of the necessary metabolic step.

The findings reported here demonstrate that only 1 of the 3 possible carboxylic acid metabolites, 3-MIC, is potent enough in the isolated adipose tissue system and the intact dog to explain the effects of 3,5-DMI in vivo. The close similarity in time course of plasma FFA effects of both 3-MIC and 3,5-DMI provides additional evidence that 3-MIC is the active species and, further, suggests that the conversion of 3,5-DMI to 3-MIC is relatively efficient.

The lack of effectiveness of 5-MIC and IDC in both the adipose tissue system and the intact dog rules out these compounds as potential active metabolites of 3,5-DMI.

Percent inhibition of the norepinephrine-induced FFA release from isolated adipose tissue by 3,5-dimethylisoxazole, 3-methyl isoxazole-5-carboxylic acid, 5-methyl isoxazole-3-carboxylic acid and isoxazole-3,5-dicarboxylic acid

	Molar concentration				
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
3,5-dimethyl-isoxazole	0	0	0	0	0
3-methylisoxazole-5-carboxylic acid	[a]81 ± 6	65 ± 6	62 ± 7	29 ± 3	0
5-methylisoxazole-3-carboxylic acid	23 ± 5	8 ± 3	0	0	0
isoxazole-3,5-di-carboxylic acid	0	0	0	0	0

[a] Mean $\pm$ S.D.; $n = 6$ experimental flasks.

Zusammenfassung. Von allen möglichen Isoxazolcarbonsäurenstoffwechselprodukten von 3,5-Dimethylisoxazol (3,5-DMI), die untersucht wurden, war nur die 3-Methylisoxazol-5-carbonsäure wirksam genug, um die auffallende Wirkung von 3,5-DMI auf unveresterte Fettsäurespiegel in normalen Hunden zu erklären.

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Fluorescence of Indole Derivatives

The identification and estimation of indolic compounds in biological material is of considerable interest. Due to the extremely low quantities in which several of these compounds occur in various tissues, fluorescence reactions are generally used. In this paper are described the different fluorescence properties of 5-methoxyindoles compared with other indolic compounds, using suitable solvent systems.

Early investigations of BOWMAN et al.[1] described a native fluorescence maximum at 330–360 nm in aqueous solution for many indoles after excitation at 275–296 nm. Using 3*N* HCl as a solvent, the 5-hydroxyindoles[2] and the 5-methoxyindoles[3] showed an additional fluorescence maximum at 550 nm. A systematic study of indole fluorescence at various pH values was made by WILLIAMS[4]. For a number of naturally occurring indoles a greater sensitivity could be reached by a chemical reaction resulting in a fluorescent product. The condensation of formaldehyde with indolealkylamines is used in histochemistry for the fluorescence microscopic localization of these compounds[5]. Serotonin[6,7] as well as its N,N-dimethyl derivative, bufotenine[8], can be determined with great sensitivity after reaction with ninhydrin. Recently, MAICKEL and MILLER[9] described the fluorescent properties of a number of indole derivatives after reaction with *o*-phthalaldehyde. Up to now no reliable technique existed for analytical differentiation between serotonin and melatonin.

According to LERNER[10] and MCISAAC[11] mammalian pineal glands appear to be a rich source of various indoles. During investigations on the presence of melatonin in pineal organs of lower vertebrates[12], the necessity was

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Relative fluorescence intensities of 3- and 5-substituted indoles in 3 solvent systems: I, 1 *N* NaOH; II, 40% formaldehyde/1 *N* NaOH (1:1); III, 1 *N* NaOH/acetic anhydride (1:1)

Compound	R_3	R_5	I	II	III	
Indole	–	–	2.4 (400)	20.3 (380)	2.1 (380)	
Indole-3-carboxylic acid	–COOH	–	13.0 (380)	70.6 (340)	7.7 (360)	
Indole-3-acetic acid	$-CH_2-COOH$	–	11.6 (420)	100 (380)	1.9 (360)	
Tryptophol	$-CH_2-CH_2OH$	–	7.4 (420)	102 (380)	2.7 (360)	
Tryptamine	$-CH_2-CH_2-NH_2$	–	8.5 (420)	158 (370)	3.0 (360)	
Tryptophan	$-CH_2-CH(COOH)-NH_2$	–	9.0 (420)	205 (360)	1.3 (360)	
5-Methyltryptophan	$-CH_2-CH(COOH)-NH_2$	$-CH_3$	11.8 (400)	186 (360)	7.1 (360)	
N-Acetyltryptamine	$-CH_2-CH_2-NH-CO-CH_3$	–	9.1 (420)	77.3 (380)	2.3 (360)	
N-Acetyltryptophan	$-CH_2-CH(COOH)-NH-CO-CH_3$	–	6.5 (420)	87.6 (380)	1.5 (340)	
5-Hydroxyindole-3-acetic acid	$-CH_2-COOH$	–OH	74.4 (460)	64.7 (380)	8.6 (340)	2.3 (560)
Serotonin (5-hydroxytryptamine)	$-CH_2-CH_2-NH_2$	–OH	6.2 (380)	32.8 (380)	9.3 (360)	2.2 (560)
5-Hydroxytryptophan	$-CH_2-CH(COOH)-NH_2$	–OH	8.0 (380)	40.9 (380)	8.3 (340)	2.1 (560)
N-Acetylserotonin	$-CH_2-CH_2-NH-CO-CH_3$	–OH	9.1 (380) 12,6 (460)	56.7 (380)	13.0 (360)	3.4 (560)
5-Methoxyindole-3-acetic acid	$-CH_2-COOH$	$-OCH_3$	64.9 (430)	455 (360)	17.8 (340)	28.9 (545)
5-Methoxytryptamine	$-CH_2-CH_2-NH_2$	$-OCH_3$	117 (430)	850 (360)	109 (340)	95.3 (545)
Melatonin	$-CH_2-CH_2-NH-CO-CH_3$	$-OCH_3$	74.8 (430)	672 (360)	81.2 (340)	61.0 (545)

Values in parentheses represent the fluorescence maximum in nm. Excitation wave-length: 307 nm. Excitation and emission slit: 1 mm

felt of having a fluorescence reaction making it possible to distinguish between the 5-methoxyindoles and the other indolic compounds present.

In attempting to obtain a more or léss specific fluorescence reaction for the 5-methoxyindoles, we studied the fluorescence properties of a number of indoles in different solvent systems. All compounds were measured in a concentration of 5×10^{-5} molar. A Zeiss spectrofluorometer (ZFM4C) was used. An excitation wave-length of 307 nm and slits of 1 mm were applied. The amplification factor of the instrument was kept constant during all measurements. Starting from the fluorescence properties in alkaline media, a marked increase was observed in the fluorescence of the methoxyderivatives, using a mixture of 40% formaldehyde and 1 *N* NaOH (Table, column II). A far better distinction between methoxy- and hydroxyindoles was found by adding acetic anhydride to an alkaline indole solution (Table, column III). In this solvent system the methoxy compounds show a fluorescence in the yellow part of the spectrum at 545 nm. The 5-hydroxyindoles, including serotonin, do not show any appreciable fluorescence, whereas the indoles without a substituent in the 5-position also have a low fluorescence between 340 and 440 nm.

The mechanism of this reaction deserves further investigation. Work is in progress to use this visible fluorescence of 5-methoxyindoles for the localization of melatonin in pineal organs of mammals and lower vertebrates[13].

Zusammenfassung. Die Verwendung von 1 *N* Natronlauge und Essigsäureanhydrid bei der Bestimmung von Indolderivaten mit Hilfe der Fluoreszenzmethode erlaubt eine gute Differenzierung zwischen 5-Methoxyindolderivaten (z.B. Melatonin) und anderen Indolen. Versuche zur Verwendung der neuen Methode in biologischem Material, z.B. Epiphysenschnitten, sind im Gange.

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[3] The authors are indebted to Prof. J. C. van de Kamer for his encouragement and interest in these studies.

Zur Immunchemie der Klebsiella-Antigene

Bei der serologischen Typendifferenzierung der Klebsiellen sind zwei Antigene von Bedeutung, die somatischen (O-)Antigene und die Kapsel-(K-)Antigene. Mit der spezifischen Kapselreaktion oder durch Agglutination sind bis heute 80 verschiedene K-Typen gefunden worden, die 10 O-Serogruppen angehören[1–4]. Dabei sind alle K-Typen mit gleichem O-Antigen in einer O-Gruppe zusammengefasst. Immunchemische Untersuchungen zu diesem Problem liegen nur von Eriksen vor[5].

Wir haben mit einer systematischen Untersuchung der Klebsiella-Antigene begonnen und an über 70 serologisch klassifizierten unterschiedlichen Klebsiella-Stämmen Antigenextraktionen vorgenommen, die K- und O-Antigene in reiner Form isoliert und sowohl chemisch als auch serologisch näher untersucht. Eine Trennung der durch

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