Zusammenfassung. Die Atmung einzelner Pantoffeltierchen (*Paramecium aurelia*) aus Serien-isolierten Kulturen wurde mit Hilfe eines Cartesischen Tauchers gemessen. Die Messungen an Individuen vor und nach Autogamie (Selbstbefruchtung) zeigten, dass die Atmung nach Autogamie in signifikanter Weise ansteigt.

Da die Autogamie für das Überleben der Kulturen eine grosse Wichtigkeit besitzt, ergeben sich Parallelen zwischen den Stoffwechselveränderungen bei *Paramecium* und der Alterung der somatischen Zellen von Metazoen.

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Vitamin Requirements of *Nemalion multifidum*

In axenic culture, the red alga *Goniotrichum elegans* was found to be a vitamin heterotroph[1,2]. Like many other marine organisms, it required cyanocobalamin for growth. In November 1959, success was achieved in growing another red alga in axenic culture, *Nemalion multifidum* (WEBER and MOHR) J. Ag. a member of *Florideae*. The method for sterilization will be described in a later paper. The method for cultivation was the same as that earlier used by the author[2]. The alga was grown in the artificial medium ASP 6[3] with nitrate replaced by asparagine. During the first months, the alga seemed to be autotrophic. Cultures in a vitamin-containing substrate grew as rapidly as those in a vitamin-free solution. Subsequently, however, the vitamin-starved cultures became pale in colour, and additions of the vitamin solution of ASP 6, a mixture of 15 different vitamins, increased growth up to 100%. Eleven of the vitamins were sterilized together in an autoclave ('autoclaved vitamins') but the remainder, pyridoxamine, lactoflavin, folic acid and cyanocobalamin, were passed through a sterile glass filter before being added to the sterile flasks. The active substance could be located as being among these four vitamins (Tab. I). Cyanocobalamin (= B_{12}) had the strongest effect, but pyridoxamine also caused an increase in growth.

In order to determine which part of the cyanocobalamin molecule was necessary, different B_{12}-analogues were added (Tab. II). At that time, the inocula had been starved for four months and even additions of cyanocobalamin increased growth very little. However, Factor 1b (= Factor B ribosephosphate) was the more active one; while Factor B, which contains only the pseudophorphyrine ring, had no effect. *Nemalion* was very little affected by such analogues as Factor III or Factor Z1, in contrast to the behaviour of *Goniotrichum elegans*.

In the early experiments, not only pyridoxamine but also pyridoxine increased the growth of Nemalion. When added together with B_{12} or Factor 1b, pyridoxamine gave a higher yield than these compounds alone. When the inocula became more starved this effect did not appear (Tab. III).

In all these experiments, some growth occurred also in the controls; and thus a very weak vitamin production is indicated. An addition of one vitamin can thus enhance growth while there is some production of other necessary compounds but not sufficient for optimal growth. The cyanocobalamin molecule seems to be broken down before its incorporation in an enzyme and the Factor 1b molecule without the benzimidazole ring is more conveniently utilized for this purpose. The role which the B_6 vitamins play in this connection cannot yet be explained, but a cooperation between B_{12} and B_6 is known from the methionine synthesis of a B_6-less mutant of *Escherichia coli*[4].

Tab. I. Growth of *Nemalion multifidum* with different vitamins

Substance added	Dry weight in mg of algae from 6 flasks	
	Experiment 1 Incubation time 24 days	Experiment 2 Incubation time 20 days
0	6.4	5.8
All vitamins	10.6	—
'Autoclaved vitamins'	8.6	—
Pyridoxamine, lactoflavin, folic acid, B_{12}	13.8	9.7
Pyridoxamine	12.8	8.9
Lactoflavin	5.8	—
Folic acid	7.4	7.2
B_{12}	14.2	9.9
B_{12} + pyridoxamine	—	10.8

Tab. II. Growth of *Nemalion multifidum* with different B_{12}-analogues

Additions of B_{12}-analogues 1 µg/l	Dry weight in mg of algae from 6 flasks
0	12.7
B_{12}	14.0
Factor B	13.0
Factor III	14.0
Pseudovitamin B_{12}	13.8
Factor 1b	32.9
Factor Z1	14.3
Factor Z2	14.4
Incubation time 42 days.	

Tab. III. Growth of *Nemalion* with additions of B_{12} or Factor 1b together with pyridoxamine and B_6

Addition	Dry weight in mg of algae from 6 flasks		
	Exper. 1	Exper. 2	Exper. 3
0	4.2	4.8	2.6
B_{12}	4.8	—	—
Factor 1b	17.0	11.1	9.0
B_6	4.0	—	—
Pyridoxamine	4.2	5.6	—
B_{12} + B_6	5.0	—	—
B_{12} + pyridoxamine	5.8	—	—
Factor 1b + B_6	21.4	—	—
Factor 1b + pyridoxamine	23.2	10.4	7.4
Incubation time 30 days.			

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Zusammenfassung. Es ist gelungen, *Nemalion multifidum*, eine marine Floridee, in axenische Kultur zu bekommen. Die Alge wurde in einer synthetischen Nährlösung gezüchtet. Wenn sie ohne Vitamine 2 Monate kultiviert wurde, konnte ein Zusatz einer Vitaminmischung den Zuwachs verdoppeln. Die wirksamen Vitamine waren am Anfang B_{12} oder B_6; wenn die Alge aber längere Zeit in Hungerkultur gezüchtet worden war, konnte nur die B_{12}-Analoge, Factor Ib, den Zuwachs erhöhen.

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6-Hydroxylation: An Important Metabolic Route for α-Methyltryptamine

D,L-α-methyl tryptamine (αMT) has been found to produce LSD_{25} like effect in normal volunteers[1]. We have found that the effect of 20 mg αMT is comparable in intensity to the psychotomimetic effect of about 60 mg N,N-diethyltryptamine (DET) in man with the difference that the perceptual distortions are more and the sympathomimetic vegetative changes are less pronounced than those produced by DET[2]. Since DET has been found to produce the behavioral changes through the 6-hydroxylated metabolite[3], the question arises whether or not αMT also forms 6-hydroxylated metabolites.

Although the biochemical effects of αMT on serotonin metabolism has been extensively studied[4–6], very little is known about the metabolism of the compound itself. Since the side chain of αMT is identical with the side chain of amphetamine ($-CH_2 \cdot \underset{\displaystyle CH_3}{\underset{|}{CH}} \cdot NH_2$), the compound was expected to undergo the same microsomal deamination as amphetamine[7].

Incubating αMT with rat liver microsomes, soluble supernatant fraction, TPN and Mg^{++}[7] at 37°C resulted in the formation of 3-indolylacetone as evidenced by the paper chromatographic identification of the metabolite in the neutral ether extract with synthetically prepared 3-indolylacetone[8]. Both the metabolite and the synthetic compound gave the same Rf value in three different solvent systems and produced the same purple color reaction with Ehrlich's *p*-dimethylaminobenzaldehyde. The chromatograms of the neutral ether extract also showed another new compound which gave a fast developing blue reaction with Ehrlich's reagent and instant red color with diazotized sulfanilic acid in HCl specific for 6-hydroxy indole derivatives[9]. The Rf values of this new compound (0.83 in *n*-butanol-acetic acid and 0.90 in isopropanol-ammonia) and the color reactions were identical with those of the single metabolite obtained from synthetic 3-indolylacetone in the same microsomal enzyme system. Therefore, this compound is most probably 6-hydroxy-3- indolylacetone.

The pH of the enzymatic reaction mixture (previously extracted with ether) was then adjusted to 9.5 and extracted with *n*-butanol. When the butanol extract was shaken with dilute acetic acid, the aqueous layer contained, besides unchanged αMT[10], a new apparently basic indole derivative (Rf 0.48 in butanol-acetic acid, 0.66 in butanol-ammonia) which gave the color reactions characteristic for 6-hydroxy indoles. It is most likely that this compound is 6-hydroxy-α-methyltryptamine (6-HO-αMT).

Additional evidence for the identity of 6-HO-αMT was obtained by an experiment in which the isolated compound was again incubated with the microsomal enzyme system and yielded 6-hydroxy-3-indolylacetone by deamination.

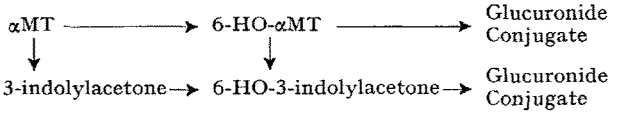
Thus the three major enzymatic products were tentatively identified as 3-indolylacetone, 6-HO-αMT and 6-hydroxy-3-indolylacetone.

The same metabolites have been found to be formed in intact animals and excreted in the urine mainly as glucuronide conjugates.

Male albino rats were injected with αMT (5 mg/kg) intraperitoneally and the urines were collected for 48 h in metabolic cages. Aliquots of the urine samples were incubated with bacterial β-glucuronidase (Sigma) in order to hydrolyze the glucuronide conjugates.

The urine samples were chromatographed without previous extraction. The chromatograms of the samples not treated with β-glucuronidase showed the presence of some unchanged αMT, a spot corresponding to 3-indolylacetone, a small spot corresponding to 6-HO-αMT and a large presumably double spot with low Rf values (0.04 to 0.20 in butanol-acetic acid and 0.20–0.45 in isopropanol-NH_3) and with typical 6-hydroxy-indole color reactions. On the chromatograms of the glucuronidase treated urine samples, this large double spot with low Rf values was absent but the spot corresponding to 6-HO-αMT was tremendously increased and another compound appeared which proved to be identical with the enzymatically prepared 6-hydroxy-3-indolylacetone.

From the evidence described above the following tentative scheme for the metabolism of αMT may be drawn:



Since 6-hydroxylated indole derivatives are more potent pharmacologically than the non-hydroxylated parent compounds[11], it is possible that one or both of the 6-hydroxy metabolites play a part in the behavioral effect of αMT.

Zusammenfassung. Der Stoffwechsel der psychotropischen Verbindung α-Methyltryptamin wurde untersucht. Die drei hauptsächlichsten Metabolite wurden *in vitro* und *in vivo* als 6-Hydroxy-α-methyltryptamin, 3-Indolylaceton und 6-Hydroxy-3-indolylaceton identifiziert.

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Clinical Neuropharmacology Research Center, Washington (D.C.), October 4, 1960.

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