Studies of the Metabolism of a Vitamin E Analog

The metabolism of vitamin E (α-tocopherol) in various animal species has been investigated by a number of workers and the results are somewhat conflicting. MELLORS and McC. BARNES[1] recently reviewed these results.

The only in vitro studies on the metabolism of α-tocopherol in rat liver homogenates were those reported by SEWARD and CORWIN[2]. Incubation of rat liver homogenates with α-tocopherol under conditions where no oxidative breakdown can occur did not lead to any transformation products.

We have studied the in vitro transformation of 6-hydroxy-2, 2, 5, 7, 8-pentamethyl chroman which differs from α-tocopherol only in having the phytyl group replaced by a methyl group. This compound was prepared with a C^{14} label in the 5-methyl group by reacting $Zn(C^{14}N)_2$, prepared from $KC^{14}N$ and $ZnCl_2$, with 6-hydroxy-2, 2, 7, 8-tetramethyl chroman to yield the 5-aldehyde which was reduced with Zn(Hg) to the desired chroman. The intermediate aldehyde was separated from unreacted starting material by preparative silica gel G chromatography (aldehyde; Rf 0.66; starting chroman, Rf 0.33) developed with $CHCl_3$. After reduction of the purified aldehyde to the chroman, it was recrystallized from petroleum ether (bp 60–110°) until only one spot showed on silica gel G thin-layer chromatography (TLC); (Rf 0.50; $CHCl_3$). This chromatographic behavior was identical with that of an authentic sample of 6-hydroxy-2, 2, 5, 7, 8-pentamethyl chroman. The IR- and NMR-spectra of the labeled and cold material were also identical. The activity was 1.62 mc/m*M* and all of the radioactivity was present at the single spot due to the chroman.

Livers from rats and rabbits that were pretreated with phenobarbital (38 mg, i.p., twice daily for 3 days) were used. The homogenate was prepared as previously reported[3]. Incubation was carried out using approximately 0.8 mg of chroman in a final volume of 2.8 ml in a 20 ml beaker for 2 h at 37 °C using a Dubnoff metabolic shaker. The incubation was terminated by adding 0.5 ml of 0.1 *N* HCl.

Isolation of metabolites was by ether extraction followed by extraction of the aqueous layer with ether: ethanol (2:1) after half saturation with ammonium sulfate.

In view of the confusion in the literature regarding true metabolites of α-tocopherol and artifacts formed by oxidation, the following experiments were performed with results seen in Tables I–VI. The conclusions from these results are that (1) transformation of the chroman is greatest in the case where cofactors are present (Table I); (2) the nonaqueous extract contains dimer or trimer model in the control experiments where no cofactors were present or the enzyme was heat denatured (Table II); (3) adding H_2O_2 to the control experiments did not result in an increase in the amounts of metabolites formed (Table III); (4) incubation under nitrogen rather than in air did not affect the control experiment but resulted in less transformation of the chroman to polar products in the experimental group (Table IV); (5) the results using rat liver are very much like those using rabbit liver (Table V); (6) tocopherolquinone model and dimer model are formed in the control experiments as well as in the experimental group where cofactor was present (Table VI); and (7) finally, new, polar metabolic products were formed (Table VI).

Incubation of the extract from the aqueous layer (Table VI) with gluculase (Endo), glucuronidase and sulfatase, in a 5.5 pH acetate buffer for 3 h at 37 °C, resulted

Table I. Incubation of 6-hydroxy-2, 2, 5, 7, 8-pentamethylchroman-5-methyl-C^{14} (rabbit liver)

Experiment	Ether extract (cpm)	Aqueous (cpm)
Control (no cofactors)	8,696,000	66,960
Control (heat denatured)	8,772,000	181,620
Experimental (with cofactors)	8,264,000	864,000

Table II. Silica gel GF thin-layer chromatography of extracts[a] ($CHCl_3$ developed) (rabbit liver)

Experiment (ether extracts)	Conclusions[b] (products present)
Control (no cofactors)	Chroman, dimer or trimer
Control (heat denatured)	Chroman, dimer or trimer
Experimental (with cofactors)	Chroman, dimer or trimer, 2 spots (0.1, 0.2)

[a] Spots were detected by means of radioautography. [b] Identification was by comparison of TLC behavior with that of authentic samples.

Table III. Incubation of 6-hydroxy-2, 2, 5, 7, 8-pentamethylchroman-5-C^{14}-methyl (rabbit liver)

Experimental	Ether extract (cpm)	Aqueous (cpm)
Control (no cofactors)	2,500,000	40,100
Control (heat denatured)	1,938,000	30,500
Control (no cofactor + H_2O_2)	2,438,000	31,050
Experimental	1,896,000	317,500

Table IV. Incubation of 6-hydroxy-2, 2, 5, 7, 8-pentamethylchroman-5-C^{14}-methyl (rabbit liver)

Experimental	Ether extract (cpm)	Aqueous (cpm)
Control (heat denatured, air)	3,076,900	45,750
Control (heat denatured, N_2)	3,333,350	58,750
Experimental (air)	3,076,900	447,000
Experimental (N_2)	3,636,350	200,000

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[2] C. R. SEWARD and L. M. CORWIN, Arch. Biochem. Biophys. *101*, 71 (1963).
[3] J. TAGG, D. YASUDA, M. TANABE and C. MITOMA, Biochem. Pharmac. *16*, 143 (1967).

Table V. Incubation of 6-hydroxy-2,2,5,7,8-pentamethylchroman-5-C^{14}-methyl (rat liver)

Experiment	Ether extract (cpm)	Aqueous (cpm)
Control (no cofactor)	2,678,600	63,125
Experimental (with cofactor)	1,810,400	755,500

Table VI. Silica gel GF thin-layer chromatography of extracts[a] (rat liver)

Experiment	Conclusions[b] (radioactive products formed)
Control-ether extract ($CHCl_3$ developed)	Origin, tocopherylquinone model, chroman, dimer
Experimental-ether extract ($CHCl_3$ developed)	Origin, tocopherylquinone model, unknown (Rf 0.20), chroman, dimer
Experimental-aqueous layer (BuOH/HOAc/H_2O, 13/3/5)	Unknown (0.34), unknown (0.39), unknown (0.49), unknown (0.55), unknown (0.64), unknown (0.71), unknown (0.79)

[a] Spots detected by means of radioautography. [b] Identification of products was by comparison of TLC behavior with authentic samples.

in splitting of 2 of the polar materials (Rf 0.49 and 0.55) to yield the original chroman and α-tocopherolquinone model which were present as glucuronides. These compounds were identified by rechromatography on TLC with $CHCl_3$ as the developing solvent along with authentic material and by comparison of their IR-spectra with those of authentic samples.

Thus, we conclude that transformation products of vitamin E formed by air oxidation or by liver peroxidases can account for many of the previously identified 'metabolites' reported. In the case of the analog of α-tocopherol used in these experiments, new water-soluble metabolites were produced. It is possible that analogous metabolites would be formed if α-tocopherol were studied in this way and we intend to investigate this possibility.

Résumé. Le métabolisme in vitro du 6-hydroxy-2, 2, 5, 7, 8-pentaméthylechromane-5-méthyle-C^{14} a été étudié sur des soies homogénéifiées de rats et de lapins. Lorsque les composés homogénéifiés n'ont pas été renforcés par un système générateur NADPH ou dénaturés par la chaleur, aucunes quantités significatives de métabolites hydrosolubles ne se sont formées. Par contre, durant les expériences de contrôle, plusieurs produits d'oxydation ont apparu.

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N-Dimethylated Indoleamines in Blood of Acute Schizophrenics

The psychotogenicity of dimethylated indoleamines is well recognized at least for N-dimethyltryptamine (DMT)[1,2]. Though there have been several studies on the urinary excretion of these N-dimethylated indoleamines in schizophrenia[3], there is no report of a similar study on blood samples. This preliminary communication presents our results on blood samples of acute and chronic schizophrenic patients.

Subjects. Five acute unmedicated schizophrenics with disturbed behavior, hallucinations and paranoid delusions, 9 chronic schizophrenics not experiencing behavioral exacerbations and with histories of long hospitalization, 2 normals from the Laboratory staff, and 1 depressive patient were chosen for this study. Blood samples were drawn from these subjects before breakfast, collected in oxalate and studied immediately.

Methods. The free amines were worked up essentially by the same procedure as described by Gross and Franzen[4]. The HCl extracts were pooled and used for spectrofluorometric examination. These extracts were then lyophilized, taken up in 5 ml of borate buffer (pH 10.2), and extracted twice with 10 ml of ethyl acetate, the extract concentrated to a volume of 100 µl and used for thin-layer chromatography (TLC) and gas-liquid chromatography (GLC).

The aqueous layer from the free amine extract was acidified to pH 2 with concentrated HCl and hydrolyzed by heating in a boiling water bath for 10 min. The conjugated amines were then extracted as described earlier.

The acid extracts were treated with 0.3 ml of concentrated HCl and read on an Aminco Bowman spectrofluorometer at activation 295 nm. A maximum at 550 nm in the emission spectrum was considered positive for 5-methoxy-N-dimethyltryptamine and bufotenin.

The final ethyl acetate concentrates were used for two-dimensional thin-layer chromatography on a silica gel G plate (0.25 mm) with chloroform-water-ammonia (12:7:1), isopropanol, ammonia (10%), water (8:1:1) as solvent systems. *p*-Dimethylaminocinnamaldehyde and diazotized *O*-tolidine were used as spray reagents.

The concentrates were also run on a 6 ft 3% SE-30 column at 180 °C isothermally with standards run separately and also mixed with the samples. Retention times and spiking of peaks of samples mixed with standards were used for identification of GLC peaks. In 2 blood samples of acute schizophrenics, we obtained large enough quantities of the free amine fractions to prepare derivatives. One of these amine fractions was used to prepare a trimethylsilyl derivative (TMS) according to the method developed here by Narasimhachari et al.[5] and the other for a heptafluorobutyril (HFB) derivative by the method

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