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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of VESSMAN et al.[6]. The TMS and HFB derivatives were run on 3% SE-30, 6 ft column under temperature programming along with even-numbered hydrocarbon mixture (C_{12}–C_{24}). The methylene unit values were calculated and compared with standard reference compounds.

Results and discussion. The identification of the amines was considered positive only when GLC and at least one of the 2 other methods used showed positive tests. N-dimethyltryptamine and 5-methoxy-N-dimethyltryptamine were identified in all 5 of the acute schizophrenic patients both in the free and conjugated amine fractions; bufotenin was identified in the free amine fraction in only 2 acute patients. In the chronic schizophrenics, the 2 normals and 1 depressive, the N-dimethyltryptamines could not be identified by the above criteria. The TMS and HFB derivatives of the free amine fractions, which were identified by GLC, showed peaks having the same methylene unit (MU) values as standard N-dimethyltryptamine and 5-methoxy-N-dimethyltryptamine and bufotenin. In the course of our investigations on the urinary excretion of dimethylated indoleamines in schizophrenia, we also examined the urine samples of 2 of the 5 acute schizophrenics described in this study. N-dimethyltryptamine, 5-methoxy-N-dimethyltryptamine and bufotenin were identified in the free amine fractions in the urine samples of both patients, by two-dimensional thin-layer chromatography, as well as by gas-liquid chromatography. The latter results will be published in a separate communication.

Resumen. En nuestros estudios en sangre de esquizofrenicos agudos, hemos encontrado, usando cromatografia en capa delgada y cromatografia gaseosa liquida, dimetiltriptamina, y 5-metoxi-N,N-dimetiltriptamina. En solo uno de ellos hallamos bufotenina. Estas substancias no fueron detectadas en nueve esquizofrenicos cronicos, dos normales y un enfermo depresivo.

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LDH Isoenzyme Spectrum in the Myocardium of Rats after Repeated Doses of Isoproterenol

When isoproterenol is administered in large doses it causes cardiac necrosis[1,2]. If, however, it is given in small doses for several days, there is an increase in the resistance of the myocardium to necrogenic doses of this catecholamine[3], and at the same time an increase in the tolerance of the right ventricle to acute anoxia[4].

Isoproterenol cardiopathy is accompanied by a marked shift in the LDH isoenzyme spectrum of the myocardium in favour of an anaerobic type of metabolism[5,6]. We therefore raised the question of whether one of the factors responsible for increased tolerance of the heart to acute anoxia and isoproterenol necrosis in rats adapted to small doses of isoproterenol is a changed capacity for anaerobic glycolysis.

Methods. The experiments were made on Wistar rats, average weight 250 g, fed on a normal laboratory diet with water ad libitum. Isoproterenol (Spofa) in doses of 1 mg/kg body weight (6 × mg/kg) was injected s.c. at 24h intervals for 6 days. 2 days after the last dose of isoproterenol, the rats were decapitated and enzymes determined in the entire myocardium.

Cardiac tissue was homogenized at 0 °C in veronal buffer at pH 8.4 and centrifuged at 15,000 rpm for 30 min, LDH isoenzymes were determined in the supernatant by electrophoresis in agar[7]. Isoenzymatic activity was detected by nitroblue tetrazolium, evaluated on an ERI 10-Zeiss Jena densitometer, and the curves constructed by planimetry. The proportion of the separate isoenzymes was expressed as a percentage of the total area of the curve. The percentage of M-units was calculated on the basis of the representation in the individual isoenzymes[8]. Total lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) activity were measured spectrophotometrically by observing decrease of extinction at 340 nm and the specific activity expressed in μM NADH/h/g wet weight.

Results. The myocardium of rats which had received injections of isoproterenol for 6 days showed a marked shift of the lactate dehydrogenase isoenzyme spectrum towards slower isoenzymes of the M-type (Figure). LDH_1 values were decreased ($P < 0.001$), whereas LDH_4 and LDH_5 were significantly increased. These changes clearly reflect an increased proportion of M-subunits of LDH, which is evidence that there was a decrease in the type of metabolism obtaining energy via oxidative cycles in favour of the type of metabolism with energy formation by anaerobic glycolysis. Total LDH and MDH activity remained unchanged.

Discussion. These results show that in rats adapted to small doses of isoproterenol there is an increase in the ratio of anaerobic to aerobic type of LDH isoenzymes. The increased anaerobiosis is probably one of the factors responsible for the greater resistance of these rats to acute

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