Glucose absorbed by small intestine, glycemia and liver glycogen in control rats, and in rats treated with ethanol (4 g/kg/die per os, 40% solution) for 2, 7 and 20 days

	No. of rats	Glucose absorbed by small intestine (mg/100 cm/h)	Glycemia (g of glucose/ 1000 ml of plasma)	Liver glycogen (g of glucose/ 100 g of wet liver)
Control rats	14	357 ± 17	6.70 ± 0.32	0.41 ± 0.08
Rats treated with ethanol for 2 days	14	294 ± 10^{b}	8.14 ± 0.20^{b}	0.67 ± 0.23
Rats treated with ethanol for 7 days	14	294 ± 60^{a}	8.87 ± 0.49^{b}	0.66 ± 0.26
Rats treated with ethanol for 20 days	14	349 ± 29	7.84 ± 0.39^{a}	0.59 ± 0.21

1 g of glucose was administered in the small intestine of all the rats. Mean values $\pm$ S.E. ^{a} $0.05 \geqslant P \geqslant 0.02$. ^{b} $0.01 \geqslant P \geqslant 0.001$.

of the small intestine. The whole mass was brought to 100 ml volume. The solution was then diluted 50 times and centrifuged. Glucose was determined in the supernatant by the HUGGET and NIXON[10] enzymatic method.

Liver glycogen expressed as glucose. The 500 mg of liver tissue taken from the live animals were added to 3 ml of 30% KOH and kept in a water-bath at 100 °C for 30 min. Glycogen was precipitated by adding 95% ethanol. It was washed with 95% ethanol and then hydrolyzed to glucose with 10 ml of $1N$ H_2SO_4 in a water-bath at 100 °C for 4 h. The mixture was neutralized with $1N$ NaOH and brought to 25 ml volume. Glucose was determined in this solution by the HUGGET and NIXON[10] enzymatic method.

Results. As shown in the Table, intestinal absorption of glucose decreases by 18% in rats treated with ethanol for 2 and 7 days, whereas it reaches almost similar values to those of the control rats in animals treated with ethanol for 20 days. As compared with the controls, glycemia increases by 21%, 32% and 17% in rats treated with ethanol for 2, 7 and 20 days respectively. Liver glycogen does not undergo statistically significant variations. Its values are very low and irregular, probably as a result of the 5 h fasting undergone by the rats before the experiment.

Discussion. The glycemia increase in rats poisoned with ethanol, in comparison with the control animals, could be caused by competitive inhibition of glucose utilization by ethanol and FFA[11]. In effect, it is known that there is an increase in plasmatic FFA caused by alcohol intoxication[2-4,12]. Intestinal absorption of glucose occurs in 2 stages: the first by means of active transport by an enzymatic mechanism, and the second by simple diffusion[13]. The enzymes which regulate the first stage of glucose absorption could either be directly or indirectly inhibited by ethanol. On the other hand, the highest glycemia levels observed in the animals treated with ethanol can slow down the diffusing process of glucose. both assumptions can explain the decrease in intestinal absorption of glucose which was observed in the rats poisoned with ethanol. Changes in glycemia and glucose intestinal absorption reached a maximum after 2 and 7 days of ethanol treatment and were less marked or even absent after 20 days of alcoholic intoxication.

The results show that the rat possesses a mechanism of ethanol inurement which is induced with time. This assumption of ours is supported by the findings of ALBERTINI et al.[14]. These authors observed that the liver alcoholdehydrogenase activity of the rat increases considerably due to both acute and chronic ethanol poisoning. Our data allow us to draw the conclusion that the changes observed both in glycemia and in intestinal absorption of glucose can be ascribed to an acute or subacute rather than to a chronic effect of alcoholic intoxication.

Riassunto. Gli Autori hanno indagato se l'intossicazione subacuta da etanolo provoca nel ratto alterazioni dell'assorbimento intestinale del glucosio. L'assorbimento intestinale del glucosio è diminuito in maniera statisticamente significativa nei ratti trattati per 2 e 7 giorni ed è risultato quasi normale in quelli trattati per 20 giorni con etanolo.

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[11] C. N. HALES and P. J. RANDLE, Lancet *1*, 790 (1963).
[12] R. H. SHAPIRO, R. L. SCHEIG, G. D. DRUMMEY, J. H. MENDELSON and K. J. ISSELBACHER, New Engl. J. Med. *272*, 610 (1965).
[13] H. W. DAVENPORT, *Physiology of the Digestive Tract* (Year Book Med. Publ. Inc., Chicago 1966), p. 189.
[14] A. ALBERTINI, E. BONERA and E. RADAELI, Lancet *1*, 1061 (1967).
[15] Istituto Simes di Cardiologia Sperimentale, Milano (Italy).

Native Fluorescence and Hallucinogenic Potency of Some Amphetamines

The native fluorescence of a molecule is a complex function of its quantum chemical properties that may include the energy of its π electrons. SNYDER and MERRILL[1], and KANG and GREEN[2] have reported that the HMO energy of methoxylated amphetamines correlates positively with their hallucinogenic potency. We have, therefore, measured the native fluorescence, at maximum activation and emission frequencies, of a number of amphetamines to see if there was any correlation with their hallucinogenic potency (as measured by SHULGIN et al.[3]). This work was carried out independently in Iowa and Edinburgh.

Measurements were taken with spectrophotofluorometer using amounts of the compound (as hydrochloride) isomolar to 10 µg of amphetamine HCl in 2 ml water volume. The degree of fluorescence is expressed in the direct readings from the Farrand (Edinburgh) and Aminco – Bowman (Iowa) and the biological activity is expressed in mescaline units, i.e. how many times the compound is as potent as mescaline as measured by SHULGIN et al.[3].

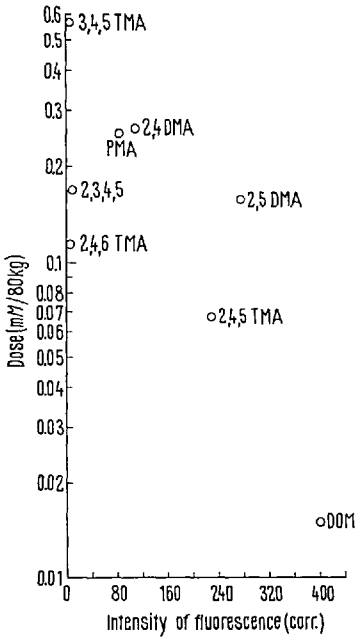
The relative intensity of emission was calibrated in the results from the 2 centres by multiplying each of the

[1] S. H. SNYDER and C. R. MERRILL, Proc. natn. Acad. Sci. *54*, 258 (1965).
[2] S. KANG and J. P. GREEN, Nature, Lond. *226*, 645 (1970).
[3] A. T. SHULGIN, T. SARGENT and C. NARANJO, Nature, Lond. *221*, 537 (1969).

Table I

Methoxy group position	Fluorescence Wave length	Degree	Hallucinogenic activity[a]
0	335/380:350/400	68:68	0
2	275/325	1012	?
3	275/325	1012	?
4	275/325	882	6
2,3	275/335	90	0
2,4	280/335	1000	5
2,5	295/340	2750	8
2,6	275/330	520	?
3,5	275/330	330	?
3,4	280/335	3080	<1
2,3,4	275/370	25	0
2,4,5	290/345	2251	17
2,4,6	275/350	41	10
2,4,5	280/360	71	2
2,3,4,5	360/440 and 295/350	48	6
2,3,4,6	330/380	20	?
DOM (2,5-dimethoxy-4-methyl)	295/340	4000	100

We also tested the following ring methylated compounds: 3; 4; 3,4; 3,5; 3,4,5; 2,3,4,5; 2,3,4,6; 2,3,5,6; 2,3,4,5,6. These all showed low fluorescence (in the range 25–85) except for 3,4 (231); 2,3,5,6 (170) and 2,3,4,6 (181). [a] Data from SHULGIN et al.[3].



Log dose (mmoles) in humans vs fluorescence; DOM, 2,5-dimethoxy-4-methyl amphetamine; TMA, trimethoxy amphetamine; DMA, dimethoxy amphetamine; PMA, paramethoxy amphetamine.

Table II. Results for bromo methoxy amphetamines

	Wave length	Degree of fluorescence
3-Bromo-4-methoxy	285/345	42
4-Bromo-3-methoxy	285/340	540
2-Bromo-5-methoxy	300/335	13.7
2-Bromo-4,5-dimethoxy	285/340	81
4-Bromo-3,5-dimethoxy	310/395	89.5
4-Bromo-2,5-dimethoxy	285/345	34

Iowa results by the ratio of values for DOM for the 2 groups. (The results are expressed in arbitrary units.) The average of the 2 readings were then taken. The results are presented in Table I.

In some cases, and in particular when scanning the bromo substituted compounds, difficulty was encountered due to intense Raman scattering peaks. 2,3,4,5-tetramethoxy and 3,4,5-trimethoxy amphetamine also showed this scattering effect. Thus, the excitation maxima is taken as the maximum absorption excepting the Raman peaks, and it is possible that an actual maxima may lie underneath but was not observable in our experimental arrangement. Water is one of the best solvents for observing Raman scattering and the use of some other solvent might eliminate this problem.

In the series of bromo methoxy substituted compounds the 3-bromo-4-methoxy isomer has been found inactive in rats, whereas the 4-bromo-2,5-dimethoxy isomer is believed to be active from results of preliminary screen in mice, but it has not been fully tested as yet.

The plot of log dose vs fluorescence (Figure) has been done using values for dose in millimoles. This gives a more accurate representation of relative potencies. These findings invite the following comments: The native fluorescence of amphetamine is much increased by adding 1 methoxy group. 2 methoxy groups had less effect than 1 (particularly the 2,3 compound) except for the 3,4 and 2,5 substitutions. 3 and 4 methoxy groups led to a progressive decrease in fluorescence except for the 2,4,5 compound where there was less reduction. Thus, there was good correlation between the degree of native fluorescence and hallucinogenic potency except for the 3,4; 2,4,6 and 2,3,4,5 compounds. The former is a feeble hallucinogen and fluoresces strongly, and the latter two are both active hallucinogens and yet fluoresce only feebly. The hallucinogenic tryptamines, as well as LSD, are in general much more potent hallucinogens than these methoxylated amphetamines, and have a notably stronger native fluorescence. The 2,4,6 and 2,3,4,5 compounds may owe their activity, in spite of their low highest occupied molecular orbital (HMO) energy, to the fact that the 2,6 (and tetra) substitutions offer maximum protection against metabolic breakdown by amine oxidase, and the 4 substitution protects against 4-hydroxylation[4].

The behavioural effects of the bromomethoxy compounds require further elucidation.

Zusammenfassung. Die native Fluoreszenz einiger Methoxy- und Bromomethoxyamphetamine wurde bestimmt. Da die Fluoreszenz eines Moleküls zum Teil von der Energie seiner π-Elektronen abhängig ist und die Stärke der halluzinogenen Aktivität dieser Verbindungen in einer positiven Beziehung zu ihrer HMP-Energie steht, wurde die Intensität der Fluoreszenz mit der Stärke der halluzinogenen Aktivität in Beziehung gebracht.

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[4] Acknowledgement. 5 of the methoxylated amphetamines tested in Iowa were the kind gift of Dr. A. T. SHULGIN.