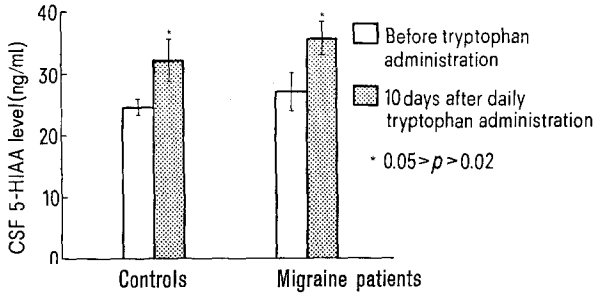
et al.[4], both spontaneous and reserpine-induced migraine attacks are relieved by the i.v. injection of 5-HT or 5-hydroxytryptophan; moreover the monoamino-oxidase inhibitors are known to alleviate headache. These effects, in our opinion, could not be merely explained on the basis of tryptophan ability to increase the synthesis of brain 5-HT.




Cerebrospinal fluid levels of 5-hydroxyindoleacetic acid (5-HIAA) immediately before and following 10 days of L-tryptophan administration (see text) in 5 migraine patients and in 2 neurological patients, taken as controls.

The possibility remains that 5-HT involvement migh occur only in some restricted areas of the brain; in that case fine CSF biochemical changes would be too difficult to evaluate.

*Riassunto.* La concentrazione di acido 5-idrossindolacetico nel liquor lombare di pazienti emicranici diminuisce lievemente e non significativamente durante l'attacco, mentre, in periodo intercritico, non è diversa da quella osservata nei soggetti normali. La somministrazione di L-triptofano porta costantemente ad un incremento dei livelli di acido 5-idrossindolacetico nel liquor lombare di soggetti emicranici, oltre ad esercitare un'azione preventiva degli accessi di cefalea.

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[15] R. W. Kimball, A. P. Friedman and E. Vallejo, Neurology *10*, 107 (1970).

## Urinary Excretion of Methylated Purines Following Inhalation of Dimethyl Sulphate

Dimethyl sulphate (DMS) is a widely used industrial and laboratory methylating agent. It has been shown to be carcinogenic in laboratory animals[1-3]. Methylation of nucleic acids of several organs has been detected in rats given i.v. injection of radioactively labelled DMS[4]. The principal site of methylation in nucleic acids by methylating agents is *N*-7 of guanine, but a number of sites, including the phosphodiester group, with less reactivity have been detected (cf. refs. [5,6]). The alkylations of nucleic acids which result in hereditary changes are not fully known, but methylation at $O^6$ in guanine seems to affect the coding property[7], whereas methylation at *N*-7 in guanine does not[8].

7-Methylguanine (7/MeGua) formed by methylation in DNA is liberated by hydrolysis[9,10] and in RNA by metabolic turnover[11]. An exogenous dose of 7MeGua is mainly excreted unchanged in the urine within a short time[12]. Urinary excretion of labelled 7MeGua has been used as an indication of methylation of nucleic acids in mammals exposed to labelled methylating agents[13-16].

Quantitative determinations of the urinary 7MeGua in relation to the administered dose of labelled methylating agents may thus give information about the overall extent of alkylation of nucleic acid constituents in the whole body. Parallel to such a study with the insecticide dimethyl dichlorovinyl phosphate (dichlorvos)[16], we have performed inhalation tests with DMS in male NMRI mice.

DMS labelled with $^3H$ with a specific activity of 150 mCi/mmole (Radiochemical Centre, Amersham) was used. 4 animals were exposed in each of the 2 tests (cf. Table) in a 6 l glass flask. In experiment 2 the labelled DMS was applied to a wad of glass fibres hanging in the flask, but for the high dose exposure (experiment 1) the DMS was applied to the wad in a glass tubing, connected with the flask, through which a slow stream of air passed; the latter arrangement made it possible to keep the animals under exposure for a longer time. The air concentration of DMS was monitored during the exposures by counting the radioactivity of air samples drawn from the flask. In experiment 1 the air concentration was, in addition, determined by reacting air samples with 4-(p-nitrobenzyl)-pyridine[17]; both types of analysis gave the same result. The maximum DMS concentrations in both experiments were about 4 times the average concentration which is given in the Table. The total exposure given in the Table have been calculated by assuming that the mice had a minute volume of 24 ml; this is obviously an underestimate in experiment 2 as the recovered urinary excretion is slightly larger.

After exposure the animals were placed in a metabolic cage with access to water and the urine was collected

[1] H. Druckrey, R. Preussmann, N. Nashed and S. Ivankovic, Z. Krebsforsch. *68*, 103 (1966).
[2] F. A. Schlögel and P. Bannasch, Naunyn-Schmiedebergs Arch. Pharmak. *266*, 441 (1970).
[3] H. Druckrey, H. Kruse, R. Preussmann, S. Ivankovic and Ch. Landschütz, Z. Krebsforsch. *74*, 241 (1970).
[4] P. F. Swann and P. N. Magee, Biochem. J. *110*, 39 (1968).
[5] P. D. Lawley and S. A. Shah, Chem. biol. Interact. *7*, 115 (1973).
[6] P. D. Lawley, Chem. biol. Interact. *7*, 127 (1973).
[7] D. B. Ludlum, J. biol. Chem. *245*, 477 (1970).
[8] L. L. Gerchman and D. B. Ludlum, Biochim. biophys. Acta *308*, 310 (1973).
[9] P. D. Lawley and P. Brookes, Biochem. J. *89*, 127 (1963).
[10] G. P. Margison, M. J. Capps, P. J. O'Connor and A. W. Craig, Chem. biol. Interact. *6*, 119 (1973).
[11] M. J. McElhone, P. J. O'Connor and A. W. Craig, Biochem. J. *125*, 821 (1971).
[12] V. M. Craddock, A. R. Mattocks and P. N. Magee, Biochem. J. *109*, 75 (1968).
[13] V. M. Craddock and P. N. Magee, Biochem. J. *104*, 435 (1967).
[14] J. L. Skibba and G. T. Bryan, Toxic. appl. Pharmac. *18*, 707 (1971).
[15] B. C. F. Chu and P. D. Lawley, Chem. biol. Interact. *8*, 65 (1974).
[16] R. Wennerberg and G. Löfroth, Chem. biol. Interact. *8*, 339 (1974).
[17] J. Epstein, R. W. Rosenthal and R. J. Ess, Analyt. Chem. *27*, 1435 (1965).

Summary of experimental data and results of urinary excretion of labelled methylated purines from mice exposed to $^3$H-labelled dimethy sulphate by inhalation

| Experiment No. | 1 | | 2 | | |
|---|---|---|---|---|---|
| Total weight of 4 exposed animals (g) | 148 | | 164 | | |
| Exposure time (min) | 135 | | 60 | | |
| Average concentration of DMS, μg/l (μCi/l) | 16.3 (19.5) | | 0.32 (0.38) | | |
| Estimated dose (μCi) | 250 | | 2.2 | | |
| Postexposure excretion period (h) | 0–24 | 24–48 | 0–24 | 24–48 | 48–72 [a] |
| Total activity in urine (μCi) | 170 | 36 | 1.76 | 0.59 | 0.10 |
| 7-Methylguanine (nCi) | 187 [b] | 98 [b] | 1.1 | 0.6 | — [c] |
| 3-Methyladenine (nCi) | 15 [b] | 6 [b] | | | — [c] |
| 1-Methyladenine (nCi) | 11 [d] | 3 | 0.1 | — [c] | — [c] |
| Methylated purines in 0–48 h urine as fraction of total urinary activity [e] | $31 \times 10^{-4}$ | | $15 \times 10^{-4}$ | | |

[a] Urine collected for an additional 24 h period; [b] relative amounts determined and identities confirmed by paper chromatography; [c] not determined; [d] identity confirmed by paper chromatography; [e] corrected for isotope dilution assuming that both methyl groups in DMS have contributed to the same extent.

much faster than the rate of loss of 7MeGua from methyl- during 2 consecutive 24 h periods. Collection of urine, isolation of urinary purines by precipitation with silver nitrate, ion exchange chromatography on Dowex-50W-X12 of the purines and determination of radioactivity were performed as described by WENNERBERG and LÖFROTH[16]. In addition, some ion exchange fractions were investigated by paper chromatography with methanol-hydrochloric acid-water (7:2:1) and *n*-butanol-ammonia-water (85:2:12) as solvents[9,18]. Fractions containing 7MeGua also showed the presence of a radioactive spot coinciding with authentic 3-methyladenine (3MeAde) (a gift from Dr. LAWLEY) in both solvents. The relative amount of 3MeAde to 7MeGua was determined by counting appropriate pieces of the chromatograms in a toluene scintillation solution. A radioactive peak appeared in the ion exchange fractions where 1-methyladenine (1MeAde) would elute; paper chromatography of these fractions showed that the radioactivity coincided with authentic 1MeAde (Sigma Chemical Co.) in both solvents. In the ion exchange fractions from experiment 1, there was also a small radioactive peak where 7-methyladenine would elute, but these fractions were not studied further; the amounts were about one-third of the 1MeAde activities. A summary of the results is given in the Table.

Although the estimation of the total dose, assuming a standard breathing, may involve a considerable error, the data indicate that the major part of the $^3$H-activity of DMS is excreted in the urine within the first 48 h after exposure; a further check on this is the low urinary excretion of radioactivity in experiment 2 during the 3rd day after exposure.

In addition to 7MeGua, the presence of 2 minor products, 1MeAde and 3MeAde, was also detected in the urine. Whereas it is known that 7MeGua is mainly excreted unchanged[12], it is not known if 1MeAde and 3MeAde are metabolized. As the amounts of the methylated purines relative to 7MeGua are of the order expected from studies on other nucleic acid systems[19,20], it seems possible to suggest that the liberated 1MeAde and 3MeAde are not metabolized extensively. 3MeAde is not a known normal constituent of nucleic acids and is not known to be present in urine; its presence in urine may thus establish unequivocally that methylation of nucleic acid constituents has occurred.

The excretion rate of 7MeGua in the present study is rapid, having an apparent $t_1/_2$ of about 1 day; this is ated rat liver DNA[10] and RNA[11] in vivo. The results indicate that 1MeAde and 3MeAde are excreted faster than 7MeGua, and this is partly in accordance with data showing that 3MeAde is lost faster than 7MeGua from methylated DNA in cell culture[21] and in rat liver in vivo[22]. However, species differences may exist and the excretion kinetics in the present study may also have been influenced by the starvation.

The total amount of urinary methylated purines originating from the methylation of nucleic acid constituents by DMS is of the order of 0.15–0.3% of the dose. There is only a small dose dependence, since a decrease of the dose by a factor of about 100 only decreases the relative amount of methylated purines by a factor of two. It is conceivable that almost any dose of DMS would methylate nucleic acid constituents in this mammalian test system. The air concentration of DMS in experiment 2 in the present study was lower than the maximum permissible concentration (MAC) for man.

*Zusammenfassung.* Nach Inhalation des alkylierenden Agens Dimethylsulfat wurden im Urin von Mäusen methylierte Purine in Mengen von etwa 0,3% der Dimethylsulfatdosis ausgeschieden. Auch bei einer 100-fachen Expositionsverminderung mit radioaktivem Dimethalsulfat wird ungefähr der gleiche relative Anteil an markierten Purinen eliminiert.

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[18] B. SINGER and H. FRAENKEL-CONRAT, Biochemistry *8*, 3260 (1969).
[19] P. D. LAWLEY and C. J. THATCHER, Biochem. J. *116*, 693 (1970).
[20] P. D. LAWLEY and S. A. SHAH, Biochem. J. *128*, 117 (1972).
[21] I. G. WALKER and D. F. EWART, Mutation Res. *19*, 331 (1973).
[22] P. J. O'CONNOR, M. J. CAPPS and A. W. CRAIG, Br. J. Cancer *27*, 153 (1973).
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