with 0·088 mg folic acid in 0·5 ml. water 1 h before injection of 46 mg urethane in 0·5 ml. water per animal; Group III, animals pretreated twice, each time with 1 mg folic acid in 0·5 ml. water at 5 h and at 1 h before injection of 46 mg urethane in 1 ml. water per animal; Group IV, animals pretreated with 0·1 mg folic acid in 0·5 ml. water 15 min before injection of 46 mg urethane in 1 ml. water per animal; Group V, animals pretreated with 0·0032 mg/ml. folic acid in drinking water for 3 days (estimated dose 0·048 mg), then 46 mg urethane in 1 ml. water per animal; Group VI, animals pretreated with 0·0032 mg/ml. folic acid in drinking water for 4 days (estimated dose 0·064 mg), then 46 mg urethane in 1 ml. water per animal.

The dose of urethane given was calculated to be the LD_{50} dose[6] for this species, weight and sex of animal.

The results of the experiment are presented in Table 1. They merely record the number of deaths observed. These all occurred within 3–12 h following injection. In all these animals narcosis proceeded in approximately the same manner. The animals became markedly ataxic within a matter of 45 sec and were completely anaesthetized within approximately 2 min. With the large dose used no differences were noted in the initial reactions of the animals; however, as can be observed, many animals in the control urethane group died whereas in certain of the folic acid treated groups full protection was afforded. Surviving animals were followed with daily blood counts. As has previously been reported, a drop in leucocyte-level occurred after 3 days followed by subsequent recovery. Administration of folic acid in no way altered this effect. Autopsy of animals that died revealed nothing of significance.

In order to examine the effect of folic acid further in relationship to the narcotic effects of urethane, an additional investigation was performed with a lower dosage of the drug. Fourteen male Swiss mice weighing 21–22 g were divided into two equal groups: one group as control and the other group pretreated with 50 mg folic acid in 1,000 ml. drinking water for 3 days (estimated dose based on the water consumption was 0·75–1 mg per animal for the 3-day period). All animals received 30 mg urethane in 0·6 ml. physiological saline intraperitoneally. It was noted that the narcotic dose for the males was slightly higher than for the females. The mice receiving the 30 mg of urethane only became markedly ataxic within 45 sec and had reached the second stage of anaesthesia within 2 min. They persisted at this level for a considerable time, the depth of anaesthesia becoming gradually deeper over a period of hours. They recovered completely within 12 h. The mice pretreated with the folic acid also became ataxic in less than 1 min. However, this ataxia was not as marked as in the urethane-treated controls. The folic acid-treated mice then proceeded gradually to recover from this mild effect and within 2 h almost no residual effects were observed. They did not reach any further stage of anaesthesia.

It is apparent from this investigation that folic acid exerts an inhibitory effect on the narcotic effect of urethane. No effect was observed on the cytotoxic action of the compound, which is somewhat surprising in view of the effects of known anti-folic acid compounds in this regard. It is possible that other experiments with different dosage relationships may reveal different findings.

Table 1. Effect of Pretreatment of Folic Acid on Toxicity in Mice treated with LD_{50} Dose of Urethane

	Treatment	No. died/ No. treated	Mortality (%)
Group I	46 mg urethane (control)	13/23	56·5
Group II	0·088 mg folic acid + 1 h, then 46 mg urethane	3/10	33
Group III	1 mg folic acid + 5 h, 1 mg folic acid + 1 h, then 46 mg urethane	3/20	15
Group IV	0·1 mg folic acid + 15 min, then 46 mg urethane	5/10	50
Group V	0·048 mg folic acid in drinking water for 3 days, then 46 mg urethane	1/13	0·8
Group VI	0·064 mg folic acid in drinking water for 4 days, then 46 mg urethane	0/3	0

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K. Y. Lee
Philippe Shubik

Division of Oncology,
Chicago Medical School, Chicago 12.

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Tryptamine, *N*,*N*-Dimethyltryptamine, *N*,*N*-Dimethyl-5-hydroxytryptamine and 5-Methoxytryptamine in Human Blood and Urine

Tryptamine is a normal constituent of human urine[1–5]; about 30–120 μg of the amine are excreted per 24 h. In blood, tryptamine has hitherto been demonstrated only qualitatively and under pathological conditions in a carcinoid patient[6]. There is no information about an occurrence of *N*,*N*-dimethyltryptamine in human beings. *N*,*N*-dimethyl-5-hydroxytryptamine (= Bufotenin) was demonstrated qualitatively as a constituent of normal human urine[4,7]; in children an excretion of 0–0·03 μg amine/100 mg creatinine has been found with, at most, semi-quantitative methods[4]. Apparently, *N*,*N*-dimethyl-5-hydroxytryptamine was still not demonstrated in blood. 5-Methoxytryptamine has been found in the urine of patients with rheumatic fever[8], and that in an order of magnitude of 30–210 μg/24 h.

After the elaboration of sufficiently selective and quantitative procedures, which are discussed elsewhere, we were able to study the occurrence of tryptamine, *N*,*N*-dimethyltryptamine, *N*,*N*-dimethyl-5-hydroxytryptamine and 5-hydroxytryptamine in normal human blood and urine. The results refer in each case to about 40–50 healthy persons or to patients without disturbances of amine metabolism as far as could be determined. For tryptamine a blood concentration of 0·005–0·02 μg/ml. has been found. In 11 of 37 probands *N*,*N*-dimethyltryptamine was demonstrated in blood, and that in a concentration of 0·008–0·055 μg/ml. In the urine 42·98 ± 8·6 μg of dimethyltryptamine/24 h were excreted. In 12 of 46 persons, *N*,*N*-dimethyl-5-hydroxytryptamine has been found in blood, in an order of magnitude of 0·001–0·04 μg/ml.; 62·8 ± 7·2 μg *N*,*N*-dimethyl-5-hydroxy tryptamine were excreted in the urine per 24 h. 5-Methoxytryptamine could be demonstrated in blood only in 8 of 57 probands, at a concentration of 0·02–0·08 μg/ml. The urine of 24 h contained 36·55 ± 5·23 μg of 5-methoxytryptamine.

Fr. Franzen
H. Gross

5 Köln-Marienburg, Ulmenallee 3.

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3′,5′-Ditritiomethotrexate as a Granulocyte Label

The folic acid antagonist methotrexate (amethopterin; 4-amino-4-deoxy-10-methylpteroylglutamate), when administered to human subjects, binds tightly to the enzyme dihydrofolate reductase, present in immature leucocytes and in developing erythrocyte forms[1–3]. Bioassay of