The Effect of 1, 3, 7-Trimethyl and 1, 3, 7, 9-Tetramethyl Uric Acids on Uric Acid Excretion in the White Rat

HAROLD J. BLUMENTHAL and RALPH C. CORLEY, Purdue University

The main end product of purine catabolism in the rat is allantoin, formed by the action of the enzyme uricase, an aerobic dehydrogenase, specific for uric acid (7). A small amount of uric acid is regularly excreted. The effect of many substances in changing the rate of excretion of uric acid and allantoin has been reviewed by Martin (8).

In their studies on the properties of uricase *in vitro*, Keilin and Hartree (7) reported that of the 16 mono-, di-, or tri-methylated or ethylated derivatives of uric acid tested, none were oxidized by the enzyme, although these compounds did inhibit the initial rate of oxidation of uric acid from 16 to 68 per cent. It has seemed of interest to determine whether the great inhibitory effect of 1, 3, 7-trimethyl uric acid *in vitro* might be demonstrable *in vivo* in the white rat, as evidenced by a change in the amount of uric acid excreted. The relatively great solubility and solubilizing effect of 1, 3, 7, 9-tetramethyl uric acid (10) suggested its inclusion in this study.

Experimental

The trimethyl uric acid (TRMUA) and tetramethyl uric acid (TEMUA) were synthesized from caffeine. The 8-chlorocaffeine was prepared according to the method of Fischer and Reese (3) and converted to 8-methoxycaffeine by a slight modification of the procedure of Huston and Allen (5). The TRMUA obtained by splitting the methoxy group with 50 per cent hydrochloric acid at 55-70°C. for 30 minutes melted sharply at 345°C., in agreement with the value in the literature (1). TEMUA was obtained by rearrangement of 8-methoxycaffeine in an open tube, similar to the procedure of Huston and Allen (6). The TEMUA thus obtained melted at 227-229°C. which agrees with the value 228°C. reported in the literature (1). The sodium salicylate, an Eastman product, was used without further purification.

The experimental animals were adult male albino rats maintained *ad libitum* on a Purina Chow diet. The animals were housed in separate metabolism cages and the 24 hour urine samples were collected under toluene. The urine and funnel washings were refrigerated until the time of analysis. Analyses were generally started within 24 hours after collection.

Compounds administered orally were mixed with the Purina Chow. Materials administered intraperitoneally were dissolved in double distilled water. If more than 10 ml. had to be injected in one day, divided doses several hours apart were used.

The uricase method (2) was used to estimate true uric acid. Transmittancy was measured with a Cenco-Sheard-Sanford filter photo-

meter using a red filter. The dilutions used during the analyses of experimental urines were kept the same as those used during the control analyses. Each animal served as its own control. The 24 hour uric acid values for three or four days preceding the administration of the experimental compound were averaged to obtain the control value for uric acid for each animal.

Results and Discussion

The average amount of uric acid excreted daily was usually reasonably constant in the successive experiments with each rat, although the excretion from day to day at times showed wide fluctuations, probably attributable in some instances to incomplete voiding of the urine on some days to which samples were referred, and to extra elimination on the days following.

Table I presents a summary of the results. In the concentrations used, TRMUA administered, orally or intraperitoneally, had no consistent effect on the excretion of uric acid as evidenced by the average values obtained, (Series 1 and 2). The intraperitoneal injection of

TABLE I. The effect of two methylated uric acids, with or without salicylate, on the urinary excretion of uric acid.

Series	Number of rats	Average control uric acid	Exptl. days	Average exptl. uric acid	Per cent change	Compound Administered
		mgm.		mgm.		mgm.
1	11	2.72	4	2.80	+ 2.9	50 to 500 TRMUA; oral on days 1 or 1 and 2
2	12	2.80	4	2.78	— 0.7	50 to 150 TRMUA; i.p. at various times
3	13	3.63	4	3.00	— 17.4	50 TEMUA; i.p. on days 1 and 2
4a	2	3.66	2	7.79	+112.8	70 SS; i.p. each day
4b	3	3.78	2	6.00	+ 58.8	70 SS and 60 TEMUA; i.p. each day
5a	2	3.97	1	6.70	+ 68.8	78 SS (av.); i.p.
5b	3	4.18	1	6.27	+ 50.0	78 SS (av.) and 56 TEMUA; i.p.
6	5	4.32	4	3.26	— 24.5	60 TEMUA; i.p. each day
6a	2	4.63	3	5.90	+ 27.6	70 SS; i.p. each day for next 3 days
6b	3	4.01	3	3.79	— 5.5	70 SS and 60 TEMUA; i.p. each day for next 3 days

TEMUA=1,3,7,9-tetramethyl uric acid
TRMUA=1,3,7-trimethyl uric acid
SS=sodium salicylate
i.p.=intraperitoneal

TEMUA was followed by a decreased excretion of uric acid in sixteen of the eighteen individual experiments (Series 3 and 6).

As the observations with TEMUA can not be related in an obvious way to the action of uricase, a relation with excretion is suggested. Sodium salicylate is known to increase the excretion of the uric acid (cf. Friedman, 4). TEMUA was found to decrease this effect of salicylate (Series 4, 5, and 6). These results are in accord with those of Quick (9) who found that "the stimulating action of salicylate and other substances could be inhibited by ingestion of 'depressing substances'". This same effect was shown in Series 6a even after an initial decrease in urate excretion following administration of TEMUA for four days.

No clear explanation appears for the effect of TEMUA in decreasing the excretion of uric acid. Inhibition of the action of uricase observed *in vitro*, or inhibition of some specific mechanism for the reabsorption of uric acid from the glomerular filtrate presumably would have led to an increase in the elimination of uric acid. However it is conceivable that TEMUA may exert an inhibition of the mechanism for active tubular excretion of uric acid if such exists in the white rat. Further work may provide evidence in this regard. Wolfson, Cohn and Shore (11) have shown the importance of tubular excretion of uric acid in the Dalmatian dog.

Conclusions

Administration of 1, 3, 7-trimethyl uric acid, either orally or intraperitoneally, had no consistent effect on the uric acid excretion of the white rat. 1, 3, 7, 9-Tetramethyl uric acid, however, when administered intraperitoneally caused a significant decrease in urate excretion. Concomitant administration of tetramethyl uric acid and sodium salicylate caused the level of urate excretion to remain below that of rats receiving salicylate alone. It is conceivable that the effect of tetramethyl uric acid in decreasing the excretion of uric acid is associated with an inhibition for active tubular excretion of uric acid.

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