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Urinary Excretion of some Purine Bases in Normal and Schizophrenic Subjects

By D. A. BOOTH and E. B. O. SMITH

Kishimoto (1958) reported quantitative and qualitative peculiarities in the absorption spectra of body fluids in schizophrenia, and considered that there were abnormalities in the oxidation of adenine through hypoxanthine and xanthine to uric acid.

The recent carefully controlled study by Bollard, Culpan, Marks, McIlwain and Shepherd (1960) failed to confirm these findings. The urinary excretion of the non-dietary purines (Weissman and Gutman, 1957), adenine, guanine, 1- and 7-methylguanines, 8-hydroxy-7-methylguanine, hypoxanthine and xanthine was measured (Weissman, Bromberg and Gutman, 1957) in pairs of schizophrenic and normal subjects. Each of a pair was similar in sex, age and weight. No correlation was found between excretion of any compound and mental status. The patients then examined, however, had all received treatment with phenothiazine derivatives, and the present work was undertaken to determine whether chlorpromazine influences the excretion pattern of these purines and could therefore account for the negative findings of Bollard *et al.* (1960). A closer study of the occurrence of 3-methylxanthine in urine was also carried out, provoked by the observation that samples from patients occasionally contained detectable amounts.

Methods

As in the previous study (Bollard *et al.*, 1960), considerable care was taken in the selection of patients. All were in-patients of the Maudsley Hospital who had been declared by the responsible physician to be suffering unequivocally from schizophrenia. Each was then assessed independently to ensure that the classical criteria laid down by Bleuler were met. Considerable difficulty was encountered in finding patients who had received no previous treatment and who had definitely not received phenothiazine derivatives in the preceding 12 months. Of the 7 new cases here examined, 4 were suffering from paranoid schizophrenia.

Each patient was paired with a normal control subject of the same sex, and similar age and weight (Table I). Medical and nursing staff of the hospital acted as control subjects and their diets approximated to those of the patients. The patients' diets were closely observed by the ward nursing staff, special attention being paid to caffeine-containing foodstuffs, abnormalities in this and the patients' general behaviour being noted. All patients and controls were fully ambulant during the periods of study.

The urine specimens were accurately collected over 24-hour periods and kept in large polythene bottles containing 10 ml. chloroform as preservative. Samples were timed so that specimens from paired subjects could enter the first stage of the assay procedure together within 6 hours of collection.

The routine assay procedure for purines and for creatinine was exactly as reported by Bollard *et al.* (1960), and their studies of recovery from and variability in the assay may be taken to apply to the present results.

In some cases, after routine spectrophotometry, xanthine and hypoxanthine solutions were estimated according to the method of Kalckar (1947) by the increase of uric acid absorption (290 mμ) with xanthine oxidase (supplied by Worthington Biochemical Corporation, New Jersey; 0·2 μmoles of uric acid/ml./hour from hypoxanthine at pH 7·5, 25°). The results of the two assay methods showed good agreement for hypoxanthine, the enzyme method averaging 3 per cent. higher than the routine absorptiometric method. For xanthine, however, enzyme assay gave values 64 per cent. of the absorptiometric method (mean of 5 different samples, standard deviation 14 per cent.). Thus some of the routinely measured "xanthine" absorption probably arose from interfering compounds, and such values were only an approximate reflection of variations in true xanthine excretion.

With most of the samples recorded in this paper, the first supernatant from centrifugation of the silver-purine precipitate was taken to pH 5–6 with sodium hydroxide and left at 0° for 6 days. Additional precipitate was formed and then was treated, as was that which formed at pH 2. A more complete recovery of 3-methylxanthine was thus attained (Cornish, 1956; Cornish and Christman, 1957).

Addition of chlorpromazine (50 mg.) to a urine aliquot (1/10 24-hour sample) before assay gave no evidence of interference with purine estimation.

Results

Purine Excretion and Drug Treatment

Up to seven non-dietary purines were estimated in 24-hour urine samples from seven newly admitted untreated schizophrenic patients and their matched pairs (Table I). The results are similar to those found for patients whose treatment had been temporarily suspended (Bollard *et al.*, 1960), and the variance between patients and controls was no greater than that attributable to experimental errors, as estimated by a classical analysis of variance.

When normal subjects whose urinary purine excretion had been monitored for three days were given a single dose of 200 mg. chlorpromazine, no significant change in the excretion of any compound was observed over the following three days.

Excretion of 3-methylxanthine

On ten occasions which arose from seven schizophrenic and three normal subjects, an ultraviolet absorbing spot appeared on chromatograms prepared from purines precipitated with silver salts at pH 2. Its R_f values corresponded to those reported for 3-methylxanthine (Weissman *et al.*, 1957), and it ran in these solvents as a single spot with a synthetic specimen of 3-methylxanthine (kindly presented by Dr. G. B. Hitchings), and showed the ultraviolet spectral characteristics of that compound (Cavalieri, 1954).

Attention in these studies had been concentrated primarily on the endogenous purines, but altogether 19 samples underwent silver-purine precipitation at pH 5–6 in addition to the routine precipitation at pH 2. The amounts of 3-methylxanthine precipitated at each pH were added to give a value for "total 3-methylxanthine".

The amounts of 3-methylxanthine precipitated at pH 2 and pH 5 were not significantly correlated ($r=0\cdot29$; $P>0\cdot1$) taking into account all 19 samples; indeed the 7 samples giving detectable precipitation at pH 2 showed a high negative correlation ($r=0\cdot80$; $0\cdot02>P>0\cdot01$) between the two precipitations, indicating that the amount of 3-methylxanthine determined by routine pH 2 precipitation bore no simple relation to the amount excreted. On the other hand, a pH 2 precipitate was never formed from urines containing less than 11·6 mg./day and was always formed above 17·5 mg./day, as though there were a critical concentration in this region. No relation could be found between the amount precipitated at pH 2 and the observed excretions of 1- or 7-methylxanthines.

The excretion of 1- and 7-methylxanthines was measured in the case of 18 of the 19 samples and the two sets of values showed no significant

Table I

Untreated Schizophrenic Patients Matched with Normal Subjects

Pair No. Control (C) or Patient (P)	Sex	Age (years)	Weight (kg.)	Urine Volume (ml.)	Creatinine (mg./kg./day)	Endogenous Purine Excretion (mg./day)						
						Adenine	6-Succino-amino-purine	Guanine	6- and 7-methyl-guanines	8-Hydroxy-7-methyl-guanine	Hypo-xanthine	Xanthine
1 C	F	25	55	1,790	20·6	1·22	<0·3	<0·1	3·11	0·48	3·25	5·44
1 P	F	28	53	1,720	25·8	0·90	<0·3	<0·1	3·19	1·56	6·47	5·77
2 C	F	58	65	1,675	19·4	1·10	0·85	0·12	2·40	<0·1	3·36	2·97
2 P	F	54	61	950	15·3	0·75	1·33	0·12	1·88	<0·1	3·49	2·75
3 C	M	27	59	1,220	30·0	1·01	<0·3	<0·1	1·73	0·65	5·64	2·39
3 P	M	26	58	1,240	35·8	1·46	<0·3	<0·1	5·22	1·79	7·47	3·33
4 C	M	31	65	1,200	28·5	2·22	<0·3	<0·1	4·97	<0·1	7·14	4·55
4 P	M	29	78	2,360	26·2	1·66	<0·3	<0·1	4·52	<0·1	4·93	4·16
5 C	M	30	73	1,770	35·8	1·08	<0·3	<0·1	4·43	<0·1	7·85	3·67
5 P	M	33	73	1,480	39·5	1·96	<0·3	<0·1	4·18	<0·1	5·98	3·02
6 C	M	23	57	1,025	25·8	1·02	<0·3	<0·1	4·21	<0·1	7·17	4·93
6 P	M	31	56	740	26·3	—	0·64	0·7	3·02	<0·1	13·9	2·90
7 C	M	38	66	1,220	24·8	1·06	<0·3	0·16	3·77	<0·1	6·64	5·46
7 P	M	47	67	1,895	26·4	0·80	<0·3	0·22	4·02	<0·1	6·24	4·31

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correlation with each other (r=0·33). "Total" 3-methylxanthine excretion was not correlated with that of 1-methylxanthine (r=0·11), but was highly correlated (positively) with that of 7-methylxanthine (r=0·88; P<0·01).

Tea, coffee and cola drinks were excluded from the diet of a normal human subject for some days. When 200 g. chocolate was then ingested, large amounts of 3- and 7-methylxanthines were excreted, but negligible 1-methylxanthine (Fig. 1).

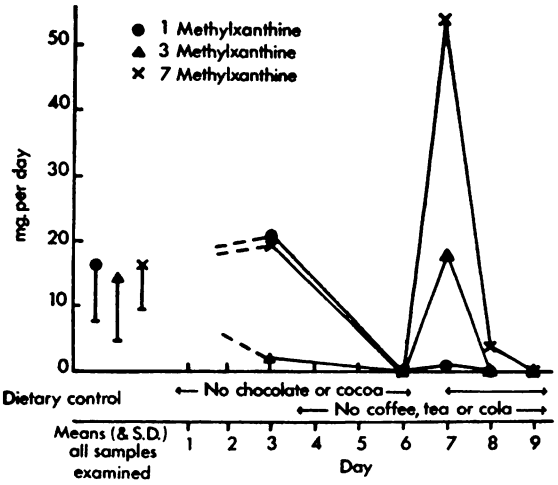

FIG. 1.—Effect of diet on a normal person's monomethylxanthine excretion. The means of all excretions determined during the investigation are compared with the daily excretion on a normal diet with selective exclusion of foods containing methylated xanthines. After completion of the 24-hour urine collection on Day 6, 200 g. of chocolate were eaten, but then cocoa products were once again excluded from the diet.

DISCUSSION

Dietary Purines

Methylated xanthines disappear from the urine of a subject deprived of tea, coffee, cocoa and cola drinks and any methylated xanthine drugs (Weissman *et al.*, 1957). Tea, coffee, and cola drinks are the normal sources of dietary caffeine, which in a 1 g. dose gives no detectable 3-methylxanthine excretion (Cornish, 1956). The trace amounts of theophylline in tea might cause excretion of a little 3-methylxanthine (Cornish, 1956), but theobromine, occurring almost exclusively in cocoa products, gives rise to appreciable 3-methylxanthine excretion, together with 7-methylxanthine.

The expectation that caffeine is a minor contributor to normal 3-methylxanthine excretion was confirmed by the small amounts observed on exclusion of cocoa products from a normal diet (Fig. 1: Day 3). It therefore seems likely that the higher 3-methylxanthine excretion by some patients may arise from ingestion of larger amounts of chocolate.

The high correlation between excretions of 3- and 7-methylxanthines probably also arises from their common origin by metabolism of cocoa theobromine. The contribution of 7-methylxanthine excretion from dietary caffeine (Cornish, 1956) could be reducing this correlation, although this effect might be counteracted by the 3-methylxanthine arising from tea theophylline.

The mean 3- and 7-methylxanthine excretion values of patients are not significantly different from those of normal subjects, indicating that greater consumption of cocoa products by some of the patients is not sufficiently gross or widespread to affect the overall comparison between the two populations.

Endogenous Purines

At the conclusion of this investigation, the purine excretion values reported in this and the previous paper (Bollard *et al.*, 1960), together with a few others analysed in this laboratory, were tested by analysis of variance for difference between the condition under which a patient was paired with a control subject for urinary purine assay and a condition in which two normal subjects were paired, similarly matched in sex, age and weight (Table II). The variance between patient/control and normal conditions was found not to be significantly above error variance (Table III), although significant differences existed between pairs in general. Thus these studies have provided no evidence to confirm Kishimoto's (1958) report of disturbances in purine metabolism in schizophrenic patients.

The purine excretions of the 70 individuals examined during this study are summarized for future reference (Table IV). Using a

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TABLE II

Matching of Pairs

Condition	Mean Differences (± s.d.)	
	Age (years)	Weight (kg.)
Patient/control subject pair	3·8 (±3·0)	5·6 (±6·0)
Pair of normal subjects	3·2 (±1·7)	3·3 (±2·0)

TABLE III

Source	Degrees of Freedom	Variance Estimate	Variance Ratio	P
Between conditions	1	40,009	1·60	>0·1
Between pairs	35	53,136	2·13	<0·01
Between purines	3	62,999	2·54	0·06
Residual	101	24,770	—	

TABLE IV

Individual Purine Excretions
(mg./day uncorrected)

	Adenine	6-Succino-aminopurine	Guanine	1- and 7-Methylguanines	8-Hydroxy-7-methylguanine
Range	0·5–2·6	0–1·4	0–1·0	0·6–8·9	0–3·9
Mean	1·3	0·3	0·3	3·6	0·8
S.D.	0·5	0·5	0·3	1·7	0·6
No. assayed	69	10	48	70	41

	Hypo-xanthine	Xanthine	1-Methyl-xanthine	3-Methyl-xanthine	7-Methyl-xanthine	1,7-Di-methyl-xanthine
Range	1·5–13·9	0·5–8·4	2·4–37·5	0–31·1	1·6–31·2	3·0–13·6
Mean	5·5	3·7	15·9	13·8	15·7	7·7
S.D.	2·8	1·1	11·7	11·2	8·7	6·4
No. assayed	70	70	35	15	34	27

The estimations summarized were performed on urine samples from 70 human subjects. 1-Methylhypoxanthine was not detectable in any of these samples (<0·1 mg./day).

colorimetric assay (Williams, 1950), Kishimoto found a mean of 7 normal subjects' urinary xanthine (3·2 mg./day) very close to ours and a mean of 17 schizophrenic subjects' (8·9 mg./day) at the upper end of the range we have observed. The excretions of the ten subjects observed by Weissman *et al.* (1956) fall within the ranges observed here, except that 1-methylhypoxanthine, N^2-methylguanine and "Spot S" were not definitely identified in this laboratory on a chromatogram from a human subject, although 1-methylhypoxanthine was detected in rat urine. Only very small excretions of these compounds were reported by Weissman *et al.* (1956) however, and the detection of N^2-methylguanine, at least, was dependent on the ultraviolet lamp used.

No advantage in matching pairs of subjects for weight or age was evident from the total data: no significant correlation was found

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between either weight or age and any purine excretion, although hypoxanthine continued to show positive correlation ($r=0\cdot26$; 44 samples; $P=0\cdot1$) with body weight between 40 and 100 kg. (cf. Bollard *et al.*, 1960). Some marked sex differences were observed, however (Table V) and so some pairing procedure is certainly necessary. The difference in hypoxanthine excretions possibly arose from the hypoxanthine/weight correlation, as the average body weight of our male subjects was greater than that of the female group. The well-known sex difference in creatinine excretion per kg. body weight was also observed.

Besides these results, our experience of the accuracy of this method is relevant to any future study of individual differences in urinary purines, and is summarized in Table VI in terms of pairs of chromatograms from one purine concentrate, pairs of aliquots from one urine sample, pairs of 24-hour samples from one subject (a calculated value from serial excretion studies), and matched normal or patient/control pairs. The variation within pairs of values generally increased in that order, indicating that the method is accurate enough to detect individual differences, although the small size of increase corresponds to a sensitivity less than ideal.

Summary

1. Ingestion of phenothiazine drugs did not detectably disturb the urinary excretion of

Table V

Sex Difference in Urinary Excretions

	Male (No. of Samples)	Female (No. of Samples)	S.D. of Whole Population	P
Xanthine (mg./day)	3·4 (34)	4·3 (28)	1·4	0·01
Hypoxanthine (mg./day) ..	6·3 (34)	4·2 (28)	1·2	<0·001
Creatinine (mg./kg./day) ..	26·8 (27)	19·3 (18)	6·4	<0·01

Table VI

Variations Within Pairs of Purine Values
(ranges as % means)

	Number of Pairs	Adenine	Guanine	1- and 7-Methyl-guanines	8-Hydroxy-7-methyl-guanine	Hypo-xanthine	Xanthine
Duplicate chromatograms	33	15	—	12	29	6	10
Duplicate aliquots of urine samples	3	26·1	—	16·1	39	9·1	17·3
Individual subjects	*	32·0	54·6	22·9	75·5	34·2	25·4
Matched normal subject pairs ..	7	21	84	29	150	32	67
(standard deviation)		(10)	(31)	(19)	(48)	(23)	(36)
Matched patient/control subject pairs	28	40	59	34	81	38	43
(standard deviation)		(26)	(75)	(36)	(63)	(21)	(28)

* 5 subjects (including the one recorded by Weissman *et al.*, 1957) for whom excretions had been estimated on 3 or more days (2 for 3, 1 for 4, 1 for 8 and 1 for 10 days). The S.D. of each subject's excretion was expressed as % his mean excretion of that purine, these coefficients of variation weighted for number of days observed, and the weighted mean of the coefficients of variation multiplied by 1·28 to give the mean range as % mean of any pair of purine samples.

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purine bases by normal subjects. No significant differences in purine excretion were found between groups of normal subjects matched with schizophrenic patients untreated with phenothiazine drugs. Drug treatment had thus not obscured differences between normal and schizophrenic populations in a previous study, and conclusions drawn then were supported.

2. Excretion of 3-methylxanthine was more frequently detected among schizophrenic patients than among normal subjects, and this fact was probably attributable to increased ingestion of cocoa products by some patients.

3. Variations and correlations among the daily urinary excretions of endogenous and dietary purine bases are collated.

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