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John H. Ayvazian, L. Fred Ayvazian

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A STUDY OF THE HYPERURICEMIA INDUCED BY HYDROCHLOROTHIAZIDE AND ACETAZOLAMIDE SEPARATELY AND IN COMBINATION *

By JOHN H. AYVAZIAN † and L. FRED AYVAZIAN

(From the Department of Medicine, New York University School of Medicine, N. Y.)

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The mechanism by which the chlorothiazide drugs produce hyperuricemia is not known, but the frequent and simultaneous reduction in renal clearance of uric acid suggests a tubular effect (1–3). Gutman, Yü and Sirota (4) have demonstrated a similar decrease in uric acid clearance after acetazolamide [1] therapy, and more extensive studies by Frascarelli, Lucidi and Cozzali (3) have not only confirmed this but have shown that levels of hyperuricemia induced by acetazolamide equal those produced by chlorothiazide [2] and hydrochlorothiazide.[3] The present studies were designed to confirm these previously reported findings, and to determine whether the effects of these drugs were additive. Complete urinary uric acid excretions and clearances were also done to test the dependence of the hyperuricemia upon diminished output.

The findings confirm those of Frascarelli and associates but differ in indicating a greater hyperuricemic activity for hydrochlorothiazide than for acetazolamide, and in demonstrating an additive effect when these drugs are used in combination. Still more significantly, these studies show that in low doses, hydrochlorothiazide increased the excretion of uric acid with an associated hyperuricemia.

METHODS

Normal, healthy medical students were used as the subjects for this study. They were required to ingest low purine diets, starting 48 hours prior to the first day of study, and their use of salicylates, vitamins and caffeine-containing beverages was curtailed. Fasting blood specimens were collected each morning at intervals of 24 hours; the plasma was separated and frozen until time for chemical analysis. For the duration of the study each subject collected his total output of urine in 24-hour aliquots, using 1-gallon plastic containers with toluene as a preservative. These specimens were analyzed daily, immediately after measurement of each total volume, except at weekends when the urine aliquots were diluted 1:10 with distilled water and stored at 4° C. The purpose of these dilutions was to prevent precipitation of uric acid with its loss to chemical quantitation.

Uric acid was measured according to Brown's modification of the urea cyanide colorimetric method, and creatinine content was determined by Phillip's modification of the alkaline picrate method (5). Duplicate analyses were performed on all specimens, and when the creatinine content varied by more than 10 per cent from the mean (or if the subject reported inadvertent or accidental loss of significant degree), the specimen was eliminated from the study. As a precaution against the possibility that a drug chromagen might interfere with these colorimetric analyses, the uricase method of Praetorius and Poulsen (6) was used to check samples of blood and urine.

RESULTS

Plasma studies. The following data refer only to *a*) the plasma uric acid levels just prior to and following medication, and *b*) the group of subjects with control uric acid levels falling below the usually accepted upper normal limit of 6.0 mg per 100 ml. The initial (control) specimen was taken after at least 48 hours of low purine diet and immediately preceding the administration of drug. The next three plasma uric acid values refer to specimens drawn 24, 48 and 72 hours after the control, during which intervals all subjects except those in the control group continued the medication. Table I lists the mean uric acid levels (calculated to two decimal places) and the range of the daily levels found in each of the four groups of subjects studied.

The control group consisted of 15 subjects

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† I. W. Held Fellow.

[1] Diamox, Lederle (2-acetylamino-1,3,4-thiadiazole-5-sulfonamide).

[2] Diuril, Merck (6-chloro-7-sulfamyl-1,2,4-benzothiadiazine-1,1-dioxide).

[3] Hydrodiuril, Merck (6-chloro-7-sulfamyl-3,4 dihydro-1,2,4 benzothiadiazine-1,1-dioxide).

TABLE I

Plasma uric acid levels: mean values and range *

Group, No.	Time in hours 0	24	48	72	Difference between 0–72 hours	Significance of difference
Control, 15	4.94 3.4–5.8	4.99 3.6–5.8	4.85 3.7–5.8	4.86 3.5–5.5	−0.08 −0.6 to +0.4	>0.05
Acetazolamide, 14	4.72 3.5–5.6	5.07 3.7–6.2	5.39 4.1–7.2	5.31 4.0–6.1	+0.59 −0.1 to +1.5	<0.01
Hydrochloro., 12	4.62 2.7–5.9	4.97 3.3–6.0	5.40 3.7–6.6	5.49 4.2–6.8	+0.87 +0.2 to +1.6	<0.01
Acet. and hydro., 11	4.77 3.4–5.4	5.37 3.3–6.4	5.99 4.2–7.1	6.53 4.8–8.1	+1.76 +0.8 to +2.8	<0.01

* Values are in mg per 100 ml plasma. All subjects were maintained on a low purine diet.

restricted to low purine diets but given no drug. These levels ranged from 3.4 to 5.8 mg per 100 ml (mean, 4.94) on the first day, and 3.5 to 5.5 mg per 100 ml (mean, 4.86) on the third and final day. Although the individual daily variations ranged up to 1.0 mg per 100 ml, these occurred at random without a discernible trend or pattern for the group.

In the second group were 14 subjects who received acetazolamide in doses of 1 g (250 mg at 9 a.m., 1 p.m., 5 p.m. and 9 p.m.) each day for 3 days. Their initial plasma uric acid levels ranged from 3.5 to 5.6 mg per 100 ml (mean, 4.72) and their levels rose after medication. The magnitude of these variations was only slightly greater than that of the control group, but a clear and consistent trend was apparent, with the plasma uric acid rising to levels of 4.0 to 6.1 mg per 100 ml (mean, 5.31) by the third day. Only 1 individual in this group of 14 failed to increase his third-day level over his control level.

Each of the 12 subjects in the third group received 50 mg hydrochlorothiazide (25 mg at 9 a.m. and 5 p.m.) daily for 3 days. Initial plasma uric acid values in this group ranged between 2.7 and 5.9 mg per 100 ml (mean, 4.62) and after 3 days of drug ingestion, all 12 rose to new levels varying from 4.2 to 6.8 mg per 100 ml (mean, 5.59).

Each of the 11 subjects in the fourth group received 50 mg hydrochlorothiazide and 500 mg acetazolamide (in divided doses at 9 a.m. and 5 p.m.) daily for 3 days. In this group the initial plasma uric acid values ranged from 3.4 to 5.4 mg per 100 ml (mean 4.77). After therapy the levels increased in all 11 subjects; the third and final readings extended from 4.8 to 8.1 mg per 100 ml (mean, 6.53).

In each of these four groups the uricase method of Praetorius and Poulsen was used to check the results of three of the subjects and to confirm the relative changes in plasma uric acid levels.

Statistical analysis of each of these groups (comparing the initial with the final plasma levels) by Wilcoxan's signed-rank test (7) shows that variations in plasma uric acid levels were insignificant in the control group, but significant with a probability of less than 0.01 for those medicated with acetazolamide and hydrochlorothiazide separately and in combination.

By using the Wilcoxan-White two-sample rank test (7) to compare each of these groups with the

TABLE II

*Uric acid renal excretion and clearance studies while receiving acetazolamide (Subject R.H.)**

Day	Plasma uric acid	Urine uric acid	Mean urine uric acid	Uric acid clearance
	mg%	*mg/24 hrs*	*mg/24 hrs*	*ml/min*
C 1	4.2	723		12.0
C 2	4.3	711		11.5
C 3	4.6	708		10.7
C 4	4.7	754	724	11.1
Rx 5	5.2	583		7.8
Rx 6	5.9	490		5.8
Rx 7	5.7	612		7.5
Rx 8	5.9	594		7.0
Rx 9	5.5	656		8.3
Rx 10	5.1			
Rx 11	5.4	697	605	9.0
PRx 12	5.4	578		7.4
PRx 13	4.4	710		11.2
PRx 14	4.2	634		10.5
PRx 15	4.4	838	690	13.2

* C: control period, low purine diet; Rx: drug period (acetazolamide, 1 g in 4 divided doses daily for 7 days); PRx: post-drug period with low purine diet continued.

TABLE III

*Uric acid renal excretion and clearance studies while receiving acetazolamide *: mean values and ranges*

Subject		Control period 4 days		Drug period 4 days		Post-drug period 4 days	
		Mean	Range	Mean	Range	Mean	Range
S.A.	Plasma uric acid†	3.6	3.5–3.7	4.1	4.0–4.2	3.8	3.3–4.1
	Urine uric acid‡	678	585–743	575	527–635	675	610–738
	Clearance§	13.1	11.3–14.0	9.8	8.7–10.8	12.6	10.3–15.5
L.R.	Plasma uric acid	3.9	3.8–3.9	4.4	4.1–4.7	3.5	3.4–3.7
	Urine uric acid	496	434–550	433	402–478	503	450–562
	Clearance	9.1	7.7–10.5	6.9	5.9–8.1	9.9	9.2–10.5
M.D.	Plasma uric acid	6.5	6.2–6.8	7.2	7.0–7.6	6.6	5.9–7.0
	Urine uric acid	622	602–632	532	458–641	559	518–635
	Clearance	6.6	6.5–6.7	5.1	4.5–6.4	6.0	5.2–7.5

* Acetazolamide, 1 g daily in 4 divided doses.
† Mg/100 ml plasma.
‡ Mg/24 hrs.
§ Ml/min.

others, no significant variation is found in the initial plasma levels of all four groups. However, the elevated third-day plasma levels in the acetazolamide, hydrochlorothiazide and combined-drug groups differ significantly from the corresponding final levels in those receiving no drug, with p values of less than 0.01. Comparison of the degree of change in plasma uric acid levels induced by acetazolamide and hydrochlorothiazide shows the increased levels in the hydrochlorothiazide group to be significant, with p equal to 0.05. Moreover, the hyperuricemia produced by combined therapy is significantly greater than that of each drug alone, with p less than 0.01.

Plasma and urine studies. The hyperuricemia induced by acetazolamide is associated with a decreased urinary excretion of uric acid. Table II lists the data obtained from Subject R.H. During the 4-day control period his calculated renal clearance of uric acid ranged from 10.7 to 12.0 ml per minute. Acetazolamide caused a relative hyperuricemia and a diminished excretion of uric acid, with a marked decrease in the renal clearance (which fell to levels ranging from 5.8 to 9.0 ml per minute). In the recovery period after the drug was discontinued, no increased excretion of uric acid was found, as one would expect if the hyperuricemia were purely the result of renal retention.

TABLE IV

Uric acid renal excretion and clearance studies while receiving hydrochlorothiazide (Subject M.G.) and hydrochlorothiazide and acetazolamide (Subject B.B.†)*

Day‡	M.G.				B.B.			
	Plasma uric acid	Urine uric acid	Mean urine uric acid	Uric acid clearance	Plasma uric acid	Urine uric acid	Mean urine uric acid	Uric acid clearance
	mg%	*mg/24 hrs*	*mg/24 hrs*	*ml/min*	*mg%*	*mg/24 hrs*	*mg/24 hrs*	*ml/min*
C 1	4.9	682		9.7	5.5	675		8.5
C 2	4.7	600		8.9	5.4	808		10.4
C 3	4.9	622		8.8	5.1	658		9.0
C 4	5.2	799	676	10.7	5.2	686	707	9.2
Rx 5	6.0	576		6.7	5.9	509		6.0
Rx 6	7.2	537		5.2	7.1	473		4.6
Rx 7	7.6	562		5.1	8.0	596		5.2
Rx 8	8.3	782	614	6.5	8.1	640	555	5.5
PRx 9	7.4	721		6.8	7.5	754		7.0
PRx 10	6.8	891		9.1	6.6	905		9.5
PRx 11	6.6	673		7.1	5.6	849		10.5
PRx 12	6.4	736	755	8.0	4.7	1,025	883	15.1

* Hydrochlorothiazide, 200 mg daily in 4 divided doses for 4 days.
† Hydrochlorothiazide (50 mg) and acetazolamide (500 mg) daily in 2 divided doses for 4 days.
‡ As in Table II.

TABLE V

*Uric acid renal excretion and clearance studies of a single subject (C.R.) receiving hydrochlorothiazide alone * (A) and later in combination with acetazolamide † (B)*

Day‡	A				B			
	Plasma uric acid	Urine uric acid	Mean urine uric acid	Uric acid clearance	Plasma uric acid	Urine uric acid	Mean urine uric acid	Uric acid clearance
	mg%	*mg/24 hrs*	*mg/24 hrs*	*ml/min*	*mg%*	*mg/24 hrs*	*mg/24 hrs*	*ml/min*
C 1	3.2	530		11.5				
C 2	3.0	396		9.2	3.7	542		10.2
C 3	3.5	543		10.8	3.7	446		8.4
C 4	3.8	540		9.9	3.4	583		11.9
C 5	3.5	504	503	10.0	3.4	506	519	10.3
Rx 6	4.1	520		8.8	3.3	553		11.6
Rx 7	4.1	598		10.1	4.2	383		6.3
Rx 8	4.2	570		9.4	4.8	518		7.5
Rx 9	5.2	586		7.8	5.6	609		7.6
Rx 10	4.5	527		8.1	5.8	558		6.7
Rx 11	5.1	598		8.1	5.2	657		8.8
Rx 12	4.7	691		10.2	5.5	512		6.5
Rx 13	4.8	652	593	9.4	5.0	656	556	9.1

* Hydrochlorothiazide, 50 mg daily in 2 divided doses for 8 days.
† As above, and acetazolamide 500 mg daily in 2 divided doses for 8 days.
‡ As in Table II.

These results are comparable with those found in three additional subjects who received the same dose of acetazolamide for 4 days each. Their data are summarized in Table III.

The potentiation of hydrochlorothiazide hyperuricemia by acetazolamide is also apparent in the data of Table IV. Subjects M.G. and B.B. were matched in age and size; M.G. received 200 mg hydrochlorothiazide daily while B.B. received 50 mg hydrochlorothiazide and 500 mg acetazolamide daily. Despite the difference in the dosage of hydrochlorothiazide, both showed similar qualitative and quantitative responses, with hyperuricemia and decreased urinary uric acid excretion and clearance. After drugs were discontinued, more complete recovery was seen in B.B. Unlike the four subjects who received acetazolamide alone, during their recovery period these two subjects increased their excretions of uric acid considerably above control levels.

TABLE VI

Uric acid renal excretion and clearance studies while receiving hydrochlorothiazide (Subjects W.Z. and J.M.†)*

Day‡	W.Z.				J.M.			
	Plasma uric acid	Urine uric acid	Mean urine uric acid	Uric acid clearance	Plasma uric acid	Urine uric acid	Mean urine uric acid	Uric acid clearance
	mg%	*mg/24 hrs*	*mg/24 hrs*	*ml/min*	*mg%*	*mg/24 hrs*	*mg/24 hrs*	*ml/min*
C 1	4.1	444		7.5	2.8	404		10.0
C 2	4.0	449		7.8	2.8	391		9.7
C 3	4.2	475		7.9	2.5	425		11.8
C 4	4.0	482	463	8.4	2.7	408	407	10.5
Rx 5	4.4	451		7.1	3.3	482		10.2
Rx 6	5.4	504		6.5	3.7	559		10.5
Rx 7	4.4	439		7.0	4.2	517		8.5
Rx 8	5.1	603		8.2	4.8	565		8.2
Rx 9	4.9	590		8.4	5.1	539		7.3
Rx 10	4.9	576	527	8.2	5.2	774		10.3
Rx 11					4.6	693	590	10.5
PRx 12	4.8	672		9.7	3.8	688		12.6
PRx 13	4.6	675		10.2	3.3	594		12.5
PRx 14	3.5	705		14.0	2.8	543		13.4
PRx 15	3.4	694	687	14.2	2.8	643	617	16.0

* Hydrochlorothiazide, 75 mg daily in 3 divided doses for 6 days.
† Hydrochlorothiazide, 50 mg daily in 2 divided doses for 7 days.
‡ As in Table II.

A similar study was done on a single subject; C.R. initially received 50 mg hydrochlorothiazide daily for 8 days, and 4 weeks later, 50 mg hydrochlorothiazide and 500 mg acetazolamide daily for 8 days (Table V). Combined therapy produced the greater degree of hyperuricemia and the greater fall in the calculated renal clearance of uric acid but, during each procedure, the uric acid excretion under medication exceeded the control. The mean control uric acid excretions were 503 and 519 mg per 24 hours, while the mean excretions during drug therapy were 593 and 556 mg per 24 hours.

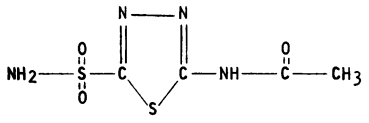
These results were confirmed in four addtional subjects, two of whom are reported here. W.Z. received 75 mg hydrochlorothiazide daily for 6 days, and J.M. received 50 mg hydrochlorothiazide daily for 7 days (Table VI). In both cases the urinary excretions and plasma levels of uric acid rose, while the calculated renal clearances diminished slightly but returned to control levels before drugs were discontinued. Moreover, and still more significantly, this hyperuricosuria persisted even after medication was discontinued, and in both instances the renal clearance of uric acid rose to levels exceeding those of the initial control periods. The plasma and urine of two of these subjects were analyzed by the uricase method as well and the results confirmed.

DISCUSSION

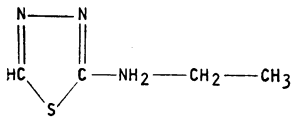
Our data support previous observations (3, 4) that acetazolamide can induce hyperuricemia and decrease the renal excretion and clearance of uric acid. Confirmatory analysis of plasma and urine by the uricase method establishes that these changes and measurements truly represent uric acid. Also confirming this activity of acetazolamide is its ability to potentiate the hyperuricemia induced by hydrochlorothiazide.

These data support the concept that this effect of acetazolamide is renally mediated. However, the observation that small doses of hydrochlorothiazide produce an increased excretion of uric acid is not compatible with the concept of renal retention as the mechanism for the associated hyperuricemia. The dual properties of the chlorothiazide drugs in increasing as well as decreasing the excretion of uric acid have previously been noted and likened to the paradoxical actions of uricosuric agents such as acetylsalicylic acid and probenecid (2, 8). Yü and Gutman feel that these drugs may exert either of two effects on the renal excretion of uric acid (9): at low concentrations they supposedly reduce the tubular secretion and consequently the excretion and clearance of uric acid; at higher dosage they inhibit the tubular reabsorption and increase the excretion and clearance. However, our data show that the increased excretion of uric acid occurs at lower doses of hydrochlorothiazide and is accompanied by hyperuricemia and a slight decrease or no change in renal clearance. This pattern of events could be produced by either mobilization of urate from the miscible pool or a decrease in the nonrenal disposal of uric acid (10), but neither of these mechanisms could account for the further increase in urinary uric acid excretion that occurred immediately after hydrochlorothiazide was discontinued. An alternative explanation is that the drug increased the production of uric acid. Uricogenesis can be produced by drug therapy, as demonstrated by Krakoff and Balis, using ethylaminothiadiazole (EATDA) (11).

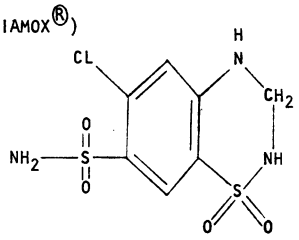
The close chemical relationship of acetazolamide (2-acetylamino-1,3,4 thiadiazole-5-sulfonamide) and EATDA (2-ethylamino-1,3,4 thiadiazole) (see Figure 1) suggests a possible mechanism by which hydrochlorothiazide, in varying doses, can increase or decrease the urinary excretion of uric acid. Krakoff and Balis found that EATDA greatly enhanced the *de novo* synthesis of purine nucleotides and did not increase the yield of uric acid from nucleic acids. Actually, their data indicate a decreased yield of uric acid from nucleic acid. EATDA may have a dual effect on uric acid metabolism, at least one of which is shared with acetazolamide. If EATDA does decrease the degradation of nucleic acids, a loss of feedback inhibition by purine or purine nucleotide salvage mechanisms could result in greatly increased *de novo* synthesis of purine nucleotides (12–14). Acetazolamide could have the same initial effect on nucleic acid degradation but could block the compensatory synthesis of purine nucleotides in a manner similar to the inhibition of bacterial nucleotide synthesis by the sulfonamide drugs (15). Hydrochlorothiazide could act similarly, with low doses allowing, and higher doses



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FIG. 1. DRUGS PRODUCING HYPERURICEMIA.

inhibiting, the compensatory *de novo* synthesis of purine nucleotides.

SUMMARY

The data presented support the concept that acetazolamide produces a relative hyperuricemia and a decreased renal excretion and clearance of uric acid. Although this hyperuricemia is not so great as that produced by hydrochlorothiazide, the two drugs together have an additive effect.

Hydrochlorothiazide, in low doses, produces a relative hyperuricemia with increased excretion of uric acid and without marked changes in its renal clearance.

The possibility is discussed that these drugs may exert an effect on uric acid through complex metabolic pathways rather than a renal tubular one.

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