*Summary.* A synthetic medium containing only cysteine, iso-leucine, d-ribose in a salt-glucose medium, buffered with Tris (hydroxymethyl aminomethane) supports the outgrowth of monkey kidney cells. The outgrowth is comparable in both rate and amount with the lactalbumin hydrolysate-calf serum (M) medium and supports the synthesis of poliovirus. Propagation of poliovirus throughout a culture in either liquid or agar medium occurs more rapidly in the presence of a simple amino acid supplement than in the serum-containing medium. Cysteine can completely replace the amino acid supplement for propagation of Type 1 in liquid cultures.

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## Effects of 2-Ethylamino-1,3,4 Thiadiazole HCl on Uric Acid Production in Man.* (22295)

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A compound having the formula, 2-ethylamino-1, 3, 4 thiadiazole HCl, was found by Oleson, *et al.*(1) to have antitumor activity in various laboratory animals. Further testing by Clarke(2) confirmed the activity against sarcoma 180 and Burchenal and Dagg (3) demonstrated a definite prolongation of the survival time of mice with transplanted leukemias 82 and 8174. Because of its antitumor effect in animals, Farber(4) initiated therapeutic trials in humans. He has supplied us with preliminary clinical dosage data.

*Materials and methods.* Clinical evaluation by our group was started in patients with far-advanced cancer. This report deals with the observations made in the 8 adult patients treated to date. The thiadiazole was given once daily by mouth, in doses of 1-4 mg/kilo/day. In most cases therapy was continued until the development of oral toxicity. Frequent physical examinations, appropriate roentgenograms, and hematological and biochemical determinations were performed before, during and after treatment. In 5 of the 8 patients herein reported daily serum and urine uric acid determinations were performed. The method employed for the determination of uric acid was that of Archibald as described in detail by Forsham, *et al.*(5). For the urine determinations this was modified as follows: Duplicate specimens were set up. The first specimen was treated in the usual manner. To the second specimen, after dilution 0.01 ml Worthington liquid uricase† was added to 5 ml diluted urine in a Coleman, Jr. cuvette and the specimen was incubated at 37°C for 90 minutes. After incubation the procedure was completed as with the first specimen. The color remaining in the second specimen represented non-uric acid chromogens and was subtracted from the total. All

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† Worthington Biochemical Co., Freehold, N. J.

TABLE I. Clinical and Uric Acid Data of Patients Treated with 2-Ethylamino-1,3,4 Thiadiazole HCl.

| Age | Sex | Diagnosis | Daily dose, mg/kg | Days | Serum U.A. (mg %) Pre Rx | Serum U.A. (mg %) During Rx | Excess urine U.A., mg |
|---|---|---|---|---|---|---|---|
| 25 | ♂ | Melano CA | 2 | 9 | — | — | — |
| | | | 4 | 3 | — | — | — |
| 38 | ♂ | LSA | 2 | 7 | 6.3 | 17.6 | — |
| 32 | ♀ | Melanoma | 2 | 7 | — | — | — |
| | | | 4 | 4 | — | 8.9 | — |
| | | | 6 | 4 | | | |
| | | | 4 | 11 | 1.9 | 6.0 | — |
| 62 | ♂ | CA tongue | 4 | 6 | 5.9 | 19.3 | 2160 |
| | | | 4 | 5 | 6.4 | 24.6 | 4270 |
| 20 | ♀ | Salivary CA | 4 | 3 | 3.9 | 13.8 | 1272 |
| | | | 4 | 4 | 4.5 | 13.2 | 1400 |
| 40 | ♀ | CA breast | 4 | 7 | 4.6 | 15.0 | 4176 |
| | | | 4* | 3 | 4.8 | 12.9 | 2509 |
| | | | 4* | 5 | 4.1 | 12.1 | 4951 |
| | | | 4* | 6 | 3.6 | 11.6 | 7216 |
| 64 | ♂ | CA kidney | 4 | 4 | 5.9 | 13.2 | 4879 |
| 39 | ♀ | CA cervix | 4 | 5 | 6.7 | 11.2 | 1469 |

* Given with equal doses of nicotinamide.

urines were analyzed in this manner. In addition occasional blood specimens were subjected to the uricase test to determine the specificity of those values reported as uric acid. In no instance was any significant amount of non-uric acid chromogen found in the blood.

*Results.* No evidence of tumor regression so far has been detected in any of the patients treated. Toxic effects have been limited to severe glossitis which occurred regularly in 4-6 days at a dose of 4 mg/kg/day. Smaller doses were tolerated for longer periods of time but similar toxicity occurred when approximately the same total dosage had been achieved. The clinical and dosage data are given in detail in Table I.

An interesting and as yet unexplained biochemical abnormality that occurred regularly was the observation that coincident with administration of the drug there was a prompt and marked elevation of serum uric acid which promptly returned to normal on cessation of administration of the agent. In order to attempt to elucidate this phenomenon, studies of urinary uric acid excretion were done in 5 of the patients. With each course of therapy there was found to be a rise in urinary uric acid excretion occurring promptly and paralleling the rise in serum uric acid. Table I indicates for each course of therapy the excess urine uric acid as compared with the average urine uric acid for a similar control period.

Fig. 1 illustrates the observations made in

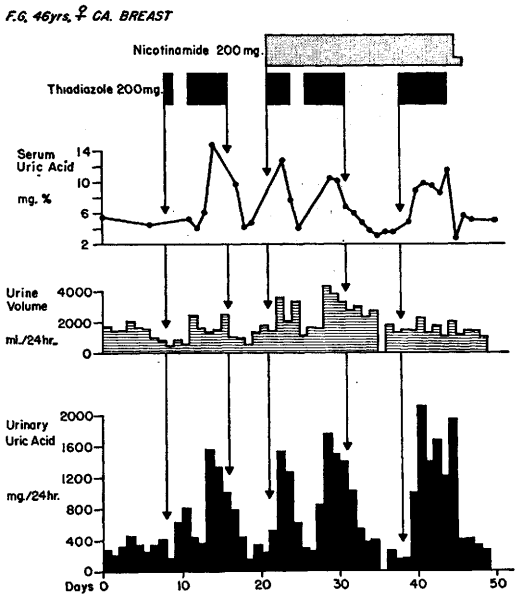


FIG. 1. Serum and urine uric acid in a patient treated with 2-ethylamino-1,3,4 thiadiazole HCl and nicotinamide.

one of the patients to whom this agent was administered. This patient, a 46-year-old woman with carcinoma of the breast and diffuse osseous metastases received 4 separate courses of medication. The dosage was 200 mg daily for 3 to 6 days in each course. On each occasion there was a rise in serum uric acid from normal levels of less than 6 mg% to levels of 10.6 to 15.0 mg%. Simultaneously the urine uric acid rose from control levels averaging 350 mg per day to levels of 1500 to 2300 mg per day. With each course severe glossitis occurred and required cessation of treatment. There was no definite evidence of regression of tumor tissue in this patient during or after treatment. Her blood urea nitrogen remained unchanged throughout this period of observation.

Inasmuch as Oleson(1) demonstrated and Clarke(2) confirmed that nicotinamide was capable of reversing the anti-tumor effect of thiadiazole, it was thought of interest to evaluate the effect of nicotinamide upon the uric acid abnormality produced by thiadiazole. Simultaneous administration of 200 mg daily of each agent produced a rise in serum and urine uric acid which was similar to that produced by thiadiazole alone. Nicotinamide did not prevent the development of oral toxicity.

*Discussion.* The mechanism of the uric acid abnormality occurring with administration of 2-ethylamino-1, 3, 4 thiadiazole HCl is not understood. The fact that urinary and serum uric acid rose in parallel would indicate that renal mechanisms are not involved. The absence of any indication of tumor regression in these patients renders unlikely but does not completely rule out the possibility that the uric acid was a product of degradation of the nucleic acid of tumor tissue. In the case illustrated an excess of approximately 7200 mg of uric acid above the pre- and post-treatment levels was found in the urine during and immediately after a single course of treatment. Since the average content of nucleic acid in tissue is 1% and since 20% of nucleic acid is composed of purines, calculation of the amount of tissue from which this 7200 mg of uric acid could derive reveals a figure of 3600 g. It might be expected that lysis of this amount of tumor or other tissue would be detectable. A third possibility is that there is a direct increase in uric acid synthesis, occurring as a result of the administration of this compound. This possibility has been neither proven nor disproven. Further studies are under way to investigate this problem and to attempt to correlate the rise in uric acid with nitrogen balance.

*Summary.* Oral administration of 2-ethylamino-1, 3, 4 thiadiazole HCl to patients with advanced cancer has been shown to cause a marked rise in serum uric acid levels and in total urinary uric acid excretion.

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