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Studies on 3, 4-Dimethyl-5-Sulfanilamido-Isoxazole (Nu-445) in Humans.*

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Sarnoff, Freedman and Hyman[1] reported on the activity of a new sulfonamide, 3,4-dimethyl-5-sulfanilamido-isoxazole (Nu-445)‡ in urinary tract infections with *B. proteus*. This sulfonamide derivative was found to be characterized by low toxicity and by a high solubility at pH 6.0 to 7.5,[2] the latter greatly diminishing the danger of crystallization in the urinary tract. The compound seemed suitable for the treatment of urinary infections because of its *in vitro* activity against the gram-negative organisms frequently occurring in urinary tract infections.[2]

Our encouraging early studies were subsequently confirmed by Haines and Micelli,[3] Narins,[4] and Lazarus and Schwarz.[5] A less optimistic report has been published by Rodgers and Colby.[6]

The clinical therapeutic work described above[1] was preceded by pharmacological studies in humans. These were mainly concerned with the absorption and elimination of the drug after oral and parenteral administration and the toxicology of the new product in human beings.

The results are presented in this paper.

Determination in blood and urine. Nu-445 may be determined by the method of Bratton and Marshall.[7] A solution of the lithium salt of Nu-445 served as the standard.

Mode of administration. Like other sulfonamides, the oral tablet is 0.5 g. Parenteral administration is facilitated to a considerable degree by the use of the lithium salt of Nu-445. Made up as a 10% solution, each ampule contains 10 cc, or 1 g, and may be injected intravenously or intramuscularly without untoward effects.

Intravenous administration. Three patients of varying weights were given 4 g (40 cc of the 10% solution) of the lithium salt of Nu-445 intravenously. The injection was completed in 3 minutes. There were no untoward reactions. Blood and urine levels were determined at regular intervals during the 24-hour period following the injection. The results of the blood level determinations are given in Table I. Renal excretion of the drug was in evidence 30 minutes after the injection, and the average level in the urine for the ensuing 24-hour period was 500 mg %. Excretion was not complete 24 hours after the injection at which time the determinations were discontinued. The figure of 500 mg % refers to total drug, 35% of which was present in the acetylated form. This is in agreement with the laboratory data of Schnitzer, *et al.*[2] who found 32% of the excreted sulfonamide to be in the acetylated form.

Intramuscular administration. Three g (30 cc of a 10% solution) of the lithium salt of Nu-445 were administered intramuscularly, 15 cc into each gluteal region. The data in Table I indicate that the concentration in the blood was somewhat lower than after the higher intravenous dose. What is ordinarily thought to be a therapeutic sulfonamide

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‡ Supplies of this compound were obtained for study from Hoffmann-La Roche, Nutley, N.J.

1 Sarnoff, S. J., Freedman, M. A., and Hyman, A. A., *J. Urol.*, 1946, **55**, 417.

2 Schnitzer, R. J., Foster, R. H. K., Ercoli, N., Soo-Hoo, G., Mangieri, C. N., and Roe, M. D., *J. Pharm.*, 1946, **88**, 47.

3 Haines, W. H., and Micelli, S., *Pennsylvania Med. J.*, 1947, **50**, 1328.

4 Narins, L., *J. Urol.*, 1948, **59**, 92.

5 Lazarus, J. A., and Schwarz, L. H., in press.

6 Rodgers, R. S., and Colby, F. H., *J. Urol.*, 1948, **59**, 659.

7 Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

TABLE I.
Blood Level of Nu-445 After Single Doses Administered by the Oral, Intramuscular, and Intravenous Route.

Dose, g	Route	No. of patients		Avg blood level (mg %) ½ hr	2-3 hr	4-5 hr	8-9 hr	24-30 hr
3	*per os*	3	free	5.8	13.8	16.0	11.0	—
			total	6.5	15.3	17.0	12.7	—
4	" "	3	free	6.8	9.0	14.6	9.2	3.8
			total	7.5	12.0	19.6	16.0	3.8
3	intramusc.	1	free	10.0	15.0	13.0	10.0	3.0
			total	10.0	16.0	16.0	14.0	6.0
4	intraven.	3	free	38.0	26.0	18.6	9.0	2.0
			total	38.0	26.0	20.0	14.0	3.3

level was, however, maintained for an 8- to 9-hour period, and even after 24 hours appreciable blood concentrations were still present. The acetylation varied from 0 to 50%. The average concentration in the urine was 150 mg %, 46.5% of which was present in the acetylated form. Excretion was not complete after 31 hours at which time observations were terminated.

Oral administration. Single oral doses of 3.0 and 4.0 g each were given to 2 groups of 3 patients. In the first group (3.0-g dose), food had been withheld for 12 hours, while the other group received the drug coincident with the noonday meal. The results of the blood levels as given in Table I do not seem to indicate that the presence of food in the stomach greatly alters the absorption. Adequate blood concentrations were maintained for a period of 2 to 9 hours. Urine determinations were carried out on specimens obtained 4 and 8 hours after the ingestion of 3 g *per os* in 3 patients. An average value of 236.6 mg % of total drug was found, 32.8% of which was in the acetylated form.

TABLE II.
Variation in Blood Level Over the 8-Hour Period. Patient Has Been Receiving 2 Grams Nu-445 *per os* Every 8 Hours for 24 Hours.

Nu-445 *per os*	Time	Patient A Free mg %	Patient A Total mg %	Patient B Free mg %	Patient B Total mg %
	12	13	17	13	18
2 g	2	10.5	14	10	16
	4	20	22	16	20
	6	16.5	20	15	19
	8	12	14	11.5	16
2 g	10	10	14	11	15

Multiple oral doses were first tried by adopting a dosage schedule of 2.0 g every 8 hours. This would confer several advantages in that it would halve the number of medications by the nursing personnel, and allow an 8-hour period during which the patient's sleep would not be disturbed. Table II indicates that such a dosage schedule does not allow the blood level to fall below the desired concentration at any time during the 8-hour period.

It seemed desirable to study dosage schedules in which a high initial dose was followed by the continued administration of smaller doses over a longer period. The blood levels observed in patients while on 3 different dosage schedules are given in Table III. Blood samples were taken midway between doses. The figures show that a consistent level was maintained for the entire period of medication. Twenty-four hours after discontinuation of the drug, the concentration of the drug in the blood was found to be quite low. Urine samples taken during the period of treatment showed an average concentration of 425.5 mg %, 34.8% of which was present in the acetylated form. Twenty-four hours after the administration of the drug was stopped, the urine concentration dropped to 12-18 mg % total drug, 59% of which was acetylated. Traces of diazotizable material could still be demonstrated in the urine of a patient 3 days after a total dose of 46.0 g, over a 7-day period, had been given.

Spinal fluid. Since the activity of Nu-445 against the meningococcus was found to be similar to that of sulfadiazine,[2] the rapidity of its entry into the spinal fluid and the

TABLE III.
Blood Level of Nu-445 After Different Oral Dosage Schedules.

Dosage	No. of patients		Avg blood level in mg % after days 1	2	3	4	5	6	7	8
4 g initial	1	free	12.0	12.5	11.0	10.0	10.0	11.0	11.0	1.5*
1 g every 4 hr		total	12.0	18.0	16.0	14.0	14.0	18.0	16.0	1.5*
3 g initial	3	free	16.0	12.3	15.0	13.8	15.8	1.7*	—	—
2 g every 8 hr		total	19.3	17.0	18.0	18.0	19.0	2.5*	—	—
3 g initial	2	free	10.7	11.0	12.3	13.0	12.0	—	—	—
1 g every 6 hr		total	16.7	16.0	17.5	17.0	17.0	—	—	—

* Medication discontinued the night before.

eventual level it attains there are of some importance. Table IV indicates that, within 2 to 3 hours after its intravenous or oral administration, the drug begins to enter the spinal fluid. In 2 patients with normal central nervous systems the eventual levels attained were low, being 1.2 mg and 1.0 mg % while blood levels were 7.5 and 11.5 mg %, respectively, of the free drug. However, in one patient with meningococcus meningitis the intravenous injections of 4 g and continued oral doses resulted in blood levels of between 28-30 mg % free and 37-40 mg % of the total drug. There was clinical and hematologic evidence of severe dehydration in this patient. At this time the spinal fluid levels were 8 mg % free and 36 mg % total Nu-445. A subsequent spinal fluid level determination revealed 5 mg % free and 12 mg % total drug. Recent evidence indicates that the barrier between blood and spinal fluid is appreciably altered in the presence of inflammation.

Toxicity in the human. Nu-445 was administered in varying doses to 37 patients. Other than the 6 patients who were given single doses at various times for the purposes of absorption studies, this group received total doses ranging from 17 to 93 g over periods ranging from 4 to 20 days. No alkali therapy was given. No attempt was made to control the hydration of the patient in order to duplicate the various conditions which one might expect to encounter clinically.

One significant fall in hemoglobin, 22%, was encountered in a patient with ulcerative colitis and a 4+ guaiaic test in the stool during the entire course of drug administration. One moderate depression of the white blood cell count occurred in a severely debilitated individual with pyelonephritis in whom a provisional diagnosis of Hodgkin's disease had been made. One patient experienced a 2° rise in temperature coincident with the onset of drug administration. He was in his 6th postoperative day, but no evidence of infection was present and the fever was attributed to the drug.

TABLE IV.
Early Spinal Fluid Levels After Oral and Parenteral Administration of Nu-445.

Dose	Hr after administration	Mg % Free	Total
4 g. p.o.	2.5	0.25	0.25
4 "	3.5	0.025	0.025
4 "	2.8	0	0.15
4 g i.v.	2.0	0.4	0.8
4 "	2.6	0.5	0.75

One patient had reacted with severe urticaria and vomiting to as little as one g of sulfadiazine on 3 different occasions, but did not react to full doses of Nu-445 over a period of 2 weeks. A second patient had experienced severe headache, nausea, and vomiting with the previous administration of either sulfadiazine or sulfathiazole. The first dose of 4 g of Nu-445 was followed by a moderate headache, but this disappeared in 24 hours and no further reaction was noted while the patient was on full doses.

Crystalluria was not observed in any patient despite the fact that no alkali was given. Hematuria attributable to the drug was not

observed, nor was there any evidence of renal irritation.

Thirty cc of a 10% solution of the lithium salt were injected intramuscularly (15 cc into each buttock) in 3 patients. The injection was tolerated well in each case and no subsequent discomfort was noted.

One patient, not included in the above group, afforded the opportunity for massive oral and intravenous therapy. Subacute bacterial endocarditis with *Bacillus proteus* had been present for several months. In view of the activity of Nu-445 against this organism in the genito-urinary tract[1] and *in vitro,* it was thought worthwhile to attempt massive therapy in this case. After a 4-g initial dose, 1 g every 4 hours was administered for 4 days, 2 g every 4 hours for 4 more days, and then 3 g every 4 hours for 10 days. No reactions were noted. While the patient was receiving this oral dose, 18 g of the lithium salt of Nu-445 were given intravenously over a period of 4 hours. Two days later, with the patient still on the same oral dose, 12 g were given intravenously in 10 minutes. Pallor and sweating were noted for a few hours after this last dose and crystalluria and microscopic hematuria were noted for the ensuing 3 days. This was accompanied by a transient rise in the blood urea nitrogen. It is doubtful if the usual clinical requirements would ever indicate the administration of even a fraction of the dose administered to this patient.

Two other patients not included in the above group who had been hospitalized because of recent coronary occlusions, had fatal recurrences while receiving drug therapy. There was post-mortem confirmation in one of these. A third patient, recovering from a recent, severe, postoperative pulmonary embolization was being treated with Nu-445 in an attempt to clear his urinary tract of a *Bacillus proteus* infection, and while under treatment sustained a fatal recurrence.

Summary. A new sulfonamide preparation has been presented which, on the basis of its high solubility in buffer solution, would be expected to greatly diminish the danger of sulfonamide crystallization in the kidney, with or without the concomitant administration of alkali. Neither crystallization nor the signs of renal irritation in the absence of crystallization were observed (except in the patient to whom enormous doses were administered). The antibacterial activity *in vivo* and *in vitro* and the low toxicity in the experimental animal combined to recommend an investigation of the clinical pharmacology of this compound. The blood and urine levels attained were found to be satisfactory and it was found possible to achieve and maintain these levels with an 8-hour dosage schedule. The entry of this drug into the spinal fluid of the normal individual is low, but in the presence of meningeal inflammation it was markedly increased in the one patient studied. The toxicity of the compound is low.

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Action of Quaternary Ammonium Salts and the Theory of Competitive Inhibition of Acetylcholine.

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In several publications[1,2,3] attempts have been made to explain local anesthesia as a competitive inhibition of acetylcholine action upon the nerve. The theory, of course, assumes acceptance of the acetylcholine theory for the transmission of impulses[4] along the nerve fibre and the nerve ends. Some assume that similarity in chemical structure enables