exerts a greater bacteriostatic and bactericidal effect on certain strains of gram negative bacilli than do other sulfonamides.

2. Clinical trial in 20 patients with urinary infections indicated excellent effectiveness of the drug in uncomplicated, monovalent infections due to *E. coli* or *B. proteus*.

3. Results of treatment in polyvalent infections and in cases with complicating pathology were poor.

4. The marked lack of toxicity of Nu 445 recommends its use in appropriate cases, in which it will occasionally be found to be more effective than other sulfonamides.

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17066. Metabolic Fate of 4,4'-Diaminodiphenylsulfone (DDS) in the Rabbit and its Isolation from Urine.

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4,4'-Diaminodiphenylsulfone (DDS) and many of its derivatives have been used with a measure of success in the treatment of experimental tuberculosis.[1,2] More recently the potentiating action of some sulfone derivatives on streptomycin has also been shown.[3] Of the various types of sulfone derivatives indirect evidence has indicated that certain disubstituted derivatives like promin, diasone and possibly sulphetrone are metabolized to the toxic parent substance DDS while the monosubstituted alkyl and hydroxyalkyl derivatives do not appear to undergo such transformation in the body to an appreciable extent.[4] Indirect evidence has also indicated that DDS itself does not change to any significant degree in the body, at least in so far as acetylation is concerned.[1,5] Obviously, direct evidence on these points can only be had by the actual isolation of DDS from the urine. The present report describes a satisfactory method for the isolation and recovery of DDS added to rabbit urine and the isolation and the identification as DDS of about 70% of the total diazotizable material in the urine of rabbits given DDS orally.

Procedure. The isolation of DDS from rabbit urine is based on the solubility of its hydrochloride in water and the relative insolubility of the base in water, but its ready solubility in ethyl acetate. Ethyl acetate appears to be the most satisfactory of several water-immiscible solvents tried.

The urine is made strongly alkaline to litmus with NaOH and chilled on ice for a while to aid precipitation of the base. It is then shaken out thoroughly 2 to 3 times in a separatory funnel with 30 to 50 cc of ethyl acetate, allowing as complete separation as possible each time. The partly emulsified combined ethyl acetate extract is cleared by the gradual addition of anhydrous sodium sulfate with stirring. The clear ethyl acetate solution is decanted and the Na_2SO_4 residue is washed 2 to 3 times with small portions of ethyl acetate.

The combined ethyl acetate extracts are washed once with about 5 cc H_2O and then thoroughly extracted with 5 cc portions of N HCl. This should be repeated 5 to 7 times. The combined acid extract is chilled on ice

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1 Smith, M. I., Emmart, E. W., and Westfall, B. B., *J. Pharm. Exp. Therapy.*, 1942, **74**, 163.

2 Smith, M. I., *N. Y. State J. Med.*, 1945, **45**, 1665.

3 Smith, M. I., McClosky, W. T., Jackson, E. L., and Bauer, H., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 261.

4 Smith, M. I., Jackson, E. L., Junge, J. M., and Bhattacharya, B. K., *Am. Rev. Tuberc.*, in press.

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TABLE I.

Fractionation of Rabbit Urine for the Isolation of 4,4'-Diaminodiphenylsulfone (DDS). Figures in first 4 fractions give DDS equivalent in mg as obtained by the Bratton and Marshall Diazotization Technic.

Fraction	30 cc normal urine with 63 mg DDS added	Urine of rabbits given 0.5 g/kg DDS orally: 30 cc urine containing 100 mg DDS equivalent	30 cc urine containing 100 mg DDS equivalent	60 cc urine containing 200 mg DDS equivalent
1. Residual urine after extraction with ethyl acetate	0.25	7.2	8.8	10.3
2. Ethyl acetate ext. after extraction with N HCl evaporation to dryness, residue taken up in dil HCl and filtered	0.25	4.8	5.8	7.1
3. Mother liquor and washings of crystals obtained by alkalinization of the N HCl extract	9.0	20.3	15.6	20.9
4. Acetone insoluble part of isolated crystals	trace	trace	trace	—
5. Acetone soluble part of isolated crystals				
(a) Weight mg	47	63	68	141
(b) % of total	74.6	63	68	70.5
(c) Melting point °C	175-6	170-2	170-2	171-2
(d) M.P. when mixed with pure DDS °C	175-6	173-4	173-4	174-5

and alkalinized by addition of about 20% NaOH solution. After being chilled on ice the crystals are collected by centrifugation, washed by centrifugation with a minimum volume of ice cold water until neutral to litmus and dried at 60-80°C. The dry residue in the centrifuge tube is dissolved for the most part in a few cc of acetone, centrifuged, the clear solution decanted into a suitable weighing bottle and the operation repeated 2 to 3 times, to separate the acetone-soluble part from the insoluble solid which is discarded. The acetone is allowed to evaporate and the residue dried *in vacuo* to constant weight. The material so obtained from urine, when pure DDS is added to it, had a sharp melting point of 175-176°C. Material so obtained from urine of rabbits given DDS *per os* assayed 100% by a modification of the Bratton and Marshal diazotization technic and usually had a melting point of 170-172°C; when mixed with pure DDS it melted at 173-175°C. Recrystallization of this material by dissolving it in a minimum volume of acetone and slowly adding 5 to 6 volumes of water, centrifugation and drying yielded crystals which had a melting point of 172-174°C. The crystals were decolorized in hot methanol solution with Norit and recovered by evaporation of the solvent. A final recrystallization from a little methanol yielded DDS melting at 175-176°C alone or mixed with authentic crystals of DDS.

Table I shows the results of fractionation of a sample rabbit urine to which 63 mg of pure DDS had been added as the hydrochloride and similar fractionation of pooled catheterized specimens of urine obtained from 3 rabbits 3 to 6 hours after the oral administration of 0.5 g/kg of DDS in aqueous suspension with gum acacia. In the latter instance the urine was assayed by a modified Bratton and Marshal technic[6] against DDS as a standard and a volume of urine corresponding to the amount of DDS equivalent indicated in the table was used for fractionation.

The data in Table I indicate that at least 63-70% of the total diazotizable material in the urine is unaltered DDS. Parallel assays of the several fractions by diazotization after acid hydrolysis at 100°C and direct diazotization at room temperature gave identical

[6] Smith, M. I., Junge, J. M., and McClosky, W. T., *J. Am. Pharm. Assn.*, Sci. ed., 1948, **37**, 461.

values except in fraction one in which the former values were from 10-30% higher, suggesting that a small amount, not over 5% of the total, had been altered to some metabolite, possibly conjugated DDS.

The technic of partition chromatography on filter paper, as originated by Consden, Gordon and Martin[7] has been adapted for the resolution of DDS and several synthetic derivatives from mixtures. Normal butanol saturated with 3% ammonia, benzene saturated with water, and equal parts of benzene and cyclohexane saturated with water were used with ascending columns of narrow (⅛ in. wide) strips of Whatman's No. 1 filter paper. The papers were hung for 3 to 24 hours in a liter graduate cylinder containing 100 cc of one of the above solvents. Colored bands of DDS or monosubstituted derivatives were produced by pipetting the Bratton and Marshal reagents (dissolved in organic solvents to minimize dispersal of the pigments produced) on the dried strips. The p-dimethylaminobenzaldehyde reagent (1% in 3% HCl) of Kühnau,[8] giving a yellow to orange precipitate, has also been found useful. It has been found that rabbit urine containing DDS or certain derivatives, may be chromatographed without prior treatment. Aliquots of 0.01 cc, containing 0.1 to 1.0 γ of each sulfone, were used.

Applying such methods to the several fractions in Table I evidence has been obtained to indicate that, in addition to the original urine, fraction 1 and possibly also fraction 2 contained another compound besides DDS. The original urine and fraction 1 gave 2 distinct bands when chromatographed from the benzene-water solvent. One of these bands, the upper, had the same R_F value as pure DDS. The other, the lower band ($R_F < 0.1$), was unlike those produced by the monoacetyl derivative of DDS or 3-hydroxy-4,4'-diaminodiphenylsulfone or the sulfide and sulfoxide analogues of DDS.

These results demonstrate that the bulk of the diazotizable material excreted in the urine following the oral administration of DDS to rabbits is unchanged DDS. The procedure described also provides a ready means for determining whether a given derivative of DDS is metabolized to the parent substance or not.

Summary. A method is described for the isolation and identification of 4,4'-diaminodiphenylsulfone (DDS) in the urine of rabbits. The method is based on the solubility of the hydrochloride of DDS in water and the relative insolubility of the base in water but its ready solubility in ethyl acetate.

Up to 70% of the total diazotizable material excreted in the urine following oral administration of DDS to rabbits has been isolated as unchanged DDS. Not more than about 5% of the diazotizable material may be in an altered form, and this probably as conjugated DDS. Partition chromatography indicated that it was not the acetylated derivative of DDS.

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[7] Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, 1944, **38**, 224.

[8] Kühnau, W. W., *Klin. Woch.*, 1938, **17**, 116.