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Renal Excretion of 5,5-Dimethyl-2,4-oxazolidinedione (Product of Demethylation of Trimethadione).* (23540)

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The antiepileptic drug, trimethadione (Tridione), is converted by metabolic demethylation to 5,5 - dimethyl - 2,4 - oxazolidinedione ("DMO"). The conversion of trimethadione to DMO is nearly complete in the dog and in man(1). When trimethadione is administered in chronic schedules, DMO accumulates until the amount in the body is many times greater than the daily dose of trimethadione. Plasma concentrations of DMO as high as 1 g/l have been observed in patients receiving therapeutic doses of trimethadione. It appears likely that DMO plays an important role in the antiepileptic effect produced by administration of trimethadione. DMO is among the most persistent of all organic compounds that have been studied, the plasma concentration in man declining by only 5 to 12% per day after discontinuation of dosage. The extraordinary persistence of DMO is indicative of a very slow rate of renal excretion. An investigation of the mechanism of renal excretion of DMO is the subject of the present report.

Methods. Concentrations of DMO in plasma were determined by the ultraviolet spectrophotometric method of Butler(1). Because of interfering materials in urine this method is not applicable to urine. For the *determination of DMO in urine* the method was modified in the following way. To 1 ml of urine in a glass stoppered tube are added 25 ml of redistilled reagent grade absolute ethyl ether saturated with water and 4 ml of 5 M NaH_2PO_4. The tube is shaken and centrifuged. The ether phase is transferred by pipette to another tube containing 50 mg of Norit. After shaking and centrifugation 20 ml of the ether is transferred to another tube, where it is shaken with 5 ml of 0.05 M borate buffer, pH 9, previously saturated with ether. The tube is centrifuged and 2 ml por-

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tions of the lower phase are transferred to each of 2 tubes. To one is added 2 ml of the buffer of pH 9 and to the other 2 ml of 0.1 N HCl. Absorption measurements are made on these 2 preparations with a Beckman DU ultraviolet spectrophotometer, the blank for the first being the buffer and for the second a mixture of equal volumes of buffer and 0.1 N HCl. Absorbancies of both preparations are measured at 215 and at 220 mμ. The absorbancies at pH 9 are corrected by subtracting the corresponding absorbancies in acid solution. The concentration of DMO is calculated from the difference between the corrected absorbancy at 215 mμ and that at 220 mμ. By the procedure of partitioning between ether and buffers as described by Butler(1), the specificity of this method has been demonstrated. *Administration of drugs* to dogs. DMO was given intravenously in aqueous solution in a dose of 50 mg/kg at least an hour before a clearance determination. Diuresis with acid urine was produced by administration of 30 ml of water per kg by stomach tube, 130 mg of meralluride sodium intravenously, or 50 to 100 ml of 0.95 M Na_2SO_4 intravenously. Excretion of alkaline urine was brought about by intravenous administration of 50 to 100 ml of 8% (w/v) $NaHCO_3$. *Collection of urine* from dogs was by catheter. Urine for pH determination was kept in glass stoppered flasks and the pH measured within 10 min. of the time of collection. *Determination of pH, measurement of protein binding, and measurement of creatinine clearances in dogs* were performed by the methods used in an earlier study of phenobarbital(2).

Results. Protein binding. In 4 g/100 ml solutions of bovine serum albumin in buffers of pH values of 6.0 to 8.5, no binding of DMO to the protein could be detected.

Extent of excretion. A 10 kg dog was given an intravenous dose of 0.5 g of DMO and the urine collected from an indwelling catheter. Excretion of DMO was accelerated by administration of several intravenous infusions of a $NaHCO_3$ solution. This treatment resulted in the plasma concentration falling to a negligible level in 2 days. Normally about 2 weeks would have been required for this to occur. The DMO as determined in the urine excreted during these 2 days accounted for 99% of the administered dose. DMO does not undergo metabolic change to any significant extent in the dog. Renal excretion is the only process of any importance in the elimination of the compound.

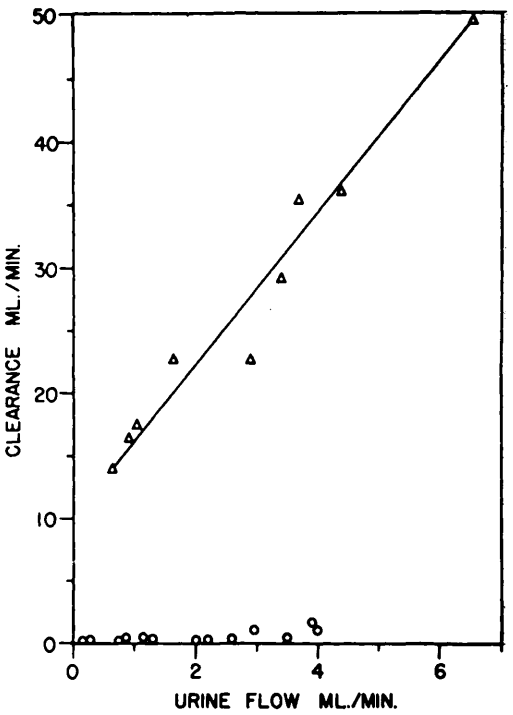

FIG. 1. Renal clearances of DMO in a dog. Values designated by circles are from experiments in which diuresis was induced by oral water, intrav. meralluride sodium, or intrav. Na_2SO_4, and in which the urinary pH was below 7.0. Values designated by triangles are from experiments in which $NaHCO_3$ was given intrav. and in which the urinary pH was 7.8 to 8.0.

Effect of urine flow and pH on excretion of DMO. Several measurements of the renal clearance of DMO were made in the same dog at different times under different conditions. The results are shown in Fig. 1. When there was no diuresis or when diuresis was brought about by water, meralluride, or Na_2SO_4, the pH of the urine was below 7 and the clearances of DMO were very low even at the highest flow rates. Urine/plasma concentration ratios as low as 0.1 were found when the urine was acid. After administration of $NaHCO_3$, the urinary pH was 7.8 to 8.0. The clearances of DMO were much higher than when the

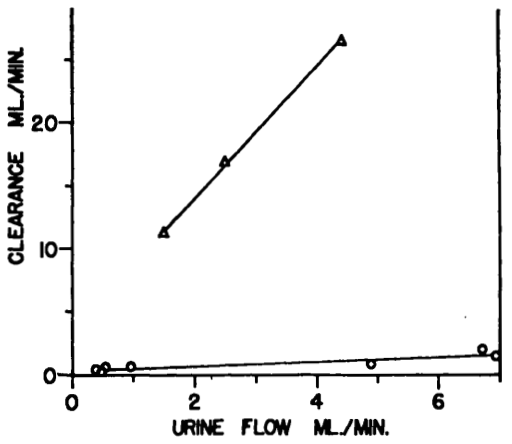

FIG. 2. Renal clearances of DMO in a man. Values designated by circles are from an exp. in which diuresis was induced by administration of 1 l of water by mouth. Urinary pH was 5.6 to 6.4. Values designated by triangles are from an exp. in which the same man was given 15 g of $NaHCO_3$ intrav. Urinary pH was 8.0.

urine was acid, and they increased with increasing rate of flow. Clearances with alkaline urine at high flow rates were as much as 250 times those with acid urine. However, the highest clearances did not equal the creatinine clearance.

Fig. 2 shows DMO clearances in a man who had been receiving 800 mg of DMO daily for several weeks. As in the dog, the clearances were very low when the urine was acid, even when the flow rate was high. The U/P ratio varied from 0.7 at low flow rates to 0.2 at high flow rates. When the urine was alkaline, the clearance was greatly increased and varied to an important extent with flow rate.

Discussion. There are a number of examples of organic acids that are excreted more rapidly in alkaline than in acid urine and of bases that are excreted more rapidly in acid than in alkaline urine (2,3). This effect has been explained in terms of a renal tubular epithelium permeable to the lipid-soluble undissociated forms of acids and bases but impermeable to the ionic forms. DMO is an acid with a pK′ value of 6.13 at 37°C and ionic strength of 0.16. The influence of urinary pH on the excretion of an acid with this value of pK′ would theoretically be predicted to be important. If an acid with pK′ = 6.13 were fully equilibrated between plasma with pH 7.4 and urine with pH 8.0, the two phases being separated by a membrane permeable only to the undissociated form, the U/P concentration ratio for the acid would be 3.8. If plasma of the same pH were equilibrated with urine of pH 6.0, the U/P ratio would be 0.09. Equilibration is never actually complete, and the amount of DMO reabsorbed is less than that predicted. The observed values of U/P are accordingly higher than the theoretical values both in acid and alkaline urine. The effect of urinary pH on the excretion of DMO can thus be explained on the assumption that the drug is filtered and then reabsorbed by a process of passive diffusion, the tubular epithelium being impermeable to the ionic form. The observed U/P ratios lower than unity in acid urine can be accounted for without the assumption of any "active transport" system.

During the greater part of the day the urine of a man on an ordinary diet is at pH values so low that the U/P ratio for DMO is at or below unity. The very extensive tubular reabsorption with acid urine together with the insusceptibility to metabolic attack account for the remarkable persistence of DMO.

Summary. A method is described for determination of 5,5-dimethyl-2,4-oxazolidinedione (DMO) in urine. DMO is not bound to serum albumin. In the dog DMO is excreted completely unchanged in the urine. In the dog and in man the renal clearance of DMO is much higher in alkaline urine than in acid urine. This is explained on the assumption that the compound is reabsorbed in the renal tubule by a process of passive diffusion, the tubular epithelium being permeable to the undissociated form and impermeable to the ionic form.

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