cle of an invertebrate (Homarus americanus) also showed activity (228 and 108 μmoles/hr x g at 37°C respectively). The liver of a hagfish (Myxine glutinosa) did not (one specimen only). Data for the rat are included in the table for comparison. The lower vertebrates show an enzyme pattern that is different from the rat: activity is highest in brain and lowest in liver. The significance of the relatively high activity in kidney of the lower vertebrates is not clear. Our success in demonstrating the enzyme was largely due to the inclusion of an ATP regenerating system in the assay and incubation for short times (< 5 min). These two factors helped overcome the known inhibitory effect of ADP which is rapidly produced by ATPases present in the crude homogenates.

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METABOLISM OF ETHYL m-AMINOBENZOATE (MS 222) IN THE DOGFISH, Squalus acanthias

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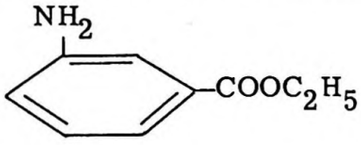
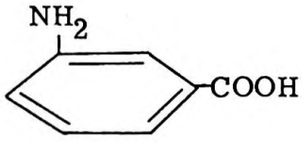
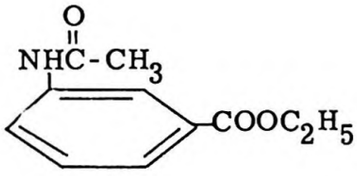
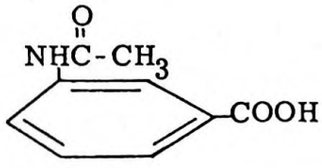
In 1967 (This Bulletin, 7:51) we established that MS 222 (I) was cleared by the gill at the rate of 10 ml/min per kg. The major portion of injected drug disappeared within 2 hours. However, a small amount of diazotizable amine was excreted in urine for longer periods, and amine was found in relatively high concentrations in the kidney. The structure of the drug suggested obvious metabolites, due to acetylation of the amine and cleavage of the ester. Table 1 shows the structures. All drugs were injected into the tail vessels.

Table 1

STRUCTURE OF MS 222 AND METABOLITES

Compound	Structure	Name
I		Ethyl-m-aminobenzoate (MS 222). MS 222 is the methane sulfonate.
II		m-aminobenzoic acid
III		Ethyl-m-acetylaminobenzoate
IV		Ethyl-m-acetylaminobenzoic acid

Experiments were done with potential metabolites m-aminobenzoic acid (II) and ethyl-m-acetylaminobenzoate (III). II was found to be impermeant at the gill, and cleared by the kidney at the mean rate of 30 ml/hr, as judged by the Bratton-Marshall reaction (see Walker and Schoettger, Investigations in Fish Control, #14, U. S. Department of Interior, P.O. Box 862, La Crosse, Wisconsin, for penetrating analysis of how the Bratton-Marshall reaction is best applied to MS 222, and presumably its congeners). U/P ratio was 1-6, the range of inulin. Half-life in plasma was 37 hrs. From the mean renal clearance and the volume of distribution of body water (66% of wt) the formula

$$t - 1/2 = \frac{\text{vol. dist.} \cdot \ln 2}{\text{clearance}}$$

yields the theoretical plasma half-life (t-1/2) in a 4 kg fish of 60 hrs, which is in fair agreement with the experimentally determined value. Tissue analysis at 1 hr after dose of 3 mg/kg shows plasma, muscle, and liver to be about the same (4-6 μg/ml) but kidney 3 x this value. Hydrolysis in acid preceding the colorimetric reaction increases the yield in urine and kidney about 20%, but not in blood, liver, or muscle. We conclude that II, the ester-hydrolyzed product of MS 222, is slowly excreted by the kidney, concentrated there and moderately acetylated. It is possible that the kidney is the site of acetylation.

Compound III was studied in the divided box, as described earlier (Comp. Biochem. & Physiol. 26:853, 1968). Analysis of blood and gill effluate and urine was carried out after 45 minutes acid hydrolysis. Clearance in the first 20 minutes was 2-8 ml/min per kg, a range slightly less than that of the parent MS 222. No drug was found in the urine in this time. We conclude that III, the acetylated product of MS 222, is excreted by the gill in the same general fashion as the parent.

MS 222 (compound I) was injected into fish and studied by Bratton-Marshall analysis with and without hydrolysis, and by the Hestrin reaction (J.B.C. 180:249, 1949) which identifies the ester group. Our earlier observations that there was a steep decline in plasma concentration in the first hour were confirmed, as was the venous/arterial ratio of about 2. There was little (20% or less) evidence of acetylation in the plasma. However, after the first hour, the plasma decay curve changed slope very radically, and persistent low levels of amine were obtained for at least 6 hours. There was, again, little difference between free amine and that after hydrolysis. The N-acetyl forms (presumably III and IV) were not found in liver and kidney. The only manifestation of tissue storage was what is presumably compound II, in kidney. Urinary data show prominent acetylation as early as 2 hours.

Table 2 shows the recoveries of free amines (I and II), esters (I and III) and total potential aromatic amine (I - IV), from the urine in one experiment, typical of five. It is clear that very little original drug appears in the urine—1% at most.

Thin layer chromatography (48% $CHCl_3$ - 48% EtOH - 2% Acetic acid) showed authentic MS 222 in small amounts for 4 hours, then a trace, but none after 17 hours. Since both esters (I and III) are rapidly cleared by the gill and not sequestered, their absence from the urine after 12 hours is expected (column 2). From the pharmacology of compound II described above, it is almost certain that this is a main component of column 1 (see also Table 3 below). Since compound III, the N-acetyl MS 222, is rapidly cleared by the gill, we would not expect it in the urine, as column 2 shows. Compound IV, along with II, then accounts for most of the urinary excretion (column 3), 5% of the dose in 2 days.

Table 2

URINARY RECOVERY OF AMINES AND ESTERS AFTER INJECTION OF 200 MG TO S. acanthias, OF MS 222

Hours	Milligrams per period		
	1 Free amine Cpds. I & II	2 Esters Cpds. I & III	3 Total amines I - IV
0 - 5	1	1.2	3.1
5 - 12	0.8	0.7	3.5
12 - 24	0.7	<0.5	1.8
24 - 48	0.8	<0.5	1.3
TOTAL	3.3	1.9	9.7

Further studies were carried out in which the urine was extracted (from pH 7.4 buffer) into chloroform (to remove the esters I and III). The aqueous (containing II and IV) and chloroform layers were then each analyzed for free and total amine. This yielded the data of Table 3.

Table 3

FRACTIONATION OF URINE OF DOGFISH RECEIVING 100 MG I.V. MS 222

Hours	Micrograms per period			
	I	II	III	IV
0 - 2	370	55	< 20	75
4 - 17	165	410	25	600
17 - 42	62	308	< 20	105

It is clear that after the first two hours the polar compounds II and IV take the ascendancy in the urine. All three metabolites were detected in the urine by thin layer chromatography; III appears more prominently than Table 3 implies; this cannot be explained, and is possibly due to contamination with a normal N-acetyl metabolite. Chromatography confirms Table 3 in showing the prominence of II and IV with increasing time.

In summary, 95% of MS 222 is rapidly excreted across the gill within the first two hours after injection, essentially intact but with possibly a small (< 10%) fraction as the N-acetyl derivative. The remaining 5% is excreted by the kidney, following cleavage of the ester bond to yield meta-aminobenzoic acid and its N-acetyl derivative. These acids are excreted at about the rate of glomerular filtration, and so persist in the body for several days.