susceptible to anesthesia with MS 222 by the intravenous route, and also showed about 20% of plasma concentration in brain and CSF (T. H. Maren and R. H. Maren, unpublished observations).

The isopropyl and isobutyl homologues of MS 222 were synthesized by the method of Adams et al (J. Am. Chem. Soc. 48:1758, 1926) and their anesthetic potency compared with that of MS 222 in goldfish. The fish were placed into beakers containing solutions of the methanesulfonates of the drugs at several different concentrations. The time interval from the first exposure of the fish to the drug solution until the disappearance of its tail reflex was measured. Figure 1 shows these time intervals—"induction times"—plotted versus concentration. The anesthetic threshold concentration is about 60 μM for the isobutyl derivative, 200 μM for the isopropyl derivative and 500 μM for MS 222. Lower concentrations produce only sedation over a 60 min period of observation. With all drugs, increasing the concentration above threshold results in a sharp shortening of induction times until at concentrations of about ten times threshold they become equipotent.

From Figure 1, the isobutyl homologue is about 8 times and the isopropyl homologue about two times more potent than MS 222.

The methane sulfonate salt of the isobutyl homologue was injected over a 10-30 second interval into dogfish via the caudal vein or artery. The fish were in the live-car during the following injection. Nine fish were used. The dosage range was 10-100 mg/kg; at the low side there was no effect, and at the high side fish died. At the 20-50 mg/kg dose range there were profound effects on the central nervous system, none of which were observed in this species following MS 222. Initially, and within 15 seconds of the end of the injection the fish made violent and rapid motions through the water, characterized by rearing and weaving of the head and twisting and plunging of the body. This lasted at most 30 seconds, and was followed by anesthesia, which lasted 1-3 hours. During this time breathing appeared regular, but the fish could not swim, and were flaccid. Most of the fish appeared normal the following day. It appeared that the degree of excitement and of toxicity was in part related to the speed of injection.

It is evident that this drug reached the brain in effective concentrations very rapidly. The difference in parenteral effectiveness between the isobutyl and ethyl esters of meta-aminobenzoic acid may be due to different gill and blood-brain barrier diffusion rates. On the other hand, the isobutyl derivative may have a greater intrinsic anesthetic activity than MS 222. Adams et al (vide supra) have shown in the goldfish that anesthetic potency correlates well with lipid solubility for a series of alkyl para-aminobenzoates. In any event, the isobutyl m-aminobenzoate may prove to be a useful parenteral anesthetic in fish, and the study of its distribution and mechanism of action will be an interesting contribution to comparative pharmacology.

1968 #24

ATPase ACTIVITY AND SODIUM GRADIENT IN THE RENAL TISSUE OF Psammomys obesus AND Rattus norwegicus

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The role of the inner zone of the renal medulla in the concentrating mechanism of the mammalian kidney is still not well understood. Experimental evidence clearly supports the hypothe-

sis that sodium is actually transported out of the thick ascending limb of the loop of Henle, and thus that the outer zone of the medulla functions as a countercurrent multiplier, with the active sodium transport being responsible for the increasing sodium gradient from cortex through the outer medulla. However, while the sodium concentration in most mammals increases from the outer medulla throughout the inner medulla, many investigations have failed to show sodium transport out of the ascending limb of the thin loop of Henle. Some recent investigations, however seem to support the hypothesis that sodium is actively transported by the thin limb of the loop of Henle. Jamison has found that the fluid of the ascending limb has a lower osmolality (50 mOs) than that of the descending limb at the same level in the medulla, and that the difference is due to the difference in sodium concentration. It has further been shown that the inulin concentration is higher in the ascending limb of the loop of Henle than in the descending and it therefore seems that the difference in osmolality is due to sodium transport out of the ascending limb, rather than movement of water into it.

Table 1

	Amount Na^+ mEq/mg UFDS*	Conc. Na^+ mEq/1	Wet weight of renal tissue g/100g b.w.
PSAMMOMYS (5 animals) Ave. ± S.E.			
Cortex	35.7 ± 2.6†	103.4 ± 8.4†	0.77 ± 0.07
Outer zone	71.0 ± 2.5	165.4 ± 9.1	0.155 ± 0.010
Inner zone 1	133.3 ± 7.4	166.6 ± 35.8	
Inner zone 2	156.8 ± 6.1	247.0 ± 23.5	
Inner zone 3	162.8 ± 14.4	325.0 ± 25.8	0.161 ± 0.003
Inner zone 4	187.5 ± 20.1	380.0 ± 146.4	
Inner zone 5	250.0 ± 21.2	425.0 ± 50.8	
RAT (10 animals) Ave. ± S.E.			
Cortex	27‡	90 ± 10‡	0.70 ± 0.01
Outer zone	42	115 ± 21	0.086 ± 0.007
Inner zone 1	78	176 ± 40	
Inner zone 2	128	252 ± 45	0.030 ± 0.003
Inner zone 3	182	470 ± 148	

*UFDS means urea free dry solids.

†Bodil Schmidt-Nielsen and Mary Ellen Trimble (in prep.).

‡Saikia, Q. J. Exptl. Physiol. 50:146 (1965).

Considerable evidence suggests that sodium and potassium activated ATPase is connected with the active transport of sodium out of red cells. Furthermore, recent evidence has connected an increase in sodium transport in the mammalian kidney with adaptive increase in ATPase activity (Epstein). If the sodium gradient in the inner zone of the medulla is indeed caused by an active transport in the ascending limb of the loop of Henle, we would therefore expect $Na^+ - K^+$ activated ATPase to be present in the inner zone of the medulla in significant amounts.

The desert rodent Psammomys obesus has a long broad inner zone of the medulla, representing 17% of the kidney weight as compared to 3% in the rat. In the antidiuretic condition the sodium concentration as well as the amount of sodium per mg dry tissue rises throughout the inner zone and shows maximum values at the tip of the papilla (Table 1). The same is true in

the rat kidney (Saikia, 1965). The concentration and amount of sodium however, are greater in the kidney of Psammomys than in the rat.

Na^+ - K^+ activated ATPase was determined in the kidneys of 5 Psammomys and 5 white rats. Kidneys were removed under ether anesthesia and the center part immediately frozen in dry ice and acetone. While still frozen the kidney was divided into zones as shown in Table 1. Care was taken to separate the zones as completely as possible. Assay for ATPase activity were carried out as described by Epstein. Protein was estimated by the method by Lowry et al (1951).

The results show that the ATPase activity is twice as high in the outer zone as in the cortex in both Psammomys and white rat. The activity is higher in the cortex and outer zone of the Psammomys than in the white rat. In the inner zone it was only 1/10 of that of the outer zone. In contrast the inner zone of white rat had about 3 times the ATPase activity as that of the Psammomys (Table 2).

Table 2

Na^+ - K^+ ACTIVATED ATPase ACTIVITY
MEAN VALUES
(μMoles of inorganic phosphate released per mg protein per hr)

Psammomys (10 kidneys)	
Cortex	24.8
Outer zone	47.4
Inner zone 1	4.6
Inner zone 2	3.8
Rat (10 kidneys)	
Cortex	16.2
Outer zone	31.4
Inner zone	12.2

For these determinations the inner zone was only divided in two portions.

If the Na^+ gradient in the inner zone were caused by Na^+ reabsorption by the ascending thin limb of the loop of Henle, we would expect higher ATPase concentrations in the inner zone of the Psammomys, and we would not expect the value to be lower than that in the white rat. Other explanations for the low ATPase activity in the inner zone are being considered.

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1968 #25

ATP–2,3 DIPHOSPHOGLYCERIC ACID RATIOS IN SEAL ERYTHROCYTES

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Substantial concentrations of 2,3 diphosphoglyceric acid (2,3 DPGA) are found almost exclusively in certain mammalian erythrocytes. The precise role of this phosphate in energy me-