obtained from the reduction of **3** with sodium borohydride. The compound melted at 246° to 247°C. Elemental analysis and ultraviolet, infrared, NMR, and mass spectral data were concordant with the assigned structures for **4** and **5**.
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16. This communication is part 94 in the series entitled "Tumor Inhibitors"; part 93 is by S. M. Kupchan (*Revista Latinoamericana de Química*, in press). Supported by grant CA-11718 from the National Cancer Institute, grant CI-102J from the American Cancer Society, and an NIH postdoctoral fellowship to R.M.S.

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Aglomerularism in Antarctic Fish

Abstract. *Urine formation in antarctic bony fish does not involve glomerular filtration. Evidence for aglomerularism came from both direct observation of kidney serial sections by light microscopy and the low concentrations of inulin labeled with carbon-14 that were excreted into the urine when this renal clearance tracer was injected into the bloodstream via a cannula implanted in the caudal vein. Aglomerularism most likely prevents urinary loss of glycoproteins with biological antifreeze properties.*

Certain of the bony fish lack glomeruli as functional units in their renal morphology and consequently have a radically different mode of urine formation from that of the other vertebrates. In its most proximal region, the nephron of these fish ends as a blind tubule with no Bowman's capsule or associated glomus; as a result no filtration process occurs. The urine is formed mainly by active secretion of material across the renal tubular epithelium into the nephron lumen. There is some reabsorption from the formative urine to conserve materials which have moved by passive diffusion from the blood into the tubule lumen, but by far the major process is active secretion (*1*).

Many species of antarctic bony fish avoid freezing because they possess a series of glycoproteins with antifreeze properties (*2–4*). The blood, coelomic fluid, and pericardial fluid all contain these glycoproteins and show a difference of as much as 1.5°C between their freezing and melting points, a phenomenon referred to as thermal hysteresis (*4*). These glycoproteins account for 4 percent of the blood (weight to volume) and they differ only in size (*2*). The concentration (by weight) of the small glycoproteins (molecular weights 8000, 3500, and 2600) is five times as great as that of the larger ones (molecular weights 10,500 to 33,000) (*5*). In the vertebrate kidney molecules with molecular weights less than approximately 40,000 are generally filtered into the formative urine at the glomerulus (*6, 7*), and in view of the high concentration of glycoprotein antifreeze molecules in the blood and their small sizes one would expect them also to be present in the urine of the antarctic fish. However, examination of antarctic teleost urine reveals that it freezes and thaws at the same temperature (approximately -1.0°C), indicating that the glycoprotein antifreeze molecules are not excreted in the urine.

There are two possible explanations for the absence of glycoprotein antifreeze in the urine of these fish: (i) the glycoproteins are filtered at the glomerulus and actively reabsorbed in the proximal tubule of the nephron in much the same manner as glucose or (ii) the kidney of the antarctic fish is aglomerular, preventing the movement of glycoproteins into the formative urine.

In the study reported here whole kidneys were dissected from live animals caught in McMurdo Sound, Antarctica, and fixed for light microscopy in Bouin's solution. The renal tissue was embedded in paraffin, serially sectioned, and stained by standard histological techniques. The kidneys of *Trematomus borchgrevinki, T. bernacchii, T. hansoni, T. lepidorhinus, T. loennbergii,* and *Gymnodraco acuticeps* were completely aglomerular (Fig. 1). Because of its large size the kidney of *Dissostichus mawsoni* could be sampled only in selected regions; however, no glomeruli were found in any of the sections.

The excretion of ^{14}C-labeled inulin (New England Nuclear) was monitored as a physiological test for the aglomerular condition. Clearance of this polysaccharide (molecular weight 5000 to 5500) is commonly employed as a measure of glomerular filtration rate because it is freely filterable at the glomerulus and is neither secreted nor reabsorbed by the tubular epithelium (*6, 8*). Specimens of *T. hansoni, T. bernacchii,* and *D. mawsoni* were fitted with urinary catheters for continuous urine collection and [^{14}C]-inulin was introduced into the bloodstream via a cannula in the caudal vein. Specimens of *T. hansoni* and *T. bernacchii* weighing approximately 200 g each were injected with 20 μc of [^{14}C]inulin, and a 10-kg specimen of *D. mawsoni* was injected with 100 μc. Urine was collected continuously and 100-μl blood samples were collected periodically. Fifty-microliter samples of urine or blood plasma were mea-

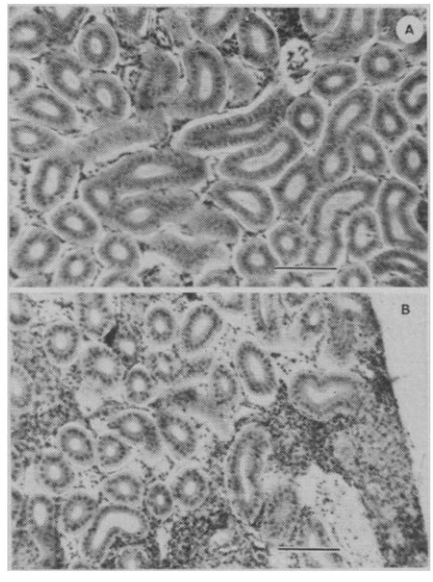

Fig. 1. (A) Photomicrograph of a representative histological section of the renal tubules of *Trematomus hansoni* in the posterior kidney region. (B) Photomicrograph of renal tissue in the trunk kidney of *Trematomus bernacchii*. The intertubular region contains hemopoietic tissue resembling that found in the head kidney. Sections in (A) and (B) were 7-μm thick and were stained with hematoxylin and eosin (scale bars, 100 μm).

Table 1. Urine/plasma ratios (U/P) for [^{14}C]inulin. Values of U/P are the ratios of ^{14}C radioactivity in 50-μl samples of urine and plasma collected 72 to 96 hours after [^{14}C]-inulin was injected. Corrections for quenching were made by adding an internal standard to each sample. After 60 hours U/P remained constant.

Species	Body weight (g)	U/P
Trematomus hansoni	180	0.040
Trematomus bernacchii	200	0.056
Dissostichus mawsoni	9966	0.031

sured into scintillation vials, and 3.5 ml of water followed by 11.5 ml of Aquasol (New England Nuclear) was added to each vial. Shaking the mixture produced a stiff gel. The amounts of radioactivity in the urine and plasma samples were determined by liquid scintillation counting (Nuclear-Chicago, Unilux II). The ratios of [^{14}C]-inulin in the urine to that in the plasma were low even after 72 to 96 hours (Table 1), indicating that these fish are indeed aglomerular. The small amounts of radioactivity in the bladder urine probably resulted from passive diffusion of labeled material into the nephron lumen, possibly in regions of cell death and renewal. Another possible explanation is that labeled inulin moved into the bladder urine by way of a localized region of irritation caused by rubbing of the catheter tip against the bladder wall.

The observed inulin urine/plasma ratios (U/P) are in marked contrast to those obtained by Lahlou *et al.* (*9*) for the aglomerular toadfish, *Opsanus tau*; these authors reported ratios as high as 0.5. Hickman and Trump (*1*) cite an average inulin U/P of 0.15 for the daddy sculpin, *Myoxocephalus scorpius*, a marine teleost described as mostly aglomerular. We interpret our low inulin U/P values as support for the findings with the light microscope.

There is strong evidence that the vertebrate glomerulus is permeable to proteins below a molecular weight of approximately 40,000 and that these filtered proteins are reabsorbed in the proximal segment of the nephron in a process that requires energy (*6*, *7*). The antifreeze glycoproteins present in the blood of antarctic fish in concentrations as high as 4 g per 100 ml of serum (*2*) would be rapidly filtered at the glomerulus if such a structure were present. Because glomerular filtration is absent, no energy expenditure is required for reabsorbing the glycoproteins or other blood proteins, and thus the metabolic cost of osmoregulation in antarctic teleosts may be considerably different from that in other marine teleosts.

All the waters south of the Antarctic Convergence are near their freezing point during the winter. For fish to exploit these food-rich waters some protective mechanism against freezing must have evolved. Aglomerularism may have played an important role because it permitted the utilization of a wide range of sizes of glycoprotein antifreeze molecules without involving energy expenditures for their tubular reabsorption.

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Neighbor Recognition in Two "Solitary" Carnivores: The Raccoon (Procyon lotor) and the Red Fox (Vulpes fulva)

Abstract. *Male raccoons and red foxes were trapped alive and exposed to each other in captivity. Animals of each species trapped close to one another demonstrated a higher frequency of initial dominance-subordinance relationships and lower frequencies of more intense aggressive interactions than did animals trapped at greater distances from each other. This suggests the existence of neighbor recognition and thus a rudimentary social structure within these free-living "solitary" species.*

When considering animal sociality, biologists often categorize each species as occupying a characteristic position along a continuum ranging from solitary to highly social (*1*). This determination is generally based on observations of physical proximity and its behavioral correlates in captive or free-living subjects. However, the ability of human (*Homo sapiens*) observers to assess such characteristics in other species must necessarily be limited by our own perceptual biases and, in the case of animal social behavior, this bias may exaggerate the importance of visually apparent factors (for example, physical proximity) at the expense of other modalities, notably auditory and olfactory. Thus, in a landmark study, Weeden and Falls (*2*) demonstrated that territorial male ovenbirds are not solitary animals arrayed in independent isolation across their habitat; rather, they constitute an interlocking social network within which animals individually recognize their neighbors. The present study extends the social concept of neighbor recognition to include two species of "solitary" mammals, the raccoon (*Procyon lotor*) and the red fox (*Vulpes fulva*).

Although scattered reports of overt social groupings occur for both species, these apparently result from attraction to artificially enhanced food sources (*3*), with some evidence of occasional sociality in the raccoon, particularly during winter denning (*4*). Adult males of both species, however, are acknowledged to be asocial relative to each other, especially during the breeding season. The basic procedure for testing neighbor recognition in these two species was as follows. Free-living animals were trapped alive in eastern King County, Washington, between 23 December 1973 and 25 February 1974, during the early stages of raccoon and fox breeding seasons in western Washington. In all, 13 male raccoons and 7 male red foxes were trapped; they were maintained singly in outdoor turkey-wire cages measuring 4 by 6 by 6 feet (1 foot = 0.3 m) and fed com-