A Possible Role of Acid Mucopolysaccharides in the Urine Concentrating Process

On theoretical grounds STEPHENSON[1] and KELMAN et al.[2] arrived at the conclusion that osmotic work must take place in the inner medulla of a concentrating kidney. The nature of this osmotic work is not as yet clarified. According to BERLINER[3] and JAMISON et al.[4] the thin limbs of the loops of Henle operate as counter-current multipliers. In the opinion of LEVER[5] the same function is carried out by the vasa recta. Recently, MARSH[6] has suggested that osmotic work is contributed by the collecting ducts. These theories seem to agree that a separation of solute from water is carried out by means of selective transport through a membrane; they differ, however, with respect to the structural element of the inner medulla that performs this function.

The purpose of this note is to emphasize that selective transport through a membrane is not the only process by which separation of solute from water can be accomplished, and that necessary means for an alternate mechanism are present in the inner medulla. A new hypothesis will be described presently which is based on two lines of evidence: (1) the renal medulla is rich in acid mucopolysaccharides, (2) solutions containing acid mucopolysaccharides (MPS, e.g. hyaluronic acid) exhibit anomalous osmotic properties.

At a recent symposium OGSTON[7] summarized the evidence which indicates that hyaluronic acid appears to be capable of binding up to 6000 ml of water/g dry substance. It was also demonstrated that from the bound water other solutes of the same solution are, to various extents, excluded, i.e. some of the water that is bound to hyaluronic acid does not serve as solvent for other molecules in the same solution. Thus, it was reported[8] that the osmotic pressures of mixtures of hyaluronic acid and serum albumin were significantly greater than the sum of the osmotic pressures of solutions containing hyaluronic acid and serum albumin separately at the same concentration. Further, in equilibrium dialysis experiments it was demonstrated[9] that the distribution volume of various solutes (e.g. inulin) does not include a significant part of the water in a hyaluronic acid solution.

In view of these observations the following mechanism may be instrumental in the urine concentrating process in the inner medulla. An MPS substance is continuously synthesized in the interstitium, and its water binding property becomes effective upon release from an intracellular site of synthesis. Due to the appearance of excess MPS in the interstitium, the vapor pressure of water is reduced, and water tends to move in from the vascular and tubular channels that penetrate this region. Swelling and loosening of the interstitial tissue follow and water-laden MPS molecules leave through lymphatic and vascular channels. It should be noted that, in the light of OGSTON's analysis[10,11], the presence of albumin in the interstitium may be conducive to an incremented osmotic pressure difference across the collecting duct membrane (which is not permeable to albumin) and to a deficit in osmotic pressure difference across the wall of the vasa recta (which is, at least partially, permeable to albumin). Consequently, water will be more effectively withdrawn from the collecting ducts than from the vasa recta. With respect to the loops of HENLE, osmolality of fluid therein will conform with the description of JAMISON et al.[4] provided that two conditions are fulfilled: (1) both thin limbs reabsorb solute to a moderate extent, and (2) the ascending limb is less permeable to water than the descending limb.

If synthesis and subsequent release of MPS were the only means by which urine is concentrated in the inner medulla, the amount of water reabsorbed from the collecting ducts should be proportional to the rate of turnover of MPS in this region. Accordingly, osmotic concentration of the collecting duct fluid in the inner medulla may attain a high level because (1) turnover of MPS is rapid, or (2) rate of delivery of tubular fluid through the collecting ducts is slow, as its volume has been reduced in more proximal parts of the tubules.

GINETSINSKII[12] has suggested that the effect of antidiuretic hormone is mediated through hyaluronidase enzyme. Although this view has not been generally accepted[13], an impressive volume of information has accumulated that links MPS substances to the urine concentrating process[14,15]. The hypothesis proposed here attempts to explain the role of MPS in a different manner.

A sine qua non of this hypothesis is that, from the maximally concentrating kidney, an excess amount of MPS must be released through the lymph and venous blood. The actual rate of release of excess MPS should depend upon (1) how much solute-free water is reabsorbed from the collecting ducts in the inner medulla (which is probably not more than a fraction of a ml/min in dog kidney) and (2) how much MPS is necessary to bind that amount of water (possibly less than 1 mg/ml). These estimates, which are hardly more than gross approximations, indicate that chemical procedures currently available for MPS determination in plasma[16] may have to be forced to or beyond the limit of sensitivity in order to verify this hypothesis. A comparison of MPS turnover rates in the renal medulla and in other organs (e.g. skin) under conditions of water loading and dehydration may reveal whether this mechanism has phylogenetically developed into a useful tool of water conservation.

In summary, by means of binding more water than solute, MPS may contribute to the urine concentrating process in the inner medulla of the mammalian kidney. This hypothetical mechanism implies that MPS is synthesized in the interstitium, adsorbs water, leaves the kidney through lymph or venous blood and is finally disposed of in another organ. Energy for the concentrating work is provided by synthesis of MPS, and the difference in permeability of the collecting duct and vas rectum membranes constitutes an asymmetry that en-

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sures unidirectional transport of water in this system[17]. Approaches for experimental verification of this hypothesis are discussed[18].

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[18] Addendum. It has been brought to the author's attention that J. KOEFOED and P. J. KNUDSEN [Proc. 1st. Int. Congr. Nephrol. Geneva/Evian 1960; pp 571–573 (1961), S. Karger, Basel] have proposed a mechanism according to which enzymatic degradation of hyaluronic acid may contribute to the creation of a hyperosmotic environment in the renal medulla.
[19] Participant of the USPHS Career Award Program.

Résumé. Par l'adsorption de plus d'eau que de corps dissous, les acides mucopolysaccharides (MPS-s) de la medulla interne du rein peuvent participer à l'élaboration d'urine concentrée. Ce nouveau mécanisme hypothétique laisse supposer que le MPS est le produit d'une synthèse cellulaire et qu'il prend l'eau d'adsorption au moment de la secrétion. Ensuite, le MPS quitte le rein par les voies veineuses et lymphatiques et enfin se détruit dans un autre organe.

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Zur Frage der Indolyl-3-acetaldehyd-Mutase in Mikroorganismen

Beim Tryptophanabbau durch Mikroorganismen und höhere Pflanzen wurde unter anderem das Auftreten von Indolyl-3-acetaldehyd (IAAld), Indolyl-3-essigsäure (IES) und β-(Indolyl-3)-äthanol (β-IÄ, Tryptophol) beobachtet[1–12].

Der biogenetische Zusammenhang dieser 3 Substanzen wird oft in einer Dismutierung des IAAld zu IES und β-IÄ gesehen. TEUSCHER[1] nimmt eine spontane Disproportionierung an, während KAPER und VELDSTRA[2] bei *Agrobacterium tumefaciens*, SCHWINN[3] bei *Phytophthora cactorum* und LIBBERT[4] bei *Pisum sativum* und andere (siehe auch GORDON[5]) eine enzymatische Dismutierung vermuten.

Die Existenz einer Indolyl-3-acetaldehyd-Mutase wurde allerdings von KLUNGSÖYR[12] angezweifelt. Sie bezog sich dabei besonders auf den Nachweis einer IAAld-Oxidase in Avena-Coleoptilen[13] und auf einige eigene Experimente beim Tryptophanstoffwechsel. Nach ihren und unseren Versuchen ist keinesfalls eine spontane Dismutierung zu erwarten. IAAld wird in wässrigen Lösungen lediglich zu einem verschwindend geringen Prozentsatz durch Luftsauerstoff[14] zu IES oxydiert. Der grösste Teil des eingesetzten IAAld geht durch Polymerisation verloren. β-IÄ ist nicht nachweisbar[10,14]. Demnach käme also höchstens eine enzymatische Disproportionierung in Frage. Die Existenz einer Acetaldehyd-Mutase zum Beispiel in Milch, die zunächst von DIXON und LUTWAK-MANN[15] angenommen wurde, ist allerdings durch Arbeiten von RACKER[16] zweifelhaft geworden. Es ist wahrscheinlicher, dass gleichzeitig eine Aldehydreductase und eine Aldehyddehydrogenase in sehr unterschiedlicher Quantität und Aktivität vorliegen.

Wir untersuchten diese Frage am Beispiel einiger Mikroorganismen, deren Tryptophanstoffwechsel zum Teil ziemlich genau bekannt ist[2,10] (Tabelle).

Bei Endomycopsis und Torulopsis findet sich nach Fütterung von IAAld nur β-IÄ und keine IES. Die Beteiligung einer Mutase am IAAld-Abbau kann damit für diese Stämme ausgeschlossen werden. Bei den beiden *Hansenula*-Stämmen und bei *Agrobacterium* lassen sich beide Indolderivate nachweisen. Da jedoch 5–20mal mehr β-IÄ als IES auftritt, müsste neben einer eventuell vorhandenen Mutase eine sehr aktive Aldehydreductase auftreten.

In zellfreien Extrakten mit IAAld und $NADH_2$ lässt sich durch die Abnahme der Extinktion bei 340 nm ein Verbrauch von $NADH_2$ durch eine Aldehydreductase[18] nachweisen (Figur 1).

In Ansätzen mit IAAld und NAD steigt dagegen die Extinktion an (Figur 1, Kurve I). Der bei der Oxydation des IAAld freiwerdende Wasserstoff wird offensichtlich auf NAD übertragen. Demnach liegen nebeneinander eine Aldehydreductase und eine Aldehyddehydrogenase vor. Werden die Enzymlösungen hochtourig zentrifugiert (> 250000 *g*), so sedimentiert die Aldehydreductase rascher als die Dehydrogenase. Die nachweisbare Reductaseaktivität ist gegenüber weniger intensiv zentrifugierten (70000 *g*) und anschliessend dialysierten Ansätzen deutlich vermindert.

Setzt man nicht dialysierten Extrakten (70000 *g*) NAD zu, so steigt die Extinktion unter Oxydation des nicht ab-

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[18] Wir wählten den Ausdruck «Aldehydreductase» anstelle des üblichen «Aldehyd-oxidoreductase», da wir bisher die Reversibilität der Reaktion nicht nachweisen konnten.