in their absence. Furthermore, the peak concentration of the SMBV ($-C^{14}$) was always lower in the tube in the presence of BMV than when SBMV(C^{14}) was sedimented by itself or with TMV. These data show that non-ideal sedimentation of a major component during density-gradient centrifugation can result in widening of the zone of a minor component that it overlaps and in a different apparent sedimentation rate of the minor component.

Sucrose density-gradient centrifugation is often used to determine the size of particles carrying a certain biological or chemical activity. Results in such experiments are obtained by determining the activity, for example, virus infectivity, enzymatic activity, or a radioactive tracer, at various depths in the column after centrifugation. Viruses and proteins form wide zones at high concentrations (*1*). Apparently non-ideal sedimentation, which causes the zone spreading, is pronounced at concentrations higher than 2 to 3 mg/ml and the sedimenting material spreads until the maximum concentration does not exceed 2 to 3 mg/ml. Our studies show that it is not the concentration of an individual component that determines the amount of non-ideal sedimentation and zone spreading, but rather the total concentration of materials sedimenting together. If this concentration is high, and followed by the determination of enzymatic or other activity, a particle present in low concentration will give neither a true indication of its homogeneity nor an accurate value of its sedimentation rate. For good resolution (*1*), the amount of sedimenting material should be less than 1 mg for the 2.5- by 7.6-cm tube of the SW 25 rotor of the Spinco model L.

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Countercurrent Multipliers in Avian Kidneys

Abstract. *The capacity to conserve urinary water by producing a concentrated urine is directly related to the number of Henle's loops in the kidneys of three terrestrial birds. This suggests that a Henle's loop countercurrent multiplier is responsible for urine concentration in these birds. Several features of the organization of the kidneys of these birds may account for the importance of multiplier number, as contrasted to multiplier length in mammals, in determining maximum urine concentration.*

Studies of salt and water balance in several races of Savannah sparrows and in the house finch showed that each of these taxa differs conspicuously in capacity to produce a concentrated urine (*1*). In addition, none of these taxa has functional salt glands (*2*). Therefore, a study of the renal and cloacal histology in representatives of these taxa was undertaken.

Two *Carpodacus mexicanus* (house finch), two *Passerculus sandwichensis brooksi*, and two *P. s. beldingi* (Savannah sparrows) were used. The drinking responses and urine-concentrating abilities of these individuals were typical for their taxa (*1*). The study showed that there are no gross differences in cloacal structure between taxa, but that there are differences in renal medullary development which are directly related to urine-concentrating ability. These differences involve the number and length of Henle's loops and therefore suggest that urine concentration is accomplished by a countercurrent multiplier system in the loop of Henle.

In birds, the medullary nephrons, characterized by the presence of Henle's loops, are scattered throughout the kidney lobes in groups called medullary lobules—that is, medullary cones (Fig. 1*A*) (*3*). There are several cortical lobules for each medullary lobule. These cortical portions, which consist of collecting tubules, and all parts of the nephron except the Henle's loop are analogous to terminal twigs in the dendritic organization within each kidney lobe. The medullary lobules are analogous to branches. Each medullary lobule unit is surrounded by a connective tissue sheath and can be recognized by a ring of collecting tubules which surrounds capillaries and thin segments of Henle's loops, and by concentric "layers" of thick segments of Henle's loops around the ring of collecting tubules (Fig. 1, *B* and *C*). The medullary lobules connect with ureteral branches which are analogous to limbs in the dendritic system. As a medullary lobule courses toward a ureteral branch, it often becomes contiguous with other lobules, forming a group of lobule units which is then enclosed in a common sheath of connective tissue (Fig. 1, *A* and *C*). Each lobule unit in the group becomes smaller as it approaches a ureteral branch because of a decrease in the number of Henle's loops (Fig. 1, *C* and D_{1-3}). Near the junction with a ureteral branch (Fig. $1D_3$) most collecting tubules have fused so that they are larger but fewer in number and only the longest Henle's loops are present. When all the collecting tubules from a lobule unit or group of lobules have fused, the resulting tubule is called a ureteral branch. These successively fuse until the trunk of the dendritic system, the ureter, is formed (Fig. 1*A*).

Because of these features of avian kidney organization, the average length of the Henle's loops in the kidney can be determined by averaging the number of concentric "layers" of thick segments of Henle's loops surrounding the ring of collecting tubules in each medullary lobule unit. An index of the number of Henle's loops in the kidney can be determined by averaging the number of medullary lobules in each kidney lobe since in birds only a few nephrons have Henle's loops (*4*). To obtain the average number of looped nephrons and the average length of the loops the relevant measurements were made in every fifth, transverse 8-μ section of the right and left pos-

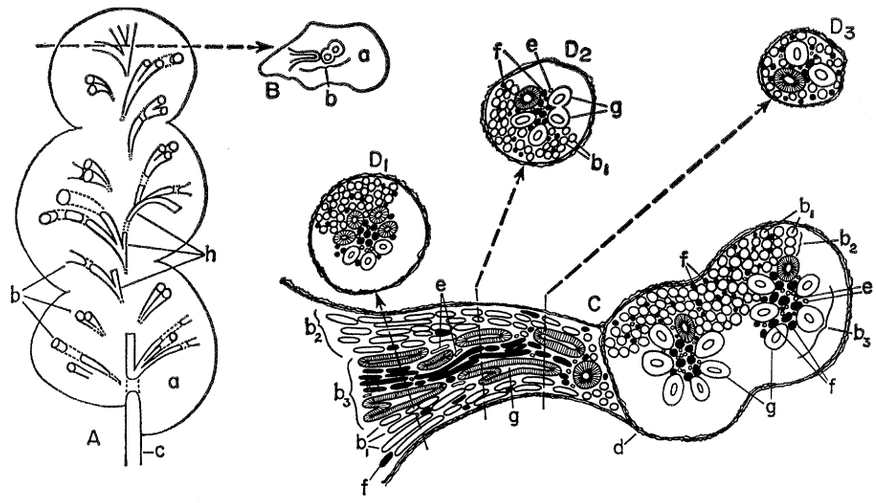

Fig. 1. Semi-diagrammatic representation of avian kidney organization as seen in *Carpodacus mexicanus*. (*A*) A parasagittal section through one kidney showing medullary portions on both sides of the plane of sectioning. (*B*) Transverse section through the anterior-most kidney lobe. (*C*) An enlarged view of the medullary portion of the kidney as shown in *B*. (D_1, D_2, D_3) Cross sections of an individual medullary lobule progressing from near the cortex toward its connection with a ureteral branch. Designations are as follows: *a*, cortex; *b*, medulla; b_1, thick segments of Henle's loops; b_2, layers of Henle's loops; b_3, ring of collecting tubules around capillaries and thin Henle's loop segments; *c*, ureter; *d*, connective tissue sheath around medullarly lobule; *e*, thin Henle's loop segment; *f*, capillary; *g*, collecting tubule; and *h*, ureteral branches.

terior kidney lobe from each bird. (A weight-specific expression for the numbers of looped nephrons was not necessary since all the birds weighed between 18 and 22 g and had similar weight-specific amounts of kidney tissue.)

The evidence suggests that the urine-concentrating ability of these birds is linearly related to the number of Henle's loops (average number of lobule units per section, Fig. 2) but is not obviously related to the average length of Henle's loops (average number of layers of loops per lobule unit, Fig. 2). The extrapolation of the urine/plasma ratio as a function of lobule units per section to a urine/plasma ratio of one at zero units per section is consistent with this because it suggests that no urine concentration can take place when there are no Henle's loops.

This evidence strongly suggests that urine concentration in the birds studied is accomplished by a countercurrent multiplier system in the loops of Henle. Such a system has been well demonstrated in mammalian kidneys (*4*). It is interesting that the urine-concentrating ability of these birds is determined by the number of nephrons having Henle's loops and not by the average length (Fig. 2) or the maximum length of the Henle's loops (*5*). In contrast, urine-concentrating ability in mammals is determined by the lengths of the long Henle's loops and not by the number of nephrons having long Henle's loops (*4*).

It is of interest to suggest some possible reasons for this apparent difference between birds and mammals, since the nature of a Henle's loop countercurrent multiplier is such that both number and length of the loop multiplier could be important determinants of maximum urine-concentrating ability (*5*). In birds, only a few of the nephrons have Henle's loops (*3*) and in the birds examined in this study the medullary lobules showed no preferential orientation (*5*) (Fig. 1*A*). Among these taxa the increase in the number of nephrons with loops has added substantially to urine-concentrating ability (units per section, Fig. 2). In contrast, all mammalian nephrons have loops and these are parallel to one another. Thus the most efficient way of adding substantially to urine-concentrating ability is to increase the length of the existing loops and this has been done (*4*), though it could also have been accomplished by increasing the proportion of nephrons with long loops. Birds are apparently limited in their potential to increase the length of loops because of the flattened and trilobate nature of their kidneys. This limitation is apparent in the lack of difference in length of the longest loops among the three taxa discussed here (*5*) and is reflected by the narrow range of values of average loop length in these taxa (loop layers per unit, Fig. 2).

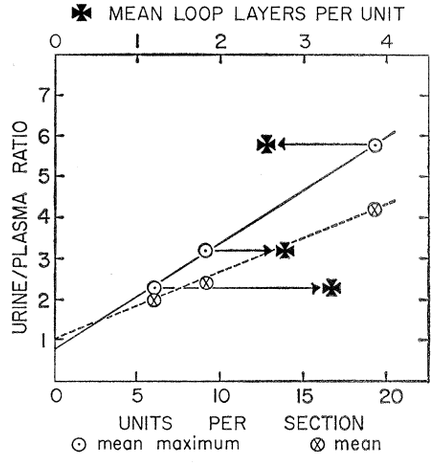

Fig. 2. Urine-concentrating ability as a function of number and length of Henle's loops (urine/plasma ratios shown are for total osmolarity; chloride urine/plasma ratios also show a linear relationship to the numbers of Henle's loops). The number of Henle's loops (indexed by units per section) is linearly related to the potential urine/plasma ratio, whereas the length of Henle's loops (indexed by loop layers per unit) is not obviously related to the potential urine/plasma ratio. The taxa represented are *Carpodacus mexicanus*, urine/plasma ratio of 2.3; *Passerculus sandwichensis brooksi*, urine/plasma ratio of 3.2; and *P. s. beldingi*, urine/plasma ratio of 5.8 (ratios from ref. *1*).

It is also of interest to examine the reasons why the presence of a good urine-concentrating mechanism in birds has not been considered previously. One reason is that, despite the presence of Henle's loops in all avian kidneys examined previously, the birds for which data on urine-concentrating ability were available show maximum urine/plasma ratios of only 1.2 to 1.8 (*2*, *3*). These birds include domestic fowl, domestic pigeon, and many marine birds with functional salt glands. In contrast, the birds reported on here are granivorous passerines and they do not have functional salt glands. They have severe problems of water economy in comparison to birds studied previously and they can produce very concentrated urine (*1*) (see urine/plasma ratios, Fig. 2). The data presented here indicate that the basis for this concentrating ability is a Henle's loop countercurrent multiplier system.

Another reason for the presence of any kind of well-developed urine-concentrating system not being considered previously is that any extensive reabsorption of water from the kidney tu-

bules could precipitate enough uric acid to clog the tubules (*3*, *6*). Actually, though it has not been suggested previously, I propose that the structure of the avian kidney makes clogging unlikely in the countercurrent multiplier method of urine concentration which depends on reabsorption of water from the collecting tubules. As the collecting tubules course toward the ureter and become ureteral branches, they fuse successively and thus gradually increase in size (*3* and Fig. 1, *C* and D_{1-3}). In effect this means that, unlike mammalian ureters, avian ureters are continuous with the collecting tubules (*3*). Thus the pronounced milking action of a bird's ureters (*7*) can pull a sludge of uric acid out of the collecting tubules. Also, the mucus produced by the glands of the collecting tubules (*3*) facilitates the passage of this uric acid through the collecting tubules.

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18 February 1965

Lactate Dehydrogenases in Trout: Evidence for a Third Subunit

Abstract. *Various tissues of the brook trout contain as many as nine forms of lactate dehydrogenase, an indication that at least three polypeptide subunits synthesized under the control of three nonallelic genes take part in the lactate dehydrogenase composition of this species. There was no evidence of a spermatozoan-specific lactate dehydrogenase.*

The occurrence of five molecular forms of lactate dehydrogenase (LDH) in tissues of most vertebrate classes has been well documented (*1*). Synthesis of the catalytically active protein requires the assembly of two classes of polypeptide subunits (A and B) into a tetramer. Since the polypeptide monomers are separable on the basis of charge, their random association would be expected to produce as many as five electrophoretically distinguishable isozymes of lactate dehydrogenase. On the basis of monomeric composition, LDH-1 (A^0B^4) and LDH-5 (A^4B^0) would thus represent two different proteins whose synthesis was controlled by two separate genetic loci. One gene regulates the synthesis of polypeptide A and the second of polypeptide B. This hypothesis is supported by chemical analysis of the amino acid composition of LDH-1 and LDH-5 (*2*), immunochemical analysis of antigenic properties of the two isozymes (*3*), and dissociation in vitro of tetramers and recombination of monomers to form LDH-2, LDH-3, and LDH-4 (*4*). Additional evidence is provided by the observations of variant isozymes in deer mice (*Peromyscus maniculatus*) and in human red blood cells, presumably produced by mutation of the gene at the B-locus (*5*), and similarly by a report of an A-locus mutation in human erythrocytes (*6*).

There is now compelling evidence for a third genetic locus active only in the testes of sexually mature mammals and birds (*7*) and responsible for the synthesis of the spermatozoan-specific LDH which was described independently by Blanco and Zinkham and by me (*8*). Thus, there is contained in spermatozoa a third class of subunit, C, which confers distinctive properties on the LDH it composes and which can combine with B monomers from LDH-1, at least in vitro (*9*). Presumably, control of synthesis of polypeptide C is associated with repression of the C-locus in immature testes as well as in the various other cell types in the animal. I now present evidence for the occurrence of an active C gene locus in all tissues of the speckled trout, *Salvelinus fontinalis*.

Multiple forms of LDH were separated by electrophoresis on polyacrylamide gels and localized on the gels as previously described (*9*). Organs were removed from the freshly killed fish, washed in ice-cold 0.1*M* sodium phosphate buffer to remove excess blood, blotted with filter paper, weighed, and then disrupted in a glass homogenizer in an amount of buffer sufficient to make a 10-percent homogenate. Spermatozoa were treated in

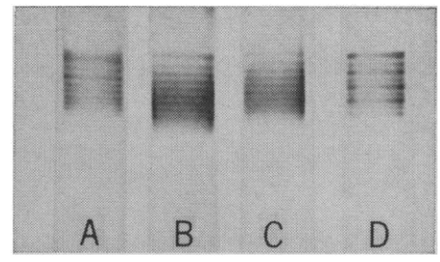

Fig. 1. LDH isozyme patterns in speckled trout tissues. The sample was applied to the top of the gel and the proteins migrated toward the anode. *A*, muscle; *B*, testes or sperm; *C*, heart; *D*, ovary.

approximately the same way except that the cells were washed by centrifugation and disrupted by high-frequency sound. Testes were homogenized and then disrupted by high-frequency sound. Skeletal muscle was first disrupted in a blender and then homogenized. All of the extracts were centrifuged at 4°C for 20 minutes at 10,000*g*, and the supernatants were used for assay. The LDH activity in the extracts was assayed spectrophotometrically by measuring the oxidation of reduced nicotinamide adenine dinucleotide (NAD) at 340 mμ with pyruvate as substrate. On this basis, it was possible to add to each gel samples of the various preparations containing the same LDH activity as measured by the changes in absorbancy per minute. Usually an amount of extract causing a decrease in absorbance of 0.10 per minute was added to a gel. Extracts were prepared from heart, muscle, testes, sperm, and ovary.

Most of the tissues examined had as many as nine isozymes of LDH but none of these isozymes were unique to the sperm cell. Tissue specificity of LDH components is reflected only by differences in relative activity of the isozymes (Fig. 1). The most rapidly migrating anodal forms were most active in the heart preparation as compared to the approximately equal activity of the testicular isozymes. The muscle LDH pattern was comparable to that of the heart, which suggests that there is no predominant muscle or heart form of the enzyme in this species (*3*). Perhaps the most distinctive pattern was observed in extracts of ovary where there were five bands indicating intense activity, with four bands of lesser enzyme activity interspersed. The mobilities of corresponding isozymes from each tissue were coincident as indicated by the appear-