the possibility of testing the thesis that histones function as gene modifiers.

Comment should also be made regarding the comparative features of the alkaline Fast Green and A-S staining procedures. As already stated, staining with alkaline Fast Green requires extraction of DNA, whereas staining with A-S requires only treatment with formalin. It would appear that alkaline Fast Green combines with those bonds of histone molecules which are ordinarily combined with DNA. In contrast, the A-S reaction involves non-DNA-bound histone groups; that is, groups which would, presumably, be free to react in the cell nucleus. Differences between the reaction of A-S and alkaline Fast Green with histones are also demonstrable when isolated histones are studied. As reported previously (*9*), A-S stains lysine-rich histones more avidly than arginine-rich histones. In contrast, alkaline Fast Green stains lysine and arginine-rich histones with almost equal intensity. The affinity for reactive sites not bound to DNA in the lysine-rich fractions of histones may account for the ability of A-S to discriminate more subtle differences in cell function and differentiation.

The addition of this information to our previous findings regarding the A-S staining of cells and histone electropheragrams leaves little doubt that A-S stains histones in a preferential manner and that the procedure is of value in cytochemical investigations. The dipteran salivary chromosomes may prove to be useful for the investigation of the biological function of histones. We submit that our findings suggest that different genes may have different histones.

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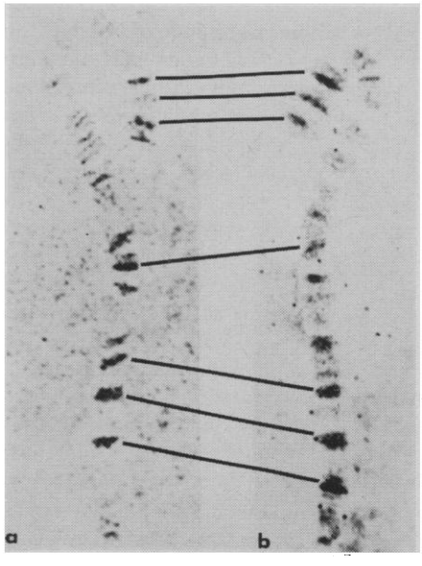

Fig. 3. Chromosome II, of *Sciara coprophila*, stained with A-S. Band-for-band comparison after (*a*) blocking and (*b*) partial extraction demonstrates differences in histone composition among different loci of the same chromosome. Feulgen staining discloses that DNA components of the bands are unaffected by either treatment.

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Autoradiographic Distribution of Radioactive Sodium in Rat Kidney

Abstract. *Autoradiographs of kidney sections from rats injected with sodium-22 showed two areas of relatively high concentration of the radioisotope. The first area appeared in the outer medulla; the second appeared as an area of increasing concentration toward the papilla. Autoradiographs of sections labeled with chloride-36 appeared essentially the same. Autoradiographs of sections labeled with inulin-$C^{14}OOH$ showed an increased concentration in the inner medulla. These results may have a bearing on the counter-current multiplier hypothesis for the production of concentrated urine.*

In 1951 Hargitay and Kuhn (*1*) described in detail their counter-current hypothesis for the production of a concentrated urine in the mammal. It was proposed that the loop of Henle functions as part of a counter-current multiplier system, yielding a progressively increasing gradient of solute concentration in the tubular fluid and surrounding interstitium from cortex to papilla. As the urine in the collecting ducts passed through the region of high solute concentration, it was proposed that water was withdrawn to form a concentrated urine. In confirmation of the general hypothesis, micropuncture studies by Gottschalk and Mylle (*2*) showed that urine in the proximal convoluted tubule was isotonic with plasma, that early distal tubular fluid was hypotonic with plasma, and that fluid in the tip of Henle's loop had the same osmolarity as fluid from the adjacent collecting ducts. From these and other considerations, it was suggested that sodium is actively transported into the interstitial fluid, taking with it chloride ions to maintain electrical neutrality, and thereby causing the pattern of increasing solute concentration. The actual site for active sodium transport has been attributed to the whole length of the ascending limb of Henle's loop (*3*), to the thick ascending part of the loop (*4*), and to both the descending and ascending limbs of the loops of Henle (*5*). Water supposedly does not move with the sodium and chloride in these areas during antidiuresis; therefore, the areas of active transport must be impermeable or less permeable to water. The uncertainty in delineating the actual site of active sodium transport is due to the difficulty in obtaining samples of urine from the outer medulla, the zone of the kidney containing the thick ascending parts of Henle's loops.

Table 1. The concentration of Na^{22} and stable sodium, and the specific activity of Na^{22} in the cortex, outer medulla, and inner medulla of rat kidney. Values represent the mean ± standard error of the mean of six rats.

Tissue sample	Na^{22} (% dose/ g wet tissue)	Stable Na (meq/ g wet tissue)	Specific activity (% dose Na^{22}/ meq stable sodium)
Cortex	.35±.04	.096±.008	3.6±0.4
Outer medulla	.54±.04	.121±.007	4.0±0.4
Inner medulla	.87±.08	.198±.021	3.7±0.4

In our investigation, the autoradiographic distribution of Na^{22}, Cl^{36}, and inulin-C^{14} in rat kidney was determined. Theoretically, this procedure would show a gradation of radioactivity from cortex to papilla if the above contentions were correct. Mature rats weighing about 400 to 500 g were injected intravenously with either carrier-free Na^{22} (32 to 40 μc), carrier-free Cl^{36} (20 to 25 μc), or inulin-C^{14} (25 μc; uniformly labeled; specific activity 0.0021 mc/mg). After various time intervals, the animals were anesthetized with ether and a midline abdominal incision was made. After ligation, the kidneys were rapidly removed and placed in isopentane at −70°C. The whole kidney was sectioned longitudinally in a cryostat microtome maintained at −20° C and 10-μ frozen sections were transferred without melting to glass slides. Non-screen x-ray film, also cooled to about −70°C, was then clamped over each section. The whole "sandwich" was transferred to a freezer kept at −20°C for exposure sufficient to give adequate autoradiographs. Adjacent sections were used for staining and histological examination.

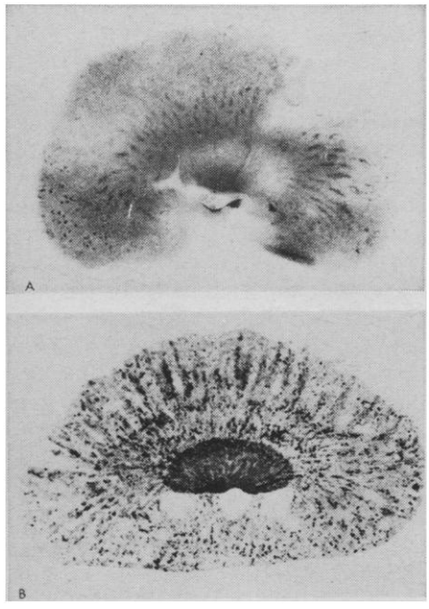

Fig. 1. Reverse autoradiographs of cross sections, parallel to the long axis of the kidney, from rats injected with Cl^{36} (*A*); and with inulin-C^{14}OOH (*B*). In each section, light areas denote deposition of the radioisotope. The most peripheral areas of exposure correspond to the cortex. In (*A*), the second area, containing the first band of increased exposure, corresponds to the outer medulla; in (*B*) this area contains streaks and spots of exposure. The third area, which contains the second band of increased exposure in (*A*), corresponds to the inner medulla; in (*B*) the entire area of increased exposure corresponds to the inner medulla.

Representative autoradiographs of sections with the three substances are shown in the cover photo and in Fig. 1. Radioactive sodium appeared to be uniformly distributed throughout the cortex with an increased concentration in the outer medulla. At the junction between the outer and inner medulla, there was a banding effect caused by a region of decreased radioactivity, followed by a region of increased deposition toward the papilla. Krakusin and Jennings (*6*) previously demonstrated the accentuated deposition of Na^{22} in the inner medulla of rat kidney, but they did not show the increased deposition within the outer medulla; the reason for the absence of this zone of Na^{22} concentration in the earlier report is not known. The autoradiograph of the Cl^{36}-labeled kidney section had essentially the same pattern as that labeled with Na^{22}. The autoradiograph of the section labeled with inulin-C^{14} revealed a different pattern of distribution: (i) the cortex and outer medulla showed a spotty distribution of inulin-C^{14} with the overall concentration being generally lower than the inner medulla; (ii) at the junction between the outer and inner medulla, there was an abrupt increase in the radioactivity of inulin-C^{14}; (iii) there was a further increase in the activity of inulin-C^{14} that occurred progressively toward the papilla.

For purposes of interpretation, it was necessary to know whether the discontinuous patterns for Na^{22} and Cl^{36} were due to technical artifacts or to a lag in equilibration of labeled ions with their stable counterparts. Autoradiographs of kidney sections from rats previously injected with I^{131}-labeled albumin and with Ca^{45} did not show the discontinuity seen with Na^{22} and Cl^{36}, indicating that the sectioning procedure could not account for the present observations. To rule out slow equilibration as a factor, both kidneys from each of six rats were removed 20 hours after the intravenous injection of Na^{22}. One kidney was sectioned as before to obtain the autoradiographic distribution pattern which was the same as shown in Fig. 1; the other kidney was also cut longitudinally in thin sections and samples from the cortex, outer medulla, and inner medulla were taken. These samples were analyzed for Na^{22} content by gamma-ray counting in a well-type scintillation detector and for stable sodium by flame photometry. Table 1 shows that there was the expected relative increase in concentration of Na^{22} and stable sodium from the cortex, through the outer medulla to the inner medulla, as previously reported. The specific activities of these areas were not significantly different and, therefore, the autoradiographic patterns could not be explained on the basis of a slower equilibration of Na^{22} in various sections of the kidney.

Any interpretation must be tempered by the fact that the exact distribution of these labeled materials between cellular and extracellular sites cannot be delineated from the present technique. However, several interesting points arise from these observations. First, there appears to be an area of high sodium chloride concentration in the outer medulla, the area where the thick ascending part of the loop of Henle and the descending part of the proximal tubule are located. Second, there is little increased water reabsorption in this area because the inulin concentration is about the same in the outer medulla as in the cortex. Third, it is only in the inner medulla, where the thin parts of the loops of Henle and the collecting ducts are located, that an increasing gradient toward the papilla of sodium, chloride, and inulin is seen. It would therefore seem that there is not a continuous gradient of sodium chloride in the rat kidney from the cortex through to the papilla as has been proposed by tissue analyses, but rather that there are two areas of concentration: one in the outer medulla in which water is not reabsorbed, and one in the inner medulla in which water is reabsorbed. Whether or not the same pattern holds for the osmotic pressure gradient cannot be deduced from the present data.

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