Incorporation of Phosphorus-32 into Salivary-type Chromosomes which exhibit 'Puffs'

A PRELIMINARY study has been made of the rate of incorporation of phosphorus-32 into the chromosomes and associated structures in the salivary gland of a Chironomid, *Metriocnemus hygropetricus*, and since there will be no immediate opportunity to carry the investigation further it seems worth while to present the results, which even at the present stage are not without interest. It has been shown by Prof. H. Bauer (who kindly made available some animals whose progeny were used for this study) that the salivary chromosomes of this species exhibit 'puffs' or Balbiani-rings of various kinds. Larvæ were grown on medium containing phosphorus-32 (as phosphate) for periods of 2–24 hr., and the incorporation was studied in autoradiographs of squashes and sections, which were stained with methyl-green-pyronin and in some cases extracted with ribonuclease or hydrochloric acid.

Both the nucleolus and the puffs stain bright red with pyronin, while the bands in the chromosomes stain with methyl-green; the pyronin staining disappears after ribonuclease digestion. Incorporation of tracer was adequate after feeding the larvæ on labelled medium for 4–8 hr., when the grain counts per unit area over the various components were:

	Cytoplasm	Puffs	Rest of chromosome	Background
4 hr.	14·2 ± 1·4	36·2 ± 2·1	4·9 ± 1·0	2·7 ± 0·1
8 hr.	33·7 ± 2·4	61·4 ± 2·7	6·1 ± 0·8	4·6 ± 0·5

The figures for 4-hr. exposures were taken from 10 cells belonging to 3 larvæ, and those for 8 hr. from 20 cells from 5 larvæ. Nearly all the activity was removed from cells treated with ribonuclease or hydrochloric acid. It is clear that the puffs are very much more active per unit area of squash than the remainder of the chromosomes, which show an incorporation very little above the background. It is not obvious what would be the relative activities of the two types of structure if one used the content of ribonucleic acid as the basis of comparison, since the staining reactions show that this substance is much more highly concentrated in the puffs (and somewhat more in the cytoplasm) than it is in the remainder of the chromosomes.

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Demonstration of Early Renal Uptake of Potassium-42 by an Autoradiographic Method for Water-soluble Isotopes of Short Half-life

STUDIES of the distribution of intravenously injected potassium-42 in rats have shown that the kidney takes up this cation from the plasma at considerably higher rates than does any other organ[1,2]. In the first few minutes after the injection, the specific activity in the renal tissues exceeds that of the plasma[2,3]. At 2 min., approximately 15 per cent[1] and 10 per cent[4] of the injected dose were found in rabbit kidneys and about 12 per cent (unpublished observations) in rat kidneys. These fractions are far greater than the expected equilibrium value. Analysis of these findings indicated that all the potassium arriving in the plasma in the kidney is exchanged with potassium from cells. Morel and Guinnebault have recently presented evidence that the renal cortex is the main site of this very rapid exchange[3]. From their experiments it appears that in the rabbit as much as 15 per cent of the total potassium present in the cortex is exchanged per minute. This was corroborated macroscopically by autoradiography in which X-ray films were exposed to 2-mm. thick frozen kidney slices. The resulting autoradiograms indicated that in the 5 min. following an intravenous injection, the intense activity emanated from a region roughly corresponding to the cortex.

The purpose of the present experiments was to obtain information microscopically about the difference in potassium-42 uptake in the various parts of the nephron. Potassium-42 bicarbonate, the chosen carrier, is highly soluble in water. This prevented the use of the traditional histological techniques. Freeze-drying followed by vacuum-embedding was precluded by the short half-life of the isotope (12·4 hr.), no useful exposure of photographic emulsion being possible after three half-lives. The technique described below circumvented these limitations.

After the intravenous injection of 25–100 μc. of potassium-42 into rats, their kidneys were excised at 2 min. and sections prepared for autoradiography or by Gomori's alkaline phosphatase technique[5]. Slices 1–2 mm. thick were promptly transferred into absolute acetone, in which they were fixed for 30 min. This was immediately followed by immersion in polyethylene glycol ('Carbowax 1000') at 44° C. for 1 hr., the shortest period found to allow complete infiltration. The tissues were then blocked in 'Carbowax 1000' and hardened at 3° C. Chemically clean slides were coated with an aqueous solution of 0·0001 per cent chrome alum and 0·005 per cent gelatin, and dried in a dust-free atmosphere. These slides were then flooded with acetone and 10-μ sections floated on to them. With acetone, the surface tension effects which cause disruption of 'Carbowax' sections when flattening is attempted on aqueous solutions did not occur. Acetone also ensured removal of 'Carbowax' without loss of potassium-42.

Groups of three adjacent sections were treated in two ways. The use of Gomori's alkaline phosphatase technique on the first and third section of a group allowed definite identification of proximal convoluted tubules. The middle section was used for autoradio-

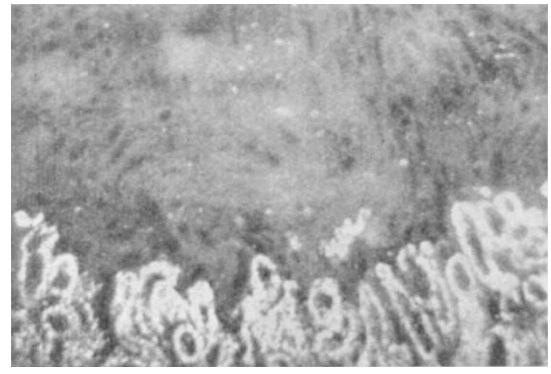

Fig. 1. Autoradiogram showing distribution of potassium-42 activity in juxtamedullary cortex. There is no evidence of medullary activity. Silver grains in emulsion showing as bright specks. Dark-ground illumination. × 60