The Deposition of Ingested Silver in the Rat Kidney at Different Ages

When silver is administered to experimental animals by replacing the drinking water by a very dilute solution of $AgNO_3$, silver is deposited in the basement membranes of a variety of tissues. This phenomenon has been used to study basement membranes in the eye[1], the brain[2–4] and in the renal glomeruli[5–10]. Since silver is almost certainly carried in the blood as a silver-protein complex and the sites of silver deposition correspond, in the adult rat at least, to the sites at which the vessels are permeable to protein[11], it was previously thought that silver administration could be used to investigate vascular permeability to protein in the renal medulla[12]. However, experiments which involved the rat and the rabbit, showed striking species differences in the distribution of silver granules in the kidney which suggested that factors other than permeability to protein might be involved. Further studies on renal vascular permeability using other methods[13] revealed that extravasation of protein occurs to a much smaller extent in immature rats (i.e. up to 3 months) than in the adult. In view of these findings, it was decided to compare the distribution of deposited silver in the kidneys of immature rats with that in adult rats which had been given silver nitrate for similar periods.

Silver was administered in the drinking water of 26 weanling rats in the form of a 0.15% aqueous silver nitrate solution for periods of from 4 to 15 weeks. The animals were weighed weekly and compared with control animals of the same age kept under identical conditions but drinking ordinary tap water. The kidneys of both groups were fixed for light and electron microscopy by intravascular perfusion of 4% cacodylate-buffered glutaraldehyde. Paraffin embedded sections were stained with haematoxylin and chromotrope for study both by transmitted and by reflected light. Silver sections were cut from post-osmicated material embedded in Epon and stained with lead citrate[14] for examination in a Siemens Elmiskop I electron microscope.

Growth and general condition of the experimental animals appeared to be completely normal when compared with that of control animals. In the mature rats, as reported previously[12], silver could be recognized both by light and electron microscopy after 5 weeks of silver ingestion. Silver granules were found in the glomeruli and around the vascular bundles and capillaries of the outer medulla. In the inner medulla they were present around the vasa recta and loops of Henle and in the interstitial cells and matrix. The density of the silver deposits increased towards the tip of the papilla and except in the earliest stages could be recognized easily by naked-eye examination. Two adult animals, however, showed an anomalous result in that no silver could be found in the medulla or the cortex after 6 weeks administration of silver. The distribution of silver in the young animals followed the same basic pattern as in the adult with two differences. Firstly, the deposition of silver took place much more slowly in the immature animals so that no silver could be seen by naked-eye examination or by light microscopy until after 12–14 weeks of administration. Secondly, electron microscopy showed that the granules were smaller and rather more sparsely distributed in immature animals than in the adults which had received silver for a corresponding period of time (Figures 1 and 2). Thus the silver granules in adult animals had a diameter between 30 and 90 nm depending on the duration of silver administration but in the immature animals, even those which had been drinking silver solution for 15 weeks, the granules never exceeded 30 nm in diameter (Figure 3).

As has been discussed in a previous paper[12], the sites of silver deposition in the kidney probably depend upon two main factors – the permeability of the blood vessels to the silver-protein complex and the chemical properties of the basal lamina which permit reduction of the complex to metallic silver. It seems probable that the former is the more important factor since there is some supporting evidence that, in the glomeruli at least, the capillaries

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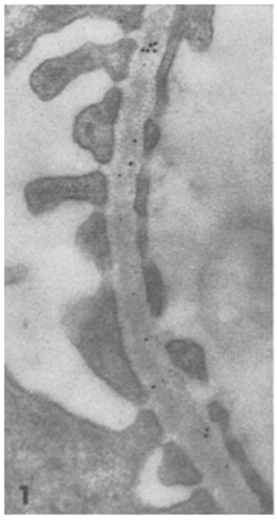

Fig. 1. Glomerulus of immature rat which had received silver for 6 weeks. ×42,000.

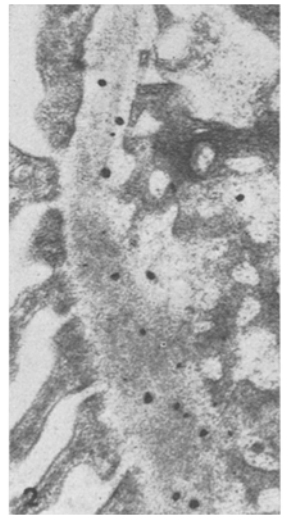

Fig. 2. Glomerulus of adult rat which had received silver for 6 weeks. ×42,000.

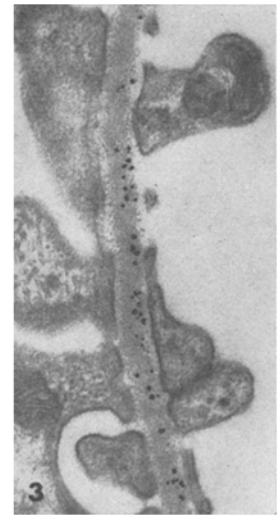

Fig. 3. Glomerulus of immature rat which had received silver for 14 weeks. ×42,000.

are less permeable in immature animals than in adults. WEBBER and BLACKBOURN[15] have shown that in the immature glomeruli at the periphery of the cortex in newborn rats there are few capillary fenestrations and these are impermeable to ferritin, although not to horseradish peroxidase. Other authors have found evidence for an increase of glomerular permeability with age in the rat[16] and in man[17]. It is also possible that age changes affecting the amount of silver deposited in the glomeruli may be due to the increase with age of the glomerular filtration rate[18–20]. This would not, however, explain the age differences noted in the medulla.

No explanation can be offered for the two adult rats in which no deposition of silver could be observed after 6 weeks administration. A similar anomaly has been reported by WALKER[21] who reviewed reports by several other workers and concluded that approximately 3 in every 100 Sprague Dawley rats deal atypically with ingested silver. The significance of the change in permeability to protein of the blood vessels in the growing rat reflected by the deposition of silver and extravasation of Evans blue[13] is difficult to understand at present since the amount of filtered protein and its subsequent fate in the kidney is controversial[22–24] and much more work is necessary before reasonable speculations can be made on its functional importance.

Résumé. Si l'eau potable est additionnée de 0,15 % de nitrate d'argent, l'argent se dépose dans les reins des rats immatures plus lentement et en moins grande quantité que chez les adultes, et ces granules sont partout plus petits. Il est probable que ces résultats indiquent un abaissement de la perméabilité des capillaires chez les immatures.

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High Nuclear DNA Content in Nervus Terminalis Ganglion Cells of *Scorpaena porcus*

A peculiar type of ganglion cell, belonging to the nervus terminalis system, was observed in the rostral half of the olfactory bulbs in Scorpaenidae (Pisces Perciformes). These neurones, 2 to 12 in number, were found within the ventro-medial wall of each bulb, were elongate in shape and often so closely in contact with one another that the boundaries between them could hardly be distinguished[1]. The most striking peculiarity of these nerve cells was the polymorphism and size of their nuclei, that (Figures 1–3) are so deeply indented as to suggest binuclearity. The nuclear diameter, as measured on paraffin sections after Bodian silver stain, was found to vary from 13,2 to 26,2 µm. One to 3 nucleoli may be present, that in electron micrographs (Figure 4) appear of remarkable size, roundish shape, and rather uniform fibrillo-granular texture.

These observations prompted us to measure the Feulgen positivity of these nuclei in order to test whether they contained a higher amount of DNA than the nuclei of other neurones in the same area[2]. Despite repeated attempts to dissociate the tissue, the extreme paucity of the cell population made it impossible to obtain a smear in which a monolayer of neurones could be studied; the histophotometric measurements were therefore performed on paraffin sections of specimens fixed in Carnoy. Serial sections of 8 µm from 10 olfactory bulbs of *Scorpaena porcus* were Feulgen stained and optical density (OD) measurements were performed by means of a Barr and Stroud GN 2 integrating microdensitometer[3]. Camera lucida drawings showed that the majority of the big nuclei under examination appeared in 2 to 3 consecutive sections: by adding the OD values for each section it was therefore possible to know the total OD for each nucleus. Within the population of nerve cells studied, only those nuclei were considered that were isolated enough, in all the sections, to guarantee that there was no overlapping or interference in the field of measurement. This choice reduced the number of acceptable values to 17. As reference elements the nuclei of the granule cells were chosen, since their Feulgen-positivity appeared constant (OD = 3) and could therefore be assumed to be diploid (2c).

From the Figure 5 it is apparent that: a) most of the OD values were found between 64c and 128c, i.e. the DNA content of most nuclei appeared to be 32 to 64 times higher than that of the granule cells; b) no value was found below 16c, although some of the measurements had been performed on the smallest elements of the cell population; c) in 2 cases the DNA content was found to approximate 256c. Although the limited number of data does not allow us to refer the values obeserved to true frequency classes, nor to express our results in terms of degrees of ploidy, the presence can be pointed out of a clear gap between the group of nuclei around 128c and those around 256c, which appears suggestive of a two-fold increase.

In the search for a functional interpretation to the striking nuclear development of the neurones described here, it is necessary to consider the more general situation of this kind of ganglion cell within the Perciformes. In this order of Teleostei, infact ROSSI and BASILE (in preparation) have observed a remarkable variability in the number and size of the nuclei, 2 parameters that seem to be inversely related, and in which respect the Scorpaenidae represent an extreme case, i.e. maximal nuclear size and extreme reduction in number. We would therefore relate the nuclear increment observed to the functional overlaod to which these elements are subject. Such an interpretation is on the other hand in accordance with

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