THE RELATIONSHIP OF THE MITOCHONDRIA TO EGG WHITE ABSORPTION DROPLETS IN RAT KIDNEY

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Foreign or native proteins which have passed through the glomerulus are reabsorbed by the cells in the middle portion of the convoluted tubule where they are concentrated as visible droplets. Oliver, MacDowell, and Lee (1) were the first to make extensive studies on the nature of these absorption droplets. Using supravital staining with dilute Janus green these authors showed that the protein droplets stained like the mitochondrial rods; they concluded from this that enzymes, known to exist in the normal mitochondrion, had been transported to the droplets. Formation of droplets they considered as a mechanism bringing together substrate, protein, and enzymes.

While the formation of protein droplets as a specific phenomenon of the metabolism of protein in the nephron has been generally accepted (2, 3), opinions have been divided on the question of the role of mitochondria in the droplet formation. The original opinion of Oliver *et al.* (1) was strongly supported by the electron microscopical observations of Rhodin (4). On the other hand Davies (5), using techniques much similar to those of Oliver's group, could not observe any participation of the mitochondria in the process of protein reabsorption. Straus, who earlier supported the view that the droplets were a combination of mitochondria and absorbed protein (6), has recently developed a new technique for the identification of foreign protein segregates (phagosomes) in the cells. By this means he has been able to differentiate the phagosomes from the mitochondria (Straus, 7).

Recent developments in the cytochemical demonstration and localization of enzymes (8), particularly those of the dehydrogenase systems (9, 10), have provided a new tool for studies on the physical state of the mitochondria. We have applied these modern techniques to the problem outlined above.

MATERIAL AND METHODS

Egg white was conjugated with a "cellulose-reactive" dye (Hess and Pearse, 11), containing the dichlorotriazine group. The dye was added at a concentration of 2 per cent of the protein content, and the conjugate dialysed in buffered saline. No free dye was removed either during the dialysis or in the subsequent operation of shaking with charcoal. Rats were injected intraperitoneally with 15 ml. of labelled egg white solution (one part of egg white, one part of saline). The animals were killed 18 hours later and parts of the kidneys were immediately frozen.

Cold microtome (cryostat) sections were cut at 6 μ. Serial pairs of sections were selected and the first section used for fluorescence microscopy. The second was used for the cytochemical demonstration either of succinate dehydrogenase or diphosphopyridine nucleotide (DPN) diaphorase. For the enzyme reactions 3,5-diphenyl-2-(4,5-dimethylthiazol-2-yl) tetrazolium bromide (MTT) was used as the final electron acceptor. The sections for examination in ultraviolet light were allowed to dry in the cryostat cabinet and then mounted in glycerine. The sections were then irradiated with UV light of between 4000 and 5000 A wave-length (mazda

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ME/D 250 watts lamp) and examined by means of a converted Beck reflecting microscope. A proximal convoluted tubule, in transverse section, was carefully mapped and registered photographically. The same tubule was then identified in the serial section after incubation for oxidative enzyme activity. By this means it was possible to observe the state of the mitochondria in the cells containing egg white droplets.

RESULTS

The results are shown in two pairs of photomicrographs; in Fig. 1 the cells of a proximal convoluted tubule are shown containing fluorescent egg white droplets. In Fig. 2 these cells are seen to exhibit normal activity for succinate dehydrogenase with a normal mitochondrial pattern. Figs. 3 and 4 show a similar tubule first containing droplets and secondly showing the normal pattern of mitochondrial activity for DPN diaphorase.

DISCUSSION

Scarpelli and Pearse (8) have demonstrated that the formazan precipitates in oxidative enzyme reactions can reflect sensitively the physical state of the mitochondria. No signs of swollen mitochondria could, however, be observed in the tubular cells during various stages of protein reabsorption. The number of formazan deposits per average mitochondrion is from three to six (12); in the present observations the number of intramitochondrial formazan droplets per cell was absolutely normal and there was thus no sign of an inversely proportional relationship between the number of protein droplets and the number of mitochondria, as found by Oliver *et al.* (1).

The evidence provided, although it is no more than presumptive, suggests that the formation of protein absorption droplets does not involve the intracellular oxidative mechanisms represented by the two enzymes studied. Two possibly unwarranted assumptions have been made. First, that all mitochondria are demonstrated by the combination of the DPN diaphorase and succinate dehydrogenase techniques and secondly, that involvement of the mitochondria in droplet formation would result in increased permeability of their limiting membranes. The first of these assumptions is likely to be correct, the second may not be.

It is necessary to observe that the zone of maximum droplet formation lies in the luminal part of the cell and that the mitochondria are in fact displaced from this region by the growing droplets (Fig. 4). Those mitochondria which are closely associated with the droplets are certainly normal in respect of the enzyme tests carried out. That is to say, their membrane permeability has neither been increased or decreased. If, however, coating of the mitochondrial membrane with protein should result in decreased permeability this might reach the stage at which extinction of the enzyme reaction occurred. If an appreciable number of mitochondria were completely disrupted the remaining fragments might continue to give positive reactions for such firmly bound enzymes as succinate dehydrogenase and DPN diaphorase. They would be expected to produce larger formazan deposits than the normal intramitochondrial ones, however. Soluble enzyme systems would be expected to give low or negative reactions if an appreciable percentage of mitochondria were damaged. No such effect was observed histochemically.

SUMMARY

Fluorescent egg white droplets, labelled with a cellulose-reactive dye containing the dichlorotriazinyl group, were produced in the renal tubules of the rat. Serial pairs of fresh frozen (cryostat) sections were examined and photo-

FIGURE 1
A tubule containing fluorescent egg white droplets. × 2000.

FIGURE 2
The same tubule in serial section incubated for succinate dehydrogenase. × 2000.

FIGURE 3
A tubule containing labelled egg white droplets. × 2000.

FIGURE 4
Serial section of the same tubule showing reaction for DPN diaphorase. × 2000.

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graphed by fluorescence microscopy, or incubated for either succinate dehydrogenase or DPN diaphorase. For both the latter a technique giving intramitochondrial localization was employed which allows appreciation of the physical state of the mitochondria.

No evidence could be obtained to suggest any mitochondrial involvement in the production of egg white droplets.

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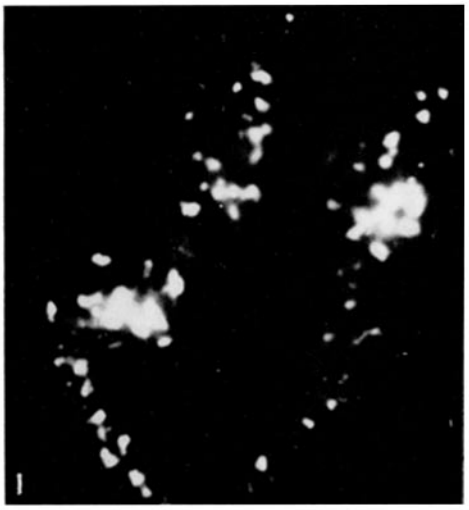

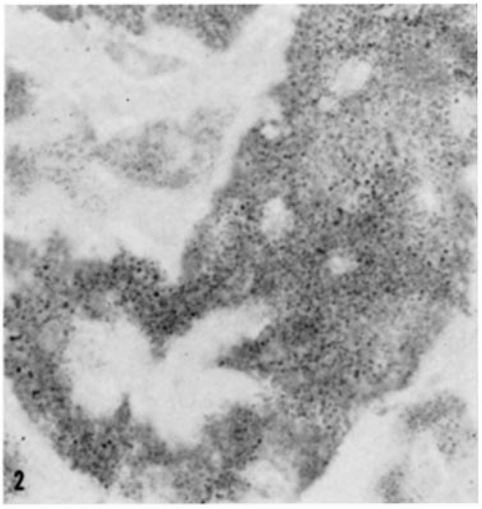

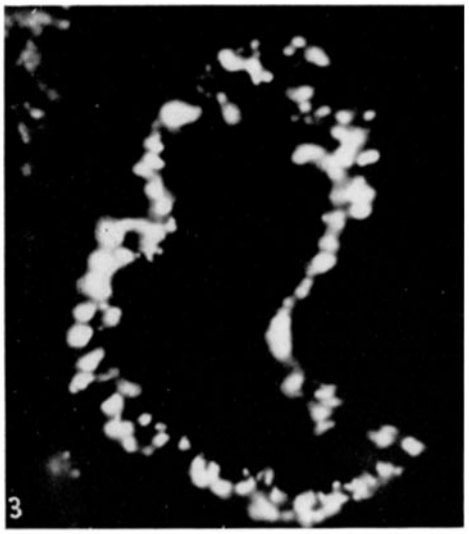

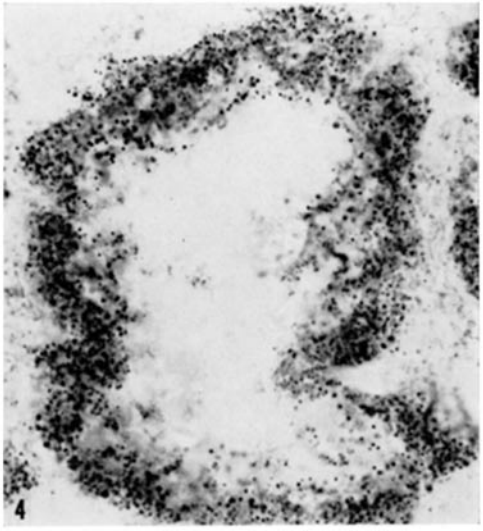

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