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THE EFFECT OF $^{2}H_2O$ ON THE MEMBRANOUS STRUCTURES OF THE PROXIMAL AND DISTAL CONVOLUTED TUBULES OF THE MOUSE

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SUMMARY

Mice were fed exclusively with $^{2}H_2O$ until they became moribund. The mice were then sacrificed and prepared for electron microscopy by conventional methods. The cells of the proximal and distal convoluted tubules have in many respects a normal appearance. However, the endoplasmic reticulum is absent, whilst a great number of peculiar inclusions are found in the cytoplasm, that consist either of a few or of many concentrically arranged membranes. Occasionally they penetrate between the brush border. There is some evidence that these membrane systems originate from short membranes found in the cytoplasm. It is suggested that the pathological changes originate from changes on the molecular level brought about by the substitution of hydrogen by deuterium.

INTRODUCTION

Chemical compounds containing deuterium differ in many respects from those containing hydrogen, *e.g.* in molecular configuration, reaction rate, vapour pressure and ionic equilibrium[1]. The effect of $^{2}H_2O$ on living organisms is of biochemical, histological and physiological interest. A number of findings have been reported in the literature: the oxidation of cholesterol is inhibited[2]; the optimal pH for enzymatic action changes[1]; the respiratory chain is affected[3]; enzyme-catalysed reactions are slowed down, possibly because ^{2}H bonds are less quickly ruptured than ^{1}H bonds[4]; proteins, presumably due to a more rigid configuration, become more heat-resistant[5]; the synthesis of DNA is presumed to be suppressed, and less RNA is produced[6]; and blockage of protein synthesis is observed in plant tissue, but not in animal tissue[6]. It is also interesting to note that the diffusion rate of $^{2}H_2O$ is different from H_2O as far as cell membranes are concerned[7], and that the viscosity of the cytoplasm containing $^{2}H_2O$ is increased[8]. At a higher level mitosis is inhibited[2,8]: this effect may be due to a suppression of DNA synthesis[6]. Small organisms, however, may become adapted to living in heavy water[6], though morphological changes have been observed. Deuterated bacteriophages T2 are larger and have a more bulbous head[9]; deuterated algae form monster cells[10]; deuterated higher plants and animals do not survive[10]. Deuterium-intoxicated mice show weakness and neuromuscular damage, the kidneys become enlarged and the tubules are destroyed, but the glomeruli are apparently not affected: a progressive decrease in glomerular filtration rate is observed[4]. Morphological changes at the electron-microscopic level are found in deuterated sympathetic ganglia isolated in culture[11]; the nuclei of the neurons and of the supporting cells show organised fibrillar components. A cytaster is formed in amphibian eggs after treatment with ^2H_2O (ref. 12).

However, to our knowledge, little work has been reported so far on the morphological effect of ^2H_2O, especially at the electron-microscopic level, and we thought that an investigation on this field might be useful. In the following, preliminary results are reported, which show that ^2H_2O brings about profound ultrastructural changes of the proximal and distal convoluted tubules of the kidney of the mouse.

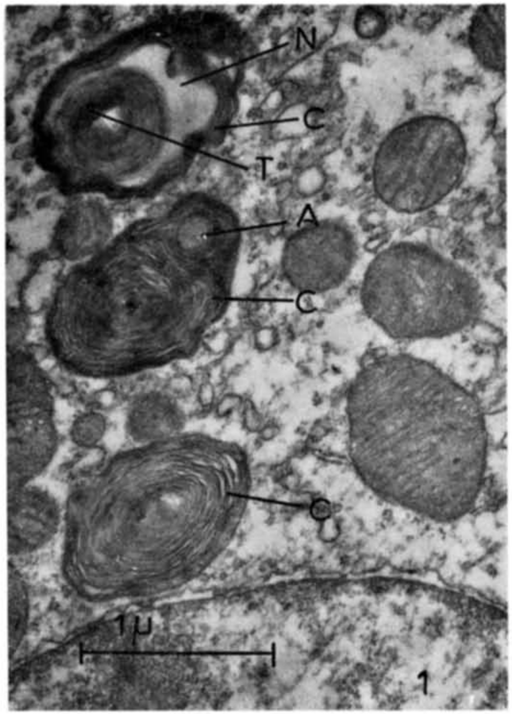
MATERIAL AND METHODS

Six mice were fed exclusively with ^2H_2O to which a small amount of Ringer's solution had been added. Six controls were fed with H_2O (deuterium oxide, 99.7 g per 100 g ^2H_2O, Norsk Hydro-Elektrisk, Kuaelstofaktieselskab, I.C.I.).

The deuterated mice became moribund after about 8 days; they were partially paralysed. The animals were sacrificed on the 7th or 8th day. The cortex of the kidney was fixed in glutaraldehyde and OsO_4, dehydrated and embedded in Epon according to standard procedure. Sections were cut on a Reichert ultramicrotome and investigated in an AEI 6 B electron microscope.

EXPERIMENTAL RESULTS AND DISCUSSION

Electron micrographs of the kidneys of mice fed with H_2O (controls) showed the conventional structural details[13]. The cells of the proximal and distal convoluted tubules of the kidney of mice fed with ^2H_2O in many respects also have a normal appearance, but the usual cytoplasmic membranes are absent and a great number of peculiar cytoplasmic inclusions are found which consist of concentrically arranged membranes (Figs. 1–6). They can be observed both in the proximal and in the distal convoluted tubules and they will be described here as concentric membrane bodies. The thickness of a single membrane was found to be about 40 Å, approximately the thickness of the two electron-dense layers of the 'unit membrane' together. The membranes of the concentric membrane bodies are more electron-dense than any other membranous structures of the cells in which they are found, *e.g.* the mitochondrial membranes (Figs. 1 and 4). Membranes of similar thickness were observed after treatment of isolated mitochondrial membranes with pepsin[14]. Similarly arranged membranes were found in the follicle cells of the thyroid gland of the hagfish[15] and in *Amoeba histolytica* after treatment with ribonuclease[16]. It should also be mentioned that structures resembling very much the concentric membrane bodies have been observed in the pigment epithelial cells of dystrophic animals[21]. The concentric membrane bodies may consist only of a few membranes (Figs. 2 and 4) or of a great number (Figs. 1–6). Frequently one or two concentric membrane bodies are enclosed by a secondary shell of concentric membranes (Fig. 3), they may also aggregate to form very large inclusions (diameter up to 7 μ (Fig. 3)). The membranes are not always closely packed (Fig. 1) and they sometimes enclose a small granular or amorphous, centrally situated body (Figs. 1 and 4). The membranes are not cylindrical, but



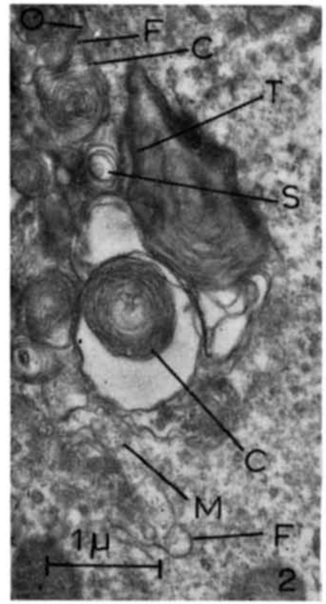
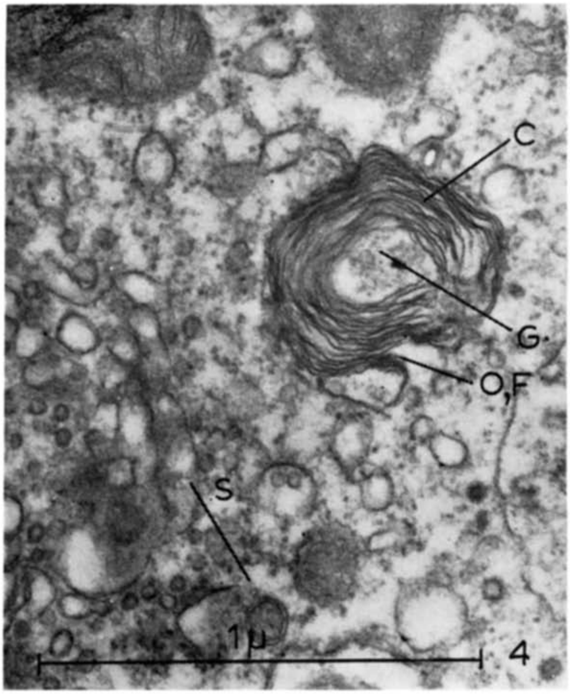


Fig. 1. Proximal convoluted tubule. Concentric membrane bodies consisting of a great number of membranes (C). Note diffuse appearance of tangentially sectioned membranes (T), membranes not closely packed (N), amorphous centre of concentric membrane bodies (A). Magnification 25000 ×.

Fig. 2. Distal convoluted tubule. Concentric membrane bodies consisting of a great number of membranes (C). Note diffuse appearance of tangentially sectioned membranes (T), concentric membrane bodies consisting of a few membranes (S), short membranes in cytoplasm (M), membranes of concentric membrane bodies with free ends (O), membranes possibly forming concentric membrane bodies (F). Magnification 15000 ×.

Fig. 3. Proximal convoluted tubule. Large aggregation of concentric membrane bodies, some jointly enclosed by secondary concentric membranes (U). Magnification 8000 ×.



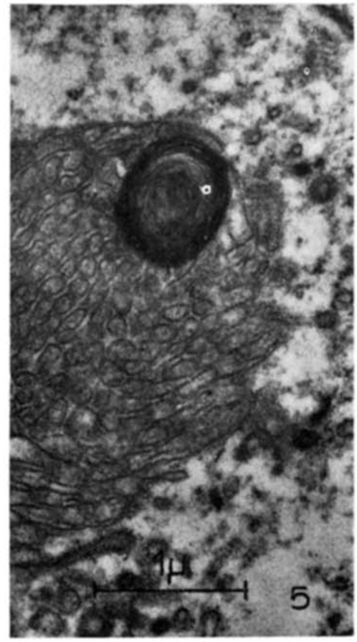


Fig. 4. Distal convoluted tubule. Concentric membrane body consisting of a great number of membranes (C), granular inclusion of concentric membrane bodies (G), concentric membrane bodies consisting only of a few membranes (S), membranes of concentric membrane bodies with free ends (O), membrane possibly forming concentric membrane bodies (F). Magnification 60000 ×.

Fig. 5. Proximal convoluted tubule. Concentric membrane body penetrating into brush border. Magnification 20000 ×.

spherical; this can be seen from tangential sections, which give a diffuse picture (Figs. 1 and 2). Furthermore, in suitable sections, cylindrical membranes would be represented by parallel straight lines, like the myelin sheath of the axon; such lines have never been observed. It is not absolutely clear how the concentric membrane bodies are formed, but certain conclusions can be drawn from the electron micrographs. Occasionally short membranes can be observed (Figs. 1 and 2) and in some concentric membrane bodies, membranes with free ends are found (Fig. 2). By aggregation of the short cytoplasmic membranes and by attachment of the short cytoplasmic membranes to the free ends of the membranes of the concentric membrane body, new concentric membrane bodies may be formed and existing ones enlarged (Figs. 2 and 4). It is also of interest that some concentric membrane bodies penetrate into the brush-border (Fig. 5) and become eventually enclosed by it (Fig. 6). Though our results cannot directly be compared with those of KATZ *et al.*[4], as the amount of ^2H_2O given differs greatly (nearly 100 % in our experiments, less than 30 % in those by KATZ *et al.*) they are in agreement in that serious pathological changes were observed. It is not remarkable that the changes bring about serious symptoms, though it cannot be decided whether these lesions or lesions of other types of cells are responsible for the death of the animals. Our results agree with other electron-

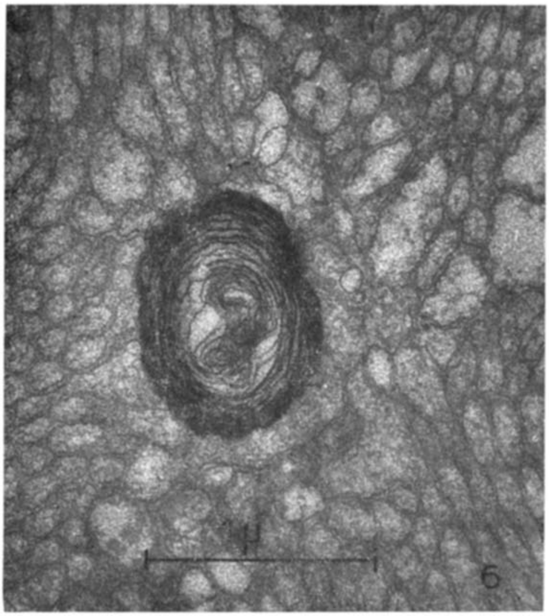


Fig. 6. Proximal convoluted tubule. Concentric membrane body enclosed by brush border. Magnification 30000 ×.

microscopic findings[11,12], in that unusual cellular inclusions were also observed. Again, a direct comparison is impossible as different types of tissue were studied. There is still no clarity concerning the arrangement of the constituent lipids and proteins in biological membranes, though many authors accept the concept of a three-layered structure[17]; there are, however, indications that the structure is more complex[18]. It has been suggested by DEUTSCH AND KRAUSE[19] that the molecular arrangement in a membrane may even depend on the functional state of the cell. The role of water in membranes is also still enigmatic[20]. The morphological changes observed by us are in the last analysis due to changes on the molecular level, but in view of our limited knowledge of normal membranes and the limitations of the electron microscope, our results cannot yet be interpreted in terms of changes of molecular arrangement. It cannot even be stated whether cellular structures which appear to be 'normal' on the electron-microscopic level are also 'normal' on the molecular level; indeed it is very likely that they are not. We are inclined to think that the molecules which form the cytoplasmic membranes, as a result of deuteration, differ structurally from the normal ones, and accordingly, on assembling, form abnormal structures. It is feasible that the aggregation of the units occurs spontaneously as a result of their geometrical configuration as it is similarly assumed that the tertiary structure of protein molecules is fundamentally determined by the sequence of the constituent amino acids. However, certain enzyme systems may determine the shape of the membranous structures and in this case the formation of concentric membrane bodies would be influenced by any changes in these systems due to deuteration.

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