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ULTRASTRUCTURAL CHANGES IN THE ISOLATED RAT KIDNEY INDUCED BY CONJUGATED BILIRUBIN AND BILE ACIDS

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Summary.—The effects of bilirubin and bile acids on the ultrastructure of proximal renal tubules have been studied using an isolated rat kidney preparation, perfused with a protein-free dextran medium. Control kidneys perfused for 1 h had a normal glomerular filtration rate and effective renal plasma flow; the ultrastructure of proximal tubular cells was well preserved, with normal mitochondria, nuclear and plasma membranes, and microvilli of the brush border.

When conjugated bilirubin, prepared from human hepatic bile, was added to the perfusion medium (5·0–7·5 mg/100 ml), marked alterations were observed in some cells, particularly with regard to the mitochondria and plasma membranes. These changes were greatly diminished by the inclusion of bovine albumin in the medium, indicating that the unbound fraction was primarily responsible for the tubular damage. The addition of taurocholate (450 μM), taurochenodeoxycholate (550 μM) or taurolithocholate (250 μM, bound to albumin) also produced plasma membrane changes, but only slight abnormalities were seen in the mitochondria and other structures.

These ultrastructural observations support the concept that the elevated plasma levels of conjugated bilirubin and to a lesser extent bile acids are related to the renal failure associated with obstructive jaundice.

PATIENTS with obstructive jaundice are more likely to develop renal failure after surgery or an episode of hypotension than non-jaundiced patients (Williams, Elliott and Zollinger, 1960; Dawson, 1965; Thal *et al.*, 1971); the incidence of renal damage appears to be related to the depth of jaundice. In bile-duct-ligated animals it has been shown that the histological lesion is focal tubular necrosis (Dawson and Stirling, 1964; Wardle, 1975). The substance responsible for the increased sensitivity of the renal parenchyma to ischaemic injury has not been identified, although there is indirect evidence that conjugated bilirubin and bile acids may be implicated (Baum, Stirling and Dawson, 1969; Aoyagi and Lowenstein, 1968). In cholestasis the excretion of these substances in urine becomes important as an alternative pathway to biliary excretion. Since only a small protein-free fraction of conjugated bilirubin and bile acids present in the plasma is available for glomerular filtration and subsequent tubular reabsorption (Ali and Billing, 1966; Weiner, Glasser and Lack, 1964; Gollan and Billing, 1975), this unbound component is the most likely potential cause of toxic injury.

In vitro studies have demonstrated that unconjugated bilirubin interferes with vital cell functions (Zetterström and Ernster, 1956). The effects, however, are greatly reduced by the addition of albumin (Mustafa, Cowger and King, 1969) and *in vivo* are relevant only to damage in the newborn brain (Diamond

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and Schmid, 1966). Because pure preparations of conjugated bilirubin are not available, its toxic effects have only been examined by comparative studies in normal and Gunn rats, since the latter animals do not form bilirubin glucuronide and this pigment does not, therefore, accumulate in the plasma with other biliary constituents after bile duct ligation. Using this experimental design, data have been obtained which suggest that an increase in plasma conjugated bilirubin is associated with the shortened red cell survival (Powell, Dunnicliff and Billing, 1968) and renal tubular damage (Baum, Stirling and Dawson, 1969) observed in obstructive jaundice.

In experimental animals, bile acids have been shown to interfere with the structure and function of a variety of cells and subcellular organelles (Palmer, 1972). The majority of investigations have employed unconjugated bile acids and it has been suggested that the degree of cytotoxicity decreases as the number of hydroxyl groups on the steroid nucleus increases (Palmer, 1969). The particularly injurious effects of the monohydroxy bile acid, lithocholic acid, are well established (Palmer, 1969) and the di-hydroxy bile acid, chenodeoxycholic acid, has also been implicated as a cause of hepatic damage (Boyd, Eastwood and MacLean, 1966; Miyai, Price and Fisher, 1971; Greim *et al.*, 1972). The trihydroxy bile acid, cholic acid, does not appear to be hepatotoxic (Boyd, Eastwood and MacLean, 1966) but has been shown to produce transient renal insufficiency following an episode of ischaemia (Aoyagi and Lowenstein, 1968). The influence of bile acids on renal ultrastructure has not been previously reported.

The development of a functional isolated rat kidney perfused with a protein-free dextran medium (Gollan and Billing, 1975) has provided a direct means of investigating the renal toxicity of substances which are extensively bound to plasma proteins. Preliminary studies established that perfusion of the isolated kidney for a period of 1 h did not result in significant ultrastructural change. It was therefore possible to use this preparation to study the effect of bilirubin and bile acids on the ultrastructure of the proximal renal tubules.

MATERIALS AND METHODS

Materials

The nuclides [^{51}Cr]EDTA (sp. activity 1 mCi/mg) and [^{125}I]Na-o-iodohippurate ([^{125}I]OIH, sp. activity 50 μCi/mg) were supplied by the Radiochemical Centre, Amersham, U.K. Sodium taurocholate, taurochenodeoxycholate and taurolithocholate were purchased from Weddell Pharmaceuticals Ltd, Wrexham, Clwyd, U.K. and unconjugated bilirubin from B.D.H. Chemicals Ltd, Poole, Dorset, U.K.

Conjugated bilirubin.—Since conjugated bilirubin is not commercially available, it was prepared from human T-tube bile by a method modified from that of Lucassen (1961). The pH of a 500-ml sample of bile which had been collected within 48 h of operation was rapidly adjusted to 6·0 with 10% oxalic acid and after centrifugation the supernatant solution was brought to pH 3·5 with 10% oxalic acid. The precipatate obtained on centrifugation was dissolved in 200 ml cold acetone and left at 4°C in the dark for 1 h. After centrifugation the pH of the supernatant was adjusted to 7·0 by the careful addition of ethanol saturated with sodium hydroxide. The resultant precipitate was dried under nitrogen and then stored in the dark at − 20°C; under these conditions it was stable for at least 6 months. The preparation used in this study contained 77% conjugated bilirubin and no detectable amounts of bile acids, cholesterol, phospholipid, or protein. It was shown to be a mixture of mono- (22%) and di- (78%) conjugates, similar to that of bile (Thompson and Hofmann, 1971). The major constituent was a β-D-diglucuronoside, but other esters were present in addition (Fevery *et al.*, 1972).

Perfusion of isolated rat kidney

The protein-free perfusion medium consisted of Krebs' improved Ringer 1 solution (Dawson and Elliott, 1959) modified by the substitution of 6·0% (w/v) dextran (mol. wt. 70,000) in 154 mM NaCl (Lomodex 70, Fisons Pharmaceuticals Ltd, Leics., U.K.) for 154 mM saline and the addition of urea. The final concentration of dextran was 3·7% (w/v). Experiments were also performed in which bovine serum albumin (fraction, V, Armour Pharmaceutical Co. Ltd, Eastbourne, Sussex, U.K.) was added to the dextran perfusate to give a final concentration

of 1·0% (w/v). The medium was warmed in the perfusion circuit to 38°C and equilibrated at pH 7·4 with 95% O_2 and 5% CO_2 before operation.

Prior to operation, male Sprague–Dawley rats weighing 350–400 g were fed *ad libitum* on a standard laboratory diet (Modified 41B, Oxoid Ltd, London, U.K.). The operative technique was essentially that described by Nishiitsutsuji-Uwo, Ross and Krebs (1967) for perfusion of the right kidney. Under ether anaesthesia the ureter was cannulated with polyethylene tubing and the kidney prepared for perfusion by the insertion of a venous cannula into the inferior vena cava and a metal arterial cannula into the renal artery (*via* the mesenteric artery). With the perfusate flowing, so that renal plasma flow was unimpaired, the kidney was dissected from the animal and placed in a plastic box. The medium (120 ml) was recirculated through the kidney at a flow rate of 30–32 ml/min and was continuously filtered by an in-line dacron filter, 14-μm pore size (type NC, Millipore (U.K.) Ltd, London, U.K.). A 10-min equilibration period was allowed for mixing of the added test substances and perfusion of the kidney then proceeded for a further 60 min. Urine was collected over 10-min intervals and midpoint samples of perfusate were taken in order to determine clearance values. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were estimated simultaneously after the addition of 4 μCi[^{51}Cr]EDTA and 12 μCi[^{125}I]OIH to the medium. The two nuclides were readily distinguished by their different radiation energies using a dual channel gamma spectrometer (GTL 300, Wallac, South Croydon, Surrey, U.K.). The clearances of EDTA and OIH were calculated from UV/P, where U and P represent the concentrations of radioactivity in urine and perfusate expressed as counts/min/ml and V the urine flow (ml/min). Previous studies have demonstrated that the GFR of the preparation remains constant for the duration of the perfusion (Gollan and Billing, 1975).

Light and Electron Microscopy

The kidney was removed immediately the perfusion was terminated, and transverse sections 1 mm thick were made through the body of the kidney. The cortical and medullary tissues were further cut into pieces 1 mm thick and fixed in ice-chilled 4% glutaraldehyde in 0·1M cacodylate buffer, pH 7·4, for 1 h and postfixed with 1% osmium tetroxide in the same buffer. The tissue blocks were dehydrated through graded ethanol, infiltrated with propylene oxide and embedded in Epon 812. Since this study was confined to the proximal tubules, sections 1 μm thick of the outer cortex were cut from multiple blocks and stained with toluidine blue for light microscopy. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope.

Experimental

Control experiments.—These were performed in order to establish whether the isolated kidney underwent any ultra-structural change during perfusion. Sections obtained from a non-perfused normal rat kidney were compared with those from kidneys perfused for 60 min with the protein-free medium (two experiments) and the medium containing 1% (w/v) bovine albumin (two experiments). In one experiment, the perfusion was terminated after only 2 min and the kidney was left to autolyse in the perfusion tray at 38°C for 1 h before the sections were obtained.

Bilirubin.—The experiments in which kidneys were perfused with bilirubin were performed in dim light. In three perfusions, conjugated bilirubin was dissolved in water (1 ml) and Millipore-filtered (pore size 0·22 μm) into the perfusate to produce concentrations of 5·0–7·5 mg bilirubin/100 ml during the perfusion. In two further experiments, kidneys were perfused with conjugated bilirubin at concentrations of 5·0–9·0 mg/100 ml in the medium containing 1·0% (w/v) albumin.

Because of the low solubility of unconjugated bilirubin in water it was only possible to perfuse the kidney with unconjugated bilirubin bound to albumin. Bilirubin (8 mg) was mixed with 1 ml 0·1M NaOH and 20 ml of perfusate containing 1·2 g bovine albumin, the pH was adjusted to 7·4 with 0·1M HCl and the solution was warmed to 38°C, filtered (type NC, Millipore) and added to 100 ml of protein-free medium in the circuit. In two experiments, the final concentration of bilirubin in the perfusate varied between 5·0 and 7·2 mg/00 ml.

Bile acids.—Two experiments were performed with each bile acid. Sodium taurocholate or taurochenodeoxycholate was dissolved in 1 ml 154 mM NaCl and added to the protein-free medium to provide concentrations of 450–475 μM and 540–600 μM respectively during perfusion. Due to its low solubility, sodium taurolithocholate (12 mg) was added with 1·2 g bovine albumin to 20 ml of perfusate and sonicated for 10 min; it was then warmed to 45°C for 1 h, filtered and added to 100 ml protein-free medium in the circuit during the equilibration period (concentration range 250–290 μM).

RESULTS

Function of the isolated kidney

The GFR and ERPF of the isolated kidney were not influenced by the addition of bilirubin, bile acids or bovine albumin

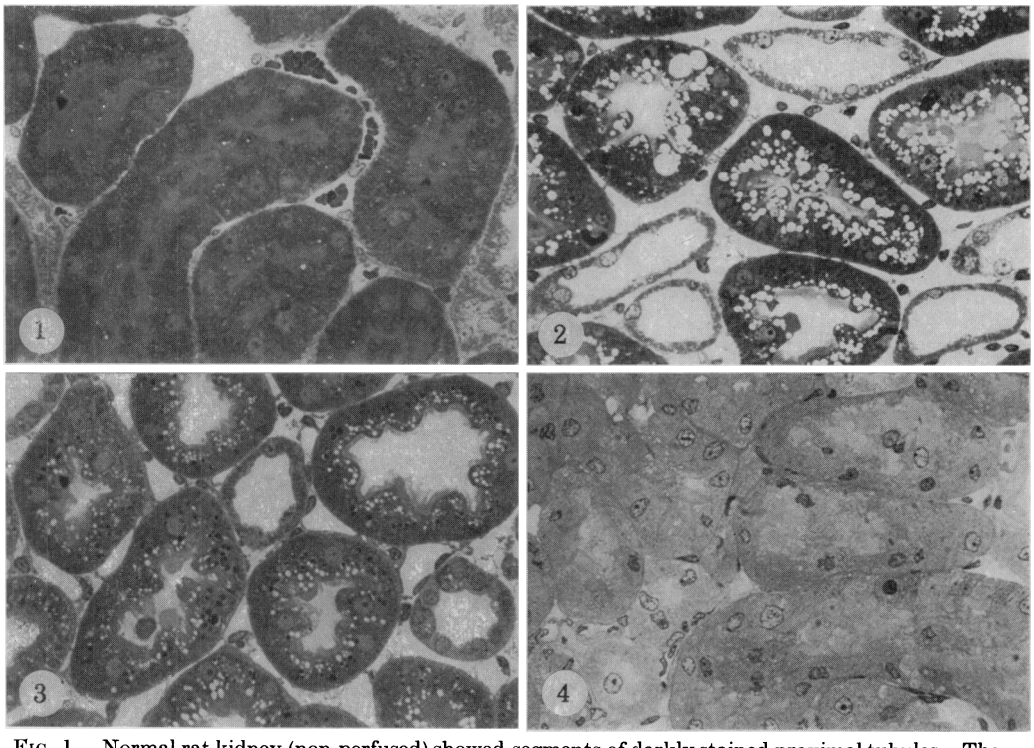

FIG. 1.—Normal rat kidney (non-perfused) showed segments of darkly stained proximal tubules. The interstitial capillaries contained red cells. × 375.

FIG. 2.—Perfusion with protein-free medium for 1 h produced distention of interstitial spaces. Apical vacuoles in proximal tubules were large and prominent. Blebbing of homogenous protoplasm into the tubular lumen was noted. × 375.

FIG. 3.—Perfusion for 1 h with the medium containing albumin produced less interstitial oedema than that seen with protein-free perfusion. Darkly stained hyaline droplets were numerous and homogenous protoplasmic blebbing was present. × 375.

FIG. 4.—Autolysis for 1 h following 2 min perfusion with protein-free medium resulted in reduction of the interstitial space and crowding of tubules. The proximal renal tubular cells showed nuclear pyknosis, poor staining and loss of protoplasmic granularity of mitochondria. The brush border was not discernible. × 375.

to the perfusion medium. The mean GFR observed after 30 min perfusion (maximum value) was 0·89 ± 0·04 (± s.e. mean) ml/min. This was within the normal range reported for the intact rat (Spector, 1956) and compared favourably with the values observed in the isolated kidney perfused with an albumin-medium (Ross, Epstein and Leaf, 1973). The ERPF was also normal, 3·73 ± 0·38 ml/min, after 30 min perfusion.

Light microscopy

In the control non-perfused rat kidney (Fig. 1), the proximal renal tubules were lined by darkly stained tall cuboidal epithelial cells with a thick brush border which appeared to obliterate the contracted lumen. A small number of clear vacuoles were present in the apical region just below the brush border. Perfusion with the protein-free medium for 1 h (Fig. 2) resulted in distention of interstitial spaces and prominent apical vacuoles, some of which became confluent. Cytoplasmic blebs were seen in the luminal surface of the epithelial cells. Kidneys perfused with the albumin containing medium (Fig. 3) for 1 h showed essentially similar features, except that the apical vacuoles were smaller and the hyaline droplets were more darkly stained.

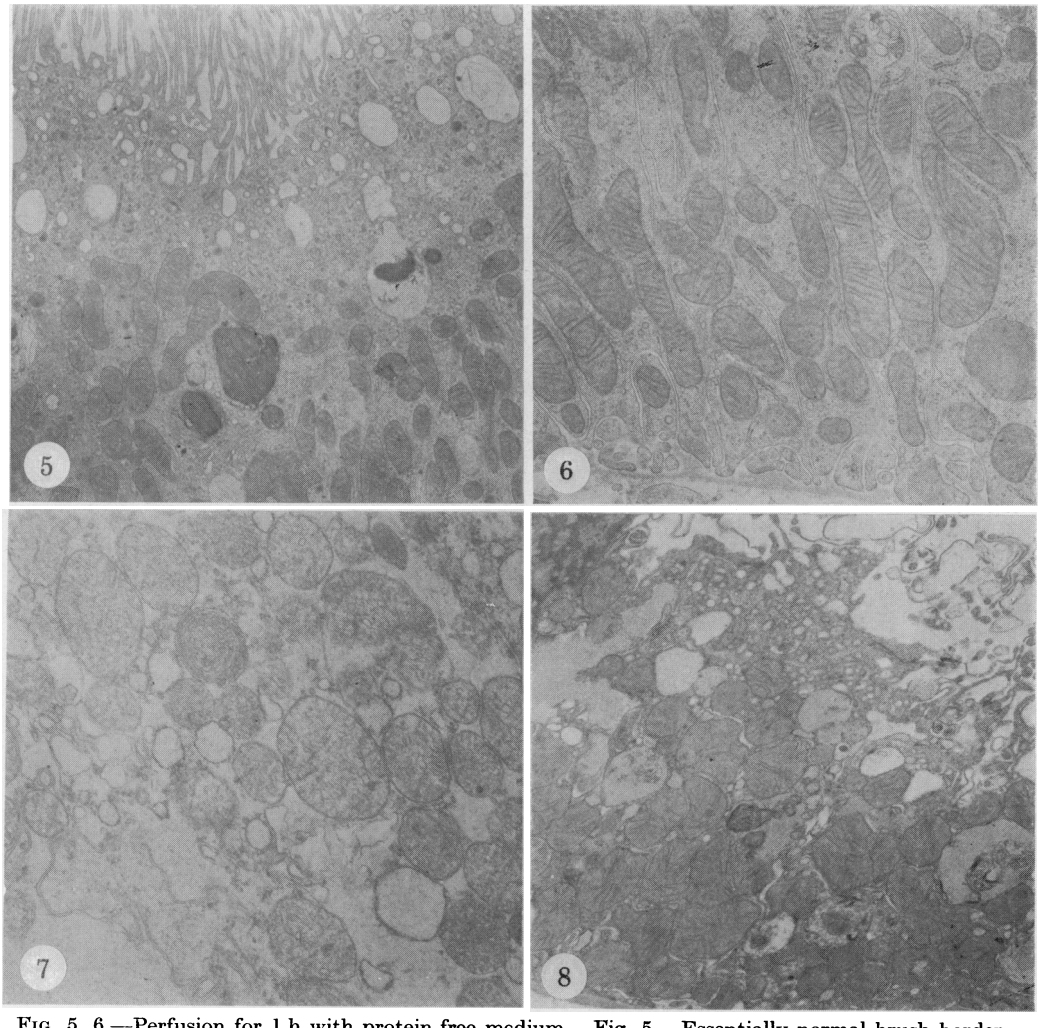

FIG. 5, 6.—Perfusion for 1 h with protein-free medium. Fig. 5.—Essentially normal brush border and upper part of proximal renal tubular cell. × 5,660. Fig. 6.—Ultrastructure of the basal part of proximal tubular cells showed good preservation. × 12,800.

FIG. 7.—Autolysis for 1 h showed cellular edema, mitochondrial swelling and prominent vesiculation of the endoplasmic reticulum. × 14,400.

FIG. 8, 9, 10.—Perfusion for 1 h with conjugated bilirubin in protein-free medium. Fig. 8.—The microvilli of the brush border showed disarray, attenuation and probable breakage. Membranous whorls were seen in phagolysosomes and the mitochondria exhibited crowding and irregularity of their contour. × 7,770. Fig. 9.—Higher magnification of distorted mitochondria. × 19,200.

Homogenous protoplasmic protrusion of the epithelial cells into the tubular lumen was also present. The kidney which was perfused with protein-free medium for only 2 min and then allowed to autolyse for 1 h (Fig. 4) showed obliteration of the interstices probably as the result of tubular swelling. The epithelial cells of the proximal tubules became homogenous and lightly stained. The nuclei were pyknotic and the brush border could not be discerned.

In kidneys perfused with conjugated bilirubin in protein-free medium the light microscopic features of the proximal tubules could not be distinguished from

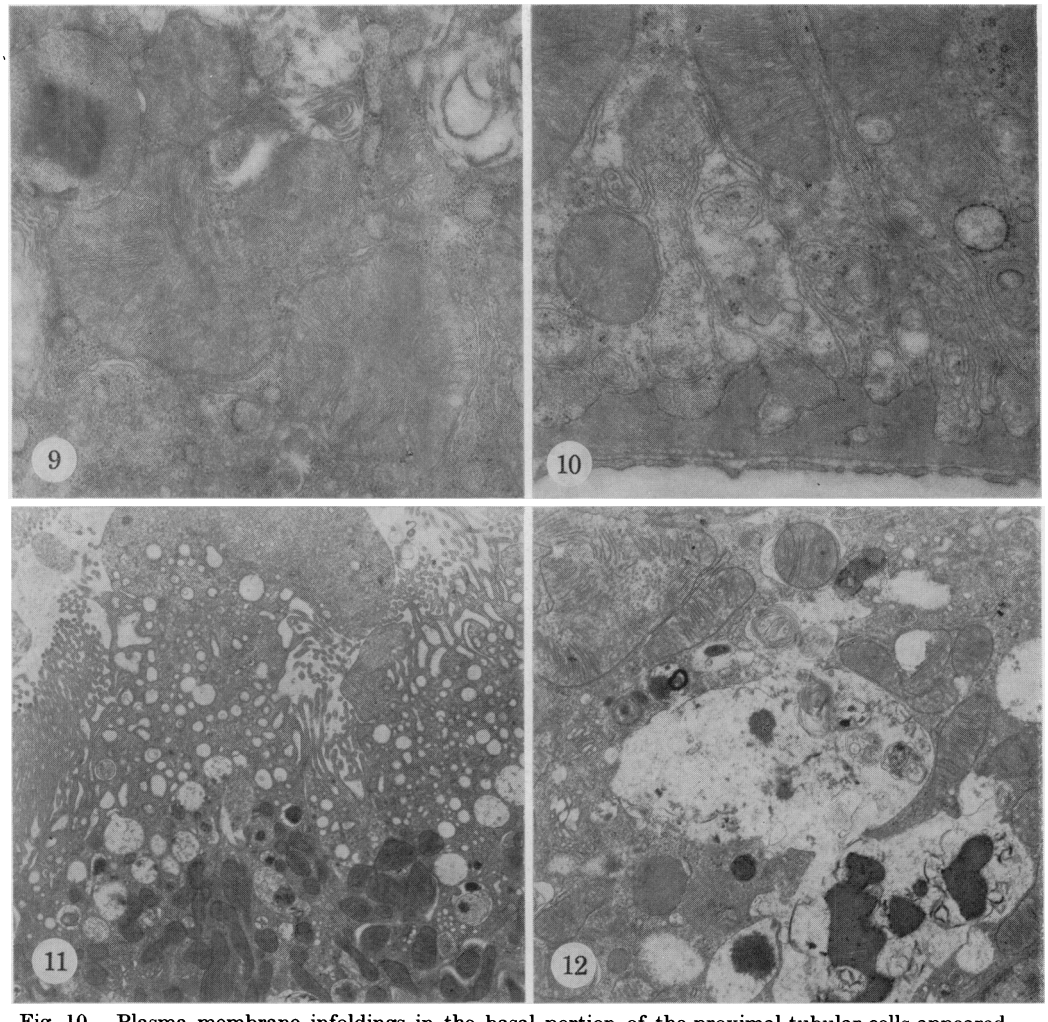

Fig. 10.—Plasma membrane infoldings in the basal portion of the proximal tubular cells appeared slightly undulant. Also slight thickening of the basement membrane was evident with focal peg-like upward projections. × 19,200.

FIG. 11, 12.—Perfusion for 1 h with conjugated bilirubin in albumin-medium. Fig. 11.—The apical portion showed mushroom-like protrusion into the tubular lumen and there was an increase in phagocytic vacuoles. Microvilli were normal × 5,700. Fig. 12.—An irregularly shaped phagolysosome contained electron-dense amorphous material and membranous whorls. A megamitochondrion is seen in the left upper corner of the illustration. × 9,500.

those of the control experiments. Similar findings were seen in the perfusion experiments with conjugated bilirubin–albumin, unconjugated bilirubin–albumin, taurocholate and taurochenodeoxycholate. However, in the taurolithocholate–albumin perfusion, desquamation and vacuolation of the tubular cells were present.

Electron microscopy

The proximal renal tubules in kidneys which had been perfused for 1 h with the dextran protein-free medium were shown to be ultrastructurally normal (Fig. 5, 6). The microvilli of the brush border were regular. The mitochondria, basal protoplasmic infoldings and all other cellular organelles were well preserved and could

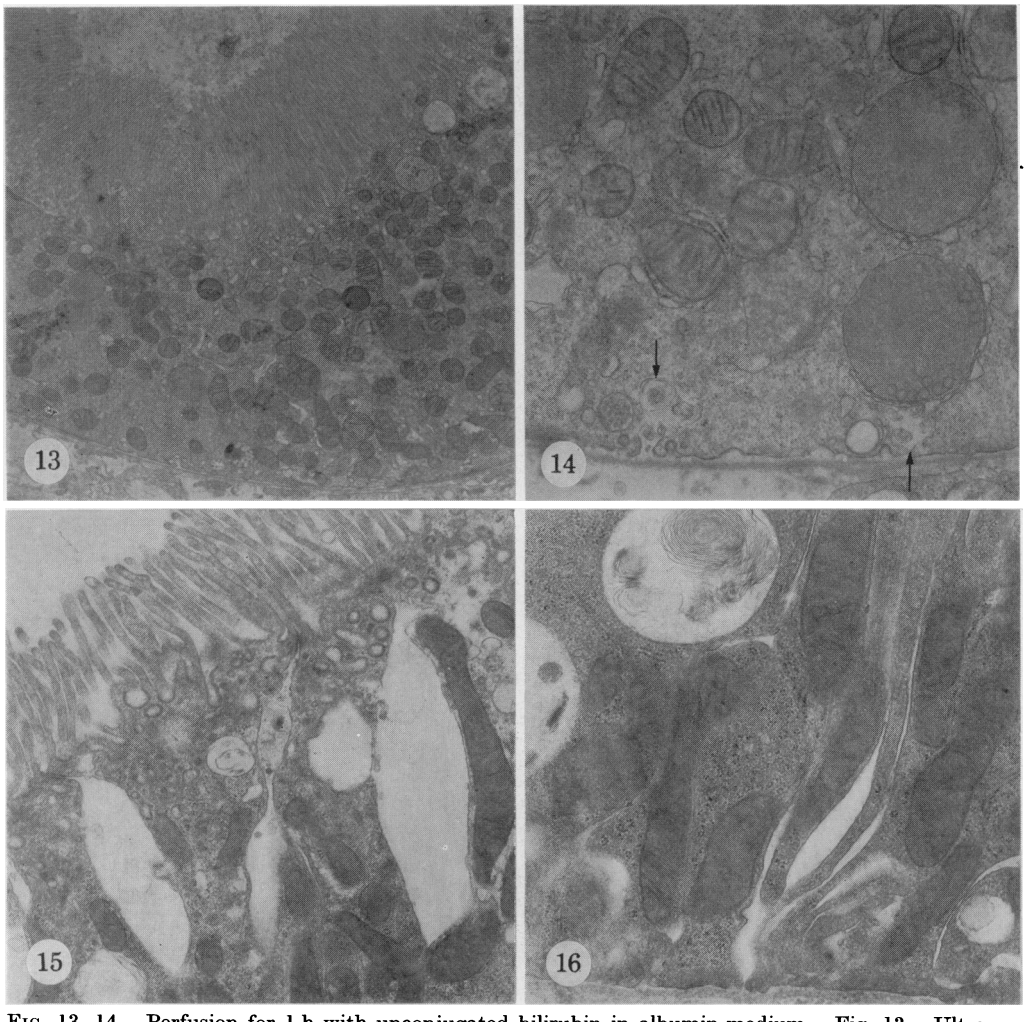

FIG. 13, 14.—Perfusion for 1 h with unconjugated bilirubin in albumin-medium. Fig. 13.—Ultrastructural morphology of the proximal tubular cells was nearly normal. × 4,600. Fig. 14.—The basal portion of some tubular cells showed focal plasma membrane alteration and whorl formation (arrows). × 16,800.

FIG. 15, 16.—Perfusion for 1 h with taurocholate in protein-free medium. Fig. 15.—Apical region of proximal tubular cell with fusiform dilatation of the intercellular space, in which flocculent and membranous materials were present. The brush border and mitochondria were normal. × 10,300. Fig. 16.—The basal portion of the proximal tubular cell also showed dilatation of the extracellular space and a phagolysosomes contained membranous whorls. An increase in free ribosomal particles was seen in the cell sap. × 15,500.

not be distinguished from those of the normal kidney. Compared with the non-perfused kidney there was an increase in the number of apical vacuoles and phagolysosomes.

Perfusion with albumin-medium resulted in increased numbers of large phagolysosomes, containing osmiophilic material, in the apical region. Otherwise, the ultrastructural morphology of the proximal tubular epithelium was entirely normal. In contrast, autolysis for 1 h resulted in cellular oedema, mitochondrial swelling, membrane disruption of some

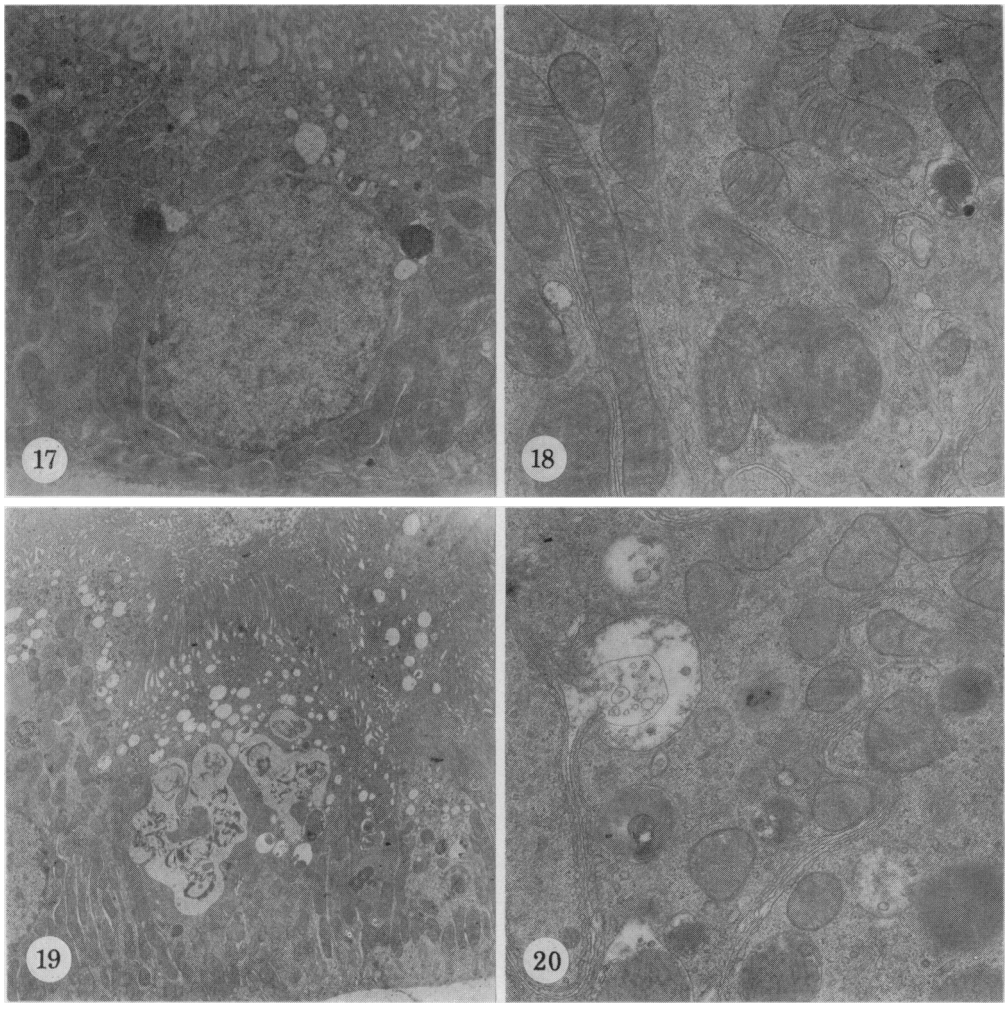

FIG. 17, 18.—Perfusion for 1 h with taurochenodeoxycholate in protein-free medium. Fig. 17.—There was slight disarray of mitochondria and irregular thickening of the basement membrane. × 6,480. Fig. 18.—Higher magnification of mitochondrial pleomorphism, × 19,400.

FIG. 19, 20.—Perfusion with taurolithocholate in albumin-medium for 1 h. Fig. 19.—There were large and irregular phagolysosomes in the upper portion of proximal tubular cells with displacement of protoplasm inward to the lumen. × 3,340. Fig. 20.—A diverticular formation was seen on one side of the basal plasma membrane fold containing vesicles and flocculent material. The smooth endoplasmic reticulum appeared as small vesicles in chains arranged parallel to the plasma membrane infoldings. × 15,500.

mitochondria and marked vesiculation of the endoplasmic reticulum (Fig. 7).

Perfusion with conjugated bilirubin in protein-free medium resulted in considerable alteration in the protoplasmic and organellar membranes of some proximal tubular cells. The microvilli of the brush border showed disarray, attenuation and disintegration with occasional whorl formation (Fig. 8). The mitochondria were enlarged and their configuration was distorted due to crowding and conformation within the available space. The mitochondrial cristae were arranged irregularly and appeared to be drawn into stacks in association with the mitochon-

drial distortion (Fig. 9). In the basal portion of the epithelial cells, the infolding plasma membrane exhibited mild undulation (Fig. 10). Free ribosomes were slightly increased in the cell sap and the basement membrane showed upward protrusion.

Perfusion with conjugated bilirubin in the albumin-medium (Fig. 11 and 12) produced more phagocytic inclusions and some focal apical protoplasmic blebbings. Apart from an occasional megamitochondrion no plasma membrane or mitochondrial membrane changes were seen. Perfusion with unconjugated bilirubin in the albumin-medium (Fig. 13, 14) caused no significant ultrastructural changes, apart from a mild degree of basal plasma membrane undulation and prominent smooth surfaced endoplasmic reticulum.

Perfusion with taurocholate resulted in minor plasma membrane changes, which were evident on the apposition surfaces of two epithelial cells. The intercellular spaces showed fusiform dilatation and contained membranous material (Fig. 15, 16). In the taurochenodeoxycholate experiments, the general ultrastructure of the proximal tubules was normal. There was mild inward protrusion of the basement membrane and some mitochondrial pleomorphism and disarray of mitochondrial cristae (Fig. 17, 18).

In the taurolithocholate experiments, with an albumin-medium, the tubular cells showed prominent phagolysosomes which contained osmiophilic and whorled membranous materials (Fig. 19). Diverticular formation was seen on the infolding plasma membrane (Fig. 20) and there was focal hyperplasia of the smooth endoplasmic reticulum.

DISCUSSION

The function of the isolated perfused kidney, assessed by the measurement of GFR and ERPF, did not reflect the presence of ultrastructural changes in the proximal tubules, since the values were not influenced by the addition of bilirubin or bile acids. Similarly, perfusion with these compounds in either the protein-free or albumin-medium did not cause significant changes on light microscopy, apart from some dilatation of the tubular lumen with flattening of tubular cells and slight expansion of the interstitial spaces. These changes are consistent with the high perfusion flow rates and the small increase in kidney weight observed after perfusion.

The control studies have demonstrated that the ultrastructure of kidneys perfused with both protein-free and albumin-medium was comparable with that of the normal kidney, particularly with regard to the mitochondria and other cytoplasmic organelles, nuclear and plasma membranes and brush border of the proximal tubular cells. The apical vacuoles, which were prominent in perfused kidneys, were considered to be physiological and consistent with normal function. The excellent ultrastructural preservation of the isolated kidney preparation justifies its use in toxicity studies, within the prescribed 1-h period.

It is well established that unconjugated bilirubin interferes with oxidative phosphorylation (Zetterström and Ernster, 1956; Ernster, 1961), and is cytotoxic to tissue culture systems (Cowger, Igo and Labbe, 1965; Lie and Bratlid, 1970) and produces swelling and structural changes in mitochondria (Mustafa, Cowger and King, 1969; Paradisi and Graziano, 1973). These effects are prevented or greatly reduced by the addition of albumin. In this study, no ultrastructural changes were seen in the kidneys perfused with unconjugated bilirubin bound to albumin and this may also reflect the protective influence of albumin.

The experiments with conjugated bilirubin and bile acids were designed so that the perfusate concentrations were similar to those achieved in the plasma of bile-duct-ligated rats. However, in the intact animal both conjugated bilirubin and bile acids are extensively bound to plasma

albumin so that, in the perfusions with conjugated bilirubin, taurocholate and taurochenodeoxycholate, the potential toxicity of the perfusate was enhanced due to the absence of protein. The ultrastructural abnormalities in plasma membranes and mitochondria observed with all these substances were most conspicuous with conjugated bilirubin. Furthermore, the abnormalities induced by conjugated bilirubin were greatly diminished by the addition of albumin to the medium. This suggests that *in vivo* the unbound fraction of conjugated bilirubin is the most likely potential cause of tubular damage. These findings are consistent with the earlier observation that the presence of obstructive jaundice sensitizes the renal parenchyma to ischaemic damage in Wistar, but not in Gunn rats (Dawson, 1964; Baum, Stirling and Dawson, 1969). Hence the elevated plasma level of conjugated bilirubin, rather than bile acids or other retained bile products, would appear to be responsible for the increased sensitivity of the tubules to ischaemic injury.

Our studies do indicate, however, that bile acids may also be cytotoxic to the proximal renal tubules, particularly with regard to the plasma membranes. Since bile acids are less tightly bound to albumin than conjugated bilirubin, relatively higher unbound concentrations are likely to occur in the plasma in obstructive jaundice and may, therefore, be associated with tubular damage. In support of this concept, renal toxicity of bile acids has been previously demonstrated by Aoyagi and Lowestein (1968); transient renal insufficiency, characterized by a fall in GFR and sodium excretion, developed in rats after an infusion of sodium cholate and taurocholate, providing this was accompanied by a 30-min period of renal ischaemia. Also, in a study of histochemical changes in the rat kidney after bile duct ligation, De Vos, De Wolf-Peeters and Desmet (1972) suggested that the appearance of alkaline phosphatase-positive vacuoles in the apical cytoplasm of proximal tubules was related, at least in part, to increased urinary bile acid excretion. In contrast to the kidney, the relationship of bile acids to liver injury has been extensively investigated (Palmer, 1972) and the ultrastructural changes in the isolated liver perfused with bile acids has been documented (Miyai, Price and Fisher, 1971). Perfusion with lithocholic acid produced injury to the bile canaliculi, whereas chenodeoxycholic acid induced widespread changes throughout the liver cells. In the present study, the mild degree of plasma membrane and mitochondrial alteration in renal tubular cells indicates that the liver and kidney probably respond differently to the injurious effects of bile acids.

Since bile acids and bilirubin both exhibit detergent-like properties, the effect of a non-ionic surface active agent (Tween 20) on the ultrastructure of the isolated kidney was examined, in an attempt to determine the mechanism underlying the toxicity (Gollan, Billing and Huang, 1976); apart from a marked vesiculation of endoplasmic reticulum and detachment of membrane-bound ribosomal particles, no mitochondrial or plasma membrane alterations were seen.

These investigations have shown that the isolated perfused kidney may be usefully employed as an experimental model to study renal toxicity under defined conditions, particularly with compounds which are normally protein-bound in plasma, such as organic anions and drugs. The results indicate that acute exposure to conjugated bilirubin and, to a lesser extent, the primary bile acids in high concentrations causes proximal tubular damage. It is, however, difficult to relate these findings to the development of renal insufficiency in obstructive jaundice, since *in vivo* the presence of albumin would greatly reduce the filtered load and hence the toxicity. It is possible that in the presence of prolonged biliary obstruction the toxic effects of these substances are additive so that the tubules become sensitized to any further

insult, such as ischaemia. These observations also support the concept that renal impairment in obstructive jaundice is multifactorial (Wardle, 1975) and it is possible that compounds other than bilirubin and bile acids, which accumulate in biliary obstruction, may also be involved.

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