the detection of RNA by autoradiography of spermatids[5] and pachytene spermatocytes[6] labeled with ^3H uridine.

The migration of the chromatoid body from the acrosomal to the caudal pole of the nucleus during spermiogenesis and its association with the Golgi apparatus in the early stages of the process have been observed by phase contrast and electron microscopy[7]. Furthermore, it has been demonstrated that multiple small vesicles accompany the body during that movement.

In the present study a cytochemical staining for acid phosphatase activity has been used in order to investigate the relationship between the Golgi apparatus and the small vesicles associated with the chromatoid body.

Materials and methods. Adult Wistar rats were anesthetized by an i.p. injection of urethane (120 mg/100 g b.wt) and the testes were perfused through the internal spermatic artery with 5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4 as previously described[8]. Sections 50–70 µm thick were obtained with a Smith Farquhar tissue sectioner or with a Lipshaw cryotome, they were then incubated for 60 min at 37 °C in Gomori's lead salt mixture[9] for acid phosphatase containing β-glycerophosphate, grade I (Sigma Chemical Co.) as substrate; postfixed in buffered OsO_4, stained with 0.5% uranyl acetate, dehydrated, and embedded in Maraglas. Sections incubated in the same medium which also contained 0.01 M sodium fluoride were used as controls, as well as mixtures lacking the substrate. Ultrathin sections were obtained with a Porter Blum microtome and the grids were observed unstained or slightly stained with uranyl acetate and lead citrate. Electron micrographs were taken with a Zeiss EM-9 A electron microscope.

Results and discussion. The presence of acid phosphatase in some lamellae, Golgi vesicles, and lysosomes resembling dense bodies in early spermatids has been reported before[10,11] and was confirmed in the present study. Furthermore, a strong positive reaction was occasionally observed in the acrosome (fig. 1).

It is a well known fact that the chromatoid body migrates during spermiogenesis from the region where the acrosome is being formed by the Golgi apparatus towards the caudal pole where the flagellum is growing out[3,7]. At the beginning of that journey, when the chromatoid body is in close association with the cisternae of the Golgi complex, many small vesicles that look like Golgi primary lysosomes and show a positive acid phosphatase reaction appear around the body; in the spaces that separate the negatively stained multiloculated structure of the chromatoid body only a few of those vesicles are seen (fig. 1).

Neither the fine structure of the chromatoid body nor its association with the small vesicles are altered during the migration to the other side of the nucleus (fig. 2). When the caudal pole of the nucleus (opposite to the acrosome) is reached, the chromatoid body is still accompanied by vesicles, some of which show lead deposits caused by acid phosphatase activity (fig. 3).

The fact that only some of the vesicles associated with the chromatoid body show the acid phosphatase reaction could be due either to functional differences[12] or to differences in enzymatic content that could, perhaps, be disclosed by another cytochemical technique.

The association of the chromatoid body with multiple small vesicles in stage 1 of spermiogenesis, as well as the contacts with the Golgi apparatus during stages 2 and 3, were also observed by Parvinen and Jokelainen[7], who proposed the possible participation of the chromatoid body in the formation of the acrosomal system; however, the present observations suggest that the transient interaction with the Golgi complex could be directed to the collection of vesicles whose hydrolytic enzymes were to be used in a later step of spermiogenesis.

It has been suggested by Clermont[13] that the smooth-surfaced vesicles frequently associated with the chromatoid body proceed from the endoplasmic reticulum; however, the presence of acid phosphatase activity in some of those vesicles indicates that some of them, at least, are provided by the Golgi apparatus.

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Structural alterations in rat kidney proximal tubules perfused with fresh autologous serum[1]

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Summary. Two min of intraluminal perfusion of the rat proximal tubules with autologous serum induced marked ultrastructural alterations including extensive cytoplasmic vesiculation due to swelling of rough endoplasmic reticulum cisternae and occasional extrusion of nuclei and cytoplasm into the lumen. Within 4 min pronounced vesiculation of mitochondria was observed. These findings are consistent with the notion that serum-induced inhibition of proximal tubular fluid absorption is due to cell lysis, presumably mediated by complement activation.

Fresh autologous serum perfused directly into the lumen of rat proximal tubule induces depolarization of intracellular potential difference, abolishes electrical resistance of the luminal cell membrane and virtually abolishes fluid transport. This effect occurs within 2 min and it is not reversible[3–5]. Although the precise mechanism for such a dramat-

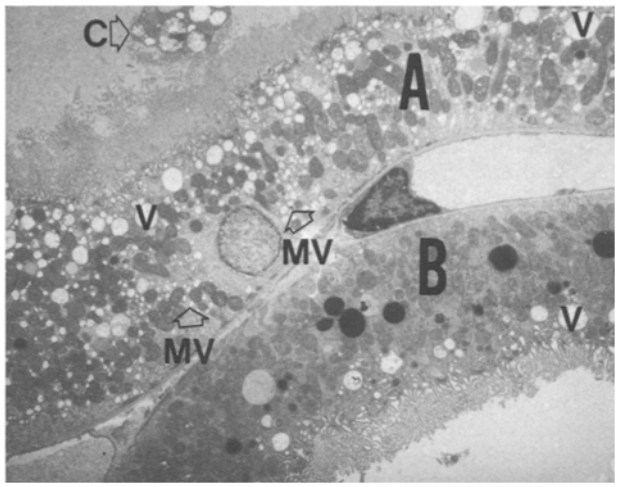

Figure 1. Electronmicrograph of a proximal tubule whose lumen was perfused for 2 min with fresh autologous serum, ×4000. A, Serum perfused tubule. B, Adjacent non-perfused tubule. C, Release of cytoplasmic mass into the lumen. MV, Microvesicles due to dilatation of rough endoplasmic reticulum. V, Large vacuoles. Note the electron dense serum protein in tubular lumen of A, the perfused tubule.

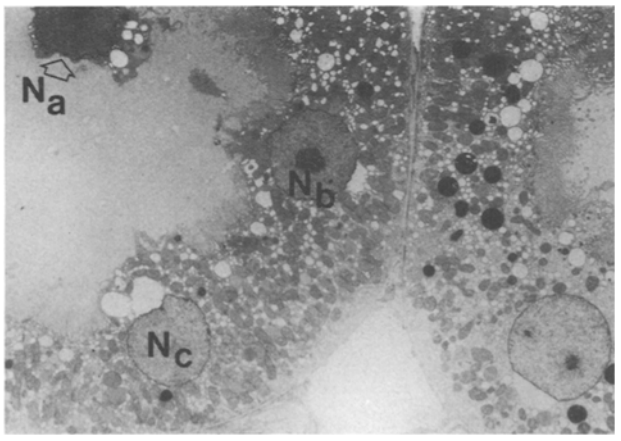

Figure 3. Electronmicrograph of proximal tubules perfused 2 min with serum, ×2500. Note release of nuclei into the lumen (N_a). Two other nuclei (N_b and N_c) appear to have migrated into a position immediately below the luminal membrane.

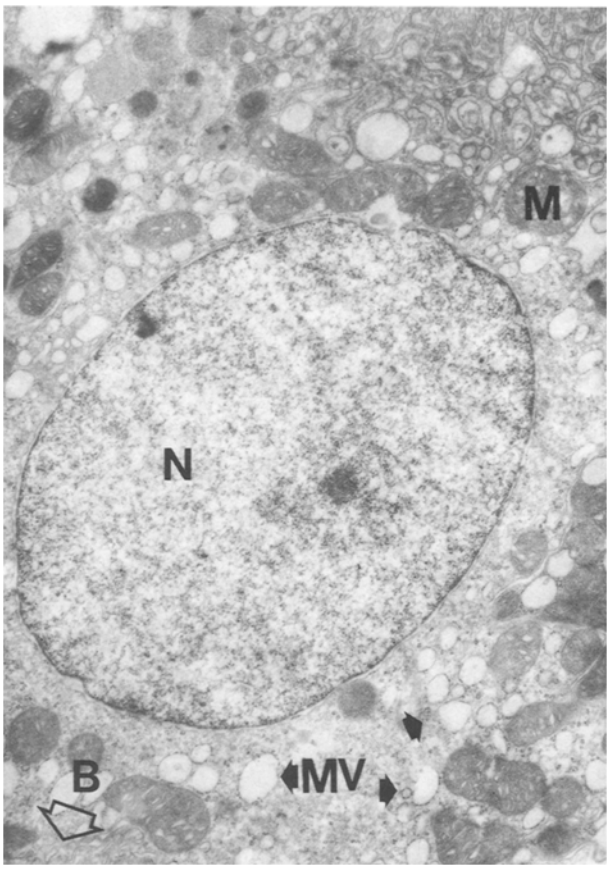

Figure 2. A higher power of the nuclear region of a cell from perfused tubule shown in figure 1, ×12,000. Note the presence of numerous small vesicles (MV), presumably due to dilatation of rough endoplasmic reticulum cisternae. BM, Tubular basement membrane. M, Mitochondria. N, Nucleus.

ic effect of fresh serum is largely unknown, available evidence indicates that the effect of serum may be due to complement-mediated cell lysis, presumably through activation of the alternative pathway[5]. Autologous serum is equally as effective as heterologous or homologous sera even after 10 times dilution with Ringer's solution[5]. Extrapolation of these findings to a clinical setting suggests the possibility that tubular atrophy, which frequently follows glomerulonephritis or the nephrotic syndrome with nonselective proteinuria[6], may be at least partially attributable to the complement-mediated tubular cell lysis by the patient's own serum. In the present study, we have described some of the ultrastructural alterations which occur during the acute state of serum-induced damage to the rat kidney proximal tubular cells.

Methods. 3 male Wister rats, 180–240 g, anesthetized with Inactin (Promonta, 80 mg/kg) were prepared for micropuncture using routine procedures[7]. Briefly, the left kidney was exposed by left flank incision and immobilized in a kidney cup. The kidney capsule was removed completely and the kidney surface covered with warm paraffin oil. Under a stereomicroscope, the proximal tubule was identified and punctured with a bevelled glass micropipette (tip diameter, 4 µm) filled with autologous serum, which was prepared from blood collected in a capillary tube from the amputated end of the tail. The tubular lumen was perfused with serum for 2 min. Dilatation of the tubular lumen during perfusion was prevented by puncturing a hole in the distal segment of the perfused proximal tubule. Serum was perfused at approximately the normal rate of tubular flow for 2 min. 2 and 4 min after completion of the intraluminal injection, perfused tubules were fixed by peritubular capillary perfusion with 3% glutaraldehyde buffered in isotonic cacodylate buffer, pH 7.4. The kidney was then removed and washed in several changes of buffered glutaraldehyde. The infused tubules and the immediately adjacent untreated tubules were microdissected and placed in 1% osmium tetroxide in 0.1 M acetate buffer, pH 7.5. The tubules perfused with heat-treated (56 °C for 30 min) serum were also included as a 2nd control. Following fixation, the tissues were dehydrated in graded concentrations of ethanol and embedded in Epon 812. Ultra-thin sections, 600–800 Å thick, were cut and stained sequentially in uranyl acetate and lead citrate and examined in a Siemens 1A electronmicroscope.

Results and discussion. As shown in figure 1, luminal perfusion of fresh serum for 2 min produced a pronounced vacuolization throughout the cytoplasm of the proximal

tubular cells. The vacuoles in these cells consist of large and small vacuoles which are more numerous than that contained in cells of the adjacent non-perfused tubules (fig. 1). At higher magnification, it can be seen that vacuoles are membrane-lined and many in the apical cytoplasm are endocytotic vesicles containing serum protein. The newly formed small vacuoles are most likely dilated cisternae of rough-surfaced endoplasmic reticulum but a few are lined by smooth membrane (fig. 2). These small vesicles are clearly distinguished from endocytotic vesicles containing electron dense serum proteins which are present only in the apical cytoplasm. Other findings include occasional clubbing of the tip of the apical microvilli with loss of microfilamentous attachments and occasional extrusion of a portion of cytoplasm (fig. 1) and nuclei (fig. 3) into the tubular lumen. The extrusion of nuclei is reminiscent of the action of cytochalasin B, a drug known to depolymerize microfilaments in vitro. Although most tight junctions appear intact morphologically, it is apparent that some tight junctions have permitted passage of electron-dense proteinaceous material into the intercellular space. Mitochondria show a mild electron translucence of intracristal spaces (fig. 2). After 4 min, tubular cells became more vesiculated and the cytoplasmic architecture appears grossly distorted. Note that most of the vesicular structures are disrupted and distorted mitochondria. By this time, many endocytotic vacuoles containing serum proteins have traveled nearly to the basal cell membrane. The fine morphology of tubules perfused with heat-inactivated serum was not significantly different from that of non-perfused control tubules and are not shown.

Discussion. These alterations of ultrastructure observed after intraluminal perfusion of fresh autologous serum are comparable to or even more pronounced than those seen after 120 min of ischemia as reported by Glaumann et al.[8]. Since the proximal tubule cells lose membrane potential and luminal membrane resistance and take up luminally perfused trypan blue[3-5] after 2 min of luminal perfusion with serum, the observed ultrastructural alterations substantiate the previous thesis that the complement-mediated cell lysis may be involved as the mechanism for serum-induced inhibition of proximal tubular fluid absorption.

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Relationship between the structure of a series of carbamate derivatives of methomyl and their biological activity

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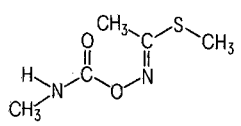
Summary. A series of 24 carbamates was obtained by structural modification of methomyl, or S-methyl-N[(methylcarbamoyl)oxy]thioacetimidate, an insecticide. The biological activity of this series and that of 7 other compounds was studied by measuring root growth of germinating corn plants with Texas and normal cytoplasm. The contribution of each substitution to phytotoxicity was determined and the sites on the molecule related to phytotoxicity and specificity localized.

Corn (*Zea mays* L.) with a mitochondrial gene for male sterility (Texas male sterile cytoplasm, or T) is highly susceptible to corn pathogens like *Helminthosporium maydis* Nisikado and Myake race T or *Phyllosticta maydis* Arny and Nelson. The insecticide methomyl or S-methyl-N[(methylcarbamoyl)oxy]thioacetimidate was found to mimic the action of pathotoxins isolated and purified from *H. maydis* and *P. maydis* cultures[2-4] both in plants[5] and on isolated mitochondria[6,7]. The study of phytotoxicity and specificity of a series of carbamates derivatives of methomyl enabled an activity/structure relationship to be determined. In the present paper we report about the action of these compounds by measuring root growth of germinating corn plants with male sterile (T) and male fertile (N) cytoplasms. The series of carbamates was obtained by progressive substitution on methomyl **1** shown below (geometric E isomer), utilized as an insecticide. (The structure presented does not indicate a preferred geometrical isomer.)



1

[a]Control root growth was normalized to 100 mm, i.e. 100%. By convention compound active and specific corresponds to 0% on Texas and 100% on Normal. [b]The 1st number corresponds to a concentration of 0.5 mg/1 ml. The 2nd number corresponds to a concentration of 1 mg/ml. The measures for each concentration were repeated twice.